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Time to act on evidence from recent large scale observational studies of the efficacy of red blood cell transfusion? Insights from a systematic review

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ABSTRACT

Objective: To carry out a systematic review of recent large observational studies on the efficacy of red blood cell transfusion (RBCT), with particular emphasis on the statistical methods used to adjust for confounding. Given the limited number of randomized trials of the efficacy of RBCT, clinicians often use evidence from observational studies. However, confounding factors, for example individuals receiving blood generally being sicker than those who do not, makes their interpretation challenging.

Design: Systematic review.

Information sources: We searched MEDLINE and EMBASE for studies published from 1 January 2006 to 31 December 2010.

Eligibility criteria for included studies: We included prospective cohort, case control studies or retrospective analyses of databases or disease registers where the effect of risk factors for mortality or survival was examined. Studies must have included more than 1000 participants receiving RBCT for any cause. We assessed the effects of RBCT versus no RBCT and different volumes and age of RBCT.

Results: Thirty two studies were included in the review; 23 assessed the effects of RBCT versus no RBCT; five assessed different volumes and four older versus newer RBCT. There was considerable variability in the patient populations, study designs and level of statistical adjustment. Overall, most studies showed a higher rate of mortality when comparing patients who received RBCT with those who did not, even when these rates were adjusted for confounding; the majority of these increases were statistically significant. The same pattern was observed in studies where protection from bias was likely to be greater, such as prospective studies.

Conclusion: Observational studies do show a consistent adverse effect of RBCT on mortality. Whether this is a true effect remains uncertain and should be addressed by conducting well designed and powered randomized controlled trials.

ARTICLE SUMMARY

Article focus

- Given the limited number of randomized trials of the efficacy of red blood cell transfusion (RBCT), clinicians often use evidence from observational studies.
- Confounding factors, for example individuals receiving blood generally being sicker than those who do not, can make their interpretation challenging.
- Our objective was to systematically review recent large observational studies (n>1000 patients) on the efficacy of RBCT, with particular emphasis on the statistical methods used to adjust for confounding.

Key messages

- We identified considerable variability in the patient populations, study designs and level of statistical adjustment.
- Most studies showed higher mortality rates when comparing patients who received RBCT with those who did not, even when adjusting for confounding. We identified similar patterns in studies where protection from bias was likely to be greatest.

Strengths and limitations of this study

- Overall, observational studies do show a consistent adverse effect of RBCT on mortality.
- However, even the best conducted adjustments for confounding cannot completely eliminate its impact, particularly when investigating the effect of RBCT on mortality.

Introduction

Randomized controlled trials are considered the gold standard with which to evaluate the efficacy of a particular health care intervention. In 2005, Blajchman (1) published a study that explored the impact that ten landmark randomized controlled trials have had on the practice of transfusion medicine. The use of randomized trials to evaluate transfusion medicine has only been established since the 1980s (1). Given the limited number of high quality randomized trials of the efficacy of blood transfusion and the challenges of conducting new trials, clinicians often rely on evidence from observational studies. In a randomized trial patients are allocated to comparison groups at random, so the level of disease is likely to be similar in each group and differences in disease severity unlikely to be the explanation for any differences in outcome seen. In an observational study whether a treatment is received or not is likely to be heavily influenced by perceived need by the treating doctor and this will be particularly true where the outcomes of transfused patients are being compared with those not transfused. In this case the groups of patients being compared are not likely to be comparable and the differences in prognostic factors may of themselves lead to difference in outcome. The impact of such "confounding" can be reduced by adjustment in the statistical analysis, but the success of this is dependent on the technique used, complete identification of the factors which might influence outcome and their accurate measurement in the patients in the study (2). As all the factors influencing outcome may never be known, adjustment is unlikely to ever completely account for the confounding occurring in observational studies. The unknown inter-dependence of multiple factors is also a major challenge.

The impact that the contribution of data from observational studies has made to the practice of transfusion medicine has not been systematically explored. However, given their publication in major journals, their impact on clinicians may be greater than is appropriate

for the types of studies and the limitations associated with their design. The aims of this systematic review were to identify recent, large observational studies on the effectiveness of red blood cell transfusion (RBCT), to critique them with particular emphasis on the statistical methods and the assumptions made in the analyses of the observational data, to consider the validity of these data as an evidence base for the practice of transfusion medicine and to inform future research in this field.

Methods

Criteria for selecting studies

Type of participants

We included both adults and children receiving RBCT for any cause. We also included studies which stated that patients received red blood cells and other blood products. When reported by the primary studies we assessed the effects of RBCT separately from other blood products.

Type of intervention and comparator

We included the following risk factors:

- RBCT versus no RBCT
- Volume 'A' of RBCT versus volume 'B' of RBCT (as defined by the primary studies)
- 'Older' RBCT versus 'newer' RBCT (as defined by the primary studies)

Type of outcome measure

Our primary outcome measure was death, mortality or survival measured at any time point.

Type of studies

We included prospective cohort, case control studies or retrospective analyses of databases or disease registers where the effect of the above risk factors on death, mortality or survival is examined. Studies must have included more than 1000 participants. This was a pragmatic limit designed to focus attention on studies most likely to have had an impact and least likely to have been affected by chance.

Search strategy

We carried out a comprehensive search of MEDLINE and EMBASE for studies published from 1 January 2006 to 31 December 2010 using the strategies in Appendix 1. We excluded conference abstracts unless they had subsequently been published as full articles.

Data collection and analysis

One review author (CD) initially screened all search results for relevance against the eligibility criteria and discarded all those that were clearly irrelevant. Thereafter, another author (SH) independently screened all remaining hits. We retrieved full text articles for all those references where we are unable to decide on eligibility based on the title and abstract alone. All full text articles were independently screened by two review authors (SH, MM) to ensure that they met the eligibility criteria.

Data extraction and management

Two review authors (SH, OO) independently extracted data from all included studies. Any disagreements were resolved by discussion or by consulting a third author if there was still uncertainty. We extracted data on the following study characteristics: the study design, how patients were recruited, the country where the study was conducted, the source of funding, the type of participants, their age, disease area, setting, the type of intervention / comparator and nature of the exposure, the number of participants in each group, whether

any formal prescribing guidance was reported, the type of outcome measure (i.e. mortality) and the time point at which it was measured.

We also extracted information on the statistical methods used to adjust for differences between study groups, in particular the number of study covariates measured, whether important covariates relating to red cell transfusion were assessed (i.e. age, sex, co-morbidity, hemoglobin) and whether these were incorporated into the analysis, whether the choice of covariates were pre-specified or data driven and the statistical model used for the statistical adjustment. We also assessed the effects of smoking as a study covariate in relation to blood transfusion and its effect on mortality. In terms of the study results we extracted data on the presentation of both the unadjusted and adjusted result for the effect of red cell transfusion on mortality as reported by each study. If not reported by the primary study we calculated (where there were sufficient data) the odds ratio for the effect of blood transfusion on mortality for unadjusted analyses using STATA (version 11). We assessed, for the unadjusted and adjusted result, whether the study reported summary statistics for each comparison group, the treatment effect, confidence interval, p value and whether the result was statistically significant. If a study reported more than one adjusted analysis we selected in order of preference (i) the main adjusted analysis mentioned in the abstract, (ii) the main adjusted analysis mentioned in the conclusions, (iii) the main adjusted analysis mentioned in the results section. If mortality was assessed for more than one time point (i.e. at 30 days and 1 year) then we used the shorter time point in our analysis.

Assessment of methodological quality

We also assessed whether studies met important methodological criteria for the reporting of observational studies: whether the samples were representative of those to whom the results might be generalised, whether important covariates in relation to RBCT and mortality

(e.g. sex, age, smoking, co-morbidity, hemoglobin level) were measured and incorporated into the analysis, whether the method of dealing with confounding between patient groups was adequate, whether a statistician was listed as an author of the study and whether the data were collected prospectively following an agreed study design.

Method of analysis

We have presented the results separately for the three different types of comparisons. Within each, due to the varied nature of the clinical conditions, study designs and level of statistical adjustment, we decided a priori not to combine the results of individual studies in a meta-analysis and instead present the results of the individual studies descriptively in the text, tables and figures.

Results

Searches of MEDLINE and EMBASE identified 4318 possible records. 4272 did not meet the eligibility criteria for this study. Full articles were retrieved for 46 studies; 14 further studies were excluded as they did not fulfil our eligibility criteria (see Figure 1). Thirty two studies were included in the review; 23 (3-26) studies assessed the effects of RBCT versus no RBCT, five studies (27-31) assessed different volumes of RBCT and four (32-35) assessed giving older versus newer RBCT.

Red blood cell transfusion versus no red blood cell transfusion

Twenty three studies (3-26) assessed the effects of RBCT versus no RBCT on mortality. Four of these studies (4;8;10;17) included both red cell transfusion and other blood products (e.g. platelets, plasma, cryoprecipitate); for one study, data were available separately for RBCT and mortality (10). For three studies it was unclear if other blood products were transfused along with red blood cells (7;9;18).

Study characteristics (Table 1)

Eight studies were prospective cohort studies following up a planned group of patients (3;4;11;12;14;20-23), the other 15 studies assessed data from a retrospective patient registry or database. Fourteen studies were conducted in the USA, two in the UK, two in Israel and the remainder in Belgium, the Netherlands, Iran and Denmark; one study was conducted in multiple countries. The time period assessed was between 1989 and 2008. Twelve of the studies (3;5;7;8;11-14;16-18;20;22) specifically looked at adults undergoing cardiac surgery, five were in patients in the intensive care unit (6;21;23;25;26), two were in adults trauma patients (4;24), two were in patients following hip fracture/replacement (9;15) one was in oncology patients (10) and the other in pediatric trauma patients (19). Three of the studies (8;16;17) specifically looked at the effects of RBCT in older adults (e.g. > 60 years). The size of the studies varied from 1,624 participants to 504,208 participants with an overall median sample size of 4344 (IQR 2085 to 11963); median 1068 (IQR 430 to 5812) for patients undergoing RBCT compared to median 2325 (IQR 1636 to 6151) for patient with no RBCT. The time period at which mortality was assessed also varied across studies from in-hospital to mortality at seven years; the most common time point being mortality at or within 30 days. Several studies reported mortality for more than one time period. Only seven of the 23 studies provided any mention of guidelines for the prescription of RBCT; two studies said no formal protocol was used (4;19), two studies stated a hemoglobin of <8g/dl (6;9), one study stated a hematocrit of less than 25-26% (18) and two studies said prescription was at the discretion of the patient care team (20;21). For full details of the characteristics of the included studies see Appendix 2.

Statistical methods (Table 2)

All 23 studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT and those who did not. However, the amount of detail and appropriateness of the method used varied across studies. In 13 studies (3;5;6;10-15;18;20;21;23;26) the choices of covariates measured were reported as pre-specified and not data driven, but this was unclear for the remaining 10 studies. The number of covariates measured and incorporated in the analysis also varied across studies with half the studies reported to assess more than 20 different covariates. Despite the high number of covariates assessed in these studies, not all measured covariates which appeared to be of specific importance in relation to RBCT. All of the 23 studies did report measuring the age and sex of the patients and 21 reported measuring patient co-morbidity. Overall, only eight (3;7;8;11-14;18;25) studies measured and incorporated the covariates age, sex, smoking, co-morbidity and haemoglobin level into the adjusted analysis.. Fourteen of the 23 studies reported using logistic regression (i.e. mortality was reported as a binary outcome) as the method of adjusting for differences in the baseline characteristics between the two patients groups; six studies reported using Cox proportional hazard (i.e. mortality was reported as a time to event outcome) and three studies reporting using both methods; in these three studies mortality was assessed for more than one time period. For full details of the statistical methods see Appendix 3.

Presentation of adjusted and unadjusted results (Table 3)

There were marked differences in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of RBCT versus no RBCT on mortality. Seven of the 23 studies reported a summary statistic for each group for both the unadjusted and adjusted analysis. Five studies reported a summary statistics for only the unadjusted analysis and one study for the adjusted analysis only; no summary statistic comparing the effects of RBCT versus no RBCT on mortality was reported in the remained 10 studies. Eight studies

reported the treatment effect (e.g. odds ratio, risk ratio, hazard ratio) and the corresponding confident interval (six studies) for both the unadjusted and adjusted analysis (3;11;12;14-16;20;22;26), whereas 12 studies reported the treatment effect and confident interval (10 studies) for adjusted analysis only and one study for the unadjusted analysis only. Where possible we calculated the odds ratio for the effect of RBCT on mortality for unadjusted analyses if it was not reported in the published article.

Seventeen of the 23 studies reported a statistically significant result for the unadjusted analysis, and 15 for the adjusted analysis (Figure 2), when comparing the effect of RBCT versus no RBCT on mortality, with more deaths occurring in patients receiving transfusion. This effect was statistically non-significant in seven studies based on the result of the adjusted analysis. Prospective studies were more likely to show a statistically significant effect for blood transfusion on mortality compared to retrospective studies for both the unadjusted and adjusted analysis. For full details see Appendix 4.

Volume 'A' red blood versus volume 'B' red blood cells

Five studies (27-31) assessed the effect of different volumes of RBCT on mortality. One of these studies (31) included both RBCT and other blood products.

Study characteristics (Table 1)

One study assessed a prospective cohort and followed up a planned group of patients (31), the other four studies assessed data from a retrospective patient registry or database. Two of the studies (29;31) specifically looked at adults undergoing cardiac surgery, one was in trauma patients (28), one was in patients undergoing major surgery (27) and one in patients in the intensive care unit (30). The size of the studies varied from 1,841 participants to 125,177 participants, with an overall median sample size of 8215 (IQR 3037 to 8799). The

volume of RBCT varied considerably across studies from 1-2 units to more than eight units. The time period at which mortality was assessed also varied across studies from in-hospital to mortality at eight years. Three of the five studies provided any mention of guidelines for the prescription of red blood cells, however only one gave any specific requirement stating a hemoglobin of <8g/dl (30) (See Appendix 2).

Statistical methods (Table 2)

All five studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received different volumes of red blood transfusion, however, as with the studies of RBCT versus no RBCT, the amount of detail and appropriateness of the method used varied across studies. In all five studies (27-31) the choices of covariates measured were reported as pre-specified. The number of covariates measured and incorporated in the analysis varied across studies with two the studies reported to assess more than 20 different covariates. Once again, despite the high number of covariates assessed in these studies, not all measured covariates seem to be of specific importance in relation to RBCT. All five studies reported measuring age and sex and patient co-morbidity, however, one (27) measured and incorporated the covariates age, sex, smoking, co-morbidity and hemoglobin level into the adjusted analysis.

Presentation of adjusted and unadjusted results (Table 3)

As with the studies of RBCT versus no RBCT, there were marked difference in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of different volumes of RBCT on mortality. Two studies reported a statistically significant result for the adjusted analysis with more deaths occurring in patients receiving larger volumes of RBCT. This effect was statistically non significant in two studies based on the result for adjusted analysis and was not reported for the remaining one study. No

studies reported on the statistical significance of the result of the unadjusted analysis (See Appendix 3 and 4).

'Older' red blood cells versus 'newer' red blood cells

Four (32-35) studies assessed the effects of age of RBCT on mortality, one of which specifically looked at leukodepleted RBCT (35).

Study characteristics (Table 1)

All four studies assessed data from a retrospective patient registry or database. Two of the studies (33;34) specifically looked at adults undergoing cardiac surgery, one was in trauma patients (35), while the other did not mention a specific patient group. The size of the studies varied from 1,813 participants to 364,037 participants, with an overall median sample size of 4358 (IQR 2264 to 185019). The period of time in which the blood was stored varied considerably across studies. Two studies (33;35) assessed RBCT stored for less than 14 days compared to those stored for more than 14 days, one study (34) compared blood stored for less than 18 days and with blood stored for more than 18 days and one study (32) looked at multiple storage periods ranging from 1 to 42 days. None of the studies provided any mention of guidelines for the prescription of red blood cells (See Appendix 2).

Statistical methods (Table 2)

All four studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT stored for different time periods, however, once again the amount of detail and appropriateness of the method used varied across studies. The number of covariates measured and incorporated in the analysis also varied across studies. All of the four studies reported measuring the age and sex of the participants. Only one study reported measuring smoking status, two studies

reported measuring patient hemoglobin levels and three studies reported assessing patient co-morbidities. Only one (33) of the four studies measured and incorporated the covariates age, sex, smoking, co-morbidity and haemoglobin level into the adjusted analysis.

Presentation of adjusted and unadjusted results (Table 3)

As with the studies of RBCT versus no RBCT and of volume 'A' red blood cells versus volume 'B' RBCT, there were marked differences in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of RBCT stored for different time periods on mortality. Two studies reported a statistically significant result for the unadjusted analysis and one study reported a statistically significant result for the adjusted analysis. In two of these three studies there were more deaths occurring in patients receiving older blood and in one study there were more deaths in patients receiving newer blood. This effect was statistically non significant in three studies based on the result for adjusted analysis (See Appendix 3 and 4).

Assessment of methodological quality (Table 4)

Overall the assessment of methodological quality varied across studies and by study group with only 10 of the 32 included studies assessing a prospective cohort following up a planned group of patients over time, the remaining two-third of the studies assessed data from a retrospective patient registry or database. In most studies the sample of patients included in the study was considered representative of those to whom the results might be generalised. Four studies (8;16;17;25) specifically focussed on older adults (>60 years) and one study (19) on children, so the findings from these studies should only be interpreted in relation to these specific patient groups. The baseline characteristics of patients who received RBCT compared to those patients who did not receive RBCT (or patients who received different volumes or age of blood) were often very different and so we wanted to

assess whether studies had adjusted for these differences when carrying out their statistical analysis. Only 10 studies measured and incorporated in the analysis covariates which we deemed of specific importance in relation to RBCT (i.e. age, sex, smoking, co-morbidity and haemoglobin level), thus we deemed the method of dealing with confounding between patient groups as adequate in only 31% of studies. Critically however, when we restricted our analysis of results to studies with adequate methods, the pattern of an increase in mortality associated with RBCT remained unchanged.

Discussion

Summary of main findings

We identified 32 observational studies of more than 1000 participants published between 2006 and 2010 assessing the effect of RBCT on mortality. Twenty three studies compared RBCT versus no RBCT, five compared different volumes and four compared different storage times. Overall there was considerable variability in the characteristics of the observational studies. However, the majority, of studies were retrospective designs assessing patients from an existing patient register or database.

We also identified considerable variability in the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT and those who did not. It was often unclear if the choice of covariates measured and used in the adjusted analyses were pre-specified at the start of the study or were driven by the underlying data. Perhaps most importantly, around half of the 32 studies did not measure and adjust for covariates which we deemed of specific importance to blood transfusion - for example, patient hemoglobin levels, age, sex and existing co-morbidities. Less than a third of studies assessed smoking which, while not directly correlated with transfusion, is an important covariate when assessing mortality.

Overall, more studies found a higher rate of mortality in patients receiving RBCT compared with those who did not, and this effect was seen in both the adjusted and unadjusted results. In general, where measured equivalently within the same study, the unadjusted estimate of risk was greater than the adjusted risk, emphasising that adverse prognostic factors are more common in patients receiving RBCT and that adjusting for them leads to a smaller estimate of risk. Considering the adjusted risks, although the size of the effect was not consistent across all studies, the direction of the effect was. Most studies suggest an increased risk of mortality associated with RBCT. Further, those studies which were designed prospectively and which used better methods of adjusting for differences in the baseline characteristics between groups were more likely to show an increase in the risk of mortality compared to studies which were based on retrospective registries or databases, although, again the size of the effect was not consistent across all studies. However, it is important to remember that even with the best methods of adjustment it cannot completely eliminate the impact of confounding (2), as the sicker the patients (thus an increased risk of mortality) the more likely they are to have received RBCT.

Comparison with other studies

We are aware of one other systematic review of observational studies looking at the effects of RBCT on mortality, which focussed specifically on critically ill adults in intensive care units and adult trauma and surgical patients (36). This systematic review by Marik and colleagues included more studies (n=45) than our review as it did not restrict its inclusion criteria to studies with >1000 patients; the median number of patients analysed was 687. They also found that RBCT was associated with an increased risk of mortality based on a meta-analysis of 12 studies (odds ratio 1.7; 95% CI 1.4 to 1.9). However there was considerable heterogeneity in the meta-analysis, suggesting that it might not have been appropriate to

combine the results of the individual studies and supports our decision not to conduct a meta-analysis.

In an overview of evidence from randomized controlled trials Wilkinson and colleagues (37) identified 142 trials in RBCT. The majority compared the effects of leucoreduced RBCT or different transfusion triggers (n=71). However, they did identify 12 trials comparing the effects of RBCT versus no transfusion, seven looking at different volumes of RBCT and 11 different ages of red blood cells. The size of the trials was very small (median 30 to 40 patients) and the overview did not specifically examine the effect of RBCT on mortality. Currently, we are aware of at least 14 ongoing or recently completed randomized controlled trials examining the effects of the age of RBCT on clinical outcomes including the ARIPI (Age of Red blood cells In Premature Infants) (38) ABLE, (Age of BLood Evaluation trial in the resuscitation of critically ill patients) (39), RECESS (REd CEll Storage duration Study) (40) and INFORM (Effects of transfusing fresh versus standard-issue red cells on in-hospital mortality) trials, for which mortality or survival is a specified outcome measure.

Limitations

Our study has several limitations. Firstly, we only included studies published in the last five years and which included more than 1,000 patients. This was because we hypothesised that studies with a larger sample size are more likely to show a truer effect of the intervention (41) and that more recent studies are more likely to use better statistical methods. It is possible therefore that the overall effect seen here might be different in older studies and/or in those carried out in smaller numbers of patients. Secondly, we decided not to combine the results of individual studies because of the variability in clinical settings and study methods, and instead presented the results of individual studies descriptively in the text and in tables and figures. More formal statistical analysis might have given a more

precise indication of the overall effect of red cell transfusion on mortality, but would have ignored the significant amount of clinical and methodological heterogeneity between studies which we identified a priori and which was very apparent in the analysis done by Marik and colleagues (36). However, in the absence of a more formal statistical analysis we have inevitably had to rely on a vote-counting approach which also has great dangers, particularly the assumption that each included study has equal weight. Our main protection against this is the very pronounced nature of the pattern we have observed and the fact that we have limited our conclusions to the direction of effect.

Finally, we limited our inclusion criteria to published articles and excluded unpublished studies or those published only as conference abstracts; thus our study could be subject to publication bias , as studies which did not show a significant effect of red cell transfusion on mortality might be less likely to be published in full (42). Outcome reporting bias may also be a problem, although difficult to combat, in the case where a risk has been measured at different time points but only those time points which are "positive" are reported. However, in the case of both publication and outcome reporting bias, the extreme nature of the pattern makes it relatively implausible that there are sufficient unpublished studies or time points to reverse it.

Conclusion

The findings from this systematic review of recent large scale observational studies show considerable variability in the patient populations and study methods when comparing the effects of RBCT on mortality. Overall, observational studies do show a consistent adverse effect of RBCT on mortality. Although it seems unlikely that this can be entirely explained by selective sampling or a predominance of poorer quality observational studies, it remains possible that even the best conducted adjustments cannot completely eliminate the impact

of confounding, particularly when investigating the effect of RBCT. We therefore believe that this can only be resolved through well designed and adequately powered randomized controlled trials. Before these can be conducted, the importance of the research question and the uncertainty of the current evidence need to be accepted. This requires clearer and more widespread presentation and understanding of the existing research evidence, to which we believe this study is a significant contribution.

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Figure 1: Flow diagram of study inclusion (1 January 2006 to 31 December 2010)

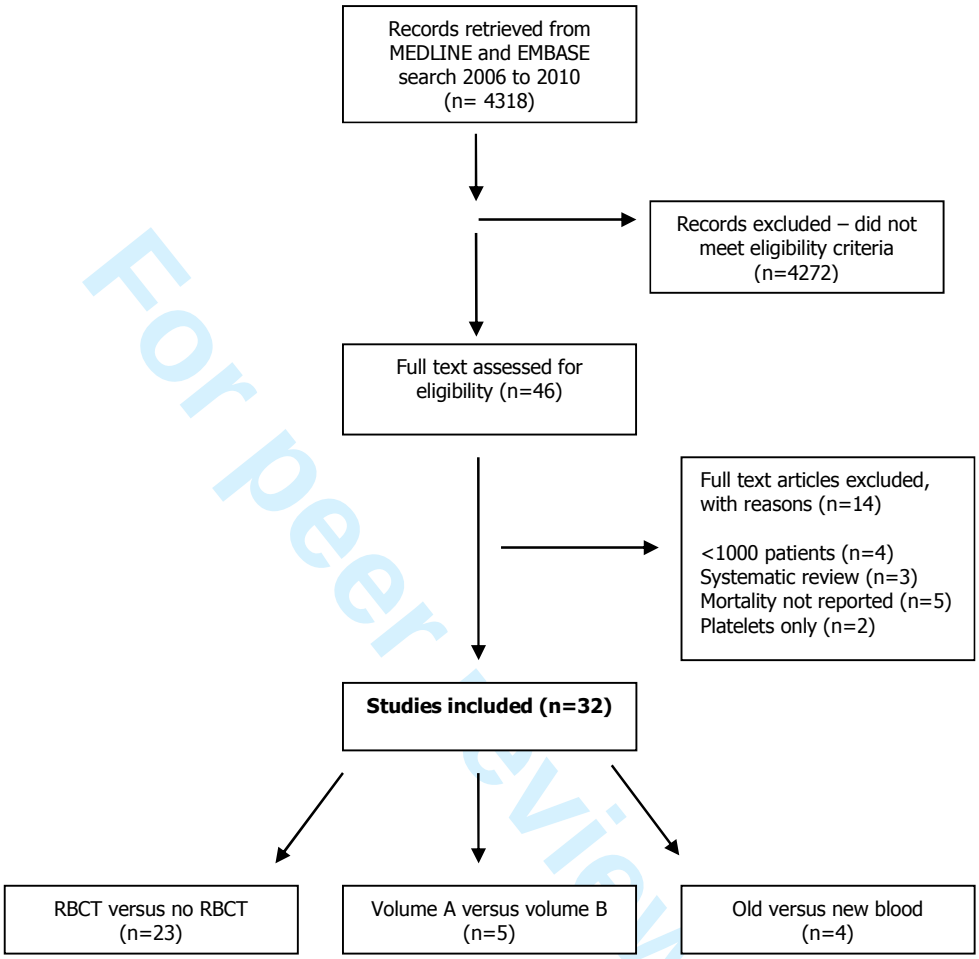

Figure 2: Effect of red blood cell transfusion versus no red blood cell transfusion on mortality (adjusted results)

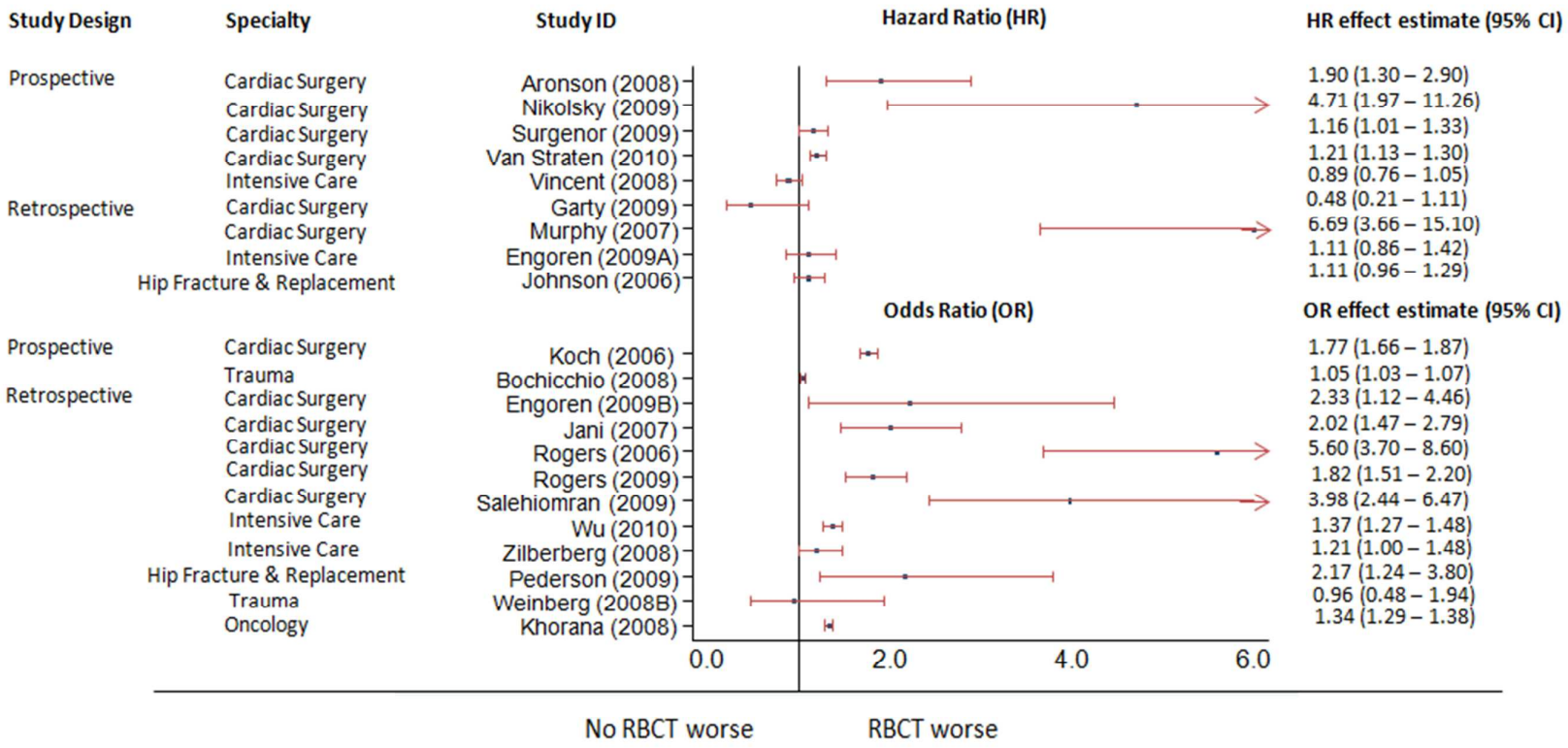

Table 1: Summary of characteristics of included studies

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B'(n=5)	Old RBC vs. new RBC (n=4)
Design			
Prospective	8 (35%)	1 (20%)	
Retrospective	15 (65%)	4 (80%)	4 (100%)
Country			
Australia		1 (20%)	
Belgium	1 (4%)		
Denmark	1 (4%)		
Germany		1 (20%)	
Iran	1 (4%)		
Israel	2 (9%)		
Netherlands	1 (4%)		1 (25%)
Sweden			1 (25%)
USA	14 (61%)	3 (60%)	2 (50%)
UK	2 (9%)		
(multiple sites)	1 (4%)		
Time period assessed	1989-2008	1993-2007	1993-2007
Sample size (median, IQR)			
All patients	4344 (IQR 2085-11963)	8215 (IQR 3037-8799)	4358 (2264-185019)
RBC transfusion	1068 (IQR 430-5812)		
No RBC transfusion	2325 (IQR 1636-6151)		
Disease area			
Cardiac surgery	12 (52%)	2 (40%)	2 (50%)
Hip fracture/replacement	2 (9%)		
Intensive care	5 (22%)	1 (20%)	
Oncology	1 (4%)		
Surgery		1 (20%)	
Trauma adults	2 (9%)	1 (20%)	1 (25%)
Trauma paediatrics	1 (4%)		
Not reported			1 (25%)
Prescribing guidance			
Reported	7 (30%)	3 (60%)	
Not reported	16 (70%)	2 (40%)	4 (100%)
Mortality assessed*			
In hospital	8	2	2
30 days	10	2	1
3 months	3		
6 months	3		
>1 year	4	1	2
Time period not specified	2		1

