PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol for an overview of systematic reviews and meta-analyses comparing mortality in restrictive and liberal haemoglobin thresholds for red cell transfusion.
AUTHORS	Trentino, Kevin; Farmer, S; Sanfilippo, Frank; Leahy, Michael; Isbister, James; Mayberry, Rhonda; Hofmann, Axel; Murray, Kevin

VERSION 1 – REVIEW

REVIEWER	Lars B Holst Copenhagen University Hospital Rigshospitalet, Copenhagen Denmark. Dept. of intensive care 4131.
REVIEW RETURNED	04-Mar-2019

GENERAL COMMENTS	Trentino et al. present af protocol for a narrative review including all systematic reviews published in the English language, between the years 2008 and 2018, including trials randomising rest. vs lib. transfusion strategies. Authors adhere to reporting standards including timely Prospero registration. Comprehensive narrative reviews as such is needed in this area of transfusion medicine to give an overview if the growing litteratur. Concernes: 1)The title does not reflect the true nature of the review : a narrative review 2) Why do authors only look at mortality outcome ? many trials report other patient important outcomes as secondary outcomes and that may be interesting to get an overview of, from a clinical point of view? 3) Could authors please speculate on the biological plausibility for any mortality outcome based on transfusions (what may be the cause effect?) 4) Please account for the way you will handle an present the results from trials in different clinical areas (e.g. pre-,intra, post-operative and critical care setting. 4) What precise trial characteristics will authors tend to extract and present, this it not entirely clear in the present protocol? 5) It would be preferable for authors to widen the search for litterature to include trials earlier than 2008 and in all language to

	counteract the inherent bias and to do a comprehensive narrative review

REVIEWER	Annemarie Docherty University of Edinburgh, UK They will be reviewing my systematic review
REVIEW RETURNED	14-Mar-2019

GENERAL COMMENTS	Thank you for the opportunity to review this protocol for an overview of systematic reviews and meta-analyses comparing mortality in restrictive and liberal haemoglobin thresholds for red cell transfusion. This is a well written protocol, in a relatively new field of overviews of systematic reviews. The authors' intentions are clear, and they have documented their search strategies. It is an interesting question, given the large number of systematic reviews that have recently been published in this field, with differing findings. Could the authors clarify whether they are using the Preferred Reporting Items for Overviews of Systematic Reviews Checklist (my understanding is that this is still a pilot form?), or PRISMA (as per the appendix), or AMSTAR 2? It would be useful to have these forms all in the appendix. The authors have said that they will descriptively report the different mortality results of their findings. These many systematic reviews cover a range of different patients, different thresholds and different clinical settings. Could the authors clarify that they will take into account the heterogeneity of the different settings of these reviews? It may not be possible to summarise evidence from these systematic reviews given these underlying differences. Will the authors look at secondary outcomes in addition to mortality?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1, Lars B Holst

- "Trentino et al. present af protocol for a narrative review including all systematic reviews published in the English language, between the years 2008 and 2018, including trials randomising rest. vs lib. transfusion strategies. Authors adhere to reporting standards including timely Prospero registration. Comprehensive narrative reviews as such is needed in this area of transfusion medicine to give an overview if the growing litteratur."

We would like to thank Reviewer 1 for their comments. We agree this review is needed as the literature on transfusion thresholds has grown considerably. We feel this overview will be valuable to readers looking for an overview summarizing the systematic reviews and meta-analyses published on red cell transfusion thresholds to date.

Concernes:

1) The title does not reflect the true nature of the review : a narrative review

We selected our title: "Protocol for an overview of systematic reviews and meta-analyses comparing mortality in restrictive and liberal haemoglobin thresholds for red cell transfusion" carefully. Our intent was to include details of the study design (overview of systematic reviews), the intervention and comparison (restrictive/liberal transfusion thresholds), the outcome of interest (mortality), and mention the manuscript is a protocol.

