Acute Normovolemic Hemodilution Reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-analysis of Randomized Trials

Anesthesia & Analgesia

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--Manuscript Draft--

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Abstract:	BACKGROUND: To better understand the role of acute normovolemic hemodilution (ANH) in a surgical setting with high risk of bleeding, we analyzed all randomized controlled trials (RCTs) in the setting of cardiac surgery which compared ANH to standard intraoperative care. The aim was to assess the incidence of ANH-related number of allogeneic red blood cell units (RBCu) transfused. Secondary outcomes included the rate of allogeneic blood transfusion and estimated total blood loss. METHODS: Twenty-nine RCTs for a total of 2439 patients (1252 patients in ANH group and 1187 in the control group) were included in our meta-analysis using PubMed/MEDLINE, Cochrane Controlled Trials Register and EMBASE. RESULTS: Patients in the ANH group received fewer allogeneic RBCu transfusions (mean difference = -0.79 , 95%CI -1.25 to -0.34 , p = 0.001 , I-square = 95.1%). Patients in the ANH group were overall transfused less with allogeneic blood when compared to controls [356/845 (42.1%) in the ANH group versus 491/876 (56.1%) in controls, risk ratio = 0.74 , 95%CI 0.62 to 0.87 , p < 0.0001 , I-square = 72.5%], and they suffered less postoperative blood loss (388 mL in ANH versus 450 mL in control, mean difference = -0.64 , 95%CI -0.97 to -0.31 , p < 0.0001 , I-square = 91.8%). CONCLUSION: Acute normovolemic hemodilution reduces the number of allogeneic

	RBCu transfused in the cardiac surgery setting together with a reduction in the rate of patients transfused with allogeneic blood and with a reduction of bleeding.
Response to Reviewers:	Dear Roman Sniecisnki, MD Executive Section Editor Anesthesia & Analgesia and Dear Jean-Francois Pittet, MD Editor-in-Chief Anesthesia & Analgesia, It is with great pleasure that we resubmit our edited manuscript, “Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials”, for consideration of publication in Anesthesia & Analgesia. We appreciate the excellent input from the Executive Section Editor and from the Statistical Editor. In addressing the considerably comments, we spent many hours editing the manuscript to better explain our results. We also carefully revised the manuscript with the help of an English mother tongue. All the authors have reviewed the paper and have approved of its resubmission. None of the data are submitted elsewhere for consideration. As the Editors and expert reviewers will see, we have taken great pains to address all comments. We hope these efforts have resulted in a manuscript viewed worthy of the high standard of Anesthesia & Analgesia. RE: MS#: AA-D-16-00021R2 "Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials" Dear Dr Monaco: Thank you for submitting your manuscript "Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials" to Anesthesia & Analgesia for consideration. Your manuscript has been reviewed by our editorial board and outside experts. Based on their reviews and my own reading of your manuscript, I would be happy to accept your manuscript for publication in Anesthesia & Analgesia, if you can provide point-by-point responses to my comments and those of the reviewers. Please see my comments and the comments from the reviewers below. Executive Section Editor Comments to the Author: Thank you for pursuing the requested statistical changes - I think this is very close to being acceptable. However, please see the below comments from the Statistical Editor which indicate several points remain unclear. We really want to make sure the reader understands all of the analysis you did, so precise wording is needed. If possible, it may help to have a native English speaker review the manuscript, paying particular attention to how the meta-analysis was constructed. I think the below comments are generally minor and should not take an exceptional amount of time. Thank you for your continuing efforts with this submission. We are sorry for the inaccuracy in the description of our analysis. We also carefully and extensively revised the manuscript with the help of an English mother tongue. _____ STATISTICAL EDITOR: Authors have responded well to many of my previous comments. However, a number of areas are still unclear and a careful further revision is therefore needed. In addition,

the manuscript could use assistance from a native English speaker on grammar and phrasing.

1. P10L13-18. Please clarify the first sensitivity analyses here. For the first one, did you perform a separate analysis after removing each trial? If so, state that more clearly. If something different, also clarify.

We thank the reviewer for his comment.
Yes, we performed the first sensitivity analysis by removing each single trial and then repeating a separate analysis for the rest of studies.
We now modified the text as: “We performed sensitivity analyses by sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies. We also performed sensitivity analyses including groups of studies with different amount of blood removed with ANH, different type of blood replacement, different type of surgery, different number of participants, the presence or not of a transfusion protocol, different year of publication and different amount of perioperative total blood loss. Finally we tested the interaction between these subgroups.”

2. P10L27. “Please clarify the meaning of "individual mean differences". Do authors mean "study-specific mean differences"?

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3. P12L4. In statistical methods you say that P < 0.10 would be significant for Egger's test, but you do not claim the P-value here of 0.074 as being statistically significant. Please explain.

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In the results section we now better write:“Visual inspection of the funnel plot did not reveal asymmetry (Figure 3) and Begg’s test was non significant (p < 0.174)” ; “Egger’s test (p < 0.074) became non significant (p = 0.404) after the exclusion of an outlier trial (Hurpe et al. 29)”

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We are sorry about this mistake. We performed analyses with each study removed one at a time.
We now write:
“Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies did not change the overall mean difference of number of RBCu transfused..”

5. P12L9-13, P12L51-53, P13L11-15. It is not clear what these two sets of estimated differences and conference intervals refer to in each of the sections. Please spell it out for the reader

We thank the reviewer for identifying this unclear piece of information in the manuscript.
We now better write in each sections:
“Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies did not change the overall mean difference of number of RBCu transfused: from a minimum of −0.76 (95% CI

−1.11 to −0.41, p < 0.0001) to a maximum of −0.51 (95% CI −0.95 to −0.13, p = 0.01).";

"Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies did not change the overall risk ratio of patients transfused with allogeneic red blood cells: from a minimum of 0.71 (95%CI 0.56 to 0.91 , p = 0.005) to a maximum of 0.78 (95%CI 0.64 to 0.94 , p < 0.001)."

And

"Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies not change the overall mean differences of total amount of blood loss: from a minimum of −92 mL (95%CI −121.92 to −63.21) p < 0.00001 to a maximum of −72 mL (95%CI -102.36 to −43.51) p < 0.00001."

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"We also found a reduction in the number of RBCu transfused in the ANH patients versus control in the subgroup of CABG surgery versus valve surgery, probably related to different tendency to bleed of these type of patients."