*studies reported mortality for >1 time point based on binary only and / or time-to-event outcome

Table 2: Method of adjusted analysis

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B' (n=5)	Old RBC vs. new RBC (n=4)
Choice of covariates			
Pre-specified	13 (57%)	5 (100%)	4 (100%)
Post hoc			
Unclear	10 (43%)		
No. of covariates measured			
1-5	2 (9%)		
6-10	4 (17%)	1 (20%)	2 (50%)
11-20	3 (13%)	2 (40%)	
>20	12 (52%)	2 (40%)	1 (25%)
Unclear	2 (9%)		1 (25%)
Important covariates assessed			
Age	23 (100%)	5 (100%)	4 (100%)
Sex	23 (100%)	5 (100%)	4 (100%)
Smoking	8 (35%)	1 (20%)	1 (25%)
Co-morbidity	21 (91%)	5 (100%)	3 (75%)
Hb level	14 (61%)	4 (80%)	2 (50%)
Important covariates incorporated into analysis			
Yes	8 (35%)	1 (20%)	1 (25%)
No	15 (65%)	4 (80%)	3 (75%)
Method of adjustment			
Cox proportional hazard	6 (26%)	1 (20%)	1 (25%)
Logistic regression	14 (61%)	4 (80%)	2 (50%)
Both *	3 (13%)		
Not reported			1 (25%)

*studies reported >1 method of adjustment when mortality was assessed for >1 time point

Table 3: Presentation of results for unadjusted and adjusted analysis (mortality)

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B' (n=5)	Old RBC vs. new RBC (n=4)
Summary statistic for each group			
Unadjusted only	5 (22%)	2 (40%)	
Adjusted only	1 (4%)		
Both	7 (30%)		1 (25%)
Not reported	10 (44%)	3 (60%)	3 (75%)
Treatment effect			
Unadjusted only	1 (4%)		
Adjusted only	12 (52%)	5 (100%)	2 (50%)
Both	8 (35%)		1 (25%)
Not reported	2 (9%)		1 (25%)
Confidence interval of treatment effect			
Unadjusted only			
Adjusted only	10 (43%)	3 (60%)	2 (50%)
Both	8 (35%)		1 (25%)
Not reported	5 (22%)	2 (40%)	1 (25%)
P-value for treatment effect			
Unadjusted only	7 (30%)		
Adjusted only		1 (20%)	
Both	1 (4%)		1 (25%)
Not reported	15 (66%)	4 (80%)	3 (75%)
Unadjusted analysis*			
Statistically significant	17 (74%)		2 (50%)
Statistically non-significant	1 (4%)		
Not reported	5 (22%)	5 (100%)	2 (50%)
Adjusted analysis*			
Statistically significant	15 (65%)	2 (40%)	1 (25%)
Statistically non-significant	7 (31%)	2 (40%)	3 (75%)
Not reported	1 (4%)	1 (20%)	

*mortality outcome – if >1 time point analysed the time point with the non-significant result was recorded

Table 4: Assessment of methodological quality of the included studies

Study ID	Data collected prospectively	Sample representative	Important covariates measured	Important covariates incorporated into analysis	Method of dealing with confounding adequate*
Aronson 2008 (3)	Yes	Unclear	Yes	Yes	Yes
Bernard 2009 (27)	No	Yes	Yes	Yes	Yes
Bochicchio 2008 (4)	Yes	Yes	No	No	No
Charles 2007 (28)	No	Yes	No	No	No
Edgren 2010 (32)	No	Yes	No	No	No
Engoren 2009 (5)	No	Yes	No	No	No
Engoren 2009 (6)	No	Yes	No	No	No
Garty 2009 (7)	No	Yes	Yes	Yes	Yes
Jani 2007 (8)	No	No (>60 years)	Yes	Yes	Yes
Johnson 2006 (9)	No	Unclear	No	No	No
Khorana 2008 (10)	No	Yes	No	No	No
Koch 2006 (11,12)	Yes	Yes	Yes	Yes	Yes
Koch 2008 (33)	No	Yes	Yes	Yes	Yes
Murphy 2007 (13)	No	Yes	Yes	Yes	Yes
Nikolsky 2009 (14)	Yes	Yes	Yes	Yes	Yes
O'Keeffe 2010 (29)	Yes	Yes	No	No	No
Pederson 2009 (15)	No	Yes	No	No	No
Rogers 2006 (16)	No	No (>65 years)	No	No	No
Rogers 2009 (17)	No	No (> 65 years)	No	No	No
Ruttinger 2007 (30)	No	Yes	No	No	No
Salehiomran 2009 (18)	No	Yes	Yes	Yes	Yes
Stone 2008 (19)	No	No (< 16 years))	No	No	No
Surgenor 2009 (20)	Yes	Yes	No	No	No
Taylor 2006 (21)	Yes	Yes	No	No	No
van de Watering 2006 (34)	No	Yes	No	No	No
van Straten 2010 (22)	Yes	Yes	No	No	No
Vincent 2008 (23)	Yes	Yes	No	No	No
Weightman 2009 (31)	Yes	Yes	No	No	No
Weinburg 2008 (24)	No	Yes	No	No	No
Weinburg 2008 (35)	No	Yes	No	No	No

Wu 2010 (25)	No	No (> 65 years)	Yes	Yes	Yes
Zilberberg 2008 (26)	No	Yes	No	No	No

*The method of dealing with confounding was deemed adequate if important covariates were measured and adjusted for in the analysis.

APPENDIX 1: Search strategies

MEDLINE (Ovid)

1. ERYTHROCYTE TRANSFUSION/
2. *BLOOD TRANSFUSION/
3. (hemotransfus* or haemotransfus*).tw.
4. ((transfus* or retransfus*) adj1 (trigger* or level* or threshold* or rule* or restrict* or limit*)).tw.
5. (transfusion* adj1 (management or practice* or polic* or strateg* or guideline* or indication* or protocol* or criteri*)).tw.
6. ((blood adj1 management) or (management adj1 blood) or (blood adj1 support) or (blood adj1 requirement*)).tw.
7. ((red cell* adj1 management) or (red cell* adj1 support) or (red cell adj1 requirement*)).tw.
8. (blood adj1 need*).tw. or transfus*.ti.
9. or/1-8
10. BLOOD TRANSFUSION/
11. ERYTHROCYTES/
12. (red cell* or red blood cell* or erythrocyte* or RBC*).tw.
13. 11 or 12
14. 10 and 13
15. (critical* or intensive or trauma or surg* or injur* or postinjur* OR organ failure* OR sepsis or septic OR infection* OR infectious OR ARDS OR acute respiratory distress OR multiorgan).ti. and transfus*.ab.
16. 9 or 14 or 15
17. BLOOD PRESERVATION/
18. transfus*.mp.
19. 17 and 18
20. ((storage or stored or storing or age* or aging or old or older or duration or fresh* or preserv* or conserv*) adj2 (whole blood or red blood cell* or red cell* or RBC*)).tw. and transfus*.mp.
21. (fresh blood or new blood or old* blood or fresh red blood cells or new red blood cells or old* red blood cells or fresh red cells or new red cells or old* red cells).tw.
22. 16 or 19 or 20 or 21
23. PROGNOSIS/
24. DISEASE FREE SURVIVAL/
25. exp CRITICAL CARE/
26. TREATMENT FAILURE/
27. exp MORTALITY/
28. SURVIVAL/
29. SURVIVAL ANALYSIS/
30. RISK ASSESSMENT/ or RISK FACTORS/
31. TREATMENT OUTCOME/
32. (survival* or survivor* or nonsurvivor* or survived or surviving).ti,ab.
33. ((predictor* or prediction*) adj1 death).tw.
34. (prognos* or mortality).tw.
35. (outcome* adj2 (therap* or treatment*)).ti,ab.
36. ((risk adj assessment) or (associated adj risk)).tw.
37. (risk* or association* or causalit* or causation or cause*).ti.
38. exp POSTOPERATIVE COMPLICATIONS/
39. exp INTRAOPERATIVE COMPLICATIONS/

40. or/23- 39
41. 22 and 40
42. BLOOD TRANSFUSION/ae, co, mo, ut or ERYTHROCYTE TRANSFUSION/ae, co, mo, ut
43. ((reaction* or effect* or efficac* or complication* or risk* or adverse* or hazard* or accident* or incident* or morbid* or death* or mortalit* or outcome*) adj3 (transfus* or postransfus* or RBC* or red cell* or erythrocyte*)).tw.
44. (transfus* or posttransfus*).ti.
45. or/41-44
46. EPIDEMIOLOGIC STUDIES/
47. exp CASE CONTROL STUDIES/
48. exp COHORT STUDIES/
49. (case* adj2 control*).tw.
50. cohort*.ti,ab.
51. (follow up adj (study or studies)).tw.
52. (observational adj2 (study or studies)).tw.
53. ((controlled adj2 trial*1) or (controlled adj2 stud*) or (comparative adj trial*) or (comparative adj stud*) or (comparison adj group*) or (comparator adj group*)).tw.
54. longitudinal.tw.
55. retrospective*.tw.
56. cross sectional.tw.
57. CROSS-SECTIONAL STUDIES/
58. Controlled clinical trial.pt.
59. CROSSOVER STUDIES/
60. Comparative study.pt.
61. CLINICAL TRIALS AS TOPIC/
62. exp CONTROLLED CLINICAL TRIALS AS TOPIC/
63. (nonrandomi* or (non adj randomi*)).tw.
64. or/46-63
65. 45 and 64

EMBASE (Ovid)

1. ERYTHROCYTE TRANSFUSION/
2. *BLOOD TRANSFUSION/ or *BLOOD COMPONENT THERAPY/
3. (hemotransfus* or haemotransfus*).tw.
4. ((transfus* or retransfus*) adj1 (trigger* or level* or threshold* or rule* or restrict* or limit*)).tw.
5. (transfusion* adj1 (management or practice* or polic* or strateg* or guideline* or indication* or protocol* or criteri*)).tw.
6. ((blood adj1 management) or (management adj1 blood) or (blood adj1 support) or (blood adj1 requirement*)).tw.
7. ((red cell* adj1 management) or (red cell* adj1 support) or (red cell adj1 requirement*)).tw.
8. (blood adj1 need*).tw. or transfus*.ti.
9. or/1-8
10. BLOOD TRANSFUSION/
11. ERYTHROCYTE/
12. (red cell* or red blood cell* or erythrocyte* or RBC*).tw.
13. 11 or 12
14. 10 and 13

15. (critical* or intensive or trauma or surg* or injur* or postinjur* OR organ failure* OR sepsis or septic OR infection* OR infectious OR ARDS OR acute respiratory distress OR multiorgan).ti. and transfus*.ab.
16. 9 or 14 or 15
17. BLOOD STORAGE/ or ERYTHROCYTE PRESERVATION/
18. transfus*.mp.
19. 17 and 18
20. ((storage or stored or storing or age* or aging or old or older or duration or fresh* or preserv* or conserv*) adj2 (whole blood or red blood cell* or red cell* or RBC*)).tw. and transfus*.mp.
21. (fresh blood or new blood or old* blood or fresh red blood cells or new red blood cells or old* red blood cells or fresh red cells or new red cells or old* red cells).tw.
22. 16 or 19 or 20 or 21
23. PROGNOSIS/
24. exp SURVIVAL/
25. exp INTENSIVE CARE/
26. exp TREATMENT OUTCOME/
27. exp EPIDEMIOLOGY/
28. RISK ASSESSMENT/ or
29. RISK BENEFIT ANALYSIS/ or RISK FACTOR/
30. RISK MANAGEMENT/
31. RISK REDUCTION/
32. (survival* or survivor* or nonsurvivor* or survived or surviving).ti,ab.
33. ((predictor* or prediction*) adj1 death).tw.
34. (prognos* or mortality).tw.
35. (outcome* adj2 (therap* or treatment*)).ti,ab.
36. (risk assessment or associated risk).tw.
37. (risk* or association* or causalit* or causation or cause*).ti.
38. exp POSTOPERATIVE COMPLICATION/
39. PEROPERATIVE COMPLICATION/
40. or/23-39
41. 22 and 40
42. ((reaction* or effect* or efficac* or complication* or risk* or adverse* or hazard* or accident* or incident* or morbid* or death* or mortalit* or outcome*) adj3 (transfus* or postransfus* or RBC* or red cell* or erythrocyte*)).tw.
43. (transfus* and posttransfus*).ti.
44. or/41-43
45. Clinical Study/
46. exp Case Control Study/
47. Family Study/
48. Longitudinal Study/
49. Retrospective Study/
50. Prospective Study/
51. Randomized Controlled Trials/
52. 50 not 51
53. Cohort Analysis/
54. Comparative Study/
55. cohort*.ti,ab.
56. (case* adj2 control*).tw.
57. (follow up adj (study or studies)).tw.
58. (observational adj2 (study or studies)).tw.

59. (epidemiologic* adj (study or studies)).tw.
60. (cross sectional adj (study or studies)).tw.
61. (retrospective* or longitudinal*).tw.
62. ((controlled adj2 trial*1) or (controlled adj2 stud*) or (comparative adj trial*) or (comparative adj stud*) or (comparison adj group*) or (comparator adj group*)).tw.
63. (nonrandomi* or (non adj randomi*)).tw.
64. or/45-49, 52-63
65. 44 and 64

APPENDIX 2: Characteristics of included studies

Study ID	Design	Objective	Participants	Intervention (exposure)	Comparator (control)	Outcome
Red blood cells versus no red blood cells – prospective studies						
Aronson 2008 (3)	Design: prospective cohort How pts recruited: admitted to intensive care unit Country: Israel Year: 2000 to 2006 Funding: not reported	Effects of RBCT in patients with acute myocardial infarction	Adults with acute MI in an intensive coronary care unit (n=2358)	RBCT (n=192) Prescribing guidance: not reported	No RBCT(n=2134)	Mortality at 6 months #
Bochicchio 2008 (4)	Design: prospective cohort How pts recruited: admitted to Adams Cowley Shock Trauma Centre Country: USA Year: 2002 to 2004 Funding: not reported	Effects of RBC and other blood product transfusion (RBC and FFP) on outcome in trauma patients	Adults admitted to intensive care unit (n=1172)	RBC and other blood product transfusion (n= 786); RBC only (n=246) Prescribing guidance: no formal protocol used	No RBC or other blood product transfusion (n=386).	Mortality (time period not specified)
Koch 2006 (11,12)	Design: prospective cohort How pts recruited: admitted to large tertiary hospital (Cleveland Clinic) Country: USA Year: 1995 to 2002 Funding: Non industry funded	Effect of RBCT on mortality in patients undergoing coronary artery bypass surgery	Adults undergoing coronary artery bypass grafting (n=11963)	RBCT (n=5812) Prescribing guidance: not reported	No RBCT(n=6151)	Mortality in-hospital
Nikolsky 2009 (14)	Design: prospective cohort How pts recruited: part of CADILLAC randomized trial comparing different mechanical reperfusion strategies Country: multi centre Year: 1997 to 1999 Funding: not reported	Effect of RBCT in patients undergoing angioplasty for acute myocardial infarction	Adults undergoing angioplasty for acute myocardial infarction (n=2060)	RBCT (n=82) Prescribing guidance: not reported	No RBCT (n=1978)	Mortality at 30 days and 1 year #
Surgenor 2009 (20)	Design: prospective cohort How pts recruited: admitted to one of eight medical centres as part of the Northern New	Effect of RBCT on mortality in patients undergoing coronary artery bypass surgery	Adults undergoing coronary artery bypass surgery (n=9079)	RBCT (n=3254) Prescribing guidance: reported as at the	No RBCT (n=5825)	Mortality over 5 years #

	England Cardiovascular Disease Study Group Country: USA Year: 2001 to 2004 Funding: not reported			discretion of the patient care team		
Taylor 2006 (21)	Design: prospective cohort How pts recruited: admitted to intensive care unit at the St John's Mercy Medical Centre Country: USA Year: 2001 to 2003 Funding: not reported	Effect of RBCT on nosocomial infection and mortality in critically ill patients	Adults admitted to critical care unit (n=2085)	RBCT (n=449) Prescribing guidance: reported as at the discretion of the patient care team	No RBCT (n=1636)	Mortality (time period not specified)
Van Straten 2010 (22)	Design: prospective cohort How pts recruited: admitted to hospital (centre not specified) Country: Netherlands Year: 1998 to 2007 Funding: not reported	Effect of RBCT on long and short term survival in patients undergoing coronary artery bypass grafting	Patients undergoing coronary artery bypass grafting (n=10425)	RBCT (n=3597) Prescribing guidance: not reported	No RBCT (n=6828)	Mortality ≤ 30 days and mortality > 30 days #
Vincent 2008 (23)	Design: prospective cohort How pts recruited: admitted to European intensive care unit (n=198 units) Country: Belgium Year: 1 May to 15 May 2002 Funding: industry supported	Effect of RBCT on mortality in European intensive care units	Adults admitted intensive care unit (n=3147)	RBCT (n=1040) Prescribing guidance: not reported	No RBCT (n=2107)	Mortality in hospital at 30 days #
Red blood cells versus no red blood cells – retrospective studies						
Engoren 2009 (5)	Design: retrospective database How pts recruited: admitted to St Vincent Mercy Medical Centre intensive care unit Country: USA Year: 2001 to 2002 Funding: not reported	Effects of RBCT in critically ill patients (excluded cardiac surgery patients)	Adults admitted to intensive care unit (n=2213)	RBCT (n=404) Prescribing guidance: haemoglobin <8 g/dl	No RBCT (n=1809)	Mortality at 30 days and 180 days #
Engoren 2009 (6)	Design: retrospective database How pts recruited: admitted to St Vincent Mercy Medical Centre	Effects of RBCT in cardiac surgery patients	Adults admitted for cardiac surgery (n=1823)	RBCT (n=378) CABG and value	No RBCT (n=615) CABG and value	Mortality within 30 days >30 days

	for cardiac surgery Country: USA Year: 1991 to 2007 Funding: not reported			RBCT (n=534) Prescribing guidance: not reported	No RBCT (n=296)	
Garty 2009 (7)	Design: retrospective database How pts recruited: admitted to cardiac or internal medicine ward in 25 public hospitals Country: Israel Year: 2003 Funding: Non industry funded	Effect of RBCT (unclear if included other blood products) on patients with acute decompressed heart failure	Adults with acute decompressed heart failure (n=2335)	RBCT (n=166) Prescribing guidance: not reported	No RBCT(n=2169)	Mortality in-hospital, 30 days, 1 year and 4 years #
Jani 2007 (8)	Design: retrospective database (Blue Cross Blue Shield of Michigan Cardiovascular Consortium) How pts recruited: admitted to academic medical centres Country: USA Year: 1997 to 2004 Funding: Blue Cross Blue Shield of Michigan	Effect of RBCT and other blood product on in-patient mortality in anaemic patients undergoing percutaneous coronary intervention (PCI) for myocardial infarction (MI)	Adults (>60 years) with anaemia undergoing PCI within 7 days for having a MI (n=4623).	RBCT and other blood product (n=1033) Prescribing guidance: no formal protocol used	No RBCT or other blood product (n=3590).	Mortality in-hospital
Johnson 2006 (9)	Design: retrospective database How pts recruited: admitted to orthopaedic unit (District General Hospital, Peterbourgh) Country: UK Year: 1989 to 2002 Funding: Non industry funded	Effect of RBCT (unclear if included other blood products) on mortality in patients with hip fracture	Adults admitted to orthopaedic unit with hip fracture (n=3625)	RBCT (n=1068) Prescribing guidance: haemoglobin <8 g/dl	No RBCT (n=2503)	Mortality at 30, 120 and 365 days #
Khorana 2008 (10)	Design: retrospective database (University Health System Consortium) How pts recruited: admitted to academic medical centres Country: USA Year: 1995 to 2003 Funding: National Cancer Institute and National Heart, Lung and Blood Institute	Effect of RBCT and other blood product on thrombosis and mortality in hospitalised patients with cancer	Adults with cancer admitted to hospital (n=504208)	RBCT and other blood product (n=74051); RBC only (n=58814) Prescribing guidance: not reported	No RBCT or other blood product (n=430157)	Mortality in-hospital

Murphy 2007 (13)	Design: 3 retrospective databases (PATS (Patient analysis and Tracking System), haematological and blood bank studys) How pts recruited: admitted to Bristol Royal Infirmary for adult cardiac surgery Country: UK Year: 1996 to 2003 Funding: British Heart Foundation	Effect of RBCT on mortality, post operative morbidity and cost in patients undergoing cardiac surgery	Adults> 16 years undergoing cardiac surgery (n=8598)	RBCT (n=4909) Prescribing guidance: not reported	No RBCT (n=3689)	Mortality up to 7 years post surgery (median 4.15 years) #
Pederson 2009 (15)	Design: retrospective database (Danish Hip Arthroplasty Registry) How pts recruited: admitted from 20 orthopaedic departments Country: Denmark Year: 1999 to 2007 Funding: not reported	Effect of RBCT on mortality in patients undergoing total hip replacement	Adults undergoing surgery for total hip replacement (n=28087)	RBCT (n=9063) Prescribing guidance: not reported	No RBCT (n=19024)	Mortality at 90 days
Rogers 2006 (16)	Design: retrospective database (Center for Medicare and Medicaid Services) How pts recruited: Medicare beneficiaries hospitalised for coronary artery bypass surgery Country: USA Year: 1997 to 1998 Funding: non industry funded	Effect of RBCT on mortality in patients undergoing coronary artery bypass surgery	Older adults (> 65 Years) undergoing coronary artery bypass surgery (n=9218)	RBCT (n=6893) Prescribing guidance: not reported	No RBCT (n=2325)	Mortality within 100 days
Rogers 2009 (17)	Design: retrospective database How pts recruited: Medicare beneficiaries who received coronary artery bypass surgery Country: USA Year: 2003 to 2006 Funding: Michigan Foundation	Effect of RBCT and other blood product on infection and mortality in patients undergoing coronary artery bypass surgery	Older adults (> 65 years) undergoing coronary artery bypass surgery (n=24789)	RBCT and other blood product (n=20789) Prescribing guidance: not reported	No RBCT or other blood product (n=4000)	Mortality in hospital and at 30 days post discharge

Salehiomran 2009 (18)	Design: retrospective database How pts recruited: patients admitted to Tehran Heart Centre who received coronary artery bypass surgery Country: Iran Year: 2002 to 2008 Funding: not reported	Effect of RBCT (unclear if included other blood products) on mortality in patients undergoing coronary artery bypass surgery	Adults undergoing coronary artery bypass surgery (n=14152)	RBCT (n=2333) Prescribing guidance: hematocrit <25-26%	No RBCT (n=11773)	Mortality at 30 days
Stone 2008 (19)	Design: retrospective database How pts recruited: admitted to paediatric trauma centre Country: USA Year: 1998 to 2006 Funding: not reported	Effect of RBCT on mortality in paediatric trauma patients	Children with blunt or penetrating injury admitted to trauma centre (n=1639)	RBCT (n=106) Prescribing guidance: reported no specific transfusion protocol was used	No RBCT (n=1533)	Mortality in-hospital
Weinberg 2008 (24)	Design: retrospective database How pts recruited: admitted to trauma centre at University of Alabama Country: USA Year: 2000 to 2007 Funding: not reported	Effect of RBCT on mortality in trauma patients	Less severely injured adults admitted to trauma centre (n=1624)	RBCT (n=430) Prescribing guidance: not reported	No RBCT (n=1194)	Mortality in hospital
Wu 2010 (25)	Design: retrospective database (Department of Veteran Affairs and National Surgical Quality Improvement Program) How pts recruited: admitted to one of 142 veteran hospitals and requiring major non cardiac surgery Country: USA Year: 1997 to 2004 Funding: not reported	Effect of RBCT on mortality in older adults after major non cardiac surgery	Older adults (>65 years) undergoing major non cardiac surgery (n=239286)	RBCT(n=22515) Prescribing guidance: not reported	No RBCT (n=216771)	Mortality at 30 days
Zilberberg 2008 (26)	Design: retrospective database (Henry Ford Health System includes data from 7 hospitals in USA) How pts recruited: admitted to hospital and requiring	Effect of RBCT on mortality in critically ill patients requiring prolonged ventilation	Adults critically ill and requiring prolonged ventilation 96 ≥hours (n=4344)	RBCT (n=2912) Prescribing guidance: not reported	No RBCT (n=1432)	Mortality in-hospital

	ventilation Country: USA Year: 2000 to 2005 Funding: industry supported					
Volume 'A' red blood cells versus volume 'B' red blood cells						
Bernard 2009 (27)	Design: retrospective database How pts recruited: admitted to hospital and requiring major surgery Country: USA Year: 2005 to 2006 Funding: industry supported	Effect of RBCT and volume of blood in patients undergoing major surgery	Adults undergoing major surgery (n=125177)	Volume of RBCT (classified as: 0 units RBC, 1 unit, 2 units, 3-4 units, 5-9 units >10 units intra operative and >4 units post operative) Prescribing guidance: no formal protocol used	Another volume of RBCT (classified as: 0 units RBC, 1 unit, 2 units, 3-4 units, 5-9 units >10 units intra operative and >4 units post operative) Prescribing guidance: no formal protocol used	Mortality at 30 days
Charles 2007 (28)	Design: retrospective database (NTRACS trauma registry) How pts recruited: admitted to Level 2 trauma centre Country: USA Year: 1994 to 2004 Funding: not reported	Effect of RBCT and volume of blood in patients with blunt trauma injuries	Adults >18 yrs with blunt trauma injuries admitted to trauma centre (n=8215)	Volume of RBCT (classified as: 0 units RBC, 1-2 units, 3-5 units, >6 units) Prescribing guidance: no formal protocol used	Another volume of RBCT (classified as: 0 units RBC, 1-2 units, 3-5 units, >6 units) Prescribing guidance: no formal protocol used	Mortality at 24 hours
O'Keefle 2010 (29)	Design: retrospective database (American College of Surgeons National Quality Improvement Program) How pts recruited: admitted from 173 hospitals and undergoing vascular surgery Country: USA Year: 2005 to 2007 Funding: not reported	Effect of RBCT on mortality in patients undergoing lower extremity revascularization	Adults with peripheral arterial disease (n=8799)	Volume of RBCT (classified as: 0 units RBC, 1 to 2 and >3 units) Prescribing guidance: not reported	Another volume of RBCT (classified as: 0 units RBC, 1 to 2 and >3 units) Prescribing guidance: not reported	Mortality at 30 days
Ruttinger 2007 (30)	Design: retrospective database How pts recruited: admitted to surgical intensive care unit LMU University Hospital., Munich Country: Germany	Effect of RBCT on mortality in critically ill patients	Patients admitted to intensive care unit (n=3037)	Volume of RBCT (classified as: 1 to 2 units (n=676) RBC, 3 to 4 (n=345), 5 to 8 (n=301) and >8 units	Another volume of RBCT (classified as: 1 to 2 units RBC, 3 to 4, 5 to 8 and >8 units)	Mortality in-hospital

