PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A systematic review of the effect of red blood cell transfusion on mortality: evidence from large scale observational studies published between 2006 and 2010
AUTHORS	Hopewell, Sally; Omar, Omar; Yu, Ly-Mee; Hyde, Chris; Doree, Carolyn; Murphy, Mike

VERSION 1 - REVIEW

REVIEWER	David Henry Institute for Clinical Evaluative Sciences, ICES I believe that I have no competing interests
REVIEW RETURNED	27-Oct-2012

GENERAL COMMENTS	The authors address a longstanding and important issue – the impact of red cell transfusion. Enthusiasm for RBCT has been blunted somewhat because of concerns about adverse effects, some with fatal outcomes. There have been a number of systematic reviews of a variety of interventions that modify exposure to RBCT. Some studies have pointed to the differences between outcomes of transfused and non-transfused individuals and the problems of adjusting for the many factors that may confound the relationship between RBCT and major health outcomes. The authors provide a fairly contemporary review of the field concentrating on recently published (2006 - 2010) larger (n>1000) controlled observational studies. They conclude unsurprisingly that there is likely to be a large degree of residual confounding of these studies and there is a need for large well controlled randomised trials, some of which are underway. They criticise the existing observational studies for inadequate adjustments, in particular non-inclusion of some factors that might confound the relationship between transfusion and clinical outcomes. My concerns about the manuscript fall into three main areas: 1) The focus of the review is broad in certain respects and narrow in others and I don't understand the logic that lies behind some of the authors' decisions about scope. 2) The assessment of the quality of the component studies 3) In looking at the adjustments for confounding they have focused more on the nature and the number of confounders and less on the methods used for adjustment . 1) The review includes observational studies of RBCT versus no RBCT; Volume 'A' of RBCT versus volume 'B' of RBCT (as defined by the primary studies) and 'Older' RBCT versus 'newer' RBCT. They have selected larger studies published between 2006 and 2010.

	a. This inclusion list suggests that the authors are interested in both control subjects who received no red cells OR received a smaller volume of allogeneic RBC. If so, there are a range of interventions that would effectively provide the same effect (less transfusions or smaller volumes) – studies of autologous RBCT, and a range of blood sparing techniques that reduce the frequency of transfusion or transfused volumes (eg cell salvage, normovolemic hemodilution, various sealants, some drugs and so on). Why have they not included studies of these interventions in their review? All of them appear to reduce allogeneic RBC exposure to some degree . b. I don't think the justification for limiting studies to publication between 2006 and 2010 is sound. There is no direct evidence that the methodological approaches improved in 2006 and I believe there may be relevant studies published both before and after the dates used by the authors. c. I am unclear why this review is confined to observational studies. For instance, there are a significant number of RCTs of transfusion thresholds (and other interventions to reduce allogeneic RBC volumes mentioned above) and there are published systematic reviews of these studies, which could be updated. d. Importantly (in my view) the authors have not considered a growing literature that looks at variation in transfusion rates between institutions and the impact on mortality (see below). 2) The authors state criteria for assessing the 'quality' of the observational studies they retrieved and tabulate their assessment of individual studies. On what basis did they choose these criteria? The field of quality assessment of non-randomised studies is not well developed but there are some (partially) validated instruments (eg Newcastle Ottawa Scale). A related issue is the reporting quality of the review. Some journals require the MOOSE instrument rather than PRISMA, which the authors provide. I don't have strong feelings but the former is directed more at observational studies. 3) The authors limit their study design considerations to an assessment of the choice of patient level covariates but they give limited consideration to the method of adjustment for confounders. This may be a reflection of the literature and authors have used mainly logistic regression and proportional hazards models. I would be interested to know whether any authors were able to derive propensity scores using a priori or high dimensional approaches and whether such analyses gave different outcomes? I am assuming not. But there is another relevant approach – the study of outcomes in institutions with very different RBCT rates. I am aware of a paper, about to be published, which describes a large observational study of RBCT in subjects having hip and knee replacement surgery. The authors found an adverse effect on 30 day and 1 year mortality of red cells using the 'traditional' patient level logistic regression approach. However, because they tracked transfusions across more than 60 institutions with marked inter-centre variation in rates of transfusion they were able to use the latter as an instrumental variable. The IVA found no adverse effect of RBCT on mortality, in contrast to the logistic regression analysis. A study published in 2010 (within the term of the present review) is also relevant - Bennett-Guerrero E, et al. Variation in use of blood transfusion in coronary artery bypass graft surgery. JAMA 2010; 304:1568–75. These authors found that transfusion rates across hospitals varied widely, but they found no associa¬tion between hospital-specific transfusion rates and postop¬erative mortality. There may be more studies like this in the literature and perhaps they should be studied.