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To address heterogeneity we first visually analysed the forest plot and we found that the vast majority of the included trials had the same direction in the estimated effect in favour of ANH, and that only the magnitude (size) of the effect was different between the studies.
Visual inspection of the funnel plot did not reveal asymmetry (Figure 3) and Begg's test was non significant (p < 0.174) suggesting that studies with little precision (studies with few participants) did not give different results from studies with greater precision (studies with more participants). Egger's test (p < 0.074) became non significant (p = 0.404) after the exclusion of an outlier trial (Hurpe et al29).

The vast majority of the sub-analyses confirmed the results of the meta-analysis. Testing the interaction between subgroups we revealed the differences between groups of trials with different amount of blood removed (< 650 mL >) with ANH, between trials published before and after 2000 year, between trials with only valve surgery and CABG surgery, and between trials with or without a transfusion protocol could partially explain heterogeneity.
Thus, based on the results of the above-mentioned sensitivity analyses, we could conclude that the one of the reasons for the observed heterogeneity was the different magnitude in the estimated effects between the individual trials while there was strong consistency in direction of the estimated effects in favour of ANH. Additional explanation of heterogeneity could be the observed inter subgroup differences between studies.

We now report "p values for interaction between subgroups" in the Supplemental material table 2

We now write in the methods: "We performed sensitivity analyses by sequentially removing each study one at a time and then repeating a separate analysis for the rest

of the studies. We also performed sensitivity analyses including groups of studies with different amount of blood removed with ANH, different type of blood replacement, different type of surgery, different number of participants, the presence or not of a transfusion protocol, different year of publication and different amount of perioperative total blood loss. Finally we tested the interaction between these subgroups."

We write in the Results section:"Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies did not change the overall mean difference of number of RBCu transfused: from a minimum of −0.76 (95% CI −1.11 to −0.41, $p < 0.0001$) to a maximum of −0.51 (95% CI −0.95 to −0.13, $p = 0.01$).
In exploring the reasons for heterogeneity across RCTs we found differences between subgroups, that could partially explain the observed heterogeneity, in the following categories: amount of blood removed with ANH; the type of surgery; the year of publication; and the presence or not of a transfusion protocol . The test for differences between subgroups did not reveal variability in effect estimates within the following categories: type of blood replacement; the number of participants; and the amount of blood loss. P values for the interaction between subgroups are reported in Supplemental material Table 2."

Furthermore we write in the Discussion: "The forest plot of RBCu transfused clearly demonstrated that there is strong consistency in the direction of the estimated effect among the all analysed studies, and the vast majority of the sub-analyses also confirmed the primary result. Different magnitude of the effect among the studies could partially explain the observed heterogeneity together with the observed differences between subgroups across trials with less or more than 650 mL of blood removed, between trials published before and after 2000 year, between trials with valve surgery and CABG surgery, and between trials with or without a transfusion protocol."

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We thank the reviewer for this pertinent comment.
We modified it as follow: "The forest plot of RBCu transfused clearly demonstrated that there is consistency in the direction of the estimated effect among the all analysed studies, and the vast majority of the sub-analyses also confirmed the primary result. Different magnitude of the effect among the studies could partially explain the observed heterogeneity together with the observed differences between subgroups across trials with less or more than 650 mL of blood removed, between trials published before and after 2000 year, between trials with valve surgery and CABG surgery, and between trials with or without a transfusion protocol."

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We appreciate these observations and we are sorry about this inexact information in the previous version of the manuscript.
We now write: "We performed sensitivity analyses by sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies."

10. P16L9-11. "In fact the only reason for that was the difference in the magnitude and not in the direction of the effect." Please be clear on what you are saying here. Do you mean that the reason why the test for heterogeneity was significant is because of different magnitudes of the treatment effect and not of different directions? If so, please say more clearly.

We appreciate this observation. We have done some changes to clarify this as follows: "On the base of absence of the publication bias, absence of small study effect, and the supportive results from the majority of the sensitivity analyses, by sequential removing each study one at a time and also repeating analysis for group of studies, we

confirmed the primary result despite heterogeneity. In fact the reason for that was not only different magnitude in the estimated effect but also the differences between subgroup of trials."

11. P16L24-29. The phrasing here is awkward and not so clear. I assume you are talking about the effect of ANH versus standard care, but you do not say that the sentence. Also, I am not clear what you mean by "obtain more supportive data for a reduction in RBCu transfusion".

We thank the reviewer for his comment
We now better write: To date this is the metanalysis with the largest cardiac surgery population considered. More conclusive data are still lacking, therefore to obtain more strong data for a reduction in RBCu transfusion with the use of ANH versus standard care in cardiac surgery we probably need RCT designed for a specific surgery setting (CABG or valve surgery), in which the amount of blood removed is at least 650 mL and with a precise transfusion protocol."

If we do not receive a revised manuscript from you within the 4 weeks, I will assume that you have elected to decline to revise your manuscript.
Please revise your paper as guided by the reviewers' suggestions and provide a point-by-point description of how you responded to their suggestions and concerns. Submit your revision via Editorial Manager by logging in to your author account and clicking the link "Submissions Needing Revision." Be sure you have pasted your response to the reviewers into the appropriate box on the online submission site.

With all good wishes,
Roman Sniecinski, MD
Executive Section Editor
Anesthesia & Analgesia

Jean-Francois Pittet, MD
Editor-in-Chief
Anesthesia & Analgesia

We greatly thank the reviewers for their very valuable and useful comments and for their important contribution to our work.

With best wishes,

Fabrizio Monaco and colleagues

Department of Anesthesia and Intensive Care,
IRCCS San Raffaele Scientific Institute,
Via Olgettina 60, 20132 Milan, Italy

Dear Jean-Francois Pittet,

and

Dear Roman Sniecisnki

On behalf of my co-authors, I am submitting the enclosed material with the title: "**Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials"** for possible publication in Anesthesia & Analgesia.

Our work meta-analysis, performed on 29 randomized controlled trials in cardiac surgery, shows that acute normovolemic hemodilution reduces the number of red blood cell unit transfused in cardiac surgery. Furthermore there is a reduction in the rate of patients transfused with allogeneic blood and in the extent of bleeding, supporting our main results.

Our work is the most comprehensive review regarding all randomized controlled trials on acute normovolemic hemodilution published in the cardiac surgery setting, providing extensive evaluation of the efficacy of such protocol in cardiac surgery patients and adding important and clinically relevant information to the previous meta-analysis on the same topic.