	Year: 1993 to 2005 Funding: not reported			(n=471)) Prescribing guidance: haemoglobin <8-9 g/dl	Prescribing guidance: haemoglobin <8-9 g/dl	
Weightman 2009 (31)	Design: prospective cohort How pts recruited: admitted to Sir Charles Gairdner hospital Country: Australia Year: 1993 to 2000 Funding: not reported	Effect of RBCT and other blood product on long term survival in patients undergoing coronary artery bypass grafting	Patients undergoing coronary artery bypass grafting (n=1841) and survived longer than 60 days	Volume of RBCT and other blood product (classified as: 0 units (n=779), 1-2 units (n=402), 3-6 units (n=333) and > 6units (n=327)) Prescribing guidance: not reported	Another volume of RBCT and other blood product (classified as: 0 units, 1-2 units, 3-6 units and > 6units) Prescribing guidance: not reported	Mortality (mean follow up 8.1 years)#
'Older' red blood cells versus 'newer' red blood cell						
Edgren 2010 (32)	Design: retrospective database How pts recruited: received blood transfusion as recorded in Scandinavian Donations and Transfusion Study Country: Sweden and Denmark Year: 1995 to 2002 Funding: National Heart, Lung and Blood Institute of NIH	Effect of RBCT duration of storage on mortality	Adults receiving ≥ 1 RBC transfusion (n=364037)	RBCT stored for 0-9 days, 10-19 days, 20-29 days, 30-42 days Prescribing guidance: no formal protocol used	RBCT stored for 0-9 days , 10-19 days, 20-29 days, 30-42 days Prescribing guidance: no formal protocol used	Mortality ≤ 7 days and mortality 8 to 730 days#
Koch 2008 (33)	Design: retrospective database (Cleveland clinic blood bank and cardiac registries) How pts recruited: admitted to Cleveland Clinic Country: USA Year: 1998 to 2006 Funding: National Institute for Health Research and Joseph Drown Foundation	Effect of RBCT duration of storage on mortality and serious complication in patients undergoing cardiac surgery	Adults >18 years undergoing coronary-artery bypass grafting, cardiac-value surgery, or both (n=6002)	RBCT stored for ≤14 days (n=2872) Prescribing guidance: no formal protocol used	RBCT stored for >14 days (n=3130) Prescribing guidance: no formal protocol used	Mortality in-hospital and at 1 year
Van de Watering 2006 (34)	Design: retrospective database (Leiden University Medical Centre)	Effect of RBCT duration of storage on mortality in	Adults undergoing cardiothoracic surgery (n=2715)	RBCT stored for <18 days (n=942)	RBCT stored for >18 days (n=941)	Mortality at 30 days#

	How pts recruited: admitted to cardiothoracic surgery unit Country: Netherlands Year: 1993 to 1999 Funding: none industry	cardiac patients		Prescribing guidance: no formal protocol used	Prescribing guidance: no formal protocol used	
Weinberg 2008 (35)	Design: retrospective database How pts recruited: admitted to trauma centre at University of Alabama Country: USA Year: 2000 to 2007 Funding: not reported	Effect of leukodepleted RBCT transfusion and duration of storage on mortality in trauma patients	Severely injured adults admitted to trauma centre (n=1813)	RBCT stored for <14 days Prescribing guidance: no formal protocol used	RBCT stored for ≥14 days Prescribing guidance: no formal protocol used	Mortality (time period not specified)

RBCT=red blood cell transfusion; NR: not reported; #time-to-event outcome

APPENDIX 3: Statistical methods and presentation of unadjusted and adjusted results of the included studies

Study ID	Study covariates	Comparison	Unadjusted results	Adjusted results	Method of adjustment
Red blood cells versus no red blood cells – prospective studies					
Aronson 2008 (3)	Number covariates: 16 Age: Yes Sex: Yes Smoking: Yes Co-morbidity: Yes Hb level: Yes Covariates pre-specified	RBCT versus no RBCT Mortality at 6 months#	RBCT (n): NR No RBCT (n): NR Hazard ratio 4.4 (95% CI 3.2 to 5.9)	RBCT (n): NR No RBCT (n): NR Hazard ratio 1.9 (95% CI 1.3 to 2.9)	Nature of adjustment: transfusion propensity, baseline characteristics, nadir haemoglobin Type of model used: logistic regression Number covariates in model: 16
Bochicchio 2008 (4)	Number covariates: 5 Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: No Unclear if covariates pre-specified or data driven	RBCT and other blood product versus no RBCT or other blood products Mortality (time period not specified)	RBCT: 147/786 No RBCT: 32/386 p<0.001 (Odds ratio not reported)	RBCT: 147/786 No RBCT: 32/386 Odds ratio 1.05 (95% CI 1.03 to 1.07)	Nature of adjustment: age, ISS, admission GCS Type of model used: logistic regression Number covariates in model: 3
Koch 2006 (11,12)	Number covariates: multiple Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates were pre-specified	RBCT versus no RBCT Mortality in hospital	RBCT (n): NR No RBCT (n): NR Odds ratio 1.78 (95% CI 1.70 to 1.87)	RBCT (n): NR No RBCT (n): NR Odds ratio 1.77 (1.67 to 1.87)	Nature of adjustment: multiple covariates Type of model used: logistic regression Number covariate in model: multiple covariates
Nikolsky 2009 (14)	Number covariates: 25 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT versus no RBCT Mortality at 30 days and 1 year#	Not reported	Mortality at 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 4.71(95% CI 1.97 to 11.26) Mortality at 1 year RBCT (n): NR	Nature of adjustment: transfusion propensity Type of model used: Cox proportional hazards model Number covariate in model: 19

				No RBCT (n): NR Hazard ratio 3.16 (95% CI 1.66 to 6.03)	
Surgenor 2009 (20)	Number covariates: multiple Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	RBCT versus no RBCT Mortality within 5 years#	RBCT (n): NR No RBCT (n): NR Hazard ratio 1.94 (95% CI 1.71 to 2.20)	RBCT (n): NR No RBCT (n): NR Hazard ratio 1.16 (95% CI 1.01 to 1.33)	Nature of adjustment: propensity model Type of model used: Cox proportion hazard model Number covariates in model: 13
Taylor 2006 (21)	Number covariates: 5 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: no Covariates pre-specified	RBCT versus no RBCT Mortality (time period not specified)	RBCT: 98/449 No RBCT: 166/1636 p<0.001 (only p value reported)	POS ≤ 25% RBCT: 47/147 No RBCT: 105/336 p=0.88 POS 25% ≤ 50% RBCT: 17/126 No RBCT: 23/358 p=0.013 POS 50% ≤ 75% RBCT: 14/94 No RBCT: 100/390 P<0.0001 POS >75% RBCT: 3/39 No RBCT: 4/444 p=0.14 (only p value reported)	Nature of adjustment: mortality predication model (POS) Type of model used: logistic regression Number covariates in model: NR
Van Straten 2010 (22)	Number covariates: 16 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: no Unclear if covariates pre-	RBCT versus no RBCT Mortality ≤ 30 days and mortality > 30 days#	Mortality ≤ 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 1.31 (95% CI 1.27 to 1.35) Mortality > 30 days	Mortality ≤ 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 1.21 (95% CI 1.13 to 1.30) Mortality > 30 days	Nature of adjustment: unclear Type of model used: Cox proportional hazards model Number covariates in model: NR

	specified or data driven		Hazard ratio 1.16 (95% CI 1.13 to 1.20)	Hazard ratio 1.04 (95% CI 0.99 to 1.07)	
Vincent 2008 (23)	Number covariates: 8 Age: yes Sex: yes Smoking :no Co-morbidity: yes Hb level: no Covariates pre-specified	RBCT versus no RBCT Mortality at 30 days in hospital#	RBCT: 311/1040 No RBCT: 436/2107 p<0.001 (only p value reported)	RBCT: NR No RBCT: NR Hazard ratio 0.89 (95% CI 0.76 to 1.05) p=0.16	Nature of adjustment: multiple covariates Type of model used: Cox proportional hazards model Number covariates in model: 8
Red blood cells versus no red blood cells – retrospective studies					
Engoren 2009 (5)	Number covariates: 25 Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: Yes Covariates pre-specified	RBCT versus no RBCT Mortality at 30 days and 180 days#	Mortality 30 days RBCT: 101/404 No RBCT: 265/1809 Mortality 180 days RBCT: 150/404 No RBCT: 414/1809 p<0.01 (Hazard ratios not reported)	Mortality 30 days RBCT: NR No RBCT: NR Hazard ratio 1.11 (95% CI 0.86 to 1.42) Mortality 180 days RBCT: NR No RBCT: NR Hazard ratio 1.14 (95% CI 0.83 to 1.58)	Nature of adjustment: multiple variables Type of model used: Cox proportional hazard modelling Number covariates in model: NR
Engoren 2009 (6)	Number covariates: multiple Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: Yes Covariates pre-specified	RBCT versus no RBCT Mortality within 30 days and >30 days	Mortality within 30 days Value only: RBCT: 26/993 No RBCT: 16/993 CABG and value: RBCT: 69/830 No RBCT: 14/830 Mortality >30 days Value only: RBCT: 160/993 No RBCT: 165/993 CABG and value: RBCT: 279/830 No RBCT: 113/830	Mortality within 30 days Value only: Odds ratio 1.95 (95% CI 0.97 to 3.91) CABG and value: Odds ratio 2.23 (95% CI 1.12 to 4.46) Mortality >30 days Value only: Risk ratio 1.25 (95% CI 0.97 to 1.61) CABG and value: Risk ratio 1.44 (95% CI 1.13 to 1.84)	Nature of adjustment: propensity score Type of model used: Cox proportional hazard modelling (mortality >30 days) and logistic regression (mortality within 30 days) Number covariates in model: NR
Garty 2009	Number covariates:	RBCT (unclear if included	Mortality in hospital	Mortality in hospital	Nature of adjustment: propensity

(7)	unclear Age: Yes Sex: Yes Smoking: Yes Co-morbidity: Yes Hb level: Yes Unclear if covariates pre-specified or data driven	other blood product) versus no RBCT Mortality in hospital, 30 days, 1 year and 4 years#	RBCT: 18/166 (10.8%) No RBCT: 113/2169 (5.2%) Mortality 30 days RBCT: 18/166 (11%) No RBCT: 183/2169 (8.5%) Mortality 1 year RBCT: 65/166 (39.6%) No RBCT: 616/2169 (28.5%) Mortality 4 years RBCT: 114/166 (69.5%) No RBCT: 1284/2169 (59.5%)	RBCT: 9/103 (8.7%) No RBCT: 15/103 (14.6%) Hazard ratio: 0.48 (95% CI 0.21 to 1.11) Mortality 30 days RBCT: 10/103 (9.7%) No RBCT: 19/103 (18.4%) Hazard ratio: 0.29 (95% CI 0.13 to 0.64) Mortality 1 year RBCT: 40/103 (38.8%) No RBCT: 44/103 (42.7%) Hazard ratio: 0.74 (95% CI 0.50 to 1.09) Mortality 4 years RBCT: 75/103 (72.8%) No RBCT: 79/103 (76.7%) Hazard ratio: 0.86 (95% CI 0.64 to 1.14)	score Type of model used: Cox proportional hazard modelling (1-4 year mortality) and logistic regression (mortality up to 30 days) Number covariates in model: 9
Jani 2007 (8)	Number covariates: 31 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Unclear if covariates pre-specified or data driven	RBCT and other blood product versus no RBCT Mortality in hospital	RBCT: 150/1033 No RBCT: 108/3590 p<0.001 (only p value reported)	RBCT: 76/598 No RBCT: 44/598 Odds ratio 2.02 (95% CI 1.47 to 2.79)	Nature of adjustment: transfusion propensity and co morbidities Type of model used: logistic regression Number covariate in model: 10
Johnson 2006 (9)	Number covariates: 7 Age: yes Sex: yes Smoking: no Co-morbidity: no HB level: yes Unclear if covariates pre-specified or data driven	RBCT (unclear if included other blood product) versus no RBCT Mortality at 30, 120, 365 days#	Mortality 30 days RBCT: 95/1068 No RBCT: 181/2503 p=0.10 Mortality 120 days RBCT: 247/1068 No RBCT: 374/2503 p<0.0001 Mortality 365 days	Mortality 30 days (not reported) Mortality 120 days (not reported) Mortality 365 days	Nature of adjustment: age, sex, ASA grade, preoperative haemoglobin, residential status, mobility score Type of model used: Cox regression Number covariate in model: 7

			RBCT : 381/1068 No RBCT: 626/2503 p<0.001 (only p values reported)	RBCT: 381/1068 No RBCT: 626/2503 Hazard ratio 1.11 (95% CI 0.96 to 1.29)	
Khorana 2008 (10)	Number covariates: Unclear Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: no Covariates pre-specified	RBCT and other blood product versus no RBCT Mortality in hospital	RBCT (n): 11.9% No RBCT (n): NR	RBCT (n): NR No RBCT (n): NR Odds ratio 1.34 (95% 1.29 to 1.38)	Nature of adjustment: NR Type of model used: multivariate logistic regression Number covariate in model: NR
Murphy 2007 (13)	Number covariates: 21 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT versus no RBCT Mortality up to 7 years post surgery#	Not reported	Mortality 0 - 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 6.69(95% CI 3.66 to 15.1) Mortality 31 days to 1 year Hazard ratio 2.59 (95% CI 1.68 to 4.18) Mortality > 1 year Hazard ratio 1.32 (95% CI 1.08 to 1.64)	Nature of adjustment: transfusion propensity Type of model used: logistic regression and Cox proportional hazards regression Number covariate in model: NR
Pederson 2009 (15)	Number covariates: 69 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT versus no RBCT Mortality at 90 day	RBCT (n): NR No RBCT (n): NR Odds ratio 2.17 (95% CI 1.24 to 3.79)	RBCT: 39/2254 No RBCT: 18/2254 Odds ratio 2.17 (95% CI 1.24 to 3.80)	Nature of adjustment: transfusion propensity Type of model used: multivariate logistic regression Number covariate in model: NR
Rogers 2006 (16)	Number covariates: 33 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: unclear	RBCT versus no RBCT Mortality within 100 days	RBCT: 648/6893 No RBCT: 44/2325 Odds ratio 6.6 (95% CI 4.4 to 9.9)	RBCT: 648/6893 No RBCT: 44/2325 Odds ratio 5.6 (95% CI 3.7 to 8.6)	Nature of adjustment: sex, age, race, co morbidity, urgency of admission Type of model used: generalised linear regression Number covariate in model: 5

	Unclear if covariates pre-specified or data driven				
Rogers 2009 (17)	Number covariates: 13 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: no Unclear if covariates pre-specified or data driven	RBCT and other blood product versus no RBCT Mortality in hospital and at 30 days	Not reported	Mortality in hospital RBCT (n): NR No RBCT (n): NR Elective surgery: Odds ratio 4.67 (95% CI 2.38 to 9.18) Urgent surgery: Odds ratio 1.82 (95% CI 1.51 to 2.20) Mortality 30 days post discharge Elective surgery: Odds ratio 2.88 (95% CI 1.38 to 5.98) Urgent surgery: Odds ratio 4.65 (95% CI 1.90 to 11.39)	Nature of adjustment: propensity score, surgical volume, hospital volume Type of model used: multivariate mixed effect logistic regression Number covariate in model: 3
Salehiomran 2009 (18)	Number covariates: 31 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT (unclear if included other blood products) versus no RBCT Mortality at 30 days	RBCT: 60/2333 No RBCT: 42/11773 p<0.001 (Odds ratio not reported)	RBCT: 60/2333 No RBCT: 42/11773 Odds ratio 3.98 (95% CI 2.44 to 6.47)	Nature of adjustment: not reported Type of model used: multivariate logistic regression Number covariate in model: 13
Stone 2008 (19)	Number covariates: 7 Age: yes Sex: yes Smoking: N/A Co-morbidity: yes Hb level: no Unclear if covariates pre-specified or data driven	RBCT versus no RBCT Mortality in hospital	RBCT: 31/106 No RBCT: 42/1533 Odds ratio 14.67 (95% CI not reported)	Not reported (authors said statistical model was to unreliable to provide reliable conclusions)	Nature of adjustment: injury severity Type of model used: logistic regression Number covariate in model: NR
Weinberg 2008 (24)	Number covariates: 9 Age: yes Sex: yes Smoking: no Co-morbidity: no Hb level: no Unclear if covariates pre-specified or data driven	RBCT versus no RBCT Mortality in hospital	RBCT (n): 4.2% No RBCT (n): 2.3% p=0.04	RBCT (n): NR No RBCT (n): NR Odds ratio 0.96 (95% CI 0.48 to 1.94)	Nature of adjustment: age, gender, ISS, injury, ventilation, transfusion volume Type of model used: logistic regression Number of covariates in model: 5

Wu 2010 (25)	Number covariates: multiple Age: yes Sex: yes Smoking: yes Co-morbidity: yes Hb level: yes Unclear if covariates pre-specified or data driven	RBCT versus no RBCT Mortality at 30 days	Not reported	RBCT (n): NR No RBCT (n): NR Odds ratio 1.37 (95% CI 1.27 to 1.48)	Nature of adjustment: mean operative time, ASA classification, rate of general anaesthesia Type of model used: logistic regression Number covariates in model: NR
Zilberberg 2008 (26)	Number covariates: multiple Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	RBCT versus no RBCT Mortality in hospital	RBCT: 938/2912 No RBCT: 342/1432 Odds ratio 1.51 (95% CI 1.31 to 1.75)	RBCT : 938/2912 No RBCT: 342/1432 Odds ratio 1.21 (95% CI 1.00 to 1.48)	Nature of adjustment: multiple variables Type of model used: logistic regression Number covariates in model: 13
Volume 'A' red blood cells versus volume 'B' red blood cells					
Bernard 2009 (27)	Number covariates: multiple Age: Yes Sex: Yes Smoking: Yes Co-morbidity: Yes Hb level: Yes Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality at 30 days	Intra operative 1 unit: 136/1343 2 units: 194/1903 3-4 units: 151/977 5-9 units: 67/412 >10 units: 45/153 Post operative >4 units: 153/575 (Odds ratios not reported)	Intra operative 1 unit: Odds ratio 1.32 2 units: Odds ratio 1.38 3-4 units: Odds ratio 1.97 5-9 units: Odds ratio 2.17 >10 units: Odds ratio 9.83 Post operative >4 units: Odds ratio 2.65 (95% CI not reported)	Nature of adjustment: transfusion propensity, type of procedure, wound class, operative duration Type of model used: logistic regression Number covariates in model: multiple
Charles 2007 (28)	Number covariates: 7 Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: No Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality at 24 hours	0 RBCT: 1.8% 1-2 units: 6.5% 3-5 units: 16.1% ≥6 units: 29.8% (Odds ratios not reported)	1-2 units: p=0.18 3-5 units: Odds ratio 3.22 p=0.002 ≥6 units: Odds ratio 4.87 p=0.000 (95% CI not reported)	Nature of adjustment: age, gender, ISS score, SI Type of model used: logistic regression Number covariates in model: 4

O'Keeffe 2010 (29)	Number covariates: 23 Age: yes Sex: yes Smoking: unclear Co-morbidity: yes HB level: yes Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality at 30 days	Not reported	1-2 units: Odds ratio 1.92 (95% CI 1.36 to 2.70) >3 units: Odds ratio 2.48 (95% CI 1.55 to 3.98)	Nature of adjustment: transfusion propensity Type of model used: logistic regression Number covariate in model: 19
Ruttinger 2007 (30)	Number covariates: 14 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: yes Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality in hospital	% reported in figure only (Odds ratios not reported)	1-2 units: Odds ratio 0.68 (95% CI 0.35 to 1.28) 3-4 units: Odds ratio 1.11 (95% CI 0.52 to 2.39) 5-8 units: Odds ratio 1.16 (95% CI 0.60 to 2.26) 8 units: Odds ratio 0.74 (95% CI 0.36 to 1.51)	Nature of adjustment: extended analysis Type of model used: logistic regression Number covariate in model: NR
Weightman 2009 (31)	Number covariates: 16 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	Volume of RBCT and other blood product (classified as: 0 units, 1-2 units, 3-6 units and > 6units) Mortality (mean follow up 8.1 years)	0 units: 80/779 1-2 units: 56/402 3-6 units: 58/333 > 6 units: 72/327	1-2 units: Hazard ratio 1.00 (95% CI 0.70 to 1.44) 3-6 units: Hazard ratio 0.98 (95% CI 0.67 to 1.41) > 6 units: Hazard ratio 1.25 (95% CI 0.87 to 1.79)	Nature of adjustment: multiple measures Type of model used: Cox proportional hazard model Number covariates in model: 12
'Older red blood cells versus 'newer' red blood cells					
Edgren 2010 (32)	Number covariates: unclear Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: No Covariates pre-specified	RBCT storage for 0- days, 10-19 days, 20-29 and 30-42 days Mortality ≤ 7 days and mortality 8 to 730 days#	Not reported	Mortality 1 to 7 days Stored 0- 9 days: Hazard ratio 0.96 (95% CI 0.91 to 1.00) Stored 10-19 days: Hazard ratio 1.00 (95% CI not reported) Stored 20-29 days: Hazard ratio 1.06 (95% CI 0.96 to 1.06) Stored 30-42 days: Hazard ratio 1.05 (95% CI 0.97 to 1.12) Mortality 8 to 730 days Stored 0- 9 days: Hazard ratio 1.01 (95% CI 0.99 to 1.02) Stored 10-19 days: Hazard ratio 1.00 (95% CI not reported)	Nature of adjustment: number transfusions, age, sex, blood group, calendar period, season, weekday, hospital, indication Type of model used: Cox proportional hazards regression Number covariates in model: 9

				Stored 20-29 days: Hazard ratio 0.99 (95% CI 0.97 to 1.01) Stored 30-42 days: Hazard ratio 1.05 (95% CI 1.02 to 1.08)	
Koch 2008 (33)	Number covariates: multiple Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT stored for ≤ 14 days versus RBCT stored for > 14 days Mortality in hospital and at 1 year	Mortality in hospital Stored ≤ 14 days: 49/2872 Stored > 14 days: 88/3130 p=0.004 (only p value reported)	Mortality at 1 year Stored ≤ 14 days: 7.4% Stored > 14 days: 11% p<0.001 (only p value reported)	Nature of adjustment: transfusion propensity Type of model used: logistic regression Number covariate in model: NR
Van de Watering 2006 (34)	Number covariates: 7 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	RBCT stored for <18 days versus RBCT stored for >18 days Mortality at 30 days#	Stored <18 days (n): NR Hazard ratio 1.33 (95% CI 1.04 to 1.68) Stored > 18 days (n): NR Hazard ratio: 0.85 (95% CI 0.69 to 1.05)	Stored <18 days (n): NR Hazard ratio 0.93 (95% CI 0.71 to 1.23) Stored > 18 days (n): NR Hazard ratio 0.98 (95% CI 0.76 to 1.25)	Nature of adjustment: number of transfusions, duration of surgery, previous CABG, number of distal anatomises, age, sex, Hb level Type of model used: NR Number covariates in model: 7
Weinberg 2008 (35)	Number covariates: 6 Age: yes Sex: yes Smoking: no Co-morbidity: no Hb level: no Covariates pre-specified	RBCT stored for <14 days versus RBCT stored for >14 days Mortality (time period not specified)	Not reported	Stored <14 days: 1-2 units: Odds ratio 1.65 (95% CI 1.01 to 2.70) ≥ 3 units: Odds ratio 1.70 (95% CI 0.96 to 2.99) Stored ≥ 14 days: 1-2 units: Odds ratio 1.78 (95% CI 1.06 to 2.98) ≥ 3 units: Odds ratio 2.78 (95% CI 1.58 to 4.88)	Nature of adjustment: age, gender, ISS, type injury, number units transfused first 24 hours, length of hospital stay Type of model used: logistic regression Number covariates in model: 6

RBCT=red blood cell transfusion; NR: not reported; OR = odds ratio; RR = risk ratio; HR = hazard ratio; #time-to-event outcome

APPENDIX 4: Summary of unadjusted and adjusted results of the included studies

Study ID	Disease area	Comparison	Mortality	Unadjusted results	Adjusted result
Red blood cells versus no red blood cells – prospective studies					
Aronson 2008 (3)	Cardiac surgery	RBCT versus no RBCT	6 months	HR 4.4 (95% CI 3.2 to 5.9)	HR 1.9 (95% CI 1.3 to 2.9)
Bochicchio 2008 (4)	Trauma	RBCT and other blood product versus no RBCT	Time period not specified	OR 2.54 (95% CI 1.70 to 3.81)*	OR 1.05 (95% CI 1.03 to 1.07)
Koch 2006 (11,12)	Cardiac surgery	RBCT versus no RBCT	In hospital	OR 1.78 (95% CI 1.70 to 1.87)	OR 1.77 (1.67 to 1.87)
Nikolsky 2009 (14)	Cardiac surgery	RBCT versus no RBCT	30 days and 1 year	Not reported	Mortality at 30 days HR 4.71(95% CI 1.97 to 11.26) Mortality at 1 year HR 3.16 (95% CI 1.66 to 6.03)
Surgenor 2009 (20)	Cardiac surgery	RBCT versus no RBCT	≤ 5 years	HR 1.94 (95% CI 1.71 to 2.20)	HR 1.16 (95% CI 1.01 to 1.33)
Taylor 2006 (21)	Intensive care	RBCT versus no RBCT	Time period not specified	OR 2.47 (95% CI 1.88 to 3.26)*	POS ≤ 25% p=0.88 POS 25% ≤ 50% p=0.013 POS 50% ≤ 75% p<0.0001 POS >75% p=0.14
Van Straten 2010 (22)	Cardiac surgery	RBCT versus no RBCT	≤ 30 days and > 30 days	Mortality ≤ 30 days HR 1.31 (95% CI 1.27 to 1.35) Mortality > 30 days HR 1.16 (95% CI 1.13 to 1.20)	Mortality ≤ 30 days HR 1.21 (95% CI 1.13 to 1.30) Mortality > 30 days HR 1.04 (95% CI 0.99 to 1.07)
Vincent 2008 (23)	Intensive care	RBCT versus no RBCT	30 days in hospital	OR 1.64 (95% CI 1.38 to 1.94)*	HR 0.89 (95% CI 0.76 to 1.05)

Red blood cells versus no red blood cells – retrospective studies					
Engoren 2009 (5)	Intensive care	RBCT versus no RBCT	30 and 180 days	Mortality 30 days OR 1.94 (95% CI 1.50 to 2.52)* Mortality 180 days OR 1.99 (95% CI 1.58 to 2.50)*	Mortality 30 days HR 1.11 (95% CI 0.86 to 1.42) Mortality 180 days HR 1.14 (95% CI 0.83 to 1.58)
Engoren 2009 (6)	Cardiac surgery	RBCT versus no RBCT	≤ 30 days and >30 days	Mortality ≤30 days Valve only: OR 1.65 (95% CI 0.88 to 3.08)* CABG and valve: OR 5.28 (95% CI 2.95 to 9.47)* Mortality >30 days Valve only: RR 0.97 (95% CI 0.79 to 1.18)* CABG and valve: RR 2.47 (95% CI 2.03 to 3.00)*	Mortality ≤30 days Valve only: OR 1.95 (95% CI 0.97 to 3.91) CABG and valve: OR 2.23 (95% CI 1.12 to 4.46) Mortality >30 days Valve only: RR 1.25 (95% CI 0.97 to 1.61) CABG and valve: RR 1.44 (95% CI 1.13 to 1.84)
Garty 2009 (7)	Cardiac surgery	RBCT (unclear if other blood product) versus no RBCT	In hospital, 30 days, 1 year and 4 years	Mortality in hospital OR 0.77 (95% CI 0.46 to 1.31)* Mortality 30 days OR 2.21 (95% CI 1.31 to 3.74)* Mortality 1 year OR 1.62 (95% CI 1.17 to 2.25)* Mortality 4 years OR 1.51 (95% CI 1.08 to 2.12)*	Mortality in hospital HR 0.48 (95% CI 0.21 to 1.11) Mortality 30 days HR 0.29 (95% CI 0.13 to 0.64) Mortality 1 year HR 0.74 (95% CI 0.50 to 1.09) Mortality 4 years HR 0.86 (95% CI 0.64 to 1.14)
Jani 2007 (8)	Cardiac surgery	RBCT and other blood product versus no RBCT	In hospital	OR 5.48 (95% CI 4.23 to 7.09)*	OR 2.02 (95% CI 1.47 to 2.79)
Johnson 2006 (9)	Hip fracture and replacement	RBCT (unclear if other blood product) versus no RBCT	30 days, 120 days, 365 days	Mortality 30 days OR 1.84 (95% CI 1.42 to 2.38)* Mortality 120 days OR 1.71 (95% CI 1.43 to 2.05)*	Mortality 365 days HR 1.11 (95% CI 0.96 to 1.29)