In the Abstract section of our protocol, we explicitly state that the aim of our review is "…systematically collate, appraise and synthesise the results of these systematic reviews and meta-analyses…" Based on this definition we are confident that the best description of our study is an overview of systematic reviews.

This is consistent with the definition supplied by the Cochrane Handbook where it mentions overviews are: "reviews designed to compile evidence from multiple systematic reviews of interventions into one accessible and usable document" [https://handbook-5-1.cochrane.org/chapter_22/22_1_1_definition_of_cochrane_overviews_of_reviews.htm]
In addition, a recent paper entitled An introduction to overviews of reviews: planning a relevant research question and objective for an overview by Hunt et al. define an 'overview' as a "systematic summaries of systematic review evidence…" [Hunt H et al. An introduction to overviews of reviews: planning a relevant research question and objective for an overview. Syst Rev. 2018;7(1):39.]
Could we please have clarified whether Reviewer 1's concern is that our protocol states that we plan to present a "narrative synthesis of the findings"? If so, we draw attention to the point highlighted by Hunt et al. "often the nature of overviews results in narrative synthesis which can draw on either quantitative or qualitative data within included systematic reviews." [Hunt H, et al. An introduction to overviews of reviews: planning a relevant research question and objective for an overview. Syst Rev. 2018 Mar 1;7(1):39.]

2) Why do authors only look at mortality outcome ? many trials report other patient important outcomes as secondary outcomes and that may be interesting to get an overview of, from a clinical point of view?

This is an important point and we would like to thank Reviewer 1 for raising it as it allows us the opportunity to improve the clarity of our protocol. We are aware that many trials report a variety of morbidity outcomes in addition to mortality. We decided a priori to include only mortality as an outcome of our overview, because our aim is to focus on a consistently reported endpoint. The limitation of reporting on pooled morbidity events is that definitions, and grades and severity of morbidity events can vary considerably across trials. Various authors have brought this out, for example:
In their systematic review Holst et al. state "The definitions of overall morbidity and adverse events were heterogeneous and should be taken into account when interpreting these data" [Holst et al. Restrictive versus liberal transfusion strategy for red blood cell transfusion: systematic review of randomised trials with meta-analysis and trial sequential analysis. BMJ. 2015;350:h1354.]
Rohde et al. conducted a systematic review focusing on infection as an outcome. They noted that the "reporting of infectious outcomes varied across studies. In some trials, all infections were listed whereas in others only specific types of infections were reported." [Rohde et al. Health care-associated infection after red blood cell transfusion: a systematic review and meta-analysis. JAMA. 2014;311(13):1317-26.]
In their Cochrane review, Carson et al highlighted that "Different grades of severity of cardiovascular events, such as myocardial infarction, congestive heart failure or stroke, or risk of overall infection, will occur in participants and may present in ways that are not always clinically overt and so are more subjective in interpretation." [Carson et al. Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion. Cochrane Database Syst Rev. 2016;10:CD002042.]
To reduce these limitations, authors have recommended future studies uniformly measure morbidity using established robust definitions. We agree this is an important future step to improve the quality of studies. For this reason, we made a conscious decision to focus our review on a consistently reported patient outcome (mortality). We feel that as both Reviewer 1 and Reviewer 2 have made a similar point in this regard we have the opportunity to improve the clarity of our decision. Therefore, we have updated our protocol to include the following comments to the Outcomes section of our protocol:
"Our overview will not include morbidity outcomes. Though these outcomes are important, and often reported as secondary outcomes, they are not without limitations. For example, the definition, grade

and severity of morbidity events pooled by systematic reviews and meta-analyses vary considerably, and as a result are more subjective in interpretation."

3) Could authors please speculate on the biological plausibility for any mortality outcome based on transfusions (what may be the cause effect?)