Our work has not been submitted for publication nor has it been published in whole or in part elsewhere. I attest to the fact that all authors listed on the title page have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to Anesthesia & Analgesia.

Fabrizio Monaco

Dear Roman Sniecisnki, MD
Executive Section Editor
Anesthesia & Analgesia

and

Dear Jean-Francois Pittet, MD
Editor-in-Chief
Anesthesia & Analgesia,

It is with great pleasure that we resubmit our edited manuscript, "Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials", for consideration of publication in Anesthesia & Analgesia.

We appreciate the excellent input from the Executive Section Editor and from the Statistical Editor. In addressing the considerably comments, we spent many hours editing the manuscript to better explain our results. We also carefully revised the manuscript with the help of an English mother tongue.

All the authors have reviewed the paper and have approved of its resubmission. None of the data are submitted elsewhere for consideration.

As the Editors and expert reviewers will see, we have taken great pains to address all comments. We hope these efforts have resulted in a manuscript viewed worthy of the high standard of Anesthesia & Analgesia.

RE: MS#: AA-D-16-00021R2 "Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials"

Dear Dr Monaco:
Thank you for submitting your manuscript "Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials" to Anesthesia & Analgesia for consideration. Your manuscript has been reviewed by our editorial board and outside experts. Based on their reviews and my own reading of your manuscript, I would be happy to accept your manuscript for publication in Anesthesia & Analgesia, if you can provide point-by-point responses to my comments and those of the reviewers. Please see my comments and the comments from the reviewers below.

Executive Section Editor Comments to the Author:

Thank you for pursuing the requested statistical changes - I think this is very close to being acceptable. However, please see the below comments from the Statistical Editor which indicate several points remain unclear. We really want to make sure the reader understands all of the analysis you did, so precise wording is needed. If possible, it may help to have a native English speaker review the manuscript, paying particular attention to how the meta-analysis was

constructed. I think the below comments are generally minor and should not take an exceptional amount of time. Thank you for your continuing efforts with this submission.

We are sorry for the inaccuracy in the description of our analysis. We also carefully and extensively revised the manuscript with the help of an English mother tongue.

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STATISTICAL EDITOR:

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We now modified the text as: "We performed sensitivity analyses by sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies. We also performed sensitivity analyses including groups of studies with different amount of blood removed with ANH, different type of blood replacement, different type of surgery, different number of participants, the presence or not of a transfusion protocol, different year of publication and different amount of perioperative total blood loss. Finally we tested the interaction between these subgroups."

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“We also found a reduction in the number of RBCu transfused in the ANH patients versus control in the subgroup of CABG surgery versus valve surgery, probably related to different tendency to bleed of these type of patients.”

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lines, please clarify how you are making your conclusions about explaining heterogeneity.

To address heterogeneity we first visually analysed the forest plot and we found that the vast majority of the included trials had the same direction in the estimated effect in favour of ANH, and that only the magnitude (size) of the effect was different between the studies.
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The vast majority of the sub-analyses confirmed the results of the meta-analysis.
Testing the interaction between subgroups we revealed the differences between groups of trials with different amount of blood removed (< 650 mL >) with ANH, between trials published before and after 2000 year, between trials with only valve surgery and CABG surgery, and between trials with or without a transfusion protocol could partially explain heterogeneity.
Thus, based on the results of the above-mentioned sensitivity analyses, we could conclude that the one of the reasons for the observed heterogeneity was the different magnitude in the estimated effects between the individual trials while there was strong consistency in direction of the estimated effects in favour of ANH. Additional explanation of heterogeneity could be the observed inter subgroup differences between studies.

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We write in the Results section:"Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies did not change the overall mean difference of number of RBCu transfused: from a minimum of −0.76 (95% CI −1.11 to −0.41, $p < 0.0001$) to a maximum of −0.51 (95% CI −0.95 to −0.13, $p = 0.01$).
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magnitude of the effect among the studies could partially explain the observed heterogeneity together with the observed differences between subgroups across trials with less or more than 650 mL of blood removed, between trials published before and after 2000 year, between trials with valve surgery and CABG surgery, and between trials with or without a transfusion protocol."

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probably need RCT designed for a specific surgery setting (CABG or valve surgery), in which the amount of blood removed is at least 650 mL and with a precise transfusion protocol."

If we do not receive a revised manuscript from you within the 4 weeks, I will assume that you have elected to decline to revise your manuscript.
Please revise your paper as guided by the reviewers' suggestions and provide a point-by-point description of how you responded to their suggestions and concerns. Submit your revision via Editorial Manager by logging in to your author account and clicking the link "Submissions Needing Revision." Be sure you have pasted your response to the reviewers into the appropriate box on the online submission site.

With all good wishes,
Roman Sniecinski, MD
Executive Section Editor
Anesthesia & Analgesia

Jean-Francois Pittet, MD
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With best wishes,

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Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials

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ABSTRACT

BACKGROUND: To better understand the role of acute normovolemic hemodilution (ANH) in a surgical setting with high risk of bleeding, we analyzed all randomized controlled trials (RCTs) in the setting of cardiac surgery which compared ANH to standard intraoperative care. The aim was to assess the incidence of ANH-related number of allogeneic red blood cell units (RBCu) transfused. Secondary outcomes included the rate of allogeneic blood transfusion and estimated total blood loss.

METHODS: Twenty-nine RCTs for a total of 2439 patients (1252 patients in ANH group and 1187 in the control group) were included in our meta-analysis using PubMed/MEDLINE, Cochrane Controlled Trials Register and EMBASE.

RESULTS: Patients in the ANH group received fewer allogeneic RBCu transfusions (mean difference = -0.79 , 95%CI -1.25 to -0.34 , p = 0.001 , I-square = 95.1%). Patients in the ANH group were overall transfused less with allogeneic blood when compared to controls [356/845 (42.1%) in the ANH group versus 491/876 (56.1%) in controls, risk ratio = 0.74 , 95%CI 0.62 to 0.87 , p < 0.0001 , I-square = 72.5%], and they suffered less postoperative blood loss (388 mL in ANH versus 450 mL in control, mean difference = -0.64 , 95%CI -0.97 to -0.31 , p < 0.0001 , I-square = 91.8%).