				Mortality 365 days OR 1.66 (95% CI 1.42 to 1.94)*	
Khorana 2008 (10)	Oncology	RBCT and other blood product versus no RBCT	In hospital	Not reported	OR 1.34 (95% 1.29 to 1.38)
Murphy 2007 (13)	Cardiac surgery	RBCT versus no RBCT	≤7 years	Not reported	Mortality 0 - 30 days HR 6.69 (95% CI 3.66 to 15.1) Mortality 31 days to 1 year HR 2.59 (95% CI 1.68 to 4.18) Mortality > 1 year HR 1.32 (95% CI 1.08 to 1.64)
Pederson 2009 (15)	Hip fracture and replacement	RBCT versus no RBCT	90 day	OR 2.17 (95% CI 1.24 to 3.79)	OR 2.17 (95% CI 1.24 to 3.80)
Rogers 2006 (16)	Cardiac surgery	RBCT versus no RBCT	≤100 days	OR 6.6 (95% CI 4.4 to 9.9)	OR 5.6 (95% CI 3.7 to 8.6)
Rogers 2009 (17)	Cardiac surgery	RBCT and other blood product versus no RBCT	In hospital and 30 days	Not reported	Mortality in hospital Elective surgery: OR 4.67 (95% CI 2.38 to 9.18) Urgent surgery: OR 1.82 (95% CI 1.51 to 2.20) Mortality 30 days post discharge Elective surgery: OR 2.88 (95% CI 1.38 to 5.98) Urgent surgery: OR 4.65 (95% CI 1.90 to 11.39)
Salehiomran 2009 (18)	Cardiac surgery	RBCT (unclear if other blood product) versus no RBCT	30 days	OR 1.55 (95% CI 1.04 to 2.30)*	OR 3.98 (95% CI 2.44 to 6.47)
Stone 2008 (19)	Paediatric trauma	RBCT versus no RBCT	In hospital	OR 14.67 (95% CI not reported)	Not reported
Weinberg 2008 (24)	Adult trauma	RBCT versus no RBCT	In hospital	OR 1.89 (95% CI 0.97 to 3.60)*	OR 0.96 (95% CI 0.48 to 1.94)
Wu 2010 (25)	Intensive care	RBCT versus no RBCT	30 days	Not reported	OR 1.37 (95% CI 1.27 to 1.48)

Zilberberg 2008 (26)	Intensive care	RBCT versus no RBCT	In hospital	OR 1.51 (95% CI 1.31 to 1.75)	OR 1.21 (95% CI 1.00 to 1.48)
Volume 'A' red blood cells versus volume 'B' red blood cells					
Bernard 2009 (27)	Surgery	Volume RBCT versus another volume RBCT	30 days	Not reported	Intra operative 1 unit: OR 1.32(95% CI not reported) 2 units: OR 1.38(95% CI not reported) 3-4 units: OR 1.97(95% CI not reported) 5-10 units: OR 2.17(95% CI not reported) >10 units: OR 9.83(95% CI not reported) Post operative >4 units: OR 2.65 (95% CI not reported)
Charles 2007 (28)	Trauma	Volume RBCT versus another volume RBCT	24 hours	Not reported	3-5 units: OR 3.22 (95% CI not reported) ≥6 units: OR 4.87 (95% CI not reported)
O'Keeffe 2010 (29)	Cardiac surgery	Volume RBCT versus another volume RBCT	30 days	Not reported	1-2 units: OR 1.92 (95% CI 1.36 to 2.70) >3 units: OR 2.48 (95% CI 1.55 to 3.98)
Ruttinger 2007 (30)	Intensive care	Volume RBCT versus another volume of RBCT	In hospital	Not reported	1-2 units: OR 0.68 (95% CI 0.35 to 1.28) 3-4 units: OR 1.11 (95% CI 0.52 to 2.39) 5-8 units: OR 1.16 (95% CI 0.60 to 2.26) 8 units: OR 0.74 (95% CI 0.36 to 1.51)
Weightman	Cardiac surgery	Volume RBCT and other blood	Mean 8.1 year follow up	Not reported	1-2 units:

2009 (31)		product versus another volume RBCT			HR 1.00 (95% CI 0.70 to 1.44) 3-6 units: HR 0.98 (95% CI 0.67 to 1.41) > 6 units: HR 1.25 (95% CI 0.87 to 1.79)
'Older red blood cells versus 'newer' red blood cells					
Edgren 2010 (32)	Not specified	RBCT stored for 0- days, 10-19 days, 20-29 and 30-42 days	≤ 7 days and 8 to 730 days	Not reported	Mortality 1 to 7 days Stored 0- 9 days: HR 0.96 (95% CI 0.91 to 1.00) Stored 10-19 days: HR 1.00 (95% CI not reported) Stored 20-29 days: HR 1.06 (95% CI 0.96 to 1.06) Stored 30-42 days: HR 1.05 (95% CI 0.97 to 1.12) Mortality 8 to 730 days Stored 0- 9 days: HR 1.01 (95% CI 0.99 to 1.02) Stored 10-19 days: HR 1.00 (95% CI not reported) Stored 20-29 days: HR 0.99 (95% CI 0.97 to 1.01) Stored 30-42 days: HR 1.05 (95% CI 1.02 to 1.08)
Koch 2008 (33)	Cardiac surgery	RBCT stored for ≤ 14 days versus RBCT stored for > 14 days	In hospital and 1 year	Mortality in hospital OR 0.60 (95% CI 0.42 to 0.85)*	Mortality at 1 year p<0.001
Van de Watering 2006 (34)	Cardiac surgery	RBCT stored for <18 days versus RBCT stored for >18 days	30 days	Stored <18 days HR 1.33 (95% CI 1.04 to 1.68) Stored > 18 days HR 0.85 (95% CI 0.69 to 1.05)	Stored <18 days HR 0.93 (95% CI 0.71 to 1.23) Stored > 18 days HR 0.98 (95% CI 0.76 to 1.25)
Weinberg 2008 (35)	Trauma	RBCT stored for <14 days versus RBCT stored for >14 days	Time period not specified	Not reported	Stored <14 days 1-2 units: OR 1.65 (95% CI 1.01 to 2.70)

					≥ 3 units: OR 1.70 (95% CI 0.96 to 2.99) Stored ≥ 14 days 1-2 units: OR 1.78 (95% CI 1.06 to 2.98) ≥ 3 units: OR 2.78 (95% CI 1.58 to 4.88)

RBCT=red blood cell transfusion; *calculated from raw data; OR = odds ratio; RR = risk ratio; HR = hazard ratio

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7-8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Appendix 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	14 & table 4
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8-14 & figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Figure 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8-14 & table 4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	15-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16-17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	17-18
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	19

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

PRISMA 2009 Checklist

BMJ open

A systematic review of the effect of red blood cell transfusion on mortality: evidence from large scale observational studies published between 2006 and 2010

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Primary Subject Heading:	Evidence based practice
Secondary Subject Heading:	Haematology (incl blood transfusion)
Keywords:	EPIDEMIOLOGY, Blood bank & transfusion medicine < HAEMATOLOGY, INTENSIVE & CRITICAL CARE

A systematic review of the effect of red blood cell transfusion on mortality: evidence from arge scale observational studies published between 2006 and 2010

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Keywords

Systematic review, observational studies, transfusion, mortality.

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ABSTRACT

Objective: To carry out a systematic review of recently published large scale observational studies assessing the effect of red blood cell transfusion (RBCT) on mortality , with particular emphasis on the statistical methods used to adjust for confounding. Given the limited number of randomized trials of the efficacy of RBCT, clinicians often use evidence from observational studies. However, confounding factors, for example individuals receiving blood generally being sicker than those who do not, makes their interpretation challenging.

Design: Systematic review.

Information sources: We searched MEDLINE and EMBASE for studies published from 1 January 2006 to 31 December 2010.

Eligibility criteria for included studies: We included prospective cohort, case control studies or retrospective analyses of databases or disease registers where the effect of risk factors for mortality or survival was examined. Studies must have included more than 1000 participants receiving RBCT for any cause. We assessed the effects of RBCT versus no RBCT and different volumes and age of RBCT.

Results: Thirty two studies were included in the review; 23 assessed the effects of RBCT versus no RBCT; five assessed different volumes and four older versus newer RBCT. There was considerable variability in the patient populations, study designs and level of statistical adjustment. Overall, most studies showed a higher rate of mortality when comparing patients who received RBCT with those who did not, even when these rates were adjusted for confounding; the majority of these increases were statistically significant. The same pattern was observed in studies where protection from bias was likely to be greater, such as prospective studies.

Conclusion: Recent observational studies do show a consistent adverse effect of RBCT on mortality. Whether this is a true effect remains uncertain as it is possible that even the best conducted adjustments cannot completely eliminate the impact of confounding.

ARTICLE SUMMARY

Article focus

- Given the limited number of randomized trials of the efficacy of red blood cell transfusion (RBCT), clinicians often use evidence from observational studies.
- Confounding factors, for example individuals receiving blood generally being sicker than those who do not, can make their interpretation challenging.
- Our objective was to systematically review large observational studies (n>1000 patients) published in the last five years assessing the effect of RBCT on mortality, with particular emphasis on the statistical methods used to adjust for confounding.

Key messages

- We identified considerable variability in the patient populations, study designs and level of statistical adjustment.
- Most studies showed higher mortality rates when comparing patients who received RBCT with those who did not, even when adjusting for confounding. We identified similar patterns in studies where protection from bias was likely to be greatest.

Strengths and limitations of this study

- Overall, observational studies do show a consistent adverse effect of RBCT on mortality.
- However, even the best conducted adjustments for confounding cannot completely eliminate its impact, particularly when investigating the effect of RBCT on mortality.

Introduction

Randomized controlled trials are considered the gold standard with which to evaluate the efficacy of a particular health care intervention. In 2005, Blajchman (1) published a study that explored the impact that ten landmark randomized controlled trials have had on the practice of transfusion medicine. The use of randomized trials to evaluate transfusion medicine has only been established since the 1980s (1). Given the limited number of high quality randomized trials of the efficacy of blood transfusion and the challenges of conducting new trials, clinicians often rely on evidence from observational studies. In a randomized trial patients are allocated to comparison groups at random, so the level of disease is likely to be similar in each group and differences in disease severity unlikely to be the explanation for any differences in outcome seen. In an observational study, the groups of patients being compared are not likely to be comparable and the differences in prognostic factors may of themselves lead to difference in outcome. The impact of such "confounding" can be reduced by adjustment in the statistical analysis, but the success of this is dependent on the technique used, complete identification of the factors which might influence outcome and their accurate measurement in the patients in the study (2). As all the factors influencing outcome may never be known, adjustment is unlikely to ever completely account for the confounding occurring in observational studies. The unknown inter-dependence of multiple factors is also a major challenge.

There is increasing implementation of restrictive policies for transfusion, and evidence of reduction in blood use in several countries such as the United Kingdom (UK) and the United States of America (USA) with no evidence of poorer clinical outcomes(3;4). However, there remains considerable variation between hospitals in blood reduction in the UK (5) and elsewhere (6) suggesting that overall blood usage could be further reduced without compromising patient safety. Observational studies may have influenced these changes in transfusion practice along with evidence from randomized controlled trials, national

guidelines, and process driven initiatives, but the impact that the contribution of data from observational studies has made to the practice of transfusion medicine has not been systematically explored. The aims of this systematic review were therefore to identify recently published (2006 to 2010), large scale observational studies assessing the effects of red blood cell transfusion (RBCT) on mortality. In particular we aimed to critique the statistical methods, and the assumptions made in the analyses of the observational data, and to consider the validity of these data as an evidence base for the practice of transfusion medicine and to inform future research in this field.

Methods

Criteria for selecting studies

Type of participants

We included both adults and children receiving RBCT for any cause. We also included studies which stated that patients received red blood cells and other blood products. When reported by the primary studies we assessed the effects of RBCT separately from other blood products.

Type of intervention and comparator

We included the following risk factors:

- RBCT versus no RBCT
- Volume 'A' of RBCT versus volume 'B' of RBCT (as defined by the primary studies)
- 'Older' RBCT versus 'newer' RBCT (as defined by the primary studies)

Type of outcome measure

Our primary outcome measure was death, mortality or survival measured at any time point.

Type of studies

We included prospective cohort, case control studies or retrospective analyses of databases or disease registers where the effect of the above risk factors on death, mortality or survival is examined. Studies must have included more than 1000 participants. This was a pragmatic limit designed to focus attention on studies most likely to have had an impact and least likely to have been affected by chance.

Search strategy

We carried out a comprehensive search (21 January 2011) of MEDLINE and EMBASE for studies published from 1 January 2006 to 31 December 2010 using the strategies in Appendix 1. Again we chose to use a pragmatic approach and limited our search to studies published in the last five years. We also excluded conference abstracts unless they had subsequently been published as full articles.

Data collection and analysis

One review author (CD) initially screened all search results for relevance against the eligibility criteria and discarded all those that were clearly irrelevant. Thereafter, another author (SH) independently screened all remaining hits. We retrieved full text articles for all those references where we are unable to decide on eligibility based on the title and abstract alone. All full text articles were independently screened by two review authors (SH, MM) to ensure that they met the eligibility criteria.

Data extraction and management

Two review authors (SH, OO) independently extracted data from all included studies. Any disagreements were resolved by discussion or by consulting a third author if there was still uncertainty. We extracted data on the following study characteristics: the study design, how

patients were recruited, the country where the study was conducted, the source of funding, the type of participants, their age, disease area, setting, the type of intervention / comparator and nature of the exposure, the number of participants in each group, whether any formal prescribing guidance was reported, the type of outcome measure (i.e. mortality) and the time point at which it was measured.

We also extracted information on the statistical methods used to adjust for differences between study groups, in particular the number of study covariates measured, whether important covariates relating to red cell transfusion were assessed (i.e. age, sex, co-morbidity, hemoglobin) and whether these were incorporated into the analysis, whether the choice of covariates were pre-specified or data driven and the statistical model used for the statistical adjustment. We also assessed the effects of smoking as a study covariate in relation to blood transfusion and its effect on mortality. In terms of the study results we extracted data on the presentation of both the unadjusted and adjusted result for the effect of red cell transfusion on mortality as reported by each study. If not reported by the primary study we calculated (where there were sufficient data) the odds ratio for the effect of blood transfusion on mortality for unadjusted analyses using STATA (version 11). We assessed, for the unadjusted and adjusted result, whether the study reported summary statistics for each comparison group, the treatment effect, confidence interval, p value and whether the result was statistically significant. If a study reported more than one adjusted analysis we selected in order of preference (i) the main adjusted analysis mentioned in the abstract, (ii) the main adjusted analysis mentioned in the conclusions, (iii) the main adjusted analysis mentioned in the results section. If mortality was assessed for more than one time point (i.e. at 30 days and 1 year) then we used the shorter time point in our analysis.

Assessment of methodological quality

We also assessed whether studies met important methodological criteria for the reporting of observational studies (2): whether the samples were representative of those to whom the results might be generalised, whether important covariates in relation to RBCT and mortality (e.g. sex, age, smoking, co-morbidity, hemoglobin level) were measured and incorporated into the analysis, whether the method of dealing with confounding between patient groups was adequate, whether a statistician was listed as an author of the study and whether the data were collected prospectively following an agreed study design. We included smoking as a covariate as, while not directly correlated with transfusion, it is considered important when assessing mortality.

Method of analysis

We have presented the results separately for the three different types of comparisons. Within each, due to the varied nature of the clinical conditions, study designs and level of statistical adjustment, we decided a priori not to combine the results of individual studies in a meta-analysis and instead present the results of the individual studies descriptively in the text, tables and figures.

Results

Searches of MEDLINE and EMBASE identified 4318 possible records. 4272 did not meet the eligibility criteria for this study. Full articles were retrieved for 45 studies; 13 further studies were excluded as they did not fulfil our eligibility criteria (see Appendix 2 for list of excluded studies). Thirty two studies were included in the review; 23 (7-30) studies assessed the effects of RBCT versus no RBCT, five studies (31-35) assessed different volumes of RBCT and four (36-39) assessed giving older versus newer RBCT (see Figure 1).

Red blood cell transfusion versus no red blood cell transfusion

Twenty three studies (7-30) assessed the effects of RBCT versus no RBCT on mortality. Four of these studies (8;12;14;21) included both red cell transfusion and other blood products (e.g. platelets, plasma, cryoprecipitate); for one study, data were available separately for RBCT and mortality (14). For three studies it was unclear if other blood products were transfused along with red blood cells (11;13;22).

Study characteristics (Table 1)

Eight studies were prospective cohort studies following up a planned group of patients (7;8;15;16;18;24-27), the other 15 studies assessed data from a retrospective patient registry or database. Fourteen studies were conducted in the USA, two in the UK, two in Israel and the remainder in Belgium, the Netherlands, Iran and Denmark; one study was conducted in multiple countries. The time period assessed was between 1989 and 2008. Twelve of the studies (7;9;11;12;15-18;20-22;24;26) specifically looked at adults undergoing cardiac surgery, five were in patients in the intensive care unit (10;25;27;29;30), two were in adults trauma patients (8;28), two were in patients following hip fracture/replacement (13;19) one was in oncology patients (14) and the other in pediatric trauma patients (23). Three of the studies (12;20;21) specifically looked at the effects of RBCT in older adults (e.g. > 60 years). The size of the studies varied from 1,624 participants to 504,208 participants with an overall median sample size of 4344 (IQR 2085 to 11963); median 1068 (IQR 430 to 5812) for patients undergoing RBCT compared to median 2325 (IQR 1636 to 6151) for patient with no RBCT. The time period at which mortality was assessed also varied across studies from in-hospital to mortality at seven years; the most common time point being mortality at or within 30 days. Several studies reported mortality for more than one time period. Only seven of the 23 studies provided any mention of guidelines for the prescription of RBCT; two studies said no formal protocol was used (8;23), two studies stated a hemoglobin of <8g/dl (10;13), one study stated a hematocrit of less than 25-26% (22) and

two studies said prescription was at the discretion of the patient care team (24;25). For full details of the characteristics of the included studies see Appendix 3.

Statistical methods (Table 2)

All 23 studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT and those who did not. However, the amount of detail and appropriateness of the method used varied across studies. In 13 studies (7;9;10;14-19;22;24;25;27;30) the choices of covariates measured were reported as pre-specified and not data driven, but this was unclear for the remaining 10 studies. The number of covariates measured and incorporated in the analysis also varied across studies with half the studies reported to assess more than 20 different covariates. Despite the high number of covariates assessed in these studies, not all measured covariates which appeared to be of specific importance in relation to RBCT. All of the 23 studies did report measuring the age and sex of the patients and 21 reported measuring patient co-morbidity. Overall, only eight (7;11;12;15-18;22;29) studies measured and incorporated the covariates age, sex, smoking, co-morbidity and haemoglobin level into the adjusted analysis.. Fourteen of the 23 studies reported using logistic regression (i.e. mortality was reported as a binary outcome) as the method of adjusting for differences in the baseline characteristics between the two patients groups; six studies reported using Cox proportional hazard (i.e. mortality was reported as a time to event outcome) and three studies reporting using both methods; in these three studies mortality was assessed for more than one time period. Nine studies (7;12;17-19;21;27;29) reported using propensity scores prior to adjusting for confounders, however, sometimes this matching was only using a much smaller subgroup of patients. For full details of the statistical methods see Appendix 4.

Presentation of adjusted and unadjusted results (Table 3)

There were marked differences in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of RBCT versus no RBCT on mortality. Seven of the 23 studies reported a summary statistic for each group for both the unadjusted and adjusted analysis. Five studies reported a summary statistics for only the unadjusted analysis and one study for the adjusted analysis only; no summary statistic comparing the effects of RBCT versus no RBCT on mortality was reported in the remained 10 studies. Eight studies reported the treatment effect (e.g. odds ratio, risk ratio, hazard ratio) and the corresponding confident interval (six studies) for both the unadjusted and adjusted analysis (7;15;16;18-20;24;26;30), whereas 12 studies reported the treatment effect and confident interval (10 studies) for adjusted analysis only and one study for the unadjusted analysis only. Where possible we calculated the odds ratio for the effect of RBCT on mortality for unadjusted analyses if it was not reported in the published article.

Seventeen of the 23 studies reported a statistically significant result for the unadjusted analysis, and 15 for the adjusted analysis (Figure 2), when comparing the effect of RBCT versus no RBCT on mortality, with more deaths occurring in patients receiving transfusion. This effect was statistically non-significant in seven studies based on the result of the adjusted analysis. Prospective studies were more likely to show a statistically significant effect for blood transfusion on mortality compared to retrospective studies for both the unadjusted and adjusted analysis. For full details see Appendix 5.

Volume 'A' red blood versus volume 'B' red blood cells

Five studies (31-35) assessed the effect of different volumes of RBCT on mortality. One of these studies (35) included both RBCT and other blood products.

Study characteristics (Table 1)

One study assessed a prospective cohort and followed up a planned group of patients (35), the other four studies assessed data from a retrospective patient registry or database. Two of the studies (33;35) specifically looked at adults undergoing cardiac surgery, one was in trauma patients (32), one was in patients undergoing major surgery (31) and one in patients in the intensive care unit (34). The size of the studies varied from 1,841 participants to 125,177 participants, with an overall median sample size of 8215 (IQR 3037 to 8799). The volume of RBCT varied considerably across studies from 1-2 units to more than eight units. The time period at which mortality was assessed also varied across studies from in-hospital to mortality at eight years. Three of the five studies provided any mention of guidelines for the prescription of red blood cells, however only one gave any specific requirement stating a hemoglobin of <8g/dl (34) (See Appendix 3).

Statistical methods (Table 2)

All five studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received different volumes of red blood transfusion, however, as with the studies of RBCT versus no RBCT, the amount of detail and appropriateness of the method used varied across studies. In all five studies (31-35) the choices of covariates measured were reported as pre-specified. The number of covariates measured and incorporated in the analysis varied across studies with two the studies reported to assess more than 20 different covariates. Once again, despite the high number of covariates assessed in these studies, not all measured covariates seem to be of specific importance in relation to RBCT. All five studies reported measuring age and sex and patient co-morbidity, however, one (31) measured and incorporated the covariates age, sex, smoking, co-morbidity and hemoglobin level into the adjusted analysis.

Presentation of adjusted and unadjusted results (Table 3)

As with the studies of RBCT versus no RBCT, there were marked difference in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of different volumes of RBCT on mortality. Two studies reported a statistically significant result for the adjusted analysis with more deaths occurring in patients receiving larger volumes of RBCT. This effect was statistically non significant in two studies based on the result for adjusted analysis and was not reported for the remaining one study. No studies reported on the statistical significance of the result of the unadjusted analysis (See Appendix 4 and 5).

'Older' red blood cells versus 'newer' red blood cells

Four (36-39) studies assessed the effects of age of RBCT on mortality, one of which specifically looked at leukodepleted RBCT (39).

Study characteristics (Table 1)

All four studies assessed data from a retrospective patient registry or database. Two of the studies (37;38) specifically looked at adults undergoing cardiac surgery, one was in trauma patients (39), while the other did not mention a specific patient group. The size of the studies varied from 1,813 participants to 364,037 participants, with an overall median sample size of 4358 (IQR 2264 to 185019). The period of time in which the blood was stored varied considerably across studies. Two studies (37;39) assessed RBCT stored for less than 14 days compared to those stored for more than 14 days, one study (38) compared blood stored for less than 18 days and with blood stored for more than 18 days and one study (36) looked at multiple storage periods ranging from 1 to 42 days. None of the studies provided any mention of guidelines for the prescription of red blood cells (See Appendix 2).

Statistical methods (Table 2)

All four studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT stored for different time periods, however, once again the amount of detail and appropriateness of the method used varied across studies. The number of covariates measured and incorporated in the analysis also varied across studies. All of the four studies reported measuring the age and sex of the participants. Only two studies reported measuring patient hemoglobin levels and three studies reported assessing patient co-morbidities. Only one (37) of the four studies measured and incorporated the covariates age, sex, smoking, co-morbidity and haemoglobin level into the adjusted analysis.

Presentation of adjusted and unadjusted results (Table 3)

As with the studies of RBCT versus no RBCT and of volume 'A' red blood cells versus volume 'B' RBCT, there were marked differences in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of RBCT stored for different time periods on mortality. Two studies reported a statistically significant result for the unadjusted analysis and one study reported a statistically significant result for the adjusted analysis. In two of these three studies there were more deaths occurring in patients receiving older blood and in one study there were more deaths in patients receiving newer blood. This effect was statistically non significant in three studies based on the result for adjusted analysis (See Appendix 4 and 5).

Assessment of methodological quality (Table 4)

Overall the assessment of methodological quality varied across studies and by study group with only 10 of the 32 included studies assessing a prospective cohort following up a planned group of patients over time, the remaining two-third of the studies assessed data from a retrospective patient registry or database. In most studies the sample of patients

included in the study was considered representative of those to whom the results might be generalised. Four studies (12;20;21;29) specifically focussed on older adults (>60 years) and one study (23) on children, so the findings from these studies should only be interpreted in relation to these specific patient groups. The baseline characteristics of patients who received RBCT compared to those patients who did not receive RBCT (or patients who received different volumes or age of blood) were often very different and so we wanted to assess whether studies had adjusted for these differences when carrying out their statistical analysis. Only 10 studies measured and incorporated in the analysis covariates which we deemed of specific importance in relation to RBCT (i.e. age, sex, smoking, co-morbidity and haemoglobin level), thus we deemed the method of dealing with confounding between patient groups as adequate in only 31% of studies. Critically however, when we restricted our analysis of results to studies with adequate methods, the pattern of an increase in mortality associated with RBCT remained unchanged.

Discussion

Summary of main findings

We identified 32 observational studies of more than 1000 participants published between 2006 and 2010 assessing the effect of RBCT on mortality. Twenty three studies compared RBCT versus no RBCT, five compared different volumes and four compared different storage times. Overall there was considerable variability in the characteristics of the observational studies. However, the majority, of studies were retrospective designs assessing patients from an existing patient register or database.

We also identified considerable variability in the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT and those who did not. It was often unclear if the choice of covariates measured and used in the adjusted

analyses were pre-specified at the start of the study or were driven by the underlying data. Perhaps most importantly, around half of the 32 studies did not measure and adjust for covariates which we deemed of specific importance to blood transfusion..

Overall, more studies found a higher rate of mortality in patients receiving RBCT compared with those who did not, and this effect was seen in both the adjusted and unadjusted results. In general, where measured equivalently within the same study, the unadjusted estimate of risk was greater than the adjusted risk, emphasising that adverse prognostic factors are more common in patients receiving RBCT and that adjusting for them leads to a smaller estimate of risk. Considering the adjusted risks, although the size of the effect was not consistent across all studies, the direction of the effect was. Most studies suggest an increased risk of mortality associated with RBCT. Further, those studies which were designed prospectively and which used better methods of adjusting for differences in the baseline characteristics between groups were more likely to show an increase in the risk of mortality compared to studies which were based on retrospective registries or databases, although, again the size of the effect was not consistent across all studies. However, it is important to remember that even with the best methods of adjustment it cannot completely eliminate the impact of confounding (2), as the sicker the patients (thus an increased risk of mortality) the more likely they are to have received RBCT.

Comparison with other studies

We are aware of one other systematic review of observational studies looking at the effects of RBCT on mortality, which focussed specifically on critically ill adults in intensive care units and adult trauma and surgical patients (40). This systematic review by Marik and colleagues included more studies (n=45) than our review as it did not restrict its inclusion criteria to studies with >1000 patients; the median number of patients analysed was 687. They also

found that RBCT was associated with an increased risk of mortality based on a meta-analysis of 12 studies (odds ratio 1.7; 95% CI 1.4 to 1.9). However there was considerable heterogeneity in the meta-analysis, suggesting that it might not have been appropriate to combine the results of the individual studies and supports our decision not to conduct a meta-analysis.

In an overview of evidence from randomized controlled trials Wilkinson and colleagues (41) identified 142 trials in RBCT. The majority compared the effects of leucoreduced RBCT or different transfusion triggers (n=71). However, they did identify 12 trials comparing the effects of RBCT versus no transfusion, seven looking at different volumes of RBCT and 11 different ages of red blood cells. The size of the trials was very small (median 30 to 40 patients) and the overview did not specifically examine the effect of RBCT on mortality. Currently, we are aware of at least 14 ongoing or recently completed randomized controlled trials examining the effects of the age of RBCT on clinical outcomes including the ARIPI (Age of Red blood cells In Premature Infants) (42) ABLE, (Age of BLood Evaluation trial in the resuscitation of critically ill patients) (43), RECESS (REd CEll Storage duration Study) (44) and INFORM (Effects of transfusing fresh versus standard-issue red cells on in-hospital mortality) trials, for which mortality or survival is a specified outcome measure.

Limitations

Our study has several limitations. Firstly, we only included studies published in the last five years and which included more than 1,000 patients. This was because we took a pragmatic approach as we hypothesised that more recent studies were more likely to use better statistical methods and also that studies with a larger sample size were more likely to show a truer effect of the intervention (45) . Thus we aimed to provide a “snap shot” of current practice rather provide a comprehensive review of all available evidence. It is possible

therefore that the overall effect seen here might be different in older studies and/or in those carried out in smaller numbers of patients. Secondly, we decided not to combine the results of individual studies because of the variability in clinical settings and study methods, and instead presented the results of individual studies descriptively in the text and in tables and figures. More formal statistical analysis might have given a more precise indication of the overall effect of red cell transfusion on mortality, but would have ignored the significant amount of clinical and methodological heterogeneity between studies which we identified a priori and which was very apparent in the analysis done by Marik and colleagues (40). However, in the absence of a more formal statistical analysis we have inevitably had to rely on a vote-counting approach which also has great dangers, particularly the assumption that each included study has equal weight. Our main protection against this is the very pronounced nature of the pattern we have observed and the fact that we have limited our conclusions to the direction of effect.