Reviewer 1 raises an interesting comment. As confirmed with Reviewer 2, our study protocol was reported in alignment with the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) 2015 checklist." We have now included this checklist in our appendix, and this does not suggest reporting on the biological plausibility of the relationship between intervention and outcome. However, we plan to report our overview of systematic reviews according to the Preferred Reporting Items for Overviews of systematic reviews (PRIO) 2017 checklist. For the introduction section, checklist item 3b states: "Provide a balanced presentation of potential benefits and harms of the intervention(s)". Therefore, we intend to address Reviewer 1's comments by including these comments in our final overview manuscript. We feel that these checklists confirm our decision to reserve our comments on biological plausibility to the reporting of our overview of systematic reviews. As such, we have made a decision not to add this information to our protocol.

4) Please account for the way you will handle an present the results from trials in different clinical areas (e.g. pre-,intra, post-operative and critical care setting.

We would like to thank Reviewer 1 for highlighting this important point. Not all trials randomise patients at the same time. Some randomise pre-operatively, others commence post-operatively, and others only during a subsection of the patients admission. We too feel this is important and that is why we will collect information on the timing of intervention, as well as relevant information on the clinical setting. This information will be made clear in the main text of the article and in the forest plot presenting the mortality risk ratios. As per Reviewer 1's comments below, we have now added more detail on what will be presented in our Data Synthesis section.

5) What precise trial characteristics will authors tend to extract and present, this it not entirely clear in the present protocol?

We do make mention of the trial characteristics that we will extract under the Data Items subsection. To be more specific we have made the paragraph more detailed as below:

"Data items to be collected will include first author details, year of publication, databases searched, database search dates, population description, clinical setting (clinical specialty), inclusion criteria, exclusion criteria, total number of patients randomised, total number of trials pooled, subgroups measured (for mortality outcomes), subgroups reported (for mortality outcomes), study funding sources, conflicts of interests, and whether review authors co-author any trials included.

We will extract the following information specific to the intervention: description of the planned intervention haemoglobin thresholds pooled, differences in actual haemoglobin thresholds pooled between trials, post transfusion haemoglobin targets or units of red cells, description of the timing of intervention pooled between trials.

For our mortality outcomes we will collect: mortality time points pooled, mortality time points reported, total number of patients randomised in pooled mortality analysis, total number of trials pooled in mortality analysis, the total number of deaths in restrictive and liberal arms, the total number of patients randomised to liberal and restrictive arms, and heterogeneity (as measured by the review authors). In terms of transfusion results, we will collect the proportion of patients receiving red blood cells including in restrictive and liberal arms, and the mean and standard deviation number of units transfused in restrictive and liberal arms."

In addition, the Data Synthesis section of our protocol provides details of our data presentation. Again in line with Reviewer 1's suggestion we have made this more detailed.

6) It would be preferable for authors to widen the search for litterature to include trials earlier than 2008 and in all language to counteract the inherent bias and to do a comprehensive narrative review

We would like to clarify that our study will include trials from before 2008. We are limiting the results of our literature search to systematic reviews published after 2008 and this does not mean trials published before 2008 will be excluded. We can highlight the difference using the 2018 Cochrane review: Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion. This systematic review was published in 2000 and then updated in 2002, 2005, 2010, 2012, and 2016. Our literature search will pick up only the versions published after 2008. However, all the versions published after 2008 pool trials with no date restriction (the earliest trial pooled appears to be from 1956).

We made this decision to restrict systematic reviews to those published after 2008 because systematic reviews and meta-analyses are frequently updated as new trials are published. This point is referred to in our protocol: "The reason for restricting our search dates is because we aim to assess the most recent literature, and earlier meta-analyses are likely to be updated."

However we feel we could make this point more clear. Therefore in line with Reviewer 1's comments we have added the following sentence: "This restriction does not mean trials published prior to 2008 will be excluded, as updated systematic reviews and meta-analyses are likely to pool trials without date restrictions."

In terms of language, our protocol mentions "Our search will be restricted to reviews published in the English language as we do not have access to professional translators." Unfortunately, we are unable to change this. In line with the example above however, we would like to make clear that our review will not exclude any trials published in languages other than English, rather systematic reviews published in languages other than English. We note that many large systematic reviews do not include language restrictions in their search; this would mean that the results of any RCTs published in languages other than English would be included in our overview.