	I accept that the analytical techniques used in the component studies in the present review – which looks at patients not institutions - may have been limited, but the general discussion on methodological issues is superficial, particularly given the stated intention of the paper to explore these issues.

REVIEWER	Prof James Isbister Consultant in Haematology and Transfusion Medicine Clinical Professor of Medicine, Sydney Medical School Royal North Shore Hospital of Sydney Adjunct Professor, University of Technology, Sydney Adjunct Professor of Medicine, Monash University, Melbourne
REVIEW RETURNED	10-Feb-2013

THE STUDY	In particular the authors opine that “well designed and adequately powered randomized controlled trials” are required to establish the “truth” of the observational studies. It is all very well to make these recommendations. However, in my view, it behoves the authors to clearly define what RCTs they demand. The authors are clearly qualified to make these recommendations and readers would appreciate clear guidelines on how equipoise would be established, what would be adequate powering, and what ethical, logistic and funding issues need to be addressed.
GENERAL COMMENTS	I make it clear in my review that in general I regard this as a good systematic review, although slightly dated. My main criticisms relative to the placing of the results in the context of the current state of knowledge and clinical practice. The author's introduction, conclusions and recommendations require major revision for their detailed review to be useful in progressing the issues surrounding the quality and safety of allogeneic red cell transfusion. In particular the authors need to clearly define what RCTs they suggest are required. Readers would appreciate clear guidelines on how equipoise would be established, what would be adequate powering, and what ethical, logistic and funding issues need to be addressed. **General Comments** This systemic review has been well conducted and I have no major questions on the validity of the analysis. My comments mainly relate to the introduction, discussion and recommendations. In particular these aspects of the manuscript do not place conclusions from the systematic review in the context of what is occurring at the clinical workface and the policy actions underway in many countries to progress concerns surrounding allogeneic blood transfusion outcomes. The authors have a limited perspective on how to strengthen the evidence base from observational data for red cell transfusions being an independent causal factor for adverse clinical outcomes. In their statements that RCTs are the only way forward they do not make specific, succinct nor practical recommendations as to exactly what RCTs they are suggesting and realistically addressing issues surrounding the feasibility of such trials ever being conducted. **Specific Comments**

	The title needs reconsideration. The observational data the authors include focuses on clinical outcomes not specifically on efficacy. Clinical outcomes relate to a combination of the presumed efficacy of red cell transfusion and the potential hazards, ie risk-benefit ratio. I say “presumed efficacy” as red cell transfusion have been grandfathered into clinical medicine not having to fulfil the rigid efficacy and safety criteria that would be demanded today for the introduction and registration of a new therapeutic. In the introduction and conclusion sections of the manuscript the authors make some questionable statements and come to debatable conclusions and recommendations. In my opinion the reasons for this could relate to the authors having not made a broader review of the transfusion medicine and patient blood management literature, taken a limited statistical frequentist view of evidence based medicine or failure to consider a “bigger picture” of the evidence and clinical practices in transfusion medicine and patient blood management. The authors state:- “*The impact that the contribution of data from observational studies has* *made to the practice of transfusion medicine has not been systematically explored.”* *“……“their impact on clinicians may be greater than is appropriate*” In my view this statement is difficult to substantiate and suggests to me an unawareness of what is happening in the real world of transfusion medicine and patient blood management. A brief review of the literature supports my contention as illustrated by these references: Freedman el al. Experience of a network of transfusion coordinators for blood conservation (Ontario Transfusion Coordinators [ONTraC]). Transfusion. 2008;48(2):237-50. Helm RE et al. Comprehensive multimodality blood conservation: 100 consecutive CABG operations without transfusion. The Annals of thoracic surgery. 1998;65(1):125-36. Kotze A et al. Effect of a patient blood management programme on preoperative anaemia, transfusion rate, and outcome after primary hip or knee arthroplasty: a quality improvement cycle. British journal of anaesthesia. 2012;108(6):943-52. The authors state:- *“This requires clearer and more widespread presentation and understanding of the existing* *research evidence, to which we believe this study is a significant contribution”.* I agree, but these opinions seem to ignore the reality of what is and has been happening for several years in current clinical practice, health sector policy development and in quality and safety initiatives in many countries. I contest the authors’ conclusions and opinions for several reasons:

	Although anaemia in haemodynamically stable patients is a risk factor for poorer clinical outcomes in a limited number of settings, (ie in patients with cardiovascular and pulmonary comorbidities) RBC transfusion has not be demonstrated to improve clinical outcomes. 1. The RCTs of restrictive transfusion policies have all confirmed the restrictive arms have had similar or better outcomes than the liberal RBC transfusion arms. What is commonly forgotten when considering the results of RCTs of restrictive transfusion, including by the authors of these papers, is exposing a patient to hazards of an intervention, for which the RCT has confirmed lack of clinical benefit, cannot been condoned. To state there is no difference in clinical outcomes for the two arms of such trials and to reassure clinicians they are not doing harm is unacceptable. These restrictive transfusion RCTs ignore the rare, but potential lethal hazards of allogeneic blood transfusions, as identified by haemovigilance programs (eg SHOT). These serious hazard include incompatibility, infection transfusion, transfusion associated GVHD and others. 2. There are studies in several counties that have demonstrated reduction in the use of labile blood components, in particular RBCs with no evidence of poorer clinical outcomes. Indeed most of these studies have demonstrated improved clinical outcomes, shorter lengths of hospital and ICU stay, and less or shorter periods of assisted ventilation. This is not to mention the cost-benefits that can be achieved. 3. Studies of Jehovah Witness patients have challenged dogmas surrounding the indications, efficacy and safety of red cell transfusion in elective surgical settings and in haemodynamically stable anaemic patients. One particular study warrants consideration (Reyes G et al Bloodless Cardiac Surgery in Jehovah's Witnesses: Outcomes Compared With a Control Group. Rev esp cardiol 2007;60(7):727-31.) The authors state:- *"In an observational study whether a treatment is received or not is likely to be heavily influenced by perceived need by the treating doctor……"* Experience and audit reviews demonstrate that the majority of RBC transfusions for stable anaemic patients are not administered on the basis of perceived need by the treating doctor, but rather a culturally imbedded default clinical practice with enormous variability between clinicians, specialties and hospitals. I reference only two studies in support this contention.

	Frank SM et al. Variability in blood and blood component utilization as assessed by an anesthesia information management system. Anesthesiology. 2012;117(1):99-106. Gombotz H et al. Blood use in elective surgery: the Austrian benchmark study. Transfusion. 2007;47(8):1468-80. Assessing the effects of smoking as a covariate in relation to blood transfusion and its effect on mortality is problematic. This is a difficult variable to assess in this context as cigarette smoking impacts directly on haemoglobin levels and blood volume in the short and longterm. For example the sudden cessation of smoking in relation to hospital admission or elective surgery may result in immediate falls in haemoglobin levels due to plasma volume expansion. In the conclusion the author's state: "*Observational studies do show a consistent adverse effect of RBCT on mortality. Whether this is a true effect remains uncertain……. 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*." The authors assume that the only valid method for determining the "*true effect*" should be addressed by well-designed and powerful randomized controlled trials. I agree this is true in some circumstances, but the authors do not consider that such trials may never be possible or ethical for several reasons. For example, RCTs addressing transfusion versus no transfusion would be challenging if not impossible. If assessment of mortality is to be the primary endpoint of these RCTs, enormous trials would be required as the mortality rates for most surgical settings in which transfusions are used are extremely low. Equipose also presents significant problems when designing RCTs in this field and has really only been achieved (with difficulty) in restriction versus liberal transfusion trials and age of stored blood used in transfusion. The authors seem to ignore in their discussion the fact that higher probability for causation from observational data can be strengthened by applying a Bayesian approach to the evidence as advocated by Austin Bradford Hill in his critera (Hill AB. The Environment and Disease: Association or Causation? Proc Roy Soc Med. 1965;58:295-300). In particular no mention is made of the importance of mechanistic evidence for adverse effects of blood transfusion. The extensive *in vitro* and *in vivo* data, especially from some of the recent larger animal research, provides supportive evidence for the adverse impacts of