CONCLUSION: Acute normovolemic hemodilution reduces the number of allogeneic RBCu transfused in the cardiac surgery setting together with a reduction in the rate of patients transfused with allogeneic blood and with a reduction of bleeding.

INTRODUCTION

In cardiac surgery, postoperative bleeding is one of the most relevant complications and, within a national blood supply range, it accounts for 15-20% of total transfusion requests. Allogeneic blood transfusions are associated with a worse short and long term outcome.[1,2] Most of the transfusions are represented by red blood cell units (RBCu) and for each unit transfused there is an additive risk of mortality and cardiac adverse events.[3-5] Careful control of major bleeding and management of blood losses can reduce the proportion of transfused patients and the number of surgical re-interventions.[6] Despite current guidelines indications and despite numerous approaches to reduce bleeding and to reduce hemoglobin transfusion threshold, more than 50% of the patients undergoing cardiac surgery receive transfusions.[7-9] This calls for different approaches and acute normovolemic hemodilution (ANH) could be a valid alternative. However doubts remain as to whether ANH is capable of reducing the need of allogeneic blood and of exerting a positive effect on morbidity and mortality.

Acute normovolemic hemodilution is performed by drawing a specific amount of blood volume from the patient, hydrating the patient to maintain isovolemia, storing patient's blood in storing bags at room temperature with anticoagulants, and re-administering it during surgery, usually following cardiopulmonary bypass (CPB) or according to patient's need. The beneficial effects of ANH are: reduced risk of adverse reactions related to transfusion of allogeneic blood products; preservation of erythrocytes from CPB damage; enhancing coagulation with the possibility of re-administering the patient's whole blood containing clotting factors and platelets; improved perfusion during CPB through a decrease of blood viscosity resulting in an increased tissue oxygen delivery above the critical anaerobic threshold.[10,11] Acute normovolemic hemodilution also is a simple and low cost procedure, with no evidence of coagulation, hemolysis, fibrinolysis or immunological activity in the collected blood.[11]

Cardiac surgery can be the ideal setting for ANH.[13-14] In fact, administering fresh whole blood after CPB allows to prevent the alteration induced by heparin administration, cardiotomy suction, and cellular activation during CPB which typically result in hemolysis, platelet activation and

consumption, complement activation and stimulation of the inflammatory cascade.[15-17] Furthermore, a reduction in blood viscosity during CPB seems to improve blood flow through stenotic and collateral vessels of the myocardium and counteracts the reduced blood oxygen-carrying capacity due to hemodilution.[18,19]

Our study is the first meta-analysis of randomized controlled trials (RCTs) conducted in adult patients undergoing any type of cardiac surgery aimed at comparing the intraoperative use of ANH versus control patients treated according to standard intraoperative care.

METHODS

Search strategies

A systematic review using PubMed/MEDLINE, Cochrane Controlled Trials Register, and EMBASE was performed by four trained investigators.[20] Additional studies were identified by manual research of references identified from original studies. In addition, authors employed backward snowballing (scanning through references of retrieved articles and pertinent reviews) and contacted international experts. Corresponding authors were contacted for missing data. The full PubMed search strategy was developed according to Biondi-Zoccai et al.[21] using the following key words: acute normovolemic hemodilution, intraoperative anemia, intraoperative autologous blood donation and cardiac surgery, search strategies are found in the Supplemental material Appendix 1 (updated November 2015). No language restrictions were enforced.

Study Selection

References were initially examined independently, by four investigators, at the title and abstract level. Divergences were resolved by consensus, and potentially relevant articles were retrieved in full formats. Inclusion criteria were: human studies performed in cardiac surgery (all types of procedures), random allocation to treatment, and comparison of ANH versus standard treatment.

Exclusion criteria were: overlapping publications, studies not conducted in adult population, and non-RCT studies. Two investigators independently assessed compliance with selection criteria and isolated the studies for final analysis (Figure 1). The authors' judgments regarding methodological quality for each included study are described in the supplemental material Supplemental Table 1.

Data Extraction

The primary end-point of this review was the number of allogeneic red blood cell units (RBCu) transfused for each patient. Secondary end-points were: the rate of allogeneic blood transfusion and estimated total blood loss.

Computations were performed with Stata (release 11, College Station, TX).[22] This study was performed in compliance with PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses).[23,24] Statistical heterogeneity hypothesis was tested with statistical significance set at two-tailed 0.1 levels, whereas extent of statistical consistency was measured with Higgins and Thompson's I-square. According to Higgins et al.[25], I-square values around 25, 50, and 75% were considered to represent low, moderate, and severe statistical heterogenicity respectively. We performed sensitivity analyses by sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies. We also performed sensitivity analyses including groups of studies with different amount of blood removed with ANH, different type of blood replacement, different type of surgery, different number of participants, the presence or not of a transfusion protocol, different year of publication and different amount of perioperative total blood loss. Finally we tested the interaction between these subgroups. We reported unadjusted P values throughout the paper. Study-specific risk ratio (RR) and its 95% confidence intervals (CI) were calculated for binary outcomes, whereas continuous variables were analyzed to compute study-specific mean differences with 95% CI. Pooled data were analyzed using the inverse variance method, either with a fixed-effect model in cases of low-moderate (I-square< 50%) statistical inconsistency, or with a random-effect model in case of moderate-high (I-square> 50%) statistical inconsistency.[26] Publication bias was assessed by visually inspecting funnel plots of primary outcomes, by analytical appraisal based on the Begg's adjusted-rank correlation test and on Egger's linear regression test (a two-sided p-value of 0.10 or less was regarded as significant).