Finally, we limited our inclusion criteria to published articles and excluded unpublished studies or those published only as conference abstracts; thus our study could be subject to publication bias , as studies which did not show a significant effect of red cell transfusion on mortality might be less likely to be published in full (46). Outcome reporting bias may also be a problem, although difficult to combat, in the case where a risk has been measured at different time points but only those time points which are “positive” are reported. However, in the case of both publication and outcome reporting bias, the extreme nature of the pattern makes it relatively implausible that there are sufficient unpublished studies or time points to reverse it.

Implications for clinical practice

In recent years, many developed countries including the UK, USA and Australia have developed national initiatives for better blood transfusion practice, sometimes called 'patient blood management' (4;5). These include the development of guidelines on blood usage promoting restrictive transfusion strategies and initiatives for using alternatives to transfusion (e.g. cell salvage techniques; improvements in the education and training of clinical staff prescribing blood; the provision of mechanisms for reviewing blood use with feedback of data to clinicians). National data on blood usage in the USA suggests an estimated decline of 3% over each of the last two years (2009-2010) (4), and similar data are available in the UK where the demand for red cell units, which steadily increased during the 1990s, has decreased by about 20% in the last 10 years. However, there remains considerable variation between hospitals in blood reduction, and national audits of blood components in the UK and elsewhere suggest that overall blood usage could be further reduced without compromising patient safety (3).

It is difficult to assess how observational studies may have influenced these changes in transfusion practice in comparison to evidence from randomized controlled trials, national guidelines, and process driven initiatives. The most likely answer is that they have all played a role in changing practice. Randomized controlled trials have found that 'restrictive' transfusion strategies are associated with similar or improved clinical outcomes compared to 'liberal' transfusion strategies (47). Many national guidelines have adopted restrictive transfusion strategies (47), while needing to make assumptions about the generalisability of the findings of randomized controlled trials in specific clinical groups of patients. There have been many smaller observational studies of process initiatives to reduce transfusion that also indicate reductions in the use of blood without any significant impact on clinical outcomes (48-50).

Conclusion

The findings from this systematic review of recent large scale observational studies show considerable variability in the patient populations and study methods when comparing the effects of RBCT on mortality. Overall, observational studies do show a consistent adverse effect of RBCT on mortality. Although it seems unlikely that this can be entirely explained by selective sampling or a predominance of poorer quality observational studies, it remains possible that even the best conducted adjustments cannot completely eliminate the impact of confounding. ..

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Figure 1: Flow diagram of study inclusion (1 January 2006 to 31 December 2010)

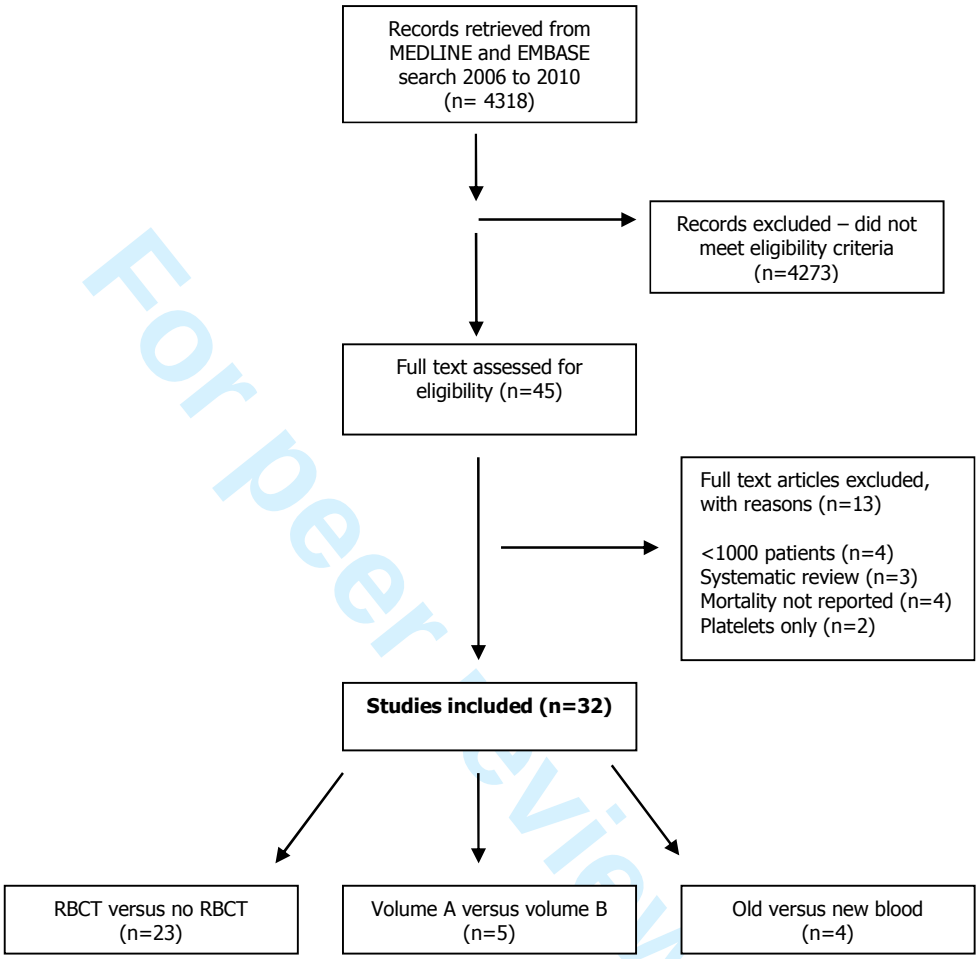

Figure 2: Effect of red blood cell transfusion versus no red blood cell transfusion on mortality (adjusted results)

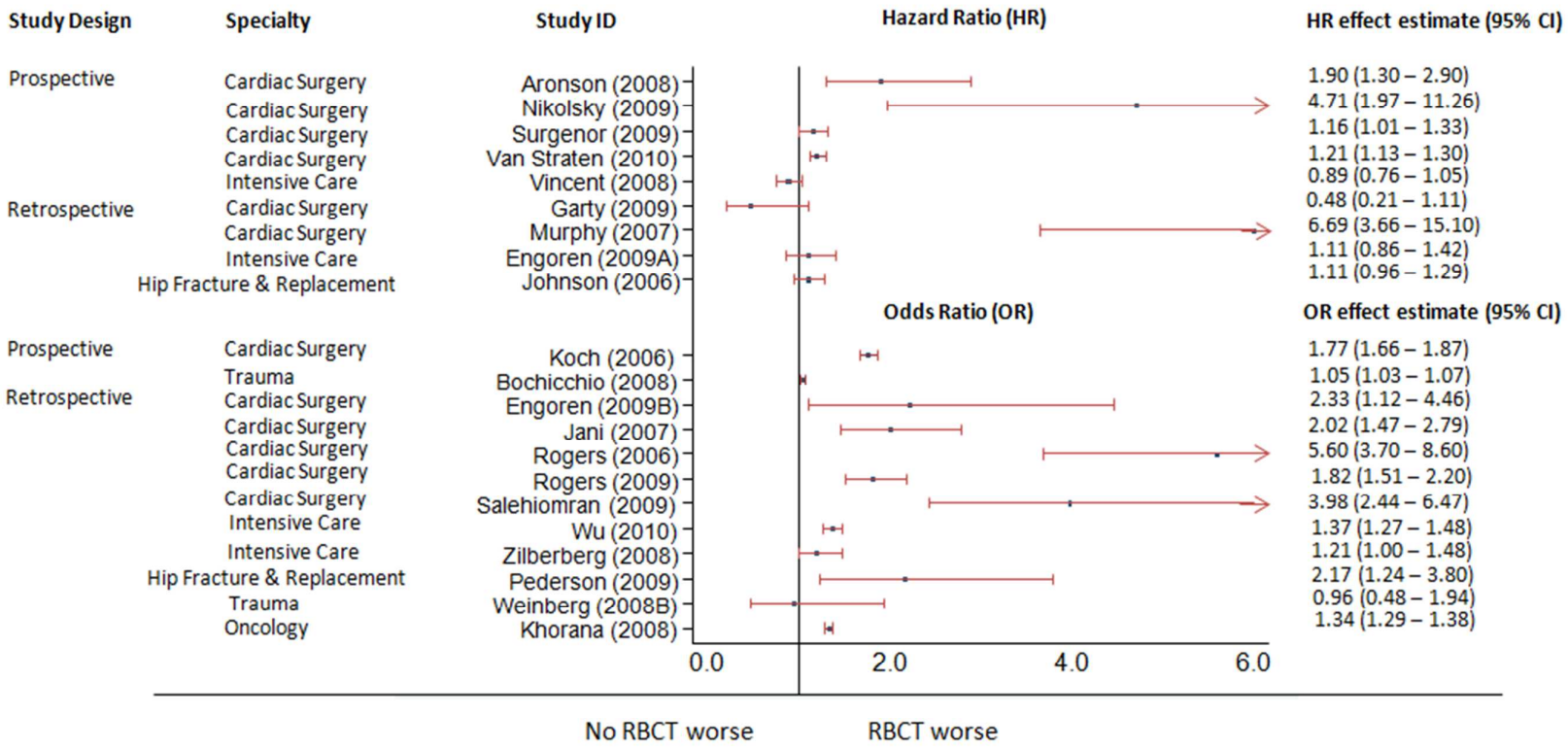

Table 1: Summary of characteristics of included studies

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B'(n=5)	Old RBC vs. new RBC (n=4)
Design			
Prospective	8 (35%)	1 (20%)	
Retrospective	15 (65%)	4 (80%)	4 (100%)
Country			
Australia		1 (20%)	
Belgium	1 (4%)		
Denmark	1 (4%)		
Germany		1 (20%)	
Iran	1 (4%)		
Israel	2 (9%)		
Netherlands	1 (4%)		1 (25%)
Sweden			1 (25%)
USA	14 (61%)	3 (60%)	2 (50%)
UK	2 (9%)		
(multiple sites)	1 (4%)		
Time period assessed	1989-2008	1993-2007	1993-2007
Sample size (median, IQR)			
All patients	4344 (IQR 2085-11963)	8215 (IQR 3037-8799)	4358 (2264-185019)
RBC transfusion	1068 (IQR 430-5812)		
No RBC transfusion	2325 (IQR 1636-6151)		
Disease area			
Cardiac surgery	12 (52%)	2 (40%)	2 (50%)
Hip fracture/replacement	2 (9%)		
Intensive care	5 (22%)	1 (20%)	
Oncology	1 (4%)		
Surgery		1 (20%)	
Trauma adults	2 (9%)	1 (20%)	1 (25%)
Trauma paediatrics	1 (4%)		
Not reported			1 (25%)
Prescribing guidance			
Reported	7 (30%)	3 (60%)	
Not reported	16 (70%)	2 (40%)	4 (100%)
Mortality assessed*			
In hospital	8	2	2
30 days	10	2	1
3 months	3		
6 months	3		
>1 year	4	1	2
Time period not specified	2		1

*studies reported mortality for >1 time point based on binary only and / or time-to-event outcome

Table 2: Method of adjusted analysis

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B' (n=5)	Old RBC vs. new RBC (n=4)
Choice of covariates			
Pre-specified	13 (57%)	5 (100%)	4 (100%)
Post hoc			
Unclear	10 (43%)		
No. of covariates measured			
1-5	2 (9%)		
6-10	4 (17%)	1 (20%)	2 (50%)
11-20	3 (13%)	2 (40%)	
>20	12 (52%)	2 (40%)	1 (25%)
Unclear	2 (9%)		1 (25%)
Important covariates assessed			
Age	23 (100%)	5 (100%)	4 (100%)
Sex	23 (100%)	5 (100%)	4 (100%)
Smoking	8 (35%)	1 (20%)	1 (25%)
Co-morbidity	21 (91%)	5 (100%)	3 (75%)
Hb level	14 (61%)	4 (80%)	2 (50%)
Important covariates incorporated into analysis			
Yes	8 (35%)	1 (20%)	1 (25%)
No	15 (65%)	4 (80%)	3 (75%)
Method of adjustment			
Cox proportional hazard	6 (26%)	1 (20%)	1 (25%)
Logistic regression	14 (61%)	4 (80%)	2 (50%)
Both *	3 (13%)		
Not reported			1 (25%)

*studies reported >1 method of adjustment when mortality was assessed for >1 time point

Table 3: Presentation of results for unadjusted and adjusted analysis (mortality)

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B' (n=5)	Old RBC vs. new RBC (n=4)
Summary statistic for each group			
Unadjusted only	5 (22%)	2 (40%)	
Adjusted only	1 (4%)		
Both	7 (30%)		1 (25%)
Not reported	10 (44%)	3 (60%)	3 (75%)
Treatment effect			
Unadjusted only	1 (4%)		
Adjusted only	12 (52%)	5 (100%)	2 (50%)
Both	8 (35%)		1 (25%)
Not reported	2 (9%)		1 (25%)
Confidence interval of treatment effect			
Unadjusted only			
Adjusted only	10 (43%)	3 (60%)	2 (50%)
Both	8 (35%)		1 (25%)
Not reported	5 (22%)	2 (40%)	1 (25%)
P-value for treatment effect			
Unadjusted only	7 (30%)		
Adjusted only		1 (20%)	
Both	1 (4%)		1 (25%)
Not reported	15 (66%)	4 (80%)	3 (75%)
Unadjusted analysis*			
Statistically significant	17 (74%)		2 (50%)
Statistically non-significant	1 (4%)		
Not reported	5 (22%)	5 (100%)	2 (50%)
Adjusted analysis*			
Statistically significant	15 (65%)	2 (40%)	1 (25%)
Statistically non-significant	7 (31%)	2 (40%)	3 (75%)
Not reported	1 (4%)	1 (20%)	

*mortality outcome – if >1 time point analysed the time point with the non-significant result was recorded

Table 4: Assessment of methodological quality of the included studies

Study ID	Data collected prospectively	Sample representative	Important covariates measured	Important covariates incorporated into analysis	Method of dealing with confounding adequate*
Aronson 2008 (3)	Yes	Unclear	Yes	Yes	Yes
Bernard 2009 (27)	No	Yes	Yes	Yes	Yes
Bochicchio 2008 (4)	Yes	Yes	No	No	No
Charles 2007 (28)	No	Yes	No	No	No
Edgren 2010 (32)	No	Yes	No	No	No
Engoren 2009 (5)	No	Yes	No	No	No
Engoren 2009 (6)	No	Yes	No	No	No
Garty 2009 (7)	No	Yes	Yes	Yes	Yes
Jani 2007 (8)	No	No (>60 years)	Yes	Yes	Yes
Johnson 2006 (9)	No	Unclear	No	No	No
Khorana 2008 (10)	No	Yes	No	No	No
Koch 2006 (11,12)	Yes	Yes	Yes	Yes	Yes
Koch 2008 (33)	No	Yes	Yes	Yes	Yes
Murphy 2007 (13)	No	Yes	Yes	Yes	Yes
Nikolsky 2009 (14)	Yes	Yes	Yes	Yes	Yes
O'Keeffe 2010 (29)	Yes	Yes	No	No	No
Pederson 2009 (15)	No	Yes	No	No	No
Rogers 2006 (16)	No	No (>65 years)	No	No	No
Rogers 2009 (17)	No	No (> 65 years)	No	No	No
Ruttinger 2007 (30)	No	Yes	No	No	No
Salehiomran 2009 (18)	No	Yes	Yes	Yes	Yes
Stone 2008 (19)	No	No (< 16 years))	No	No	No
Surgenor 2009 (20)	Yes	Yes	No	No	No
Taylor 2006 (21)	Yes	Yes	No	No	No
van de Watering 2006 (34)	No	Yes	No	No	No
van Straten 2010 (22)	Yes	Yes	No	No	No
Vincent 2008 (23)	Yes	Yes	No	No	No
Weightman 2009 (31)	Yes	Yes	No	No	No
Weinburg 2008 (24)	No	Yes	No	No	No
Weinburg 2008 (35)	No	Yes	No	No	No

Wu 2010 (25)	No	No (> 65 years)	Yes	Yes	Yes
Zilberberg 2008 (26)	No	Yes	No	No	No

*The method of dealing with confounding was deemed adequate if important covariates were measured and adjusted for in the analysis.

~~Time to act on evidence from recent~~ A systematic review of the effect of red blood cell transfusion on mortality: evidence from ~~l~~arge scale observational studies published between 2006 and 2010 ~~of the clinical outcomes efficacy of red blood cell transfusions? Insights from a systematic review~~

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Keywords

Systematic review, observational studies, transfusion, mortality.

Word count 4324

ABSTRACT

Objective: To carry out a systematic review of recently published large scale observational studies assessing the effect of red blood cell transfusion (RBCT) on mortality ~~the efficacy~~clinical outcomes ~~of red blood cell transfusion (RBCT)~~, with particular emphasis on the statistical methods used to adjust for confounding. Given the limited number of randomized trials of the efficacy of RBCT, clinicians often use evidence from observational studies. However, confounding factors, for example individuals receiving blood generally being sicker than those who do not, makes their interpretation challenging.

Design: Systematic review.

Information sources: We searched MEDLINE and EMBASE for studies published from 1 January 2006 to 31 December 2010.

Eligibility criteria for included studies: We included prospective cohort, case control studies or retrospective analyses of databases or disease registers where the effect of risk factors for mortality or survival was examined. Studies must have included more than 1000 participants receiving RBCT for any cause. We assessed the effects of RBCT versus no RBCT and different volumes and age of RBCT.

Results: Thirty two studies were included in the review; 23 assessed the effects of RBCT versus no RBCT; five assessed different volumes and four older versus newer RBCT. There was considerable variability in the patient populations, study designs and level of statistical adjustment. Overall, most studies showed a higher rate of mortality when comparing patients who received RBCT with those who did not, even when these rates were adjusted for confounding; the majority of these increases were statistically significant. The same pattern was observed in studies where protection from bias was likely to be greater, such as prospective studies.

Conclusion: Recent o~~O~~bservational studies do show a consistent adverse effect of RBCT on mortality. Whether this is a true effect remains uncertain as it is possible that even the best

conducted adjustments cannot completely eliminate the impact of confounding. ~~and should be addressed by conducting~~ further well designed and powered clinical studies, and where ~~possible well designed and powered randomized controlled trials.~~

ARTICLE SUMMARY

Article focus

- Given the limited number of randomized trials of the efficacy of red blood cell transfusion (RBCT), clinicians often use evidence from observational studies.
- Confounding factors, for example individuals receiving blood generally being sicker than those who do not, can make their interpretation challenging.
- Our objective was to systematically review ~~recent~~ large observational studies (n>1000 patients) published in the last five years ~~assessing~~~~on~~ the effect~~icacy~~ of RBCT on mortality, with particular emphasis on the statistical methods used to adjust for confounding.

Key messages

- We identified considerable variability in the patient populations, study designs and level of statistical adjustment.
- Most studies showed higher mortality rates when comparing patients who received RBCT with those who did not, even when adjusting for confounding. We identified similar patterns in studies where protection from bias was likely to be greatest.

Strengths and limitations of this study

- Overall, observational studies do show a consistent adverse effect of RBCT on mortality.
- However, even the best conducted adjustments for confounding cannot completely eliminate its impact, particularly when investigating the effect of RBCT on mortality.

Introduction

Randomized controlled trials are considered the gold standard with which to evaluate the efficacy of a particular health care intervention. In 2005, Blajchman (1) published a study that explored the impact that ten landmark randomized controlled trials have had on the practice of transfusion medicine. The use of randomized trials to evaluate transfusion medicine has only been established since the 1980s (1). Given the limited number of high quality randomized trials of the efficacy of blood transfusion and the challenges of conducting new trials, clinicians often rely on evidence from observational studies. In a randomized trial patients are allocated to comparison groups at random, so the level of disease is likely to be similar in each group and differences in disease severity unlikely to be the explanation for any differences in outcome seen. In an observational study, ~~whether a treatment is received or not is likely to be heavily influenced by perceived need by the treating doctor and this will be particularly true where the outcomes of transfused patients are being compared with those not transfused. In this case~~ the groups of patients being compared are not likely to be comparable and the differences in prognostic factors may of themselves lead to difference in outcome. The impact of such "confounding" can be reduced by adjustment in the statistical analysis, but the success of this is dependent on the technique used, complete identification of the factors which might influence outcome and their accurate measurement in the patients in the study (2). As all the factors influencing outcome may never be known, adjustment is unlikely to ever completely account for the confounding occurring in observational studies. The unknown inter-dependence of multiple factors is also a major challenge.

~~The impact that the contribution of data from observational studies has made to the practice of transfusion medicine has not been systematically explored.~~ There is increasing implementation of restrictive policies for transfusion, and evidence of reduction in blood use

in several countries such as the United Kingdom (UK) and the United States of America (USA) with no evidence of poorer clinical outcomes(3;4). However, there remains considerable variation between hospitals in blood reduction in the UK (5) and elsewhere (6) suggesting that overall blood usage could be further reduced without compromising patient safety. ~~However,~~ Observational studies may have influenced these changes in transfusion practice along with evidence from randomized controlled trials, national guidelines, and process driven initiatives, but the impact that the contribution of data from observational studies has made to the practice of transfusion medicine has not been systematically explored. ~~given their publication in major journals, their impact on clinicians may be greater than is appropriate for the types of studies and the limitations associated with their design.~~ The aims of this systematic review were therefore to identify recently published (2006 to 2010), large scale observational studies assessing~~on~~ the effect~~siveness~~ of red blood cell transfusion (RBCT) on mortality. In particular ,we aimed to critique ~~them with particular emphasis on~~ the statistical methods, and the assumptions made in the analyses of the observational data, and to consider the validity of these data as an evidence base for the practice of transfusion medicine and to inform future research in this field.

Methods

Criteria for selecting studies

Type of participants

We included both adults and children receiving RBCT for any cause. We also included studies which stated that patients received red blood cells and other blood products. When reported by the primary studies we assessed the effects of RBCT separately from other blood products.

Type of intervention and comparator

We included the following risk factors:

- RBCT versus no RBCT
- Volume 'A' of RBCT versus volume 'B' of RBCT (as defined by the primary studies)
- 'Older' RBCT versus 'newer' RBCT (as defined by the primary studies)

Type of outcome measure

Our primary outcome measure was death, mortality or survival measured at any time point.

Type of studies

We included prospective cohort, case control studies or retrospective analyses of databases or disease registers where the effect of the above risk factors on death, mortality or survival is examined. Studies must have included more than 1000 participants. This was a pragmatic limit designed to focus attention on studies most likely to have had an impact and least likely to have been affected by chance.

Search strategy

We carried out a comprehensive search (21 January 2011) of MEDLINE and EMBASE for studies published from 1 January 2006 to 31 December 2010 using the strategies in Appendix 1. Again we chose to use a pragmatic approach and limited our search to studies published in the last five years. We also excluded conference abstracts unless they had subsequently been published as full articles.

Data collection and analysis

One review author (CD) initially screened all search results for relevance against the eligibility criteria and discarded all those that were clearly irrelevant. Thereafter, another author (SH) independently screened all remaining hits. We retrieved full text articles for all

those references where we are unable to decide on eligibility based on the title and abstract alone. All full text articles were independently screened by two review authors (SH, MM) to ensure that they met the eligibility criteria.

Data extraction and management

Two review authors (SH, OO) independently extracted data from all included studies. Any disagreements were resolved by discussion or by consulting a third author if there was still uncertainty. We extracted data on the following study characteristics: the study design, how patients were recruited, the country where the study was conducted, the source of funding, the type of participants, their age, disease area, setting, the type of intervention / comparator and nature of the exposure, the number of participants in each group, whether any formal prescribing guidance was reported, the type of outcome measure (i.e. mortality) and the time point at which it was measured.

We also extracted information on the statistical methods used to adjust for differences between study groups, in particular the number of study covariates measured, whether important covariates relating to red cell transfusion were assessed (i.e. age, sex, co-morbidity, hemoglobin) and whether these were incorporated into the analysis, whether the choice of covariates were pre-specified or data driven and the statistical model used for the statistical adjustment. We also assessed the effects of smoking as a study covariate in relation to blood transfusion and its effect on mortality. In terms of the study results we extracted data on the presentation of both the unadjusted and adjusted result for the effect of red cell transfusion on mortality as reported by each study. If not reported by the primary study we calculated (where there were sufficient data) the odds ratio for the effect of blood transfusion on mortality for unadjusted analyses using STATA (version 11). We assessed, for the unadjusted and adjusted result, whether the study reported summary statistics for each

comparison group, the treatment effect, confidence interval, p value and whether the result was statistically significant. If a study reported more than one adjusted analysis we selected in order of preference (i) the main adjusted analysis mentioned in the abstract, (ii) the main adjusted analysis mentioned in the conclusions, (iii) the main adjusted analysis mentioned in the results section. If mortality was assessed for more than one time point (i.e. at 30 days and 1 year) then we used the shorter time point in our analysis.

Assessment of methodological quality

We also assessed whether studies met important methodological criteria for the reporting of observational studies (2): whether the samples were representative of those to whom the results might be generalised, whether important covariates in relation to RBCT and mortality (e.g. sex, age, smoking, co-morbidity, hemoglobin level) were measured and incorporated into the analysis, whether the method of dealing with confounding between patient groups was adequate, whether a statistician was listed as an author of the study and whether the data were collected prospectively following an agreed study design. We included smoking as a covariate as, while not directly correlated with transfusion, it is considered important when assessing mortality.

Method of analysis

We have presented the results separately for the three different types of comparisons. Within each, due to the varied nature of the clinical conditions, study designs and level of statistical adjustment, we decided a priori not to combine the results of individual studies in a meta-analysis and instead present the results of the individual studies descriptively in the text, tables and figures.

Results

Searches of MEDLINE and EMBASE identified 4318 possible records. 4272 did not meet the eligibility criteria for this study. Full articles were retrieved for 45 studies; 13 further studies were excluded as they did not fulfil our eligibility criteria (see Appendix 2 for list of excluded studies~~Figure 1~~). Thirty two studies were included in the review; 23 (7-30) studies assessed the effects of RBCT versus no RBCT, five studies (31-35) assessed different volumes of RBCT and four (36-39) assessed giving older versus newer RBCT~~.~~ (see Figure 1).

Red blood cell transfusion versus no red blood cell transfusion

Twenty three studies (7-30) assessed the effects of RBCT versus no RBCT on mortality. Four of these studies (8;12;14;21) included both red cell transfusion and other blood products (e.g. platelets, plasma, cryoprecipitate); for one study, data were available separately for RBCT and mortality (14). For three studies it was unclear if other blood products were transfused along with red blood cells (11;13;22).

Study characteristics (Table 1)

Eight studies were prospective cohort studies following up a planned group of patients (7;8;15;16;18;24-27), the other 15 studies assessed data from a retrospective patient registry or database. Fourteen studies were conducted in the USA, two in the UK, two in Israel and the remainder in Belgium, the Netherlands, Iran and Denmark; one study was conducted in multiple countries. The time period assessed was between 1989 and 2008. Twelve of the studies (7;9;11;12;15-18;20-22;24;26) specifically looked at adults undergoing cardiac surgery, five were in patients in the intensive care unit (10;25;27;29;30), two were in adults trauma patients (8;28), two were in patients following hip fracture/replacement (13;19) one was in oncology patients (14) and the other in pediatric trauma patients (23). Three of the studies (12;20;21) specifically looked at the effects of RBCT in older adults (e.g. > 60 years). The size of the studies varied from 1,624 participants to 504,208 participants

with an overall median sample size of 4344 (IQR 2085 to 11963); median 1068 (IQR 430 to 5812) for patients undergoing RBCT compared to median 2325 (IQR 1636 to 6151) for patient with no RBCT. The time period at which mortality was assessed also varied across studies from in-hospital to mortality at seven years; the most common time point being mortality at or within 30 days. Several studies reported mortality for more than one time period. Only seven of the 23 studies provided any mention of guidelines for the prescription of RBCT; two studies said no formal protocol was used (8;23), two studies stated a hemoglobin of <8g/dl (10;13), one study stated a hematocrit of less than 25-26% (22) and two studies said prescription was at the discretion of the patient care team (24;25). For full details of the characteristics of the included studies see Appendix 3~~2~~.

Statistical methods (Table 2)

All 23 studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT and those who did not. However, the amount of detail and appropriateness of the method used varied across studies. In 13 studies (7;9;10;14-19;22;24;25;27;30) the choices of covariates measured were reported as pre-specified and not data driven, but this was unclear for the remaining 10 studies. The number of covariates measured and incorporated in the analysis also varied across studies with half the studies reported to assess more than 20 different covariates. Despite the high number of covariates assessed in these studies, not all measured covariates which appeared to be of specific importance in relation to RBCT. All of the 23 studies did report measuring the age and sex of the patients and 21 reported measuring patient co-morbidity. Overall, only eight (7;11;12;15-18;22;29) studies measured and incorporated the covariates age, sex, smoking, co-morbidity and haemoglobin level into the adjusted analysis.. Fourteen of the 23 studies reported using logistic regression (i.e. mortality was reported as a binary outcome) as the method of adjusting for differences in the baseline characteristics

between the two patients groups; six studies reported using Cox proportional hazard (i.e. mortality was reported as a time to event outcome) and three studies reporting using both methods; in these three studies mortality was assessed for more than one time period. Nine studies (7;12;17-19;21;27;29) reported using propensity scores prior to adjusting for confounders, however, sometimes this matching was only using a much smaller subgroup of patients. For full details of the statistical methods see Appendix 43.