	allogeneic blood transfusion, especially the storage age of blood transfused. I thus feel the authors have a responsibility to at least consider other “ways to act” in their discussion if they have in the title of the paper *“Time to act”.* The authors conclude the only way to act should focus on RCTs and not on the bigger picture of what can and should be done to improve the evidence base of RBC transfusion and what policy changes in the practice of transfusion medicine are important on the basis of the precautionary principle. As there is poor evidence for efficacy of RBC transfusions in anaemic haemodynamically stable patients and there is evidence for adverse clinical outcomes from RBC transfusions action has been occurring for several years. The views expressed by the authors are behind the times to suggest that now is the “time to act”. **Conclusion Comments** This systemic review has been well conducted and I have no major questions on the validity of the analysis. Unfortunately, there are more studies since 2010 addressing clinical outcomes from allogeneic red blood cell transfusion which tends to date this review. I also question the decision not to use some of the smaller studies as there are some that demonstrate substantial adverse outcomes of RBC transfusion with smaller patient numbers. I may seem harsh in my general criticisms of the introduction, discussion and recommendations. However, I feel justified in so doing for the reasons I have already outline. With a title suggesting it is “time to act” and includes “insights from a systematic review”, I feel obliged to express a broader view and to contest several of the authors’ statements and recommendations. It is for these reasons that in my opinion the core aspects of the systematic review warrant exposure in the medical literature. However, it is a long paper, the ultimate message is clear and brief, but placing this in a broader clinical, scientific and health policy context has been poorly and inadequately addressed by the authors. To put a positive spin on my peer review, if this manuscript is to be considered for publication I would suggest a more succinct manuscript summarising the systematic review and a more current assessment of the implications of their review. Perhaps having a link to the data as an appendix would be more appropriate. The title needs to be changed as most of the views expressed in the introduction, conclusions and recommendations are out of date and do not represent what is happening in the real world of

	transfusion medicine and patient blood management. The following title I suggest would be more appropriate: *"A* *systematic review of large scale observational studies of clinical outcomes of red blood cell* *transfusions from 2006 to 2010".* The authors opine that "*well designed and adequately powered randomized controlled* *trials"* are required to establish the "truth" of the observational studies. It is all very well to make these recommendations. However, in my view, it behoves the authors to clearly define what RCTs they are suggesting. The authors are clearly qualified to make these recommendations and readers would appreciate clear guidelines on how equipoise would be established, what would be adequate powering, and what ethical, logistic and funding issues need to be addressed.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1) The review includes observational studies of RBCT versus no RBCT; Volume 'A' of RBCT versus volume 'B' of RBCT (as defined by the primary studies) and 'Older' RBCT versus 'newer' RBCT. They have selected larger studies published between 2006 and 2010.

a. This inclusion list suggests that the authors are interested in both control subjects who received no red cells OR received a smaller volume of allogeneic RBC. If so, there are a range of interventions that would effectively provide the same effect (less transfusions or smaller volumes) – studies of autologous RBCT, and a range of blood sparing techniques that reduce the frequency of transfusion or transfused volumes (eg cell salvage, normovolemic hemodilution, various sealants, some drugs and so on). Why have they not included studies of these interventions in their review? All of them appear to reduce allogeneic RBC exposure to some degree .

RESPONSE: Our review had a specific inclusion criteria which by default would exclude a number of the different types of studies you mention above, some of which have already be addressed by other systematic reviews. By narrowing the focus of our inclusion criteria it allowed us to pay greater attention to the statistical methods and assumptions made in the analysis of the observational studies. This was highlighted as a specific aim of the review.

b. I don't think the justification for limiting studies to publication between 2006 and 2010 is sound. There is no direct evidence that the methodological approaches improved in 2006 and I believe there may be relevant studies published both before and after the dates used by the authors.

RESPONSE: When conducting this study, we undertook to look at the evidence over a defined five year period. Thus we aimed to provide a snap shot of current practice rather a comprehensive review of all available evidence. We have made this explicit within the review and also acknowledged this as a limitation.

c. I am unclear why this review is confined to observational studies. For instance, there are a significant number of RCTs of transfusion thresholds (and other interventions to reduce allogeneic

RBC volumes mentioned above) and there are published systematic reviews of these studies, which could be updated.

RESPONSE: Greater rationale for only including observational studies is given in the background section.

d. Importantly (in my view) the authors have not considered a growing literature that looks at variation in transfusion rates between institutions and the impact on mortality (see below).