RESULTS

Characteristics of the Included Studies

Twenty-nine randomized control trials for a total of 2439 patients (1252 patients in the ANH group and 1187 patients in the control group) were selected.[27-55] Baseline characteristics in ANH and control group were similar in all included studies. All but one studies included only elective patients with Herregods et al. [38] enrolling semi-urgent coronary artery bypass graft (CABG). None of the studies mentioned the enrollment of patients with risk factors for increased risk of bleeding in cardiac surgery as per blood conservation guidelines.[16] Sixteen RCTs included only CABG interventions,[30,31,33,36-39,44-47,49-51,54,55] five included combined CABG and valve operations,[27,28,35,40,52] three focused on heart valve interventions[41,48,53], one studied CABG and aortic arch repair patients[34] and four took into account all types of cardiac surgery procedures.[29,32,42,43] Two CABG studies focused on "off pump" patients,[46,50] two studies did not specify if "off or on pump" CABG procedures were performed[35,37] while all other studies analyzed procedures performed with CPB. Methods used to perform ANH were different among the analyzed studies; only one among all RCTs analyzed two types of ANH methods, low and high volume hemodilution.[29] Volume replacement was performed with colloids in the majority of studies[29-31,33,37-39,42-45,47,48,50-54], crystalloids in three studies[35,49,55]; a combination of crystalloids and colloids in two studies[34,46]; crystalloids and/or plasma protein fraction in two other cases,[27,28] and albumin in two studies.[27,32] Eight studies[27-29,33,37,38,41,55] did not use specific transfusion protocols. In two studies hemodilution was done after heparin infusion[36,41]. Characteristics of the included studies are described in Table 1 and baseline data in Table 2.

Data Synthesis

Primary outcome

Number of allogeneic red blood cell units transfused

In the 21 studies reporting this data and including overall 1852 patients, the ANH group received fewer RBCu transfusions, mean difference = -0.79 , 95%CI -1.25 to -0.34 , p = 0.001 , I-square = 95.1% . (Figure 2)

Visual inspection of the funnel plot did not reveal asymmetry (Figure 3) and Begg's test was non significant ($p < 0.174$) suggesting that studies with little precision (studies with few participants) did not give different results from studies with greater precision (studies with more participants). Egger's test ($p < 0.074$) became non significant ($p = 0.404$) after the exclusion of an outlier trial (Hurpe et al[29]).

Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies did not change the overall mean difference of number of RBCu transfused: from a minimum of −0.76 (95% CI −1.11 to −0.41, $p < 0.0001$) to a maximum of −0.51 (95% CI −0.95 to −0.13, $p = 0.01$).

In exploring the reasons for heterogeneity across RCTs we found differences between subgroups, that could partially explain the observed heterogeneity, in the following categories: amount of blood removed with ANH; the type of surgery; the year of publication; and the presence or not of a transfusion protocol . The test for differences between subgroups did not reveal variability in effect estimates within the following categories: type of blood replacement; the number of participants; and the amount of blood loss. P values for the interaction between subgroups are reported in Supplemental material Table 2.

Secondary outcomes

Rate of perioperative allogeneic blood transfusion

Less ANH patients received allogeneic blood transfusions compared to controls, 356/845 (42.1%) in the ANH group versus 491/876 (56.1%) in controls, risk ratio = 0.74, 95%CI 0.62 to 0.87, $p < 0.0001$, I-square = 72.5% , with Begg's test $p = 0.940$ and Egger's test $p = 0.015$, in 18 studies with 1721 patients included. (Supplemental material Figure 1)

Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies did not change the overall risk ratio of patients transfused with allogeneic red blood cells: from a minimum of 0.71 (95%CI 0.56 to 0.91 , $p = 0.005$) to a

maximum of 0.78 (95%CI 0.64 to 0.94 , $p < 0.001$).

Estimated total blood loss

Patients managed with ANH had less estimated total blood loss, 388 mL in ANH versus 450 mL in control, mean difference = -0.64, 95%CI -0.97 to -0.31, $p < 0.0001$, I-square = 91.8% , with Begg's test p = 0.013 and Egger's test p = 0.061 , according to 23 studies analyzed, which included 2043 patients. (Supplemental material Figure 2)

Sensitivity analyses showed that sequentially removing each study one at a time and then repeating a separate analysis for the rest of the studies not change the overall mean differences of total amount of blood loss: from a minimum of −92 mL (95%CI −121.92 to −63.21) $p < 0.00001$ to a maximum of −72 mL (95%CI -102.36 to −43.51) $p < 0.00001$.

DISCUSSION

To the best of our knowledge, the present meta-analysis including 29 RCTs and investigating ANH use versus standard treatment in cardiac surgery, is the first performed specifically in this setting and focusing on patients' need of allogeneic RBCu. The main identified finding is a clinically relevant reduction in RBCu transfusions in patients receiving ANH. Further findings are a decrease in the rate of patients transfused with allogeneic blood and a reduction in estimated total blood loss. These findings are of paramount importance and may have a great clinical and economic impact. Blood products' transfusions are known to be cost-intensive and to significantly increase the risk of perioperative complications, including mortality, prolonged hospitalization and increased hospital resource utilization.[56,57] In particular, cardiac and pulmonary dysfunction, neurological impairment, renal failure (50% of patients have a significant increase in serum creatinin and among these 5% needs renal replacement therapy) and infections (nosocomial infections occur in 10-20% of cardiac surgery patients) are increased in patients receiving transfusions and are associated with an overall worse outcome, higher in-hospital mortality, longer hospitalizations, and a higher rate of discharges to chronic care facilities.[3,4] Therefore, a strategy like ANH able to minimize the exposure to blood products may reduce costs and morbidity. Moreover, as reported by Grant et al[14], cardiac surgery is the most appropriate setting in which ANH may play a relevant role, since transfusion requirements remain high despite compelling evidence of many adverse effects and even though the advances in perioperative blood conservation techniques.[6-9] Data on ANH in cardiac surgery were still greatly conflicting and inconclusive before performing this meta-analysis. As observed by Goldberg et al. in a recent observational study on patients undergoing cardiac surgery, only 17% of patients received ANH.[58] Although several studies examined the role of ANH in cardiac surgery and guidelines proposed ANH as an approved practice in selected patients with adequate preoperative hemoglobin levels (class IIb, level B), to date there were no large randomized studies or meta-analyses which systematically reviewed the role of this technique in the cardiac surgery field.[16,59]

Previous meta-analyses established a limited usefulness of ANH in terms of outcome. Bryson, Laupacis and Wells, in 1998, performed a systematic review and meta-analysis, regarding ANH

management in cardiac and non-cardiac surgery and concluded that ANH reduces the need for allogeneic red blood cell transfusions (odds ratio 0.31 , 95%CI 0.15 to 0.62) in all areas, but with a less compelling evidence in cardiac surgery (odds ratio 0.51 , 95%CI 0.26 to 0.99). Bryson's meta-analysis considered only 11 RCTs performed in cardiac surgery.[60]

Another meta-analysis performed in 2004 by Segal et al. compared ANH to standard-of-care in all surgical settings suggested a small benefit of ANH but failed to perform a specific sub-category analysis in cardiac surgery.[61]

Recently Zhou et al. showed that ANH is effective in reducing allogeneic blood loss transfusion, with a significant heterogeneity and publication bias, and only when surgical blood loss is one liter or when it exceeds 20% of the patients' blood volume. However, the authors included in their meta-analysis a case-mixed population confirming, ultimately, the findings of previous meta-analyses. Moreover, Zhou's meta-analysis considered only 23 RCTs in cardiac surgery.[13]

In the present meta-analysis in order to be more exhaustive and to further validate safety and reliability of ANH practice, the rate of patients transfused with allogeneic blood transfusion and the estimated total blood loss were considered.