Reviewer: 2, Annemarie Docherty

- Thank you for the opportunity to review this protocol for an overview of systematic reviews and meta-analyses comparing mortality in restrictive and liberal haemoglobin thresholds for red cell transfusion. This is a well written protocol, in a relatively new field of overviews of systematic reviews. The authors' intentions are clear, and they have documented their search strategies. It is an interesting question, given the large number of systematic reviews that have recently been published in this field, with differing findings.

We too would like to thank Reviewer 2 for the time they have taken to review our protocol. We are pleased to read their comments regarding the quality of our protocol, and we feel this reflects well on the time and effort we spent designing and writing our study.

1) Could the authors clarify whether they are using the Preferred Reporting Items for Overviews of Systematic Reviews Checklist (my understanding is that this is still a pilot form?), or PRISMA (as per the appendix), or AMSTAR 2? It would be useful to have these forms all in the appendix.

We would like to thank Reviewer 2 for drawing our attention to this point that will allow us to improve the clarity of our protocol. The PRISMA Checklist is for reporting Systematic Reviews and Meta-Analyses. We will not use this checklist for our overview, however we use the PRISMA flow diagram to summarise our study selection. Although the PRIO (Reporting Items for Overviews of systematic reviews) checklist was published as a pilot tool [Bougioukas et al. Preferred Reporting Items for Overviews of systematic reviews including harms checklist: A pilot tool to be used for balanced reporting of benefits and harms. J Clin Epidemiol. 2017.], it is the only tool we identified specific to Overviews of Systematic Reviews and as such we feel that it is more relevant than checklists for Systematic Reviews and Meta-analyses. For example, it includes reporting items unique to overviews

such as reporting the methodological quality of included systematic reviews, and reporting measures of overlap. To make this point more clear we have added the following sentence in our protocol: "Although this checklist has been published as a pilot tool we have chosen to apply it as it contains reporting items specific to our study design."
We have also removed the sentence in the abstract that was referring to the checklist we would use to report our overview as this potentially contributed to the confusion.
In terms of our submitted protocol, we reported this in alignment with Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines. The reason why we chose this checklist is because we found no guidelines specific to protocols for overviews of systematic reviews, hence our decision to apply the PRISMA-P checklist (where relevant). To make this point clear we have added the following sentence to our METHODS AND ANALYSIS paragraph:
"This protocol was reported in alignment with the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) 2015 checklist." As per Reviewer 2's comments this checklist was uploaded with our initial submission.

As mentioned in our protocol the AMSTAR 2 Tool is used only "To assess the methodological quality of systematic reviews and meta-analyses included in our overview". We are happy to include the AMSTAR 2 form as an appendix for our future submission; however as our current submission is a protocol, we feel the reference to the AMSTAR 2 tool (reference 27) provides the readers with sufficient detail to understand the methods we will employ for our overview.

2) The authors have said that they will descriptively report the different mortality results of their findings. These many systematic reviews cover a range of different patients, different thresholds and different clinical settings. Could the authors clarify that they will take into account the heterogeneity of the different settings of these reviews? It may not be possible to summarise evidence from these systematic reviews given these underlying differences.

As we mention in the Data Synthesis section of our protocol, we will not pool data or conduct a meta-analysis of results. We agree that the systematic reviews are likely to cover a wide variety of clinical settings, with some even including mixed settings. We will, however, be recording information on heterogeneity presented within systematic reviews. In line with Reviewer 1's comments, we have added this point to our Data Items section of our protocol.
In addition, our Data Synthesis section makes clear that we will present results as a "narrative synthesis" so no results will be pooled together. This narrative review will allow us to discuss mortality outcomes by different clinical setting.

3) Will the authors look at secondary outcomes in addition to mortality?