RESPONSE: We have tried to address this variation in transfusion rates in the discussion section.

2) The authors state criteria for assessing the 'quality' of the observational studies they retrieved and tabulate their assessment of individual studies. On what basis did they choose these criteria? The field of quality assessment of non-randomised studies is not well developed but there are some (partially) validated instruments (eg Newcastle Ottawa Scale). A related issue is the reporting quality of the review. Some journals require the MOOSE instrument rather than PRISMA, which the authors provide. I don't have strong feelings but the former is directed more at observational studies.

RESPONSE: We are aware of a number of different checklists and scale for assessing the quality of non randomized studies although as yet there is no consensus on which is the most appropriate tool to use. As such we based our assessment criteria on recommendation from the HTA report by: Deeks JJ, Dinnes J, D'Amico R, Sowden AJ, Sakarovitch C, Song F, Petticrew M, Altman DG. Evaluating non-randomised intervention studies. Health Technol Assess 2003;7(27):iii-173.

3) The authors limit their study design considerations to an assessment of the choice of patient level covariates but they give limited consideration to the method of adjustment for confounders. This may be a reflection of the literature and authors have used mainly logistic regression and proportional hazards models. I would be interested to know whether any authors were able to derive propensity scores using a priori or high dimensional approaches and whether such analyses gave different outcomes? I am assuming not. But there is another relevant approach – the study of outcomes in institutions with very different RBCT rates. I am aware of a paper, about to be published, which describes a large observational study of RBCT in subjects having hip and knee replacement surgery. The authors found an adverse effect on 30 day and 1 year mortality of red cells using the 'traditional' patient level logistic regression approach. However, because they tracked transfusions across more than 60 institutions with marked inter-centre variation in rates of transfusion they were able to use the latter as an instrumental variable. The IVA found no adverse effect of RBCT on mortality, in contrast to the logistic regression analysis. A study published in 2010 (within the term of the present review) is also relevant - Bennett-Guerrero E, et al. Variation in use of blood transfusion in coronary artery bypass graft surgery. JAMA 2010; 304:1568–75. These authors found that transfusion rates across hospitals varied widely, but they found no association between hospital-specific transfusion rates and postoperative mortality. There may be more studies like this in the literature and perhaps they should be studied. I accept that the analytical techniques used in the component studies in the present review – which looks at patients not institutions - may have been limited, but the general discussion on methodological issues is superficial, particularly given the stated intention of the paper to explore these issues.

RESPONSE: Information on the method of adjusting of confounders was limited with studies using either logistic regression or proportional hazard ratios. Details on the nature of the statistical methods used is given in Appendix Table 3 to 5. Several studies did report using propensity scores prior to adjusting for confounders, however, sometimes this matching was only in a much smaller subgroup of patients. None the studies compared the different types of analysis. We have added details of those which included a propensity score to the results section.

We acknowledge that there may well be variations in institution and country usage of RBC transfusion and have tried to address this variation in clinical practice in the discussion section.

We have added a table of excluded studies as an appendix.

Reviewer 2:

In particular the authors opine that “well designed and adequately powered randomized controlled trials” are required to establish the “truth” of the observational studies. It is all very well to make these recommendations. However, in my view, it behoves the authors to clearly define what RCTs they demand. The authors are clearly qualified to make these recommendations and readers would appreciate clear guidelines on how equipoise would be established, what would be adequate powering, and what ethical, logistic and funding issues need to be addressed.

I make it clear in my review that in general I regard this as a good systematic review, although slightly dated.

RESPONSE: Please see earlier comments regarding the time frame of this review.

My main criticisms relative to the placing of the results in the context of the current state of knowledge and clinical practice. The author's introduction, conclusions and recommendations require major revision for their detailed review to be useful in progressing the issues surrounding the quality and safety of allogeneic red cell transfusion. In particular the authors need to clearly define what RCTs they suggest are required. Readers would appreciate clear guidelines on how equipoise would be established, what would be adequate powering, and what ethical, logistic and funding issues need to be addressed.

RESPONSE: We have revised the background to place this review in the context of the existing evidence and the uncertainty surrounding the contribution of evidence from observational studies in clinical practice. We have also revised the discussion section as suggested to place the results of this review in the context of current knowledge and clinical practice. We have removed the emphasis on the importance of conducting more randomized trials as on reflection seems to be beyond the .scope and findings of our review.