We confirmed a significant reduction in the number of allogeneic RBCu transfused. These data are coherent with Goldberg's study[58], in which a significant reduction in RBCu transfusions was observed in patients undergoing cardiac surgery in whom ANH protocol was adopted. The forest plot of RBCu transfused clearly demonstrated that in our meta-analysis there is strong consistency in the direction of the estimated effect among all analyzed studies, and the vast majority of our sub-analyses confirmed the results of the primary analysis. Different magnitude of the effect among studies could partially explain the observed heterogeneity together with the observed differences in several subgroups: trials with different quantities of blood removed; trials published before and after the year 2000; trials with valve surgery and CABG surgery; and trials with or without a transfusion protocol.

When a great volume of blood was removed (more than 650 mL) there was a more pronounced reduction in RBCu transfusion, in agreement with the report of Goldberg et al.[58] Recent trials show a reduced benefit from ANH probably because of reduced blood loss in modern cardiac surgery due to the widespread use in cardiac surgery of blood conservation techniques. We also found a reduction in

the number of RBCu transfused in the ANH patients versus control in the subgroup of CABG surgery versus valve surgery, probably related to different tendency to bleed of these type of patients; ultimately the use of a transfusion protocol could also influence the risk of RBCu transfusion. For the primary outcome we noted an absence of publication bias, absence of small study effect, confirmative results from most sensitivity analyses, by sequential removing each study one at a time and also repeating analysis for specific group of studies. We therefore concluded that the reason for heterogeneity due to different magnitude in the estimated effect and by the differences between subgroup of trials.

The rate of patients transfused with allogeneic blood and the estimated total blood loss were also decreased in the ANH group, confirming the beneficial effects of ANH on clinically relevant outcomes. These findings are consistent with those reported in Goldberg's study, in which an overall decrease in red blood cell transfusion was reported in the ANH group.[58]

To date this meta-analysis included the largest cardiac surgery population ever studied. Nonetheless, conclusive data are still lacking, and to obtain definitive data and demonstrate a reduction in RBCu transfusion with the use of ANH versus standard care in cardiac surgery we will probably need a large RCT designed for a specific surgery setting (CABG or valve surgery), in which the amount of blood removed is at least 650 mL and with utilizes a precise transfusion protocol.

Limitations

The following limitations of the present meta-analysis are acknowledged: only a restricted number of trials used the same hemodilution procedure and performed volume replacement with the same substance; colloids were extensively used for volume replacement in the ANH group and since they increase the risk of bleeding when compared to crystalloids they might have worsened the coagulation, resulting in decreased ANH efficacy[62]. In two studies ANH was performed after heparin infusion,[36,41] even though no differences in the results were found with sensitivity analyses. Heterogeneity was observed for the primary and secondary outcomes suggesting that large, high-quality RCTs are necessary to reach more conclusive results.

Conclusion

This meta-analysis is the most comprehensive review regarding all RCTs on ANH published in cardiac surgery and provides an extensive evaluation of the efficacy of such technique in this setting in decreasing the number of allogeneic RBCu transfused. This is in agreement with the observed reduction in the rate of patients requiring transfusions with allogeneic blood and in the estimated total blood loss. ANH can be considered a valid technique to reduce allogeneic red blood cell transfusions in the cardiac surgery setting.

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Figure legends

Figure 1. Flow diagram of the systematic review process

Figure 2. Forest plot of the number of allogeneic red blood cell units transfused. ANH, acute normovolemic hemodilution; CI, confidence interval; SMD, mean difference

Figure 3. Funnel plot of the number of allogeneic red blood cell units transfused. SMD, mean difference