We thank Reviewer 2 for raising this important question, also raised by Reviewer 1. As mentioned in our reply to Reviewer 1 we made a conscious decision to include only mortality as an outcome of our overview. We made this decision based on our aim to focus on a consistently reported endpoint. The limitation of reporting on pooled morbidity events is that definitions vary across trials as well as the grades and severity of events included. Various authors have brought this out, for example:
Holst et al. state "The definitions of overall morbidity and adverse events were heterogeneous and should be taken into account when interpreting these data" [Holst et al. Restrictive versus liberal transfusion strategy for red blood cell transfusion: systematic review of randomised trials with meta-analysis and trial sequential analysis. BMJ. 2015;350:h1354.]
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In their Cochrane review, Carson et al highlighted that “Different grades of severity of cardiovascular events, such as myocardial infarction, congestive heart failure or stroke, or risk of overall infection, will occur in participants and may present in ways that are not always clinically overt and so are more subjective in interpretation.” [Carson et al. Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion. Cochrane Database Syst Rev. 2016;10:CD002042.]

To reduce these limitations authors have recommended future studies uniformly measure morbidity using established robust definitions. For this reason we made a conscious decision to focus our review on a common and consistently reported patient outcome (mortality). However, given both reviewers have made this point we have updated our protocol to include the following to the Outcomes section:
“Our overview will not include morbidity outcomes. Though these outcomes are important, and often reported as secondary outcomes, they have limitations. For example, the definition, grade and severity of morbidity events pooled by systematic reviews and meta-analyses vary considerably, and as a result are more subjective in interpretation.”

VERSION 2 – REVIEW

REVIEWER	Annemarie Docherty University of Edinburgh, UK
REVIEW RETURNED	29-May-2019

GENERAL COMMENTS	Thank you for the opportunity to review this revised manuscript. The authors have addressed my concerns regarding the checklists they will be using. They have acknowledged that this will be a narrative review, as the heterogeneity of the clinical settings will prevent pooling of results. They will only look at mortality outcomes - this is clear and explicit, and therefore fine for the protocol. I think not looking at secondary outcomes is a potential missed opportunity however, as this is where the nuance of blood transfusion thresholds lies - virtually all the systematic reviews show that there is no difference in mortality between thresholds. The interesting part is surely the cardiovascular events in cardiovascular patients, or cancer recurrence/infection in cancer surgery etc?

VERSION 2 – AUTHOR RESPONSE

Reviewer: Annemarie Docherty
1) The authors have addressed my concerns regarding the checklists they will be using.

They have acknowledged that this will be a narrative review, as the heterogeneity of the clinical settings will prevent pooling of results.

They will only look at mortality outcomes - this is clear and explicit, and therefore fine for the protocol. I think not looking at secondary outcomes is a potential missed opportunity however, as this is where the nuance of blood transfusion thresholds lies - virtually all the systematic reviews show that there is no difference in mortality between thresholds. The interesting part is surely the cardiovascular events in cardiovascular patients, or cancer recurrence/infection in cancer surgery etc?

We would like to thank Reviewer 2 for the time they have taken to re-review our protocol. We are pleased to read their comments. Regarding mortality comments, we appreciate the Reviewers comments of a potential missed opportunity, however as previously mentioned we made a conscious decision to include only mortality as an outcome of our overview and we made this decision based on our aim to focus on a consistently reported endpoint. The limitation of reporting on pooled morbidity events such as cardiovascular events is definitions vary across trials as well as the grades and severity of events included. Various authors of systematic reviews and meta-analyses have highlighted this limitation and recommended caution in interpretation. For this reason, we made a conscious decision to focus our review on a common and consistently reported patient outcome (mortality). Based on both Reviewers comments regarding this in a previous review we updated our protocol to include the following to the Outcomes section:
“Our overview will not include morbidity outcomes. Though these outcomes are important, and often reported as secondary outcomes, they have limitations. For example, the definition, grade and severity of morbidity events pooled by systematic reviews and meta-analyses vary considerably, and as a result are more subjective in interpretation.”
As the Reviewer states: “They will only look at mortality outcomes - this is clear and explicit, and therefore fine for the protocol.” Given this we have made no further changes to our protocol.