Response to additional reviewer 2 main comments:

The title needs reconsideration. The observational data the authors include focuses on clinical outcomes not specifically on efficacy. Clinical outcomes relate to a combination of the presumed efficacy of red cell transfusion and the potential hazards, ie risk-benefit ratio. I say “presumed efficacy” as red cell transfusion have been grandfathered into clinical medicine not having to fulfil the rigid efficacy and safety criteria that would be demanded today for the introduction and registration of a new therapeutic.

RESPONSE: We have revised the title to reflect both the nature of the clinical outcomes “mortality” and the time frame assessed within the review.

In the introduction and conclusion sections of the manuscript the authors make some questionable statements and come to debatable conclusions and recommendations. In my opinion the reasons for this could relate to the authors having not made a broader review of the transfusion medicine and patient blood management literature, taken a limited statistical frequentist view of evidence based medicine or failure to consider a “bigger picture” of the evidence and clinical practices in transfusion

medicine and patient blood management.

RESPONSE: We have revised the background to place this review in the context of the existing evidence and the uncertainty surrounding the contribution of evidence from observational studies in clinical practice. We have also revised the discussion section as suggested to place the results of this review in the context of current knowledge and clinical practice.

The authors state:- “The impact that the contribution of data from observational studies has made to the practice of transfusion medicine has not been systematically explored.” “……“their impact on clinicians may be greater than is appropriate”
In my view this statement is difficult to substantiate and suggests to me an unawareness of what is happening in the real world of transfusion medicine and patient blood management. A brief review of the literature supports my contention as illustrated by these references:
• Freedman el al. Experience of a network of transfusion coordinators for blood conservation (Ontario Transfusion Coordinators [ONTraC]). Transfusion. 2008;48(2):237-50.
• Helm RE et al. Comprehensive multimodality blood conservation: 100 consecutive CABG operations without transfusion. The Annals of thoracic surgery. 1998;65(1):125-36.
• Kotze A et al. Effect of a patient blood management programme on preoperative anaemia, transfusion rate, and outcome after primary hip or knee arthroplasty: a quality improvement cycle. British journal of anaesthesia. 2012;108(6):943-52.

RESPONSE: We have removed this sentence and also revised the background to reflect the current differences in the use of blood transfusion in different countries. We have also including these references in the revised discussion section.

The authors state:-
“This requires clearer and more widespread presentation and understanding of the existing research evidence, to which we believe this study is a significant contribution”.
I agree, but these opinions seem to ignore the reality of what is and has been happening for several years in current clinical practice, health sector policy development and in quality and safety initiatives in many countries. I contest the authors’ conclusions and opinions for several reasons:
Although anaemia in haemodynamically stable patients is a risk factor for poorer clinical outcomes in a limited number of settings, (ie in patients with cardiovascular and pulmonary comorbidities) RBC transfusion has not be demonstrated to improve clinical outcomes.
1. The RCTs of restrictive transfusion policies have all confirmed the restrictive arms have had similar or better outcomes than the liberal RBC transfusion arms. What is commonly forgotten when considering the results of RCTs of restrictive transfusion, including by the authors of these papers, is exposing a patient to hazards of an intervention, for which the RCT has confirmed lack of clinical benefit, cannot been condoned. To state there is no difference in clinical outcomes for the two arms of such trials and to reassure clinicians they are not doing harm is unacceptable. These restrictive transfusion RCTs ignore the rare, but potential lethal hazards of allogeneic blood transfusions, as identified by haemovigilance programs (eg SHOT). These serious hazard include incompatibility, infection transfusion, transfusion associated GVHD and others.
2. There are studies in several counties that have demonstrated reduction in the use of labile blood components, in particular RBCs with no evidence of poorer clinical outcomes. Indeed most of these studies have demonstrated improved clinical outcomes, shorter lengths of hospital and ICU stay, and less or shorter periods of assisted ventilation. This is not to mention the cost-benefits that can be achieved.
3. Studies of Jehovah Witness patients have challenged dogmas surrounding the indications, efficacy and safety of red cell transfusion in elective surgical settings and in haemodynamically stable anaemic patients. One particular study warrants consideration (Reyes G et al Bloodless Cardiac Surgery in Jehovah's Witnesses: Outcomes Compared With a Control Group. Rev esp cardiol

2007;60(7):727-31.)