Presentation of adjusted and unadjusted results (Table 3)

There were marked differences in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of RBCT versus no RBCT on mortality. Seven of the 23 studies reported a summary statistic for each group for both the unadjusted and adjusted analysis. Five studies reported a summary statistics for only the unadjusted analysis and one study for the adjusted analysis only; no summary statistic comparing the effects of RBCT versus no RBCT on mortality was reported in the remained 10 studies. Eight studies reported the treatment effect (e.g. odds ratio, risk ratio, hazard ratio) and the corresponding confident interval (six studies) for both the unadjusted and adjusted analysis (7;15;16;18-20;24;26;30), whereas 12 studies reported the treatment effect and confident interval (10 studies) for adjusted analysis only and one study for the unadjusted analysis only. Where possible we calculated the odds ratio for the effect of RBCT on mortality for unadjusted analyses if it was not reported in the published article.

Seventeen of the 23 studies reported a statistically significant result for the unadjusted analysis, and 15 for the adjusted analysis (Figure 2), when comparing the effect of RBCT versus no RBCT on mortality, with more deaths occurring in patients receiving transfusion. This effect was statistically non-significant in seven studies based on the result of the adjusted analysis. Prospective studies were more likely to show a statistically significant

effect for blood transfusion on mortality compared to retrospective studies for both the unadjusted and adjusted analysis. For full details see Appendix 54.

Volume 'A' red blood versus volume 'B' red blood cells

Five studies (31-35) assessed the effect of different volumes of RBCT on mortality. One of these studies (35) included both RBCT and other blood products.

Study characteristics (Table 1)

One study assessed a prospective cohort and followed up a planned group of patients (35), the other four studies assessed data from a retrospective patient registry or database. Two of the studies (33;35) specifically looked at adults undergoing cardiac surgery, one was in trauma patients (32), one was in patients undergoing major surgery (31) and one in patients in the intensive care unit (34). The size of the studies varied from 1,841 participants to 125,177 participants, with an overall median sample size of 8215 (IQR 3037 to 8799). The volume of RBCT varied considerably across studies from 1-2 units to more than eight units. The time period at which mortality was assessed also varied across studies from in-hospital to mortality at eight years. Three of the five studies provided any mention of guidelines for the prescription of red blood cells, however only one gave any specific requirement stating a hemoglobin of <8g/dl (34) (See Appendix 32).

Statistical methods (Table 2)

All five studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received different volumes of red blood transfusion, however, as with the studies of RBCT versus no RBCT, the amount of detail and appropriateness of the method used varied across studies. In all five studies (31-35) the choices of covariates measured were reported as pre-specified. The number of covariates

measured and incorporated in the analysis varied across studies with two the studies reported to assess more than 20 different covariates. Once again, despite the high number of covariates assessed in these studies, not all measured covariates seem to be of specific importance in relation to RBCT. All five studies reported measuring age and sex and patient co-morbidity, however, one (31) measured and incorporated the covariates age, sex, smoking, co-morbidity and hemoglobin level into the adjusted analysis.

Presentation of adjusted and unadjusted results (Table 3)

As with the studies of RBCT versus no RBCT, there were marked difference in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of different volumes of RBCT on mortality. Two studies reported a statistically significant result for the adjusted analysis with more deaths occurring in patients receiving larger volumes of RBCT. This effect was statistically non significant in two studies based on the result for adjusted analysis and was not reported for the remaining one study. No studies reported on the statistical significance of the result of the unadjusted analysis (See Appendix 43 and 54).

'Older' red blood cells versus 'newer' red blood cells

Four (36-39) studies assessed the effects of age of RBCT on mortality, one of which specifically looked at leukodepleted RBCT (39).

Study characteristics (Table 1)

All four studies assessed data from a retrospective patient registry or database. Two of the studies (37;38) specifically looked at adults undergoing cardiac surgery, one was in trauma patients (39), while the other did not mention a specific patient group. The size of the studies varied from 1,813 participants to 364,037 participants, with an overall median

sample size of 4358 (IQR 2264 to 185019). The period of time in which the blood was stored varied considerably across studies. Two studies (37;39) assessed RBCT stored for less than 14 days compared to those stored for more than 14 days, one study (38) compared blood stored for less than 18 days and with blood stored for more than 18 days and one study (36) looked at multiple storage periods ranging from 1 to 42 days. None of the studies provided any mention of guidelines for the prescription of red blood cells (See Appendix 2).

Statistical methods (Table 2)

All four studies provided information on the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT stored for different time periods, however, once again the amount of detail and appropriateness of the method used varied across studies. The number of covariates measured and incorporated in the analysis also varied across studies. All of the four studies reported measuring the age and sex of the participants. Only ~~one study reported measuring smoking status,~~ two studies reported measuring patient hemoglobin levels and three studies reported assessing patient co-morbidities. Only one (37) of the four studies measured and incorporated the covariates age, sex, smoking, co-morbidity and haemoglobin level into the adjusted analysis.

Presentation of adjusted and unadjusted results (Table 3)

As with the studies of RBCT versus no RBCT and of volume 'A' red blood cells versus volume 'B' RBCT, there were marked differences in the presentation and reporting of the unadjusted and adjusted results when comparing the effects of RBCT stored for different time periods on mortality. Two studies reported a statistically significant result for the unadjusted analysis and one study reported a statistically significant result for the adjusted analysis. In two of these three studies there were more deaths occurring in patients receiving older blood and in one study there were more deaths in patients receiving newer blood. This

effect was statistically non significant in three studies based on the result for adjusted analysis (See Appendix 43 and 54).

Assessment of methodological quality (Table 4)

Overall the assessment of methodological quality varied across studies and by study group with only 10 of the 32 included studies assessing a prospective cohort following up a planned group of patients over time, the remaining two-third of the studies assessed data from a retrospective patient registry or database. In most studies the sample of patients included in the study was considered representative of those to whom the results might be generalised. Four studies (12;20;21;29) specifically focussed on older adults (>60 years) and one study (23) on children, so the findings from these studies should only be interpreted in relation to these specific patient groups. The baseline characteristics of patients who received RBCT compared to those patients who did not receive RBCT (or patients who received different volumes or age of blood) were often very different and so we wanted to assess whether studies had adjusted for these differences when carrying out their statistical analysis. Only 10 studies measured and incorporated in the analysis covariates which we deemed of specific importance in relation to RBCT (i.e. age, sex, smoking, co-morbidity and haemoglobin level), thus we deemed the method of dealing with confounding between patient groups as adequate in only 31% of studies. Critically however, when we restricted our analysis of results to studies with adequate methods, the pattern of an increase in mortality associated with RBCT remained unchanged.

Discussion

Summary of main findings

We identified 32 observational studies of more than 1000 participants published between 2006 and 2010 assessing the effect of RBCT on mortality. Twenty three studies compared

RBCT versus no RBCT, five compared different volumes and four compared different storage times. Overall there was considerable variability in the characteristics of the observational studies. However, the majority, of studies were retrospective designs assessing patients from an existing patient register or database.

We also identified considerable variability in the statistical methods used to adjust for differences in the baseline characteristics of patients who received RBCT and those who did not. It was often unclear if the choice of covariates measured and used in the adjusted analyses were pre-specified at the start of the study or were driven by the underlying data. Perhaps most importantly, around half of the 32 studies did not measure and adjust for covariates which we deemed of specific importance to blood transfusion. ~~– for example, patient hemoglobin levels, age, sex and existing co-morbidities. Less than a third of studies assessed smoking which, while not directly correlated with transfusion, is an important covariate when assessing mortality.~~

Overall, more studies found a higher rate of mortality in patients receiving RBCT compared with those who did not, and this effect was seen in both the adjusted and unadjusted results. In general, where measured equivalently within the same study, the unadjusted estimate of risk was greater than the adjusted risk, emphasising that adverse prognostic factors are more common in patients receiving RBCT and that adjusting for them leads to a smaller estimate of risk. Considering the adjusted risks, although the size of the effect was not consistent across all studies, the direction of the effect was. Most studies suggest an increased risk of mortality associated with RBCT. Further, those studies which were designed prospectively and which used better methods of adjusting for differences in the baseline characteristics between groups were more likely to show an increase in the risk of mortality compared to studies which were based on retrospective registries or databases, although,

again the size of the effect was not consistent across all studies. However, it is important to remember that even with the best methods of adjustment it cannot completely eliminate the impact of confounding (2), as the sicker the patients (thus an increased risk of mortality) the more likely they are to have received RBCT.

Comparison with other studies

We are aware of one other systematic review of observational studies looking at the effects of RBCT on mortality, which focussed specifically on critically ill adults in intensive care units and adult trauma and surgical patients (40). This systematic review by Marik and colleagues included more studies (n=45) than our review as it did not restrict its inclusion criteria to studies with >1000 patients; the median number of patients analysed was 687. They also found that RBCT was associated with an increased risk of mortality based on a meta-analysis of 12 studies (odds ratio 1.7; 95% CI 1.4 to 1.9). However there was considerable heterogeneity in the meta-analysis, suggesting that it might not have been appropriate to combine the results of the individual studies and supports our decision not to conduct a meta-analysis.

In an overview of evidence from randomized controlled trials Wilkinson and colleagues (41) identified 142 trials in RBCT. The majority compared the effects of leucoreduced RBCT or different transfusion triggers (n=71). However, they did identify 12 trials comparing the effects of RBCT versus no transfusion, seven looking at different volumes of RBCT and 11 different ages of red blood cells. The size of the trials was very small (median 30 to 40 patients) and the overview did not specifically examine the effect of RBCT on mortality. Currently, we are aware of at least 14 ongoing or recently completed randomized controlled trials examining the effects of the age of RBCT on clinical outcomes including the ARIPI (Age of Red blood cells In Premature Infants) (42) ABLE, (Age of BLood Evaluation trial in the

resuscitation of critically ill patients) (43), RECESS (REd CEll Storage duration Study) (44) and INFORM (Effects of transfusing fresh versus standard-issue red cells on in-hospital mortality) trials, for which mortality or survival is a specified outcome measure.

Limitations

Our study has several limitations. Firstly, we only included studies published in the last five years and which included more than 1,000 patients. This was because we took a pragmatic approach as we hypothesised that more recent studies were more likely to use better statistical methods and also ~~hypothesised~~ that studies with a larger sample size wea~~r~~e more likely to show a truer effect of the intervention (45) ~~and that more recent studies are more likely to use better statistical methods~~. Thus we aimed to provide a "snap shot" of current practice rather provide a comprehensive review of all available evidence. It is possible therefore that the overall effect seen here might be different in older studies and/or in those carried out in smaller numbers of patients. Secondly, we decided not to combine the results of individual studies because of the variability in clinical settings and study methods, and instead presented the results of individual studies descriptively in the text and in tables and figures. More formal statistical analysis might have given a more precise indication of the overall effect of red cell transfusion on mortality, but would have ignored the significant amount of clinical and methodological heterogeneity between studies which we identified a priori and which was very apparent in the analysis done by Marik and colleagues (40). However, in the absence of a more formal statistical analysis we have inevitably had to rely on a vote-counting approach which also has great dangers, particularly the assumption that each included study has equal weight. Our main protection against this is the very pronounced nature of the pattern we have observed and the fact that we have limited our conclusions to the direction of effect.

Finally, we limited our inclusion criteria to published articles and excluded unpublished studies or those published only as conference abstracts; thus our study could be subject to publication bias , as studies which did not show a significant effect of red cell transfusion on mortality might be less likely to be published in full (46). Outcome reporting bias may also be a problem, although difficult to combat, in the case where a risk has been measured at different time points but only those time points which are "positive" are reported. However, in the case of both publication and outcome reporting bias, the extreme nature of the pattern makes it relatively implausible that there are sufficient unpublished studies or time points to reverse it.

Implications for clinical practice

In recent years, many developed countries including the UK, USA and Australia have developed national initiatives for better blood transfusion practice, sometimes called 'patient blood management' (4;5). These include the development of guidelines on blood usage promoting restrictive transfusion strategies and initiatives for using alternatives to transfusion (e.g. cell salvage techniques; improvements in the education and training of clinical staff prescribing blood; the provision of mechanisms for reviewing blood use with feedback of data to clinicians). National data on blood usage in the USA suggests an estimated decline of 3% over each of the last two years (2009-2010) (4), and similar data are available in the UK where the demand for red cell units, which steadily increased during the 1990s, has decreased by about 20% in the last 10 years. However, there remains considerable variation between hospitals in blood reduction, and national audits of blood components in the UK and elsewhere suggest that overall blood usage could be further reduced without compromising patient safety (3).

It is difficult to assess how observational studies may have influenced these changes in transfusion practice in comparison to evidence from randomized controlled trials, national guidelines, and process driven initiatives. The most likely answer is that they have all played a role in changing practice. Randomized controlled trials have found that 'restrictive' transfusion strategies are associated with similar or improved clinical outcomes compared to 'liberal' transfusion strategies (47). Many national guidelines have adopted restrictive transfusion strategies (47), while needing to make assumptions about the generalisability of the findings of randomized controlled trials in specific clinical groups of patients. There have been many smaller observational studies of process initiatives to reduce transfusion that also indicate reductions in the use of blood without any significant impact on clinical outcomes (48-50).

Conclusion

The findings from this systematic review of recent large scale observational studies show considerable variability in the patient populations and study methods when comparing the effects of RBCT on mortality. Overall, observational studies do show a consistent adverse effect of RBCT on mortality. Although it seems unlikely that this can be entirely explained by selective sampling or a predominance of poorer quality observational studies, it remains possible that even the best conducted adjustments cannot completely eliminate the impact of confounding. ~~, particularly when investigating the effect of RBCT. We therefore believe that this can only be resolved through well designed and adequately powered randomized controlled trials.~~. ~~Before these can be conducted, the importance of the research question and the uncertainty of the current evidence need to be accepted. This requires clearer and more widespread presentation and understanding of the existing research evidence, to which we believe this study is a significant contribution.~~

Author contributions: SH and OO were involved in the design, implementation, and analysis of the study and in writing the final manuscript. CH, MM and LY were involved in the design and analysis of the study and in writing the final manuscript.

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Figure 1: Flow diagram of study inclusion (1 January 2006 to 31 December 2010)

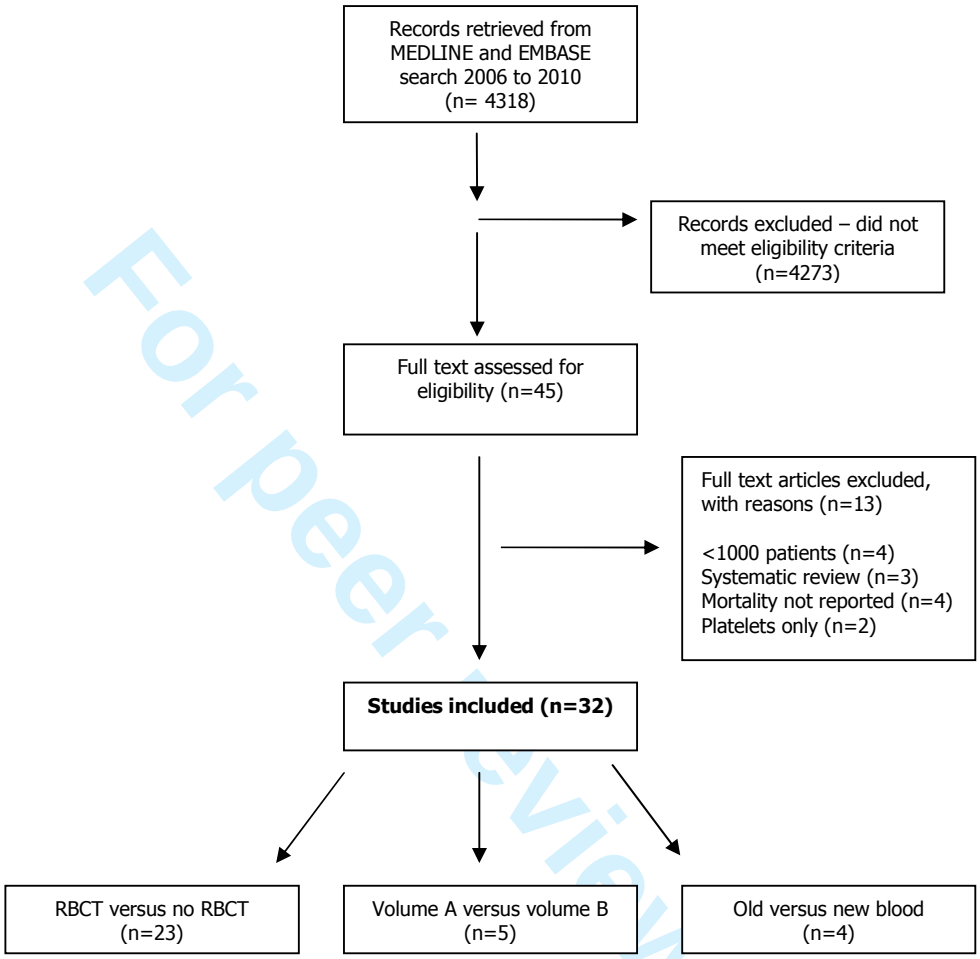

Figure 2: Effect of red blood cell transfusion versus no red blood cell transfusion on mortality (adjusted results)

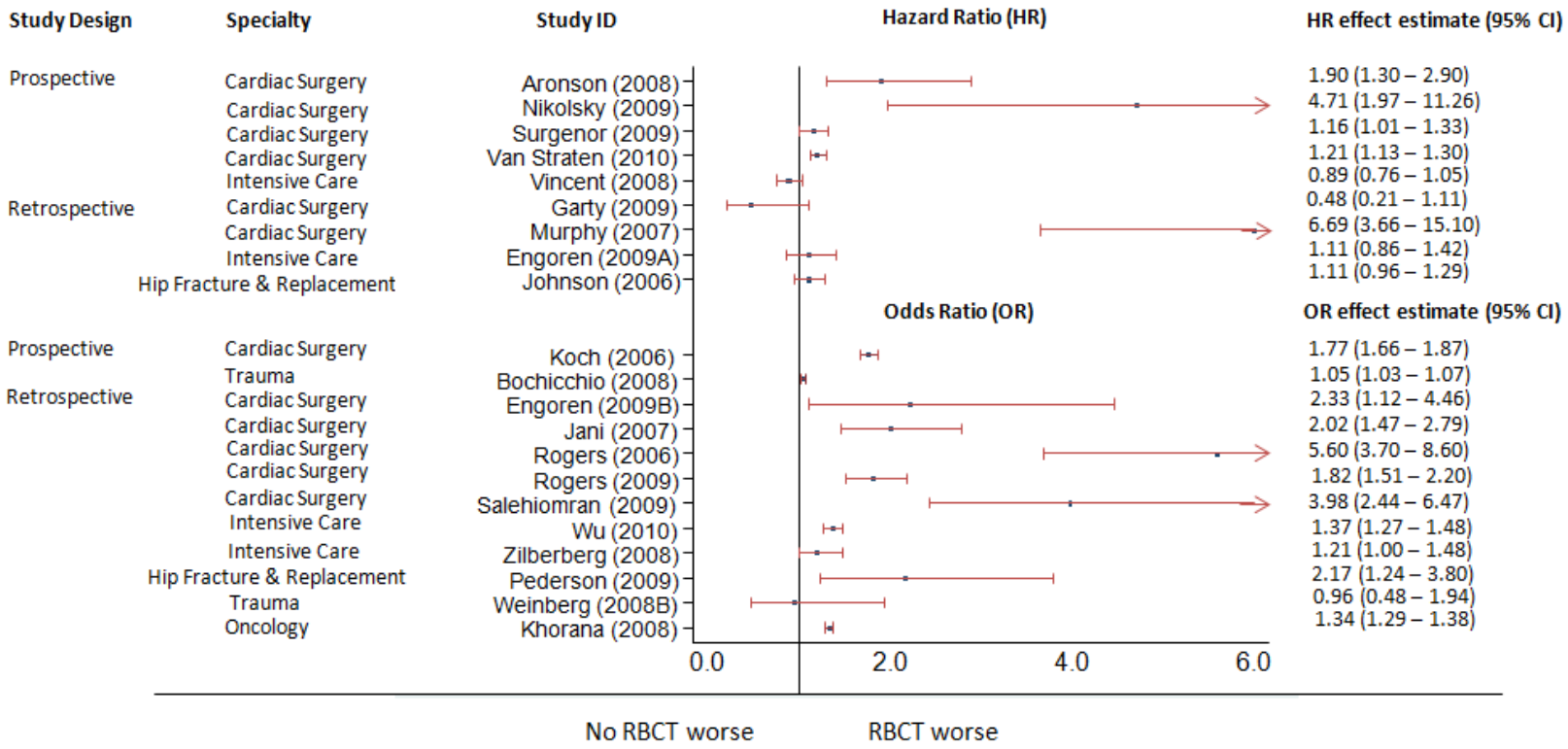

Table 1: Summary of characteristics of included studies

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B'(n=5)	Old RBC vs. new RBC (n=4)
Design			
Prospective	8 (35%)	1 (20%)	
Retrospective	15 (65%)	4 (80%)	4 (100%)
Country			
Australia		1 (20%)	
Belgium	1 (4%)		
Denmark	1 (4%)		
Germany		1 (20%)	
Iran	1 (4%)		
Israel	2 (9%)		
Netherlands	1 (4%)		1 (25%)
Sweden			1 (25%)
USA	14 (61%)	3 (60%)	2 (50%)
UK	2 (9%)		
(multiple sites)	1 (4%)		
Time period assessed	1989-2008	1993-2007	1993-2007
Sample size (median, IQR)			
All patients	4344 (IQR 2085-11963)	8215 (IQR 3037-8799)	4358 (2264-185019)
RBC transfusion	1068 (IQR 430-5812)		
No RBC transfusion	2325 (IQR 1636-6151)		
Disease area			
Cardiac surgery	12 (52%)	2 (40%)	2 (50%)
Hip fracture/replacement	2 (9%)		
Intensive care	5 (22%)	1 (20%)	
Oncology	1 (4%)		
Surgery		1 (20%)	
Trauma adults	2 (9%)	1 (20%)	1 (25%)
Trauma paediatrics	1 (4%)		
Not reported			1 (25%)
Prescribing guidance			
Reported	7 (30%)	3 (60%)	
Not reported	16 (70%)	2 (40%)	4 (100%)
Mortality assessed*			
In hospital	8	2	2
30 days	10	2	1
3 months	3		
6 months	3		
>1 year	4	1	2
Time period not specified	2		1

*studies reported mortality for >1 time point based on binary only and / or time-to-event outcome

Table 2: Method of adjusted analysis

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B' (n=5)	Old RBC vs. new RBC (n=4)
Choice of covariates			
Pre-specified	13 (57%)	5 (100%)	4 (100%)
Post hoc			
Unclear	10 (43%)		
No. of covariates measured			
1-5	2 (9%)		
6-10	4 (17%)	1 (20%)	2 (50%)
11-20	3 (13%)	2 (40%)	
>20	12 (52%)	2 (40%)	1 (25%)
Unclear	2 (9%)		1 (25%)
Important covariates assessed			
Age	23 (100%)	5 (100%)	4 (100%)
Sex	23 (100%)	5 (100%)	4 (100%)
Smoking	8 (35%)	1 (20%)	1 (25%)
Co-morbidity	21 (91%)	5 (100%)	3 (75%)
Hb level	14 (61%)	4 (80%)	2 (50%)
Important covariates incorporated into analysis			
Yes	8 (35%)	1 (20%)	1 (25%)
No	15 (65%)	4 (80%)	3 (75%)
Method of adjustment			
Cox proportional hazard	6 (26%)	1 (20%)	1 (25%)
Logistic regression	14 (61%)	4 (80%)	2 (50%)
Both *	3 (13%)		
Not reported			1 (25%)

*studies reported >1 method of adjustment when mortality was assessed for >1 time point

Table 3: Presentation of results for unadjusted and adjusted analysis (mortality)

Type of comparison	RBCT vs. no RBCT (n=23)	Volume 'A' vs. Volume 'B' (n=5)	Old RBC vs. new RBC (n=4)
Summary statistic for each group			
Unadjusted only	5 (22%)	2 (40%)	
Adjusted only	1 (4%)		
Both	7 (30%)		1 (25%)
Not reported	10 (44%)	3 (60%)	3 (75%)
Treatment effect			
Unadjusted only	1 (4%)		
Adjusted only	12 (52%)	5 (100%)	2 (50%)
Both	8 (35%)		1 (25%)
Not reported	2 (9%)		1 (25%)
Confidence interval of treatment effect			
Unadjusted only			
Adjusted only	10 (43%)	3 (60%)	2 (50%)
Both	8 (35%)		1 (25%)
Not reported	5 (22%)	2 (40%)	1 (25%)
P-value for treatment effect			
Unadjusted only	7 (30%)		
Adjusted only		1 (20%)	
Both	1 (4%)		1 (25%)
Not reported	15 (66%)	4 (80%)	3 (75%)
Unadjusted analysis*			
Statistically significant	17 (74%)		2 (50%)
Statistically non-significant	1 (4%)		
Not reported	5 (22%)	5 (100%)	2 (50%)
Adjusted analysis*			
Statistically significant	15 (65%)	2 (40%)	1 (25%)
Statistically non-significant	7 (31%)	2 (40%)	3 (75%)
Not reported	1 (4%)	1 (20%)	

*mortality outcome – if >1 time point analysed the time point with the non-significant result was recorded

Table 4: Assessment of methodological quality of the included studies

Study ID	Data collected prospectively	Sample representative	Important covariates measured	Important covariates incorporated into analysis	Method of dealing with confounding adequate*
Aronson 2008 (3)	Yes	Unclear	Yes	Yes	Yes
Bernard 2009 (27)	No	Yes	Yes	Yes	Yes
Bochicchio 2008 (4)	Yes	Yes	No	No	No
Charles 2007 (28)	No	Yes	No	No	No
Edgren 2010 (32)	No	Yes	No	No	No
Engoren 2009 (5)	No	Yes	No	No	No
Engoren 2009 (6)	No	Yes	No	No	No
Garty 2009 (7)	No	Yes	Yes	Yes	Yes
Jani 2007 (8)	No	No (>60 years)	Yes	Yes	Yes
Johnson 2006 (9)	No	Unclear	No	No	No
Khorana 2008 (10)	No	Yes	No	No	No
Koch 2006 (11,12)	Yes	Yes	Yes	Yes	Yes
Koch 2008 (33)	No	Yes	Yes	Yes	Yes
Murphy 2007 (13)	No	Yes	Yes	Yes	Yes
Nikolsky 2009 (14)	Yes	Yes	Yes	Yes	Yes
O'Keeffe 2010 (29)	Yes	Yes	No	No	No
Pederson 2009 (15)	No	Yes	No	No	No
Rogers 2006 (16)	No	No (>65 years)	No	No	No
Rogers 2009 (17)	No	No (> 65 years)	No	No	No
Ruttinger 2007 (30)	No	Yes	No	No	No
Salehiomran 2009 (18)	No	Yes	Yes	Yes	Yes
Stone 2008 (19)	No	No (< 16 years))	No	No	No
Surgenor 2009 (20)	Yes	Yes	No	No	No
Taylor 2006 (21)	Yes	Yes	No	No	No
van de Watering 2006 (34)	No	Yes	No	No	No
van Straten 2010 (22)	Yes	Yes	No	No	No
Vincent 2008 (23)	Yes	Yes	No	No	No
Weightman 2009 (31)	Yes	Yes	No	No	No
Weinburg 2008 (24)	No	Yes	No	No	No
Weinburg 2008 (35)	No	Yes	No	No	No

Wu 2010 (25)	No	No (> 65 years)	Yes	Yes	Yes
Zilberberg 2008 (26)	No	Yes	No	No	No

*The method of dealing with confounding was deemed adequate if important covariates were measured and adjusted for in the analysis.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7-8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Appendix 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	14 & table 4
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8-14 & figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Figure 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8-14 & table 4
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	15-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16-17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	17-18
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	19

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

PRISMA 2009 Checklist

APPENDIX 1: Search strategies

MEDLINE (Ovid)

1. ERYTHROCYTE TRANSFUSION/
2. *BLOOD TRANSFUSION/
3. (hemotransfus* or haemotransfus*).tw.
4. ((transfus* or retransfus*) adj1 (trigger* or level* or threshold* or rule* or restrict* or limit*)).tw.
5. (transfusion* adj1 (management or practice* or polic* or strateg* or guideline* or indication* or protocol* or criteri*)).tw.
6. ((blood adj1 management) or (management adj1 blood) or (blood adj1 support) or (blood adj1 requirement*)).tw.
7. ((red cell* adj1 management) or (red cell* adj1 support) or (red cell adj1 requirement*)).tw.
8. (blood adj1 need*).tw. or transfus*.ti.
9. or/1-8
10. BLOOD TRANSFUSION/
11. ERYTHROCYTES/
12. (red cell* or red blood cell* or erythrocyte* or RBC*).tw.
13. 11 or 12
14. 10 and 13
15. (critical* or intensive or trauma or surg* or injur* or postinjur* OR organ failure* OR sepsis or septic OR infection* OR infectious OR ARDS OR acute respiratory distress OR multiorgan).ti. and transfus*.ab.
16. 9 or 14 or 15
17. BLOOD PRESERVATION/
18. transfus*.mp.
19. 17 and 18
20. ((storage or stored or storing or age* or aging or old or older or duration or fresh* or preserv* or conserv*) adj2 (whole blood or red blood cell* or red cell* or RBC*)).tw. and transfus*.mp.
21. (fresh blood or new blood or old* blood or fresh red blood cells or new red blood cells or old* red blood cells or fresh red cells or new red cells or old* red cells).tw.
22. 16 or 19 or 20 or 21
23. PROGNOSIS/
24. DISEASE FREE SURVIVAL/
25. exp CRITICAL CARE/
26. TREATMENT FAILURE/
27. exp MORTALITY/
28. SURVIVAL/
29. SURVIVAL ANALYSIS/
30. RISK ASSESSMENT/ or RISK FACTORS/
31. TREATMENT OUTCOME/
32. (survival* or survivor* or nonsurvivor* or survived or surviving).ti,ab.
33. ((predictor* or prediction*) adj1 death).tw.
34. (prognos* or mortality).tw.
35. (outcome* adj2 (therap* or treatment*)).ti,ab.
36. ((risk adj assessment) or (associated adj risk)).tw.
37. (risk* or association* or causalit* or causation or cause*).ti.
38. exp POSTOPERATIVE COMPLICATIONS/
39. exp INTRAOPERATIVE COMPLICATIONS/