Figure 1

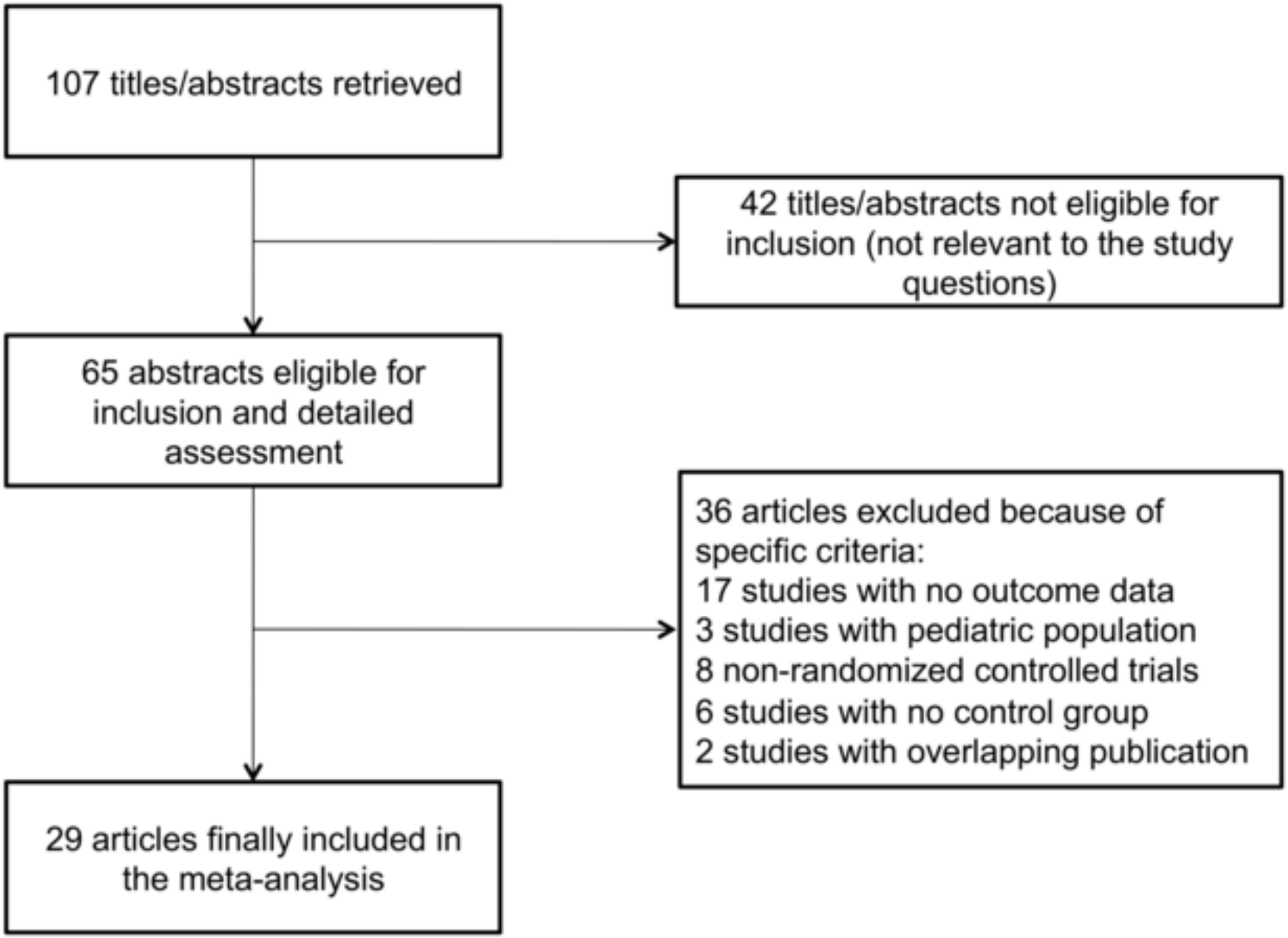

Figure 2

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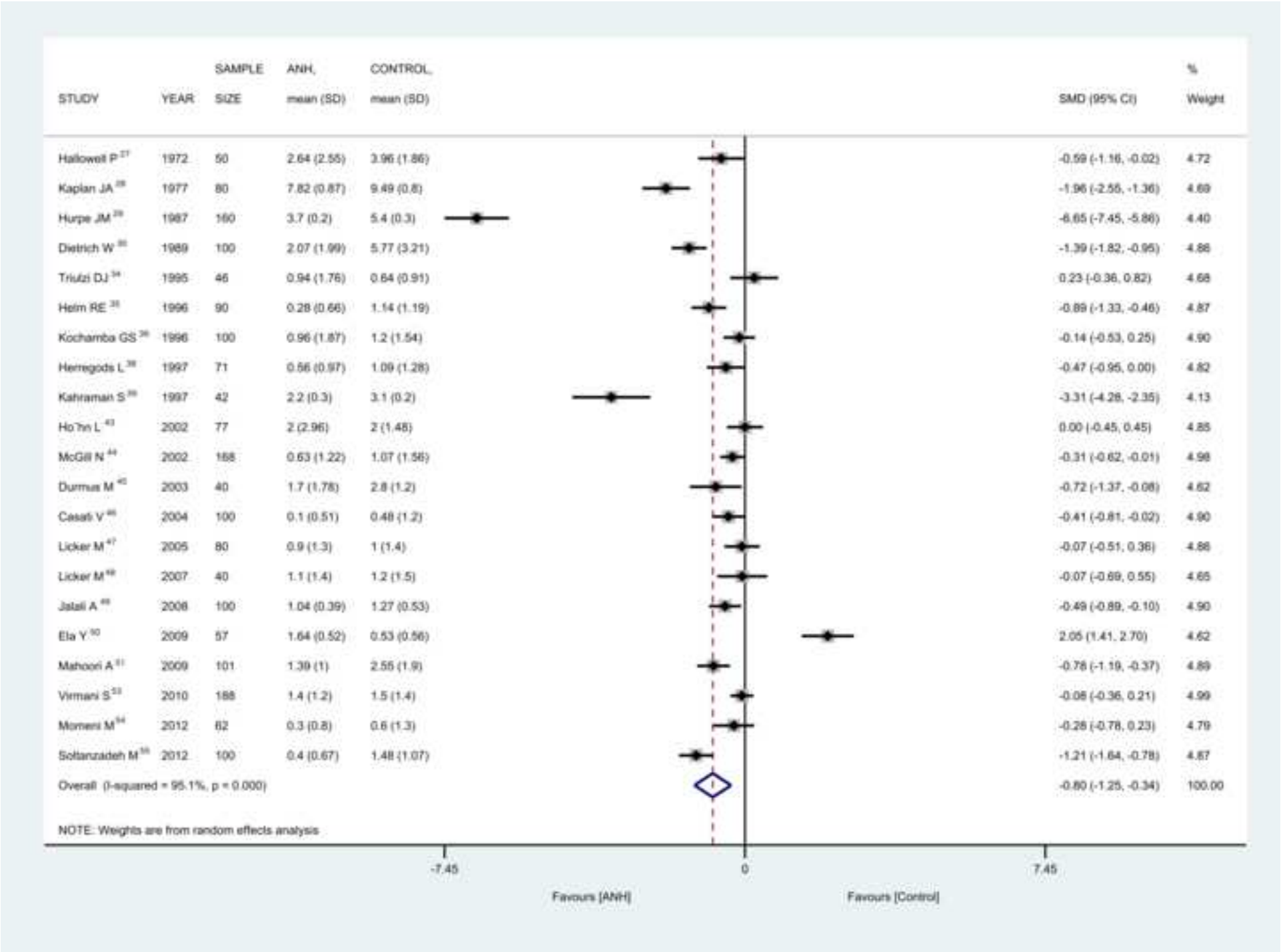