RESPONSE: We agree with many of the helpful comments made and have tried to address this in revising the discussion and rewording of the review conclusions.

The authors state:-
“In an observational study whether a treatment is received or not is likely to be heavily influenced by perceived need by the treating doctor……”
Experience and audit reviews demonstrate that the majority of RBC transfusions for stable anaemic patients are not administered on the basis of perceived need by the treating doctor, but rather a culturally imbedded default clinical practice with enormous variability between clinicians, specialties and hospitals. I reference only two studies in support this contention.
• Frank SM et al. Variability in blood and blood component utilization as assessed by an anesthesia information management system. Anesthesiology. 2012;117(1):99-106.
• Gombotz H et al. Blood use in elective surgery: the Austrian benchmark study. Transfusion. 2007;47(8):1468-80.

RESPONSE: We agree this is misleading and have deleted this sentence from the introduction.

Assessing the effects of smoking as a covariate in relation to blood transfusion and its effect on mortality is problematic. This is a difficult variable to assess in this context as cigarette smoking impacts directly on haemoglobin levels and blood volume in the short and longterm. For example the sudden cessation of smoking in relation to hospital admission or elective surgery may result in immediate falls in haemoglobin levels due to plasma volume expansion.

RESPONSE: We still believe that it is important to include smoking as a covariate when assessing mortality and have included greater rationale in the methods section. However, given the difficulties you describe above we have given in less prominence in the discussion.

In the conclusion the author’s state: “Observational studies do show a consistent adverse effect of RBCT on mortality. Whether this is a true effect remains uncertain……. 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.”
The authors assume that the only valid method for determining the “true effect” should be addressed by well-designed and powerful randomized controlled trials. I agree this is true in some circumstances, but the authors do not consider that such trials may never be possible or ethical for several reasons. For example, RCTs addressing transfusion versus no transfusion would be challenging if not impossible. If assessment of mortality is to be the primary endpoint of these RCTs, enormous trials would be required as the mortality rates for most surgical settings in which transfusions are used are extremely low. Equipose also presents significant problems when designing RCTs in this field and has really only been achieved (with difficulty) in restriction versus liberal transfusion trials and age of stored blood used in transfusion.

RESPONSE: We agree and have removed the emphasis on the importance of conducting more randomized trials as on reflection seems to be beyond the findings of our review.

The authors seem to ignore in their discussion the fact that higher probability for causation from observational data can be strengthened by applying a Bayesian approach to the evidence as advocated by Austin Bradford Hill in his critera (Hill AB. The Environment and Disease: Association or Causation? Proc Roy Soc Med. 1965;58:295-300). In particular no mention is made of the importance of mechanistic evidence for adverse effects of blood transfusion. The extensive in vitro and in vivo

data, especially from some of the recent larger animal research, provides supportive evidence for the adverse impacts of allogeneic blood transfusion, especially the storage age of blood transfused. I thus feel the authors have a responsibility to at least consider other "ways to act" in their discussion if they have in the title of the paper "Time to act". The authors conclude the only way to act should focus on RCTs and not on the bigger picture of what can and should be done to improve the evidence base of RBC transfusion and what policy changes in the practice of transfusion medicine are important on the basis of the precautionary principle. As there is poor evidence for efficacy of RBC transfusions in anaemic haemodynamically stable patients and there is evidence for adverse clinical outcomes from RBC transfusions action has been occurring for several years. The views expressed by the authors are behind the times to suggest that now is the "time to act".

RESPONSE: We agree and have revised the title of the manuscript. We have also placed less emphasis on the importance of conducting more randomized trials as on reflection seems to be beyond the findings of our review.

VERSION 2 – REVIEW

REVIEWER	Prof James Isbister Consultant in Haematology and Transfusion Medicine Clinical Professor of Medicine, Sydney Medical School Royal North Shore Hospital of Sydney Adjunct Professor, University of Technology, Sydney Adjunct Professor of Medicine, Monash University, Melbourne No Conflicts of Interest
REVIEW RETURNED	24-Mar-2013

THE STUDY	My previous concerns have been well addressed
GENERAL COMMENTS	The review of the manuscript addresses most of the issues I raised in my original review. Those that are not commented on were at the discretion of the authors and do not impact on my opinion as acceptance for publication.