40. or/23- 39
41. 22 and 40
42. BLOOD TRANSFUSION/ae, co, mo, ut or ERYTHROCYTE TRANSFUSION/ae, co, mo, ut
43. ((reaction* or effect* or efficac* or complication* or risk* or adverse* or hazard* or accident* or incident* or morbid* or death* or mortalit* or outcome*) adj3 (transfus* or postransfus* or RBC* or red cell* or erythrocyte*)).tw.
44. (transfus* or posttransfus*).ti.
45. or/41-44
46. EPIDEMIOLOGIC STUDIES/
47. exp CASE CONTROL STUDIES/
48. exp COHORT STUDIES/
49. (case* adj2 control*).tw.
50. cohort*.ti,ab.
51. (follow up adj (study or studies)).tw.
52. (observational adj2 (study or studies)).tw.
53. ((controlled adj2 trial*1) or (controlled adj2 stud*) or (comparative adj trial*) or (comparative adj stud*) or (comparison adj group*) or (comparator adj group*)).tw.
54. longitudinal.tw.
55. retrospective*.tw.
56. cross sectional.tw.
57. CROSS-SECTIONAL STUDIES/
58. Controlled clinical trial.pt.
59. CROSSOVER STUDIES/
60. Comparative study.pt.
61. CLINICAL TRIALS AS TOPIC/
62. exp CONTROLLED CLINICAL TRIALS AS TOPIC/
63. (nonrandomi* or (non adj randomi*)).tw.
64. or/46-63
65. 45 and 64

EMBASE (Ovid)

1. ERYTHROCYTE TRANSFUSION/
2. *BLOOD TRANSFUSION/ or *BLOOD COMPONENT THERAPY/
3. (hemotransfus* or haemotransfus*).tw.
4. ((transfus* or retransfus*) adj1 (trigger* or level* or threshold* or rule* or restrict* or limit*)).tw.
5. (transfusion* adj1 (management or practice* or polic* or strateg* or guideline* or indication* or protocol* or criteri*)).tw.
6. ((blood adj1 management) or (management adj1 blood) or (blood adj1 support) or (blood adj1 requirement*)).tw.
7. ((red cell* adj1 management) or (red cell* adj1 support) or (red cell adj1 requirement*)).tw.
8. (blood adj1 need*).tw. or transfus*.ti.
9. or/1-8
10. BLOOD TRANSFUSION/
11. ERYTHROCYTE/
12. (red cell* or red blood cell* or erythrocyte* or RBC*).tw.
13. 11 or 12
14. 10 and 13

15. (critical* or intensive or trauma or surg* or injur* or postinjur* OR organ failure* OR sepsis or septic OR infection* OR infectious OR ARDS OR acute respiratory distress OR multiorgan).ti. and transfus*.ab.
16. 9 or 14 or 15
17. BLOOD STORAGE/ or ERYTHROCYTE PRESERVATION/
18. transfus*.mp.
19. 17 and 18
20. ((storage or stored or storing or age* or aging or old or older or duration or fresh* or preserv* or conserv*) adj2 (whole blood or red blood cell* or red cell* or RBC*)).tw. and transfus*.mp.
21. (fresh blood or new blood or old* blood or fresh red blood cells or new red blood cells or old* red blood cells or fresh red cells or new red cells or old* red cells).tw.
22. 16 or 19 or 20 or 21
23. PROGNOSIS/
24. exp SURVIVAL/
25. exp INTENSIVE CARE/
26. exp TREATMENT OUTCOME/
27. exp EPIDEMIOLOGY/
28. RISK ASSESSMENT/ or
29. RISK BENEFIT ANALYSIS/ or RISK FACTOR/
30. RISK MANAGEMENT/
31. RISK REDUCTION/
32. (survival* or survivor* or nonsurvivor* or survived or surviving).ti,ab.
33. ((predictor* or prediction*) adj1 death).tw.
34. (prognos* or mortality).tw.
35. (outcome* adj2 (therap* or treatment*)).ti,ab.
36. (risk assessment or associated risk).tw.
37. (risk* or association* or causalit* or causation or cause*).ti.
38. exp POSTOPERATIVE COMPLICATION/
39. PEROPERATIVE COMPLICATION/
40. or/23-39
41. 22 and 40
42. ((reaction* or effect* or efficac* or complication* or risk* or adverse* or hazard* or accident* or incident* or morbid* or death* or mortalit* or outcome*) adj3 (transfus* or postransfus* or RBC* or red cell* or erythrocyte*)).tw.
43. (transfus* and posttransfus*).ti.
44. or/41-43
45. Clinical Study/
46. exp Case Control Study/
47. Family Study/
48. Longitudinal Study/
49. Retrospective Study/
50. Prospective Study/
51. Randomized Controlled Trials/
52. 50 not 51
53. Cohort Analysis/
54. Comparative Study/
55. cohort*.ti,ab.
56. (case* adj2 control*).tw.
57. (follow up adj (study or studies)).tw.
58. (observational adj2 (study or studies)).tw.

59. (epidemiologic* adj (study or studies)).tw.
60. (cross sectional adj (study or studies)).tw.
61. (retrospective* or longitudinal*).tw.
62. ((controlled adj2 trial*1) or (controlled adj2 stud*) or (comparative adj trial*) or (comparative adj stud*) or (comparison adj group*) or (comparator adj group*)).tw.
63. (nonrandomi* or (non adj randomi*)).tw.
64. or/45-49, 52-63
65. 44 and 64

APPENDIX 2: Table of excluded studies

Study ID	Reference	Reason for exclusion
Bennett-Guerrero 2010	Bennett-Guerrero E, Zhao Y, O'Brien SM, et al. Variation in use of blood transfusion in coronary artery bypass graft surgery. JAMA. 2010 Oct 13;304(14):1568-75.	Mortality data not reported
Duchesne 2008	Duchesne JC, Hunt JP, Wahl G,et al. Review of current blood transfusions strategies in a mature level I trauma center: were we wrong for the last 60 years? J Trauma. 2008Aug;65(2):272-6	<1000 patients
Fung 2006	Fung MK, Moore K, Ridenour M, et al. Clinical effects of reverting from leukoreduced to nonleukoreduced blood in cardiac surgery. Transfusion. 2006 Mar;46(3):386-91.	<1000 patients
Karkouti 2006a	Karkouti K, Wijeysundera DN, Yau TM, et al. Platelet transfusions are not associated with increased morbidity or mortality in cardiac surgery. Can J Anaesth. 2006 Mar;53(3):279-87.	Platelets only
Karkouti 2006b	Karkouti K, Yau TM, Rensburg Av, et al. The effects of a treatment protocol for cardiac surgical patients with excessive blood loss on clinical outcomes. Vox Sang. 2006 Aug;91(2):148-56.	Mortality data not reported
Kneyber 2009	Kneyber MC, Gazendam RP, Markhorst DG, et al. Length of storage of red blood cells does not affect outcome in critically ill children. Intensive Care Med. 2009 Jan;35(1):179-80.	<1000 patients
Lelubre 2009	Lelubre C, Piagnerelli M, Vincent JL. Association between duration of storage of transfused red blood cells and morbidity and mortality in adult patients: mythor reality? Transfusion. 2009 Jul;49(7):1384-94.	Systematic review
Marik 2008	Marik PE, Corwin HL. Efficacy of red blood cell transfusion in the critically ill: a systematic review of the literature. Crit Care Med. 2008 Sep;36(9):2667-74.	Systematic review
Muller 2008	Müller MH, Moubarak P, Wolf H, et al. Independent determinants of early death in critically ill surgical patients. Shock. 2008 Jul;30(1):11-6.	Mortality data not reported
Oliver 2009	Oliver E, Carrio ML, Rodríguez-Castro D, et al. Relationships among haemoglobin level, packed red cell transfusion and clinical outcomes in patients after cardiac surgery. Intensive Care Med. 2009 Sep;35(9):1548-55.	Mortality data not reported
Van de Watering 2008	van de Watering LM, Brand A. Effects of Storage of Red Cells. Transfus Med Hemother. 2008;35(5):359-367.	Systematic review
Welsby 2010	Welsby IJ, Lockhart E, Phillips-Bute B, et al. Storage age of transfused platelets and outcomes after cardiac surgery. Transfusion. 2010 Nov;50(11):2311-7.	Platelets only
Whyte 2009	Whyte RK, Kirpalani H, Asztalos EV,et al. Neurodevelopmental outcome of extremely low birth weight infants randomly assigned to restrictive or liberal hemoglobin thresholds for blood transfusion. Pediatrics. 2009 Jan;123(1):207-13	<1000 patients

APPENDIX 3~~2~~: Characteristics of included studies

Study ID	Design	Objective	Participants	Intervention (exposure)	Comparator (control)	Outcome
Red blood cells versus no red blood cells – prospective studies						
Aronson 2008 (7)	Design: prospective cohort How pts recruited: admitted to intensive care unit Country: Israel Year: 2000 to 2006 Funding: not reported	Effects of RBCT in patients with acute myocardial infarction	Adults with acute MI in an intensive coronary care unit (n=2358)	RBCT (n=192) Prescribing guidance: not reported	No RBCT(n=2134)	Mortality at 6 months #
Bochicchio 2008 (8)	Design: prospective cohort How pts recruited: admitted to Adams Cowley Shock Trauma Centre Country: USA Year: 2002 to 2004 Funding: not reported	Effects of RBC and other blood product transfusion (RBC and FFP) on outcome in trauma patients	Adults admitted to intensive care unit (n=1172)	RBC and other blood product transfusion (n= 786); RBC only (n=246) Prescribing guidance: no formal protocol used	No RBC or other blood product transfusion (n=386).	Mortality (time period not specified)
Koch 2006 (15,16)	Design: prospective cohort How pts recruited: admitted to large tertiary hospital (Cleveland Clinic) Country: USA Year: 1995 to 2002 Funding: Non industry funded	Effect of RBCT on mortality in patients undergoing coronary artery bypass surgery	Adults undergoing coronary artery bypass grafting (n=11963)	RBCT (n=5812) Prescribing guidance: not reported	No RBCT(n=6151)	Mortality in-hospital
Nikolsky 2009 (18)	Design: prospective cohort How pts recruited: part of CADILLAC randomized trial comparing different mechanical reperfusion strategies Country: multi centre Year: 1997 to 1999 Funding: not reported	Effect of RBCT in patients undergoing angioplasty for acute myocardial infarction	Adults undergoing angioplasty for acute myocardial infarction (n=2060)	RBCT (n=82) Prescribing guidance: not reported	No RBCT (n=1978)	Mortality at 30 days and 1 year #
Surgenor 2009 (24)	Design: prospective cohort How pts recruited: admitted to one of eight medical centres as part of the Northern New	Effect of RBCT on mortality in patients undergoing coronary artery bypass surgery	Adults undergoing coronary artery bypass surgery (n=9079)	RBCT (n=3254) Prescribing guidance: reported as at the	No RBCT (n=5825)	Mortality over 5 years #

	England Cardiovascular Disease Study Group Country: USA Year: 2001 to 2004 Funding: not reported			discretion of the patient care team		
Taylor 2006 (25)	Design: prospective cohort How pts recruited: admitted to intensive care unit at the St John's Mercy Medical Centre Country: USA Year: 2001 to 2003 Funding: not reported	Effect of RBCT on nosocomial infection and mortality in critically ill patients	Adults admitted to critical care unit (n=2085)	RBCT (n=449) Prescribing guidance: reported as at the discretion of the patient care team	No RBCT (n=1636)	Mortality (time period not specified)
Van Straten 2010 (26)	Design: prospective cohort How pts recruited: admitted to hospital (centre not specified) Country: Netherlands Year: 1998 to 2007 Funding: not reported	Effect of RBCT on long and short term survival in patients undergoing coronary artery bypass grafting	Patients undergoing coronary artery bypass grafting (n=10425)	RBCT (n=3597) Prescribing guidance: not reported	No RBCT (n=6828)	Mortality ≤ 30 days and mortality > 30 days #
Vincent 2008 (27)	Design: prospective cohort How pts recruited: admitted to European intensive care unit (n=198 units) Country: Belgium Year: 1 May to 15 May 2002 Funding: industry supported	Effect of RBCT on mortality in European intensive care units	Adults admitted intensive care unit (n=3147)	RBCT (n=1040) Prescribing guidance: not reported	No RBCT (n=2107)	Mortality in hospital at 30 days #
Red blood cells versus no red blood cells – retrospective studies						
Engoren 2009 (9)	Design: retrospective database How pts recruited: admitted to St Vincent Mercy Medical Centre intensive care unit Country: USA Year: 2001 to 2002 Funding: not reported	Effects of RBCT in critically ill patients (excluded cardiac surgery patients)	Adults admitted to intensive care unit (n=2213)	RBCT (n=404) Prescribing guidance: haemoglobin <8 g/dl	No RBCT (n=1809)	Mortality at 30 days and 180 days #
Engoren 2009 (10)	Design: retrospective database How pts recruited: admitted to St Vincent Mercy Medical Centre	Effects of RBCT in cardiac surgery patients	Adults admitted for cardiac surgery (n=1823)	RBCT (n=378) CABG and value	No RBCT (n=615) CABG and value	Mortality within 30 days >30 days

	for cardiac surgery Country: USA Year: 1991 to 2007 Funding: not reported			RBCT (n=534) Prescribing guidance: not reported	No RBCT (n=296)	
Garty 2009 (11)	Design: retrospective database How pts recruited: admitted to cardiac or internal medicine ward in 25 public hospitals Country: Israel Year: 2003 Funding: Non industry funded	Effect of RBCT (unclear if included other blood products) on patients with acute decompressed heart failure	Adults with acute decompressed heart failure (n=2335)	RBCT (n=166) Prescribing guidance: not reported	No RBCT(n=2169)	Mortality in-hospital, 30 days, 1 year and 4 years #
Jani 2007 (12)	Design: retrospective database (Blue Cross Blue Shield of Michigan Cardiovascular Consortium) How pts recruited: admitted to academic medical centres Country: USA Year: 1997 to 2004 Funding: Blue Cross Blue Shield of Michigan	Effect of RBCT and other blood product on in-patient mortality in anaemic patients undergoing percutaneous coronary intervention (PCI) for myocardial infarction (MI)	Adults (>60 years) with anaemia undergoing PCI within 7 days for having a MI (n=4623).	RBCT and other blood product (n=1033) Prescribing guidance: no formal protocol used	No RBCT or other blood product (n=3590).	Mortality in-hospital
Johnson 2006 (13)	Design: retrospective database How pts recruited: admitted to orthopaedic unit (District General Hospital, Peterbourgh) Country: UK Year: 1989 to 2002 Funding: Non industry funded	Effect of RBCT (unclear if included other blood products) on mortality in patients with hip fracture	Adults admitted to orthopaedic unit with hip fracture (n=3625)	RBCT (n=1068) Prescribing guidance: haemoglobin <8 g/dl	No RBCT (n=2503)	Mortality at 30, 120 and 365 days #
Khorana 2008 (14)	Design: retrospective database (University Health System Consortium) How pts recruited: admitted to academic medical centres Country: USA Year: 1995 to 2003 Funding: National Cancer Institute and National Heart, Lung and Blood Institute	Effect of RBCT and other blood product on thrombosis and mortality in hospitalised patients with cancer	Adults with cancer admitted to hospital (n=504208)	RBCT and other blood product (n=74051); RBC only (n=58814) Prescribing guidance: not reported	No RBCT or other blood product (n=430157)	Mortality in-hospital

Murphy 2007 (17)	Design: 3 retrospective databases (PATS (Patient analysis and Tracking System), haematological and blood bank studys) How pts recruited: admitted to Bristol Royal Infirmary for adult cardiac surgery Country: UK Year: 1996 to 2003 Funding: British Heart Foundation	Effect of RBCT on mortality, post operative morbidity and cost in patients undergoing cardiac surgery	Adults> 16 years undergoing cardiac surgery (n=8598)	RBCT (n=4909) Prescribing guidance: not reported	No RBCT (n=3689)	Mortality up to 7 years post surgery (median 4.15 years) #
Pederson 2009 (19)	Design: retrospective database (Danish Hip Arthroplasty Registry) How pts recruited: admitted from 20 orthopaedic departments Country: Denmark Year: 1999 to 2007 Funding: not reported	Effect of RBCT on mortality in patients undergoing total hip replacement	Adults undergoing surgery for total hip replacement (n=28087)	RBCT (n=9063) Prescribing guidance: not reported	No RBCT (n=19024)	Mortality at 90 days
Rogers 2006 (20)	Design: retrospective database (Center for Medicare and Medicaid Services) How pts recruited: Medicare beneficiaries hospitalised for coronary artery bypass surgery Country: USA Year: 1997 to 1998 Funding: non industry funded	Effect of RBCT on mortality in patients undergoing coronary artery bypass surgery	Older adults (> 65 Years) undergoing coronary artery bypass surgery (n=9218)	RBCT (n=6893) Prescribing guidance: not reported	No RBCT (n=2325)	Mortality within 100 days
Rogers 2009 (21)	Design: retrospective database How pts recruited: Medicare beneficiaries who received coronary artery bypass surgery Country: USA Year: 2003 to 2006 Funding: Michigan Foundation	Effect of RBCT and other blood product on infection and mortality in patients undergoing coronary artery bypass surgery	Older adults (> 65 years) undergoing coronary artery bypass surgery (n=24789)	RBCT and other blood product (n=20789) Prescribing guidance: not reported	No RBCT or other blood product (n=4000)	Mortality in hospital and at 30 days post discharge

Salehiomran 2009 (22)	Design: retrospective database How pts recruited: patients admitted to Tehran Heart Centre who received coronary artery bypass surgery Country: Iran Year: 2002 to 2008 Funding: not reported	Effect of RBCT (unclear if included other blood products) on mortality in patients undergoing coronary artery bypass surgery	Adults undergoing coronary artery bypass surgery (n=14152)	RBCT (n=2333) Prescribing guidance: hematocrit <25-26%	No RBCT (n=11773)	Mortality at 30 days
Stone 2008 (23)	Design: retrospective database How pts recruited: admitted to paediatric trauma centre Country: USA Year: 1998 to 2006 Funding: not reported	Effect of RBCT on mortality in paediatric trauma patients	Children with blunt or penetrating injury admitted to trauma centre (n=1639)	RBCT (n=106) Prescribing guidance: reported no specific transfusion protocol was used	No RBCT (n=1533)	Mortality in-hospital
Weinberg 2008 (28)	Design: retrospective database How pts recruited: admitted to trauma centre at University of Alabama Country: USA Year: 2000 to 2007 Funding: not reported	Effect of RBCT on mortality in trauma patients	Less severely injured adults admitted to trauma centre (n=1624)	RBCT (n=430) Prescribing guidance: not reported	No RBCT (n=1194)	Mortality in hospital
Wu 2010 (29)	Design: retrospective database (Department of Veteran Affairs and National Surgical Quality Improvement Program) How pts recruited: admitted to one of 142 veteran hospitals and requiring major non cardiac surgery Country: USA Year: 1997 to 2004 Funding: not reported	Effect of RBCT on mortality in older adults after major non cardiac surgery	Older adults (>65 years) undergoing major non cardiac surgery (n=239286)	RBCT(n=22515) Prescribing guidance: not reported	No RBCT (n=216771)	Mortality at 30 days
Zilberberg 2008 (30)	Design: retrospective database (Henry Ford Health System includes data from 7 hospitals in USA) How pts recruited: admitted to hospital and requiring	Effect of RBCT on mortality in critically ill patients requiring prolonged ventilation	Adults critically ill and requiring prolonged ventilation 96 ≥hours (n=4344)	RBCT (n=2912) Prescribing guidance: not reported	No RBCT (n=1432)	Mortality in-hospital

	ventilation Country: USA Year: 2000 to 2005 Funding: industry supported					
Volume 'A' red blood cells versus volume 'B' red blood cells						
Bernard 2009 (31)	Design: retrospective database How pts recruited: admitted to hospital and requiring major surgery Country: USA Year: 2005 to 2006 Funding: industry supported	Effect of RBCT and volume of blood in patients undergoing major surgery	Adults undergoing major surgery (n=125177)	Volume of RBCT (classified as: 0 units RBC, 1 unit, 2 units, 3-4 units, 5-9 units >10 units intra operative and >4 units post operative) Prescribing guidance: no formal protocol used	Another volume of RBCT (classified as: 0 units RBC, 1 unit, 2 units, 3-4 units, 5-9 units >10 units intra operative and >4 units post operative) Prescribing guidance: no formal protocol used	Mortality at 30 days
Charles 2007 (32)	Design: retrospective database (NTRACS trauma registry) How pts recruited: admitted to Level 2 trauma centre Country: USA Year: 1994 to 2004 Funding: not reported	Effect of RBCT and volume of blood in patients with blunt trauma injuries	Adults >18 yrs with blunt trauma injuries admitted to trauma centre (n=8215)	Volume of RBCT (classified as: 0 units RBC, 1-2 units, 3-5 units, >6 units) Prescribing guidance: no formal protocol used	Another volume of RBCT (classified as: 0 units RBC, 1-2 units, 3-5 units, >6 units) Prescribing guidance: no formal protocol used	Mortality at 24 hours
O'Keefle 2010 (33)	Design: retrospective database (American College of Surgeons National Quality Improvement Program) How pts recruited: admitted from 173 hospitals and undergoing vascular surgery Country: USA Year: 2005 to 2007 Funding: not reported	Effect of RBCT on mortality in patients undergoing lower extremity revascularization	Adults with peripheral arterial disease (n=8799)	Volume of RBCT (classified as: 0 units RBC, 1 to 2 and >3 units) Prescribing guidance: not reported	Another volume of RBCT (classified as: 0 units RBC, 1 to 2 and >3 units) Prescribing guidance: not reported	Mortality at 30 days
Ruttinger 2007 (34)	Design: retrospective database How pts recruited: admitted to surgical intensive care unit LMU University Hospital., Munich Country: Germany	Effect of RBCT on mortality in critically ill patients	Patients admitted to intensive care unit (n=3037)	Volume of RBCT (classified as: 1 to 2 units (n=676) RBC, 3 to 4 (n=345), 5 to 8 (n=301) and >8 units	Another volume of RBCT (classified as: 1 to 2 units RBC, 3 to 4, 5 to 8 and >8 units)	Mortality in-hospital

	Year: 1993 to 2005 Funding: not reported			(n=471)) Prescribing guidance: haemoglobin <8-9 g/dl	Prescribing guidance: haemoglobin <8-9 g/dl	
Weightman 2009 (35)	Design: prospective cohort How pts recruited: admitted to Sir Charles Gairdner hospital Country: Australia Year: 1993 to 2000 Funding: not reported	Effect of RBCT and other blood product on long term survival in patients undergoing coronary artery bypass grafting	Patients undergoing coronary artery bypass grafting (n=1841) and survived longer than 60 days	Volume of RBCT and other blood product (classified as: 0 units (n=779), 1-2 units (n=402), 3-6 units (n=333) and > 6units (n=327)) Prescribing guidance: not reported	Another volume of RBCT and other blood product (classified as: 0 units, 1-2 units, 3-6 units and > 6units) Prescribing guidance: not reported	Mortality (mean follow up 8.1 years)#
'Older' red blood cells versus 'newer' red blood cell						
Edgren 2010 (36)	Design: retrospective database How pts recruited: received blood transfusion as recorded in Scandinavian Donations and Transfusion Study Country: Sweden and Denmark Year: 1995 to 2002 Funding: National Heart, Lung and Blood Institute of NIH	Effect of RBCT duration of storage on mortality	Adults receiving ≥ 1 RBC transfusion (n=364037)	RBCT stored for 0-9 days, 10-19 days, 20-29 days, 30-42 days Prescribing guidance: no formal protocol used	RBCT stored for 0-9 days , 10-19 days, 20-29 days, 30-42 days Prescribing guidance: no formal protocol used	Mortality ≤ 7 days and mortality 8 to 730 days#
Koch 2008 (37)	Design: retrospective database (Cleveland clinic blood bank and cardiac registries) How pts recruited: admitted to Cleveland Clinic Country: USA Year: 1998 to 2006 Funding: National Institute for Health Research and Joseph Drown Foundation	Effect of RBCT duration of storage on mortality and serious complication in patients undergoing cardiac surgery	Adults >18 years undergoing coronary-artery bypass grafting, cardiac-value surgery, or both (n=6002)	RBCT stored for ≤14 days (n=2872) Prescribing guidance: no formal protocol used	RBCT stored for >14 days (n=3130) Prescribing guidance: no formal protocol used	Mortality in-hospital and at 1 year
Van de Watering 2006 (38)	Design: retrospective database (Leiden University Medical Centre)	Effect of RBCT duration of storage on mortality in	Adults undergoing cardiothoracic surgery (n=2715)	RBCT stored for <18 days (n=942)	RBCT stored for >18 days (n=941)	Mortality at 30 days#

	How pts recruited: admitted to cardiothoracic surgery unit Country: Netherlands Year: 1993 to 1999 Funding: none industry	cardiac patients		Prescribing guidance: no formal protocol used	Prescribing guidance: no formal protocol used	
Weinberg 2008 (39)	Design: retrospective database How pts recruited: admitted to trauma centre at University of Alabama Country: USA Year: 2000 to 2007 Funding: not reported	Effect of leukodepleted RBCT transfusion and duration of storage on mortality in trauma patients	Severely injured adults admitted to trauma centre (n=1813)	RBCT stored for <14 days Prescribing guidance: no formal protocol used	RBCT stored for ≥14 days Prescribing guidance: no formal protocol used	Mortality (time period not specified)

RBCT=red blood cell transfusion; NR: not reported; #time-to-event outcome

APPENDIX 43: Statistical methods and presentation of unadjusted and adjusted results of the included studies

Study ID	Study covariates	Comparison	Unadjusted results	Adjusted results	Method of adjustment
Red blood cells versus no red blood cells – prospective studies					
Aronson 2008 (7)	Number covariates: 16 Age: Yes Sex: Yes Smoking: Yes Co-morbidity: Yes Hb level: Yes Covariates pre-specified	RBCT versus no RBCT Mortality at 6 months#	RBCT (n): NR No RBCT (n): NR Hazard ratio 4.4 (95% CI 3.2 to 5.9)	RBCT (n): NR No RBCT (n): NR Hazard ratio 1.9 (95% CI 1.3 to 2.9)	Nature of adjustment: transfusion propensity, baseline characteristics, nadir haemoglobin Type of model used: logistic regression Number covariates in model: 16
Bochicchio 2008 (8)	Number covariates: 5 Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: No Unclear if covariates pre-specified or data driven	RBCT and other blood product versus no RBCT or other blood products Mortality (time period not specified)	RBCT: 147/786 No RBCT: 32/386 p<0.001 (Odds ratio not reported)	RBCT: 147/786 No RBCT: 32/386 Odds ratio 1.05 (95% CI 1.03 to 1.07)	Nature of adjustment: age, ISS, admission GCS Type of model used: logistic regression Number covariates in model: 3
Koch 2006 (15,16)	Number covariates: multiple Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates were pre-specified	RBCT versus no RBCT Mortality in hospital	RBCT (n): NR No RBCT (n): NR Odds ratio 1.78 (95% CI 1.70 to 1.87)	RBCT (n): NR No RBCT (n): NR Odds ratio 1.77 (1.67 to 1.87)	Nature of adjustment: multiple covariates Type of model used: logistic regression Number covariate in model: multiple covariates
Nikolsky 2009 (18)	Number covariates: 25 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT versus no RBCT Mortality at 30 days and 1 year#	Not reported	Mortality at 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 4.71(95% CI 1.97 to 11.26) Mortality at 1 year RBCT (n): NR	Nature of adjustment: transfusion propensity Type of model used: Cox proportional hazards model Number covariate in model: 19