Figure 3

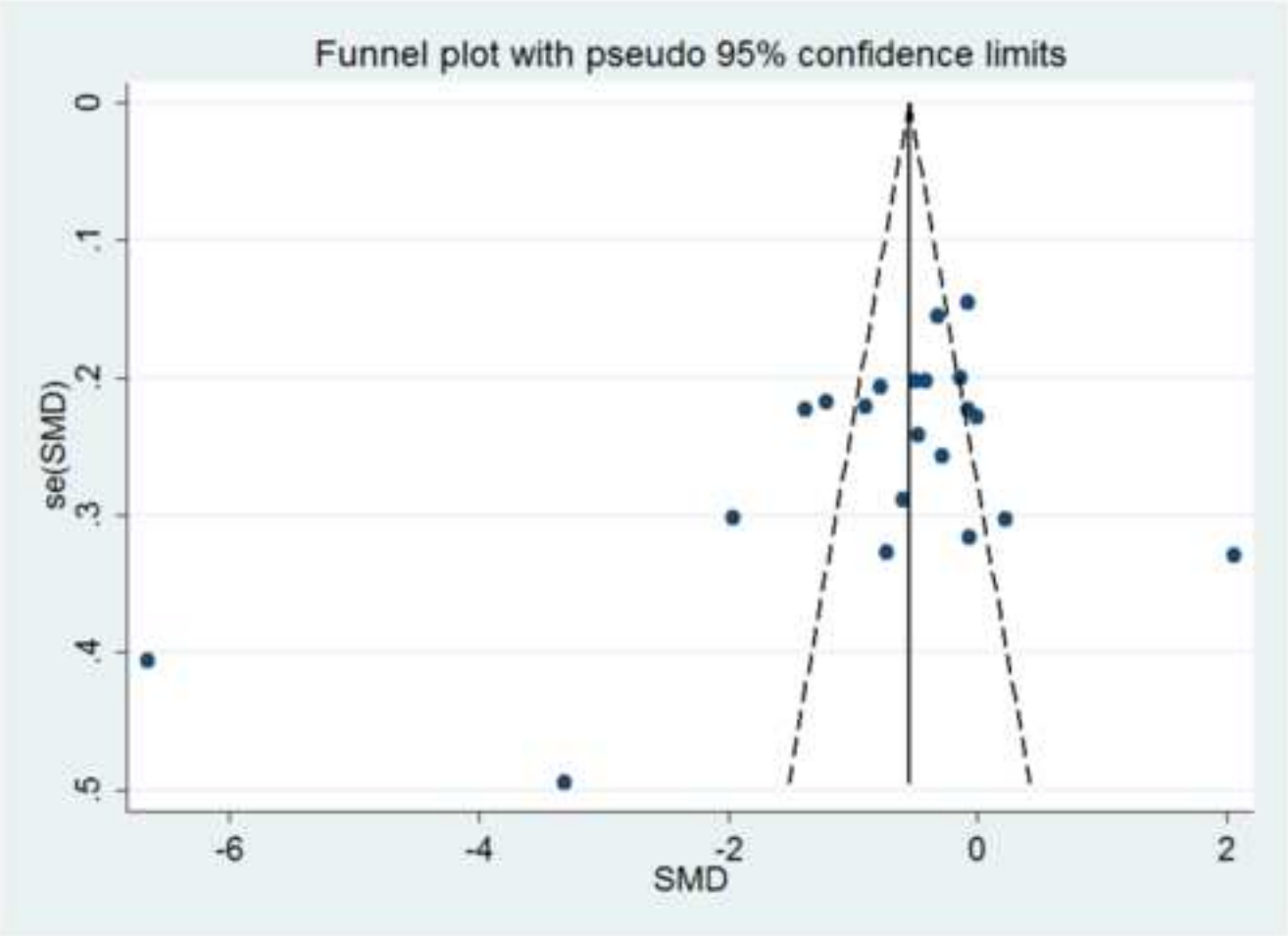

Table 1. Characteristics of included studies

First author, year	Journal	Type of cardiac surgery	Sample Size	Amount of blood removed (ANH)		Fluid replacement		Time of ANH reinfusion	Hb and/or Ht baseline		Hb and/or Ht post surgery		Transfusion protocol
				Formula	Average	Type	Ratio		ANH	Control	ANH	Control	
Hallowell P, 1972 [27]	J Thorac Cardiovasc Surg	CABG on pump and Valvular	50	≥2 unit of blood	1,252 mL	Crystalloids or PPF 5% and/or homologous blood or 5% Albumin	NR	After Protamine administration	Ht 39	Ht 38	Ht 29	Ht 30	NO
Kaplan JA, 1977 [28]	J Thorac Cardiovasc Surg	CABG on pump and Valvular	80	13-15% of total blood volume	742 mL	500 ml PPF and 1,000 mL of 5% Dextrose in Ringer Lactate	NR	After CPB	NR	NR	NR	NR	NO
Hurpe JM, 1987 [29]	Ann Fr Anesth Reanim	All cardiac surgery	160	10 mg/kg	734 mL	Gelatin	1:1	After CPB and Protamine administration	Ht 37.3	Ht 37.3	Ht 31.7	Ht 34.7	NO
Dietrich W, 1989 [30]	J Thorac Cardiovasc Surg	CABG on pump	100	10 mL/kg	731 mL	HES	NR	Before the end of operation	Ht 42	Ht 41	Ht 35	Ht 36	Ht<30
Boldt J, 1991 [31]	J Cardiothorac Vasc Anesth	CABG on pump	45	10 mL/kg	890 mL	Hypertonic saline and Colloids or Colloids	0.25:1 or 1:1	After CPB	Hb 13.6	Hb 13.5	Hb 12.3	Hb 10.6	Hb<7
Vedrinne C, 1992 [32]	J Cardiothorac Vasc Anest	All cardiac surgery	60	400 mL	400 mL	500 mL of 4% Albumin	1.25:1	After CPB	Hb 14.3	Hb 14.2	Hb 10	Hb 9.9	Ht<27 pre and after CPB; Ht<24 during CPB; Ht<30 in ICU
Herregods L, 1995 [33]	Anaesthesia	CABG	30	EBV (initial Ht- target Ht of 34%)/initial Ht	785 mL	Succinyl-linked Gelatin	NR	NR	Hb 13.6 Ht 40.3	Hb 13.2 Ht 39.1	NR	NR	NO
Triulzi DJ, 1995 [34]	Transfusion	CABG on pump and Aortic Arch Repair	46	17±2% of circulating blood volume (16-18%)	924 mL	Colloids and Crystalloids	1:1 and 3:1	After CPB and within 6 hours of collection	Hb 14.2	Hb 14.1	Hb 10	Hb 9.5	Ht<20 during CPB; Ht<24 after CPB
Helm RE, 1996 [35]	Ann Thorac Surg	CABG on pump and Valvular	90	EBV (initial Ht - Ht Pre-CPB) / Ht initial	NR	Crystalloids	2:1	30 min after Protamine