				No RBCT (n): NR Hazard ratio 3.16 (95% CI 1.66 to 6.03)	
Surgenor 2009 (24)	Number covariates: multiple Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	RBCT versus no RBCT Mortality within 5 years#	RBCT (n): NR No RBCT (n): NR Hazard ratio 1.94 (95% CI 1.71 to 2.20)	RBCT (n): NR No RBCT (n): NR Hazard ratio 1.16 (95% CI 1.01 to 1.33)	Nature of adjustment: propensity model Type of model used: Cox proportion hazard model Number covariates in model: 13
Taylor 2006 (25)	Number covariates: 5 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: no Covariates pre-specified	RBCT versus no RBCT Mortality (time period not specified)	RBCT: 98/449 No RBCT: 166/1636 p<0.001 (only p value reported)	POS ≤ 25% RBCT: 47/147 No RBCT: 105/336 p=0.88 POS 25% ≤ 50% RBCT: 17/126 No RBCT: 23/358 p=0.013 POS 50% ≤ 75% RBCT: 14/94 No RBCT: 100/390 P<0.0001 POS >75% RBCT: 3/39 No RBCT: 4/444 p=0.14 (only p value reported)	Nature of adjustment: mortality predication model (POS) Type of model used: logistic regression Number covariates in model: NR
Van Straten 2010 (26)	Number covariates: 16 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: no Unclear if covariates pre-	RBCT versus no RBCT Mortality ≤ 30 days and mortality > 30 days#	Mortality ≤ 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 1.31 (95% CI 1.27 to 1.35) Mortality > 30 days	Mortality ≤ 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 1.21 (95% CI 1.13 to 1.30) Mortality > 30 days	Nature of adjustment: unclear Type of model used: Cox proportional hazards model Number covariates in model: NR

	specified or data driven		Hazard ratio 1.16 (95% CI 1.13 to 1.20)	Hazard ratio 1.04 (95% CI 0.99 to 1.07)	
Vincent 2008 (27)	Number covariates: 8 Age: yes Sex: yes Smoking :no Co-morbidity: yes Hb level: no Covariates pre-specified	RBCT versus no RBCT Mortality at 30 days in hospital#	RBCT: 311/1040 No RBCT: 436/2107 p<0.001 (only p value reported)	RBCT: NR No RBCT: NR Hazard ratio 0.89 (95% CI 0.76 to 1.05) p=0.16	Nature of adjustment: multiple covariates Type of model used: Cox proportional hazards model Number covariates in model: 8
Red blood cells versus no red blood cells – retrospective studies					
Engoren 2009 (9)	Number covariates: 25 Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: Yes Covariates pre-specified	RBCT versus no RBCT Mortality at 30 days and 180 days#	Mortality 30 days RBCT: 101/404 No RBCT: 265/1809 Mortality 180 days RBCT: 150/404 No RBCT: 414/1809 p<0.01 (Hazard ratios not reported)	Mortality 30 days RBCT: NR No RBCT: NR Hazard ratio 1.11 (95% CI 0.86 to 1.42) Mortality 180 days RBCT: NR No RBCT: NR Hazard ratio 1.14 (95% CI 0.83 to 1.58)	Nature of adjustment: multiple variables Type of model used: Cox proportional hazard modelling Number covariates in model: NR
Engoren 2009 (10)	Number covariates: multiple Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: Yes Covariates pre-specified	RBCT versus no RBCT Mortality within 30 days and >30 days	Mortality within 30 days Value only: RBCT: 26/993 No RBCT: 16/993 CABG and value: RBCT: 69/830 No RBCT: 14/830 Mortality >30 days Value only: RBCT: 160/993 No RBCT: 165/993 CABG and value: RBCT: 279/830 No RBCT: 113/830	Mortality within 30 days Value only: Odds ratio 1.95 (95% CI 0.97 to 3.91) CABG and value: Odds ratio 2.23 (95% CI 1.12 to 4.46) Mortality >30 days Value only: Risk ratio 1.25 (95% CI 0.97 to 1.61) CABG and value: Risk ratio 1.44 (95% CI 1.13 to 1.84)	Nature of adjustment: propensity score Type of model used: Cox proportional hazard modelling (mortality >30 days) and logistic regression (mortality within 30 days) Number covariates in model: NR
Garty 2009	Number covariates:	RBCT (unclear if included	Mortality in hospital	Mortality in hospital	Nature of adjustment: propensity

(11)	unclear Age: Yes Sex: Yes Smoking: Yes Co-morbidity: Yes Hb level: Yes Unclear if covariates pre-specified or data driven	other blood product) versus no RBCT Mortality in hospital, 30 days, 1 year and 4 years#	RBCT: 18/166 (10.8%) No RBCT: 113/2169 (5.2%) Mortality 30 days RBCT: 18/166 (11%) No RBCT: 183/2169 (8.5%) Mortality 1 year RBCT: 65/166 (39.6%) No RBCT: 616/2169 (28.5%) Mortality 4 years RBCT: 114/166 (69.5%) No RBCT: 1284/2169 (59.5%)	RBCT: 9/103 (8.7%) No RBCT: 15/103 (14.6%) Hazard ratio: 0.48 (95% CI 0.21 to 1.11) Mortality 30 days RBCT: 10/103 (9.7%) No RBCT: 19/103 (18.4%) Hazard ratio: 0.29 (95% CI 0.13 to 0.64) Mortality 1 year RBCT: 40/103 (38.8%) No RBCT: 44/103 (42.7%) Hazard ratio: 0.74 (95% CI 0.50 to 1.09) Mortality 4 years RBCT: 75/103 (72.8%) No RBCT: 79/103 (76.7%) Hazard ratio: 0.86 (95% CI 0.64 to 1.14)	score Type of model used: Cox proportional hazard modelling (1-4 year mortality) and logistic regression (mortality up to 30 days) Number covariates in model: 9
Jani 2007 (12)	Number covariates: 31 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Unclear if covariates pre-specified or data driven	RBCT and other blood product versus no RBCT Mortality in hospital	RBCT: 150/1033 No RBCT: 108/3590 p<0.001 (only p value reported)	RBCT: 76/598 No RBCT: 44/598 Odds ratio 2.02 (95% CI 1.47 to 2.79)	Nature of adjustment: transfusion propensity and co morbidities Type of model used: logistic regression Number covariate in model: 10
Johnson 2006 (13)	Number covariates: 7 Age: yes Sex: yes Smoking: no Co-morbidity: no HB level: yes Unclear if covariates pre-specified or data driven	RBCT (unclear if included other blood product) versus no RBCT Mortality at 30, 120, 365 days#	Mortality 30 days RBCT: 95/1068 No RBCT: 181/2503 p=0.10 Mortality 120 days RBCT: 247/1068 No RBCT: 374/2503 p<0.0001 Mortality 365 days	Mortality 30 days (not reported) Mortality 120 days (not reported) Mortality 365 days	Nature of adjustment: age, sex, ASA grade, preoperative haemoglobin, residential status, mobility score Type of model used: Cox regression Number covariate in model: 7

			RBCT : 381/1068 No RBCT: 626/2503 p<0.001 (only p values reported)	RBCT: 381/1068 No RBCT: 626/2503 Hazard ratio 1.11 (95% CI 0.96 to 1.29)	
Khorana 2008 (14)	Number covariates: Unclear Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: no Covariates pre-specified	RBCT and other blood product versus no RBCT Mortality in hospital	RBCT (n): 11.9% No RBCT (n): NR	RBCT (n): NR No RBCT (n): NR Odds ratio 1.34 (95% 1.29 to 1.38)	Nature of adjustment: NR Type of model used: multivariate logistic regression Number covariate in model: NR
Murphy 2007 (17)	Number covariates: 21 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT versus no RBCT Mortality up to 7 years post surgery#	Not reported	Mortality 0 - 30 days RBCT (n): NR No RBCT (n): NR Hazard ratio 6.69(95% CI 3.66 to 15.1) Mortality 31 days to 1 year Hazard ratio 2.59 (95% CI 1.68 to 4.18) Mortality > 1 year Hazard ratio 1.32 (95% CI 1.08 to 1.64)	Nature of adjustment: transfusion propensity Type of model used: logistic regression and Cox proportional hazards regression Number covariate in model: NR
Pederson 2009 (19)	Number covariates: 69 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT versus no RBCT Mortality at 90 day	RBCT (n): NR No RBCT (n): NR Odds ratio 2.17 (95% CI 1.24 to 3.79)	RBCT: 39/2254 No RBCT: 18/2254 Odds ratio 2.17 (95% CI 1.24 to 3.80)	Nature of adjustment: transfusion propensity Type of model used: multivariate logistic regression Number covariate in model: NR
Rogers 2006 (20)	Number covariates: 33 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: unclear	RBCT versus no RBCT Mortality within 100 days	RBCT: 648/6893 No RBCT: 44/2325 Odds ratio 6.6 (95% CI 4.4 to 9.9)	RBCT: 648/6893 No RBCT: 44/2325 Odds ratio 5.6 (95% CI 3.7 to 8.6)	Nature of adjustment: sex, age, race, co morbidity, urgency of admission Type of model used: generalised linear regression Number covariate in model: 5

	Unclear if covariates pre-specified or data driven				
Rogers 2009 (21)	Number covariates: 13 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: no Unclear if covariates pre-specified or data driven	RBCT and other blood product versus no RBCT Mortality in hospital and at 30 days	Not reported	Mortality in hospital RBCT (n): NR No RBCT (n): NR Elective surgery: Odds ratio 4.67 (95% CI 2.38 to 9.18) Urgent surgery: Odds ratio 1.82 (95% CI 1.51 to 2.20) Mortality 30 days post discharge Elective surgery: Odds ratio 2.88 (95% CI 1.38 to 5.98) Urgent surgery: Odds ratio 4.65 (95% CI 1.90 to 11.39)	Nature of adjustment: propensity score, surgical volume, hospital volume Type of model used: multivariate mixed effect logistic regression Number covariate in model: 3
Salehiomran 2009 (22)	Number covariates: 31 Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT (unclear if included other blood products) versus no RBCT Mortality at 30 days	RBCT: 60/2333 No RBCT: 42/11773 p<0.001 (Odds ratio not reported)	RBCT: 60/2333 No RBCT: 42/11773 Odds ratio 3.98 (95% CI 2.44 to 6.47)	Nature of adjustment: not reported Type of model used: multivariate logistic regression Number covariate in model: 13
Stone 2008 (23)	Number covariates: 7 Age: yes Sex: yes Smoking: N/A Co-morbidity: yes Hb level: no Unclear if covariates pre-specified or data driven	RBCT versus no RBCT Mortality in hospital	RBCT: 31/106 No RBCT: 42/1533 Odds ratio 14.67 (95% CI not reported)	Not reported (authors said statistical model was to unreliable to provide reliable conclusions)	Nature of adjustment: injury severity Type of model used: logistic regression Number covariate in model: NR
Weinberg 2008 (28)	Number covariates: 9 Age: yes Sex: yes Smoking: no Co-morbidity: no Hb level: no Unclear if covariates pre-specified or data driven	RBCT versus no RBCT Mortality in hospital	RBCT (n): 4.2% No RBCT (n): 2.3% p=0.04	RBCT (n): NR No RBCT (n): NR Odds ratio 0.96 (95% CI 0.48 to 1.94)	Nature of adjustment: age, gender, ISS, injury, ventilation, transfusion volume Type of model used: logistic regression Number of covariates in model: 5

Wu 2010 (29)	Number covariates: multiple Age: yes Sex: yes Smoking: yes Co-morbidity: yes Hb level: yes Unclear if covariates pre-specified or data driven	RBCT versus no RBCT Mortality at 30 days	Not reported	RBCT (n): NR No RBCT (n): NR Odds ratio 1.37 (95% CI 1.27 to 1.48)	Nature of adjustment: mean operative time, ASA classification, rate of general anaesthesia Type of model used: logistic regression Number covariates in model: NR
Zilberberg 2008 (30)	Number covariates: multiple Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	RBCT versus no RBCT Mortality in hospital	RBCT: 938/2912 No RBCT: 342/1432 Odds ratio 1.51 (95% CI 1.31 to 1.75)	RBCT : 938/2912 No RBCT: 342/1432 Odds ratio 1.21 (95% CI 1.00 to 1.48)	Nature of adjustment: multiple variables Type of model used: logistic regression Number covariates in model: 13
Volume 'A' red blood cells versus volume 'B' red blood cells					
Bernard 2009 (31)	Number covariates: multiple Age: Yes Sex: Yes Smoking: Yes Co-morbidity: Yes Hb level: Yes Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality at 30 days	Intra operative 1 unit: 136/1343 2 units: 194/1903 3-4 units: 151/977 5-9 units: 67/412 >10 units: 45/153 Post operative >4 units: 153/575 (Odds ratios not reported)	Intra operative 1 unit: Odds ratio 1.32 2 units: Odds ratio 1.38 3-4 units: Odds ratio 1.97 5-9 units: Odds ratio 2.17 >10 units: Odds ratio 9.83 Post operative >4 units: Odds ratio 2.65 (95% CI not reported)	Nature of adjustment: transfusion propensity, type of procedure, wound class, operative duration Type of model used: logistic regression Number covariates in model: multiple
Charles 2007 (32)	Number covariates: 7 Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: No Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality at 24 hours	0 RBCT: 1.8% 1-2 units: 6.5% 3-5 units: 16.1% ≥6 units: 29.8% (Odds ratios not reported)	1-2 units: p=0.18 3-5 units: Odds ratio 3.22 p=0.002 ≥6 units: Odds ratio 4.87 p=0.000 (95% CI not reported)	Nature of adjustment: age, gender, ISS score, SI Type of model used: logistic regression Number covariates in model: 4

O'Keeffe 2010 (33)	Number covariates: 23 Age: yes Sex: yes Smoking: unclear Co-morbidity: yes HB level: yes Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality at 30 days	Not reported	1-2 units: Odds ratio 1.92 (95% CI 1.36 to 2.70) >3 units: Odds ratio 2.48 (95% CI 1.55 to 3.98)	Nature of adjustment: transfusion propensity Type of model used: logistic regression Number covariate in model: 19
Ruttinger 2007 (34)	Number covariates: 14 Age: yes Sex: yes Smoking: no Co-morbidity: yes HB level: yes Covariates pre-specified	Volume of RBCT versus another volume of RBCT Mortality in hospital	% reported in figure only (Odds ratios not reported)	1-2 units: Odds ratio 0.68 (95% CI 0.35 to 1.28) 3-4 units: Odds ratio 1.11 (95% CI 0.52 to 2.39) 5-8 units: Odds ratio 1.16 (95% CI 0.60 to 2.26) 8 units: Odds ratio 0.74 (95% CI 0.36 to 1.51)	Nature of adjustment: extended analysis Type of model used: logistic regression Number covariate in model: NR
Weightman 2009 (35)	Number covariates: 16 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	Volume of RBCT and other blood product (classified as: 0 units, 1-2 units, 3-6 units and > 6units) Mortality (mean follow up 8.1 years)	0 units: 80/779 1-2 units: 56/402 3-6 units: 58/333 > 6 units: 72/327	1-2 units: Hazard ratio 1.00 (95% CI 0.70 to 1.44) 3-6 units: Hazard ratio 0.98 (95% CI 0.67 to 1.41) > 6 units: Hazard ratio 1.25 (95% CI 0.87 to 1.79)	Nature of adjustment: multiple measures Type of model used: Cox proportional hazard model Number covariates in model: 12
'Older red blood cells versus 'newer' red blood cells					
Edgren 2010 (36)	Number covariates: unclear Age: Yes Sex: Yes Smoking: No Co-morbidity: Yes Hb level: No Covariates pre-specified	RBCT storage for 0- days, 10-19 days, 20-29 and 30-42 days Mortality ≤ 7 days and mortality 8 to 730 days#	Not reported	Mortality 1 to 7 days Stored 0- 9 days: Hazard ratio 0.96 (95% CI 0.91 to 1.00) Stored 10-19 days: Hazard ratio 1.00 (95% CI not reported) Stored 20-29 days: Hazard ratio 1.06 (95% CI 0.96 to 1.06) Stored 30-42 days: Hazard ratio 1.05 (95% CI 0.97 to 1.12) Mortality 8 to 730 days Stored 0- 9 days: Hazard ratio 1.01 (95% CI 0.99 to 1.02) Stored 10-19 days: Hazard ratio 1.00 (95% CI not reported)	Nature of adjustment: number transfusions, age, sex, blood group, calendar period, season, weekday, hospital, indication Type of model used: Cox proportional hazards regression Number covariates in model: 9

				Stored 20-29 days: Hazard ratio 0.99 (95% CI 0.97 to 1.01) Stored 30-42 days: Hazard ratio 1.05 (95% CI 1.02 to 1.08)	
Koch 2008 (37)	Number covariates: multiple Age: yes Sex: yes Smoking: yes Co-morbidity: yes HB level: yes Covariates pre-specified	RBCT stored for ≤ 14 days versus RBCT stored for > 14 days Mortality in hospital and at 1 year	Mortality in hospital Stored ≤ 14 days: 49/2872 Stored > 14 days: 88/3130 p=0.004 (only p value reported)	Mortality at 1 year Stored ≤ 14 days: 7.4% Stored > 14 days: 11% p<0.001 (only p value reported)	Nature of adjustment: transfusion propensity Type of model used: logistic regression Number covariate in model: NR
Van de Watering 2006 (38)	Number covariates: 7 Age: yes Sex: yes Smoking: no Co-morbidity: yes Hb level: yes Covariates pre-specified	RBCT stored for <18 days versus RBCT stored for >18 days Mortality at 30 days#	Stored <18 days (n): NR Hazard ratio 1.33 (95% CI 1.04 to 1.68) Stored > 18 days (n): NR Hazard ratio: 0.85 (95% CI 0.69 to 1.05)	Stored <18 days (n): NR Hazard ratio 0.93 (95% CI 0.71 to 1.23) Stored > 18 days (n): NR Hazard ratio 0.98 (95% CI 0.76 to 1.25)	Nature of adjustment: number of transfusions, duration of surgery, previous CABG, number of distal anatomises, age, sex, Hb level Type of model used: NR Number covariates in model: 7
Weinberg 2008 (39)	Number covariates: 6 Age: yes Sex: yes Smoking: no Co-morbidity: no Hb level: no Covariates pre-specified	RBCT stored for <14 days versus RBCT stored for >14 days Mortality (time period not specified)	Not reported	Stored <14 days: 1-2 units: Odds ratio 1.65 (95% CI 1.01 to 2.70) ≥ 3 units: Odds ratio 1.70 (95% CI 0.96 to 2.99) Stored ≥ 14 days: 1-2 units: Odds ratio 1.78 (95% CI 1.06 to 2.98) ≥ 3 units: Odds ratio 2.78 (95% CI 1.58 to 4.88)	Nature of adjustment: age, gender, ISS, type injury, number units transfused first 24 hours, length of hospital stay Type of model used: logistic regression Number covariates in model: 6

RBCT=red blood cell transfusion; NR: not reported; OR = odds ratio; RR = risk ratio; HR = hazard ratio; #time-to-event outcome

APPENDIX 54: Summary of unadjusted and adjusted results of the included studies

Study ID	Disease area	Comparison	Mortality	Unadjusted results	Adjusted result
Red blood cells versus no red blood cells – prospective studies					
Aronson 2008 (7)	Cardiac surgery	RBCT versus no RBCT	6 months	HR 4.4 (95% CI 3.2 to 5.9)	HR 1.9 (95% CI 1.3 to 2.9)
Bochicchio 2008 (8)	Trauma	RBCT and other blood product versus no RBCT	Time period not specified	OR 2.54 (95% CI 1.70 to 3.81)*	OR 1.05 (95% CI 1.03 to 1.07)
Koch 2006 (15,16)	Cardiac surgery	RBCT versus no RBCT	In hospital	OR 1.78 (95% CI 1.70 to 1.87)	OR 1.77 (1.67 to 1.87)
Nikolsky 2009 (18)	Cardiac surgery	RBCT versus no RBCT	30 days and 1 year	Not reported	Mortality at 30 days HR 4.71(95% CI 1.97 to 11.26) Mortality at 1 year HR 3.16 (95% CI 1.66 to 6.03)
Surgenor 2009 (24)	Cardiac surgery	RBCT versus no RBCT	≤ 5 years	HR 1.94 (95% CI 1.71 to 2.20)	HR 1.16 (95% CI 1.01 to 1.33)
Taylor 2006 (25)	Intensive care	RBCT versus no RBCT	Time period not specified	OR 2.47 (95% CI 1.88 to 3.26)*	POS ≤ 25% p=0.88 POS 25% ≤ 50% p=0.013 POS 50% ≤ 75% p<0.0001 POS >75% p=0.14
Van Straten 2010 (26)	Cardiac surgery	RBCT versus no RBCT	≤ 30 days and > 30 days	Mortality ≤ 30 days HR 1.31 (95% CI 1.27 to 1.35) Mortality > 30 days HR 1.16 (95% CI 1.13 to 1.20)	Mortality ≤ 30 days HR 1.21 (95% CI 1.13 to 1.30) Mortality > 30 days HR 1.04 (95% CI 0.99 to 1.07)
Vincent 2008 (27)	Intensive care	RBCT versus no RBCT	30 days in hospital	OR 1.64 (95% CI 1.38 to 1.94)*	HR 0.89 (95% CI 0.76 to 1.05)

Red blood cells versus no red blood cells – retrospective studies					
Engoren 2009 (9)	Intensive care	RBCT versus no RBCT	30 and 180 days	Mortality 30 days OR 1.94 (95% CI 1.50 to 2.52)* Mortality 180 days OR 1.99 (95% CI 1.58 to 2.50)*	Mortality 30 days HR 1.11 (95% CI 0.86 to 1.42) Mortality 180 days HR 1.14 (95% CI 0.83 to 1.58)
Engoren 2009 (10)	Cardiac surgery	RBCT versus no RBCT	≤ 30 days and >30 days	Mortality ≤30 days Valve only: OR 1.65 (95% CI 0.88 to 3.08)* CABG and valve: OR 5.28 (95% CI 2.95 to 9.47)* Mortality >30 days Valve only: RR 0.97 (95% CI 0.79 to 1.18)* CABG and valve: RR 2.47 (95% CI 2.03 to 3.00)*	Mortality ≤30 days Valve only: OR 1.95 (95% CI 0.97 to 3.91) CABG and valve: OR 2.23 (95% CI 1.12 to 4.46) Mortality >30 days Valve only: RR 1.25 (95% CI 0.97 to 1.61) CABG and valve: RR 1.44 (95% CI 1.13 to 1.84)
Garty 2009 (11)	Cardiac surgery	RBCT (unclear if other blood product) versus no RBCT	In hospital, 30 days, 1 year and 4 years	Mortality in hospital OR 0.77 (95% CI 0.46 to 1.31)* Mortality 30 days OR 2.21 (95% CI 1.31 to 3.74)* Mortality 1 year OR 1.62 (95% CI 1.17 to 2.25)* Mortality 4 years OR 1.51 (95% CI 1.08 to 2.12)*	Mortality in hospital HR 0.48 (95% CI 0.21 to 1.11) Mortality 30 days HR 0.29 (95% CI 0.13 to 0.64) Mortality 1 year HR 0.74 (95% CI 0.50 to 1.09) Mortality 4 years HR 0.86 (95% CI 0.64 to 1.14)
Jani 2007 (12)	Cardiac surgery	RBCT and other blood product versus no RBCT	In hospital	OR 5.48 (95% CI 4.23 to 7.09)*	OR 2.02 (95% CI 1.47 to 2.79)
Johnson 2006 (13)	Hip fracture and replacement	RBCT (unclear if other blood product) versus no RBCT	30 days, 120 days, 365 days	Mortality 30 days OR 1.84 (95% CI 1.42 to 2.38)* Mortality 120 days OR 1.71 (95% CI 1.43 to 2.05)*	Mortality 365 days HR 1.11 (95% CI 0.96 to 1.29)

				Mortality 365 days OR 1.66 (95% CI 1.42 to 1.94)*	
Khorana 2008 (14)	Oncology	RBCT and other blood product versus no RBCT	In hospital	Not reported	OR 1.34 (95% 1.29 to 1.38)
Murphy 2007 (17)	Cardiac surgery	RBCT versus no RBCT	≤7 years	Not reported	Mortality 0 - 30 days HR 6.69 (95% CI 3.66 to 15.1) Mortality 31 days to 1 year HR 2.59 (95% CI 1.68 to 4.18) Mortality > 1 year HR 1.32 (95% CI 1.08 to 1.64)
Pederson 2009 (19)	Hip fracture and replacement	RBCT versus no RBCT	90 day	OR 2.17 (95% CI 1.24 to 3.79)	OR 2.17 (95% CI 1.24 to 3.80)
Rogers 2006 (20)	Cardiac surgery	RBCT versus no RBCT	≤100 days	OR 6.6 (95% CI 4.4 to 9.9)	OR 5.6 (95% CI 3.7 to 8.6)
Rogers 2009 (21)	Cardiac surgery	RBCT and other blood product versus no RBCT	In hospital and 30 days	Not reported	Mortality in hospital Elective surgery: OR 4.67 (95% CI 2.38 to 9.18) Urgent surgery: OR 1.82 (95% CI 1.51 to 2.20) Mortality 30 days post discharge Elective surgery: OR 2.88 (95% CI 1.38 to 5.98) Urgent surgery: OR 4.65 (95% CI 1.90 to 11.39)
Salehiomran 2009 (22)	Cardiac surgery	RBCT (unclear if other blood product) versus no RBCT	30 days	OR 1.55 (95% CI 1.04 to 2.30)*	OR 3.98 (95% CI 2.44 to 6.47)
Stone 2008 (23)	Paediatric trauma	RBCT versus no RBCT	In hospital	OR 14.67 (95% CI not reported)	Not reported
Weinberg 2008 (28)	Adult trauma	RBCT versus no RBCT	In hospital	OR 1.89 (95% CI 0.97 to 3.60)*	OR 0.96 (95% CI 0.48 to 1.94)
Wu 2010 (29)	Intensive care	RBCT versus no RBCT	30 days	Not reported	OR 1.37 (95% CI 1.27 to 1.48)

Zilberberg 2008 (30)	Intensive care	RBCT versus no RBCT	In hospital	OR 1.51 (95% CI 1.31 to 1.75)	OR 1.21 (95% CI 1.00 to 1.48)
Volume 'A' red blood cells versus volume 'B' red blood cells					
Bernard 2009 (31)	Surgery	Volume RBCT versus another volume RBCT	30 days	Not reported	Intra operative 1 unit: OR 1.32(95% CI not reported) 2 units: OR 1.38(95% CI not reported) 3-4 units: OR 1.97(95% CI not reported) 5-10 units: OR 2.17(95% CI not reported) >10 units: OR 9.83(95% CI not reported) Post operative >4 units: OR 2.65 (95% CI not reported)
Charles 2007 (32)	Trauma	Volume RBCT versus another volume RBCT	24 hours	Not reported	3-5 units: OR 3.22 (95% CI not reported) ≥6 units: OR 4.87 (95% CI not reported)
O'Keeffe 2010 (33)	Cardiac surgery	Volume RBCT versus another volume RBCT	30 days	Not reported	1-2 units: OR 1.92 (95% CI 1.36 to 2.70) >3 units: OR 2.48 (95% CI 1.55 to 3.98)
Ruttinger 2007 (34)	Intensive care	Volume RBCT versus another volume of RBCT	In hospital	Not reported	1-2 units: OR 0.68 (95% CI 0.35 to 1.28) 3-4 units: OR 1.11 (95% CI 0.52 to 2.39) 5-8 units: OR 1.16 (95% CI 0.60 to 2.26) 8 units: OR 0.74 (95% CI 0.36 to 1.51)
Weightman	Cardiac surgery	Volume RBCT and other blood	Mean 8.1 year follow up	Not reported	1-2 units:

2009 (35)		product versus another volume RBCT			HR 1.00 (95% CI 0.70 to 1.44) 3-6 units: HR 0.98 (95% CI 0.67 to 1.41) > 6 units: HR 1.25 (95% CI 0.87 to 1.79)
'Older red blood cells versus 'newer' red blood cells					
Edgren 2010 (36)	Not specified	RBCT stored for 0- days, 10-19 days, 20-29 and 30-42 days	≤ 7 days and 8 to 730 days	Not reported	Mortality 1 to 7 days Stored 0- 9 days: HR 0.96 (95% CI 0.91 to 1.00) Stored 10-19 days: HR 1.00 (95% CI not reported) Stored 20-29 days: HR 1.06 (95% CI 0.96 to 1.06) Stored 30-42 days: HR 1.05 (95% CI 0.97 to 1.12) Mortality 8 to 730 days Stored 0- 9 days: HR 1.01 (95% CI 0.99 to 1.02) Stored 10-19 days: HR 1.00 (95% CI not reported) Stored 20-29 days: HR 0.99 (95% CI 0.97 to 1.01) Stored 30-42 days: HR 1.05 (95% CI 1.02 to 1.08)
Koch 2008 (37)	Cardiac surgery	RBCT stored for ≤ 14 days versus RBCT stored for > 14 days	In hospital and 1 year	Mortality in hospital OR 0.60 (95% CI 0.42 to 0.85)*	Mortality at 1 year p<0.001
Van de Watering 2006 (38)	Cardiac surgery	RBCT stored for <18 days versus RBCT stored for >18 days	30 days	Stored <18 days HR 1.33 (95% CI 1.04 to 1.68) Stored > 18 days HR 0.85 (95% CI 0.69 to 1.05)	Stored <18 days HR 0.93 (95% CI 0.71 to 1.23) Stored > 18 days HR 0.98 (95% CI 0.76 to 1.25)
Weinberg 2008 (39)	Trauma	RBCT stored for <14 days versus RBCT stored for >14 days	Time period not specified	Not reported	Stored <14 days 1-2 units: OR 1.65 (95% CI 1.01 to 2.70)

					≥ 3 units: OR 1.70 (95% CI 0.96 to 2.99) Stored ≥ 14 days 1-2 units: OR 1.78 (95% CI 1.06 to 2.98) ≥ 3 units: OR 2.78 (95% CI 1.58 to 4.88)

RBCT=red blood cell transfusion; *calculated from raw data; OR = odds ratio; RR = risk ratio; HR = hazard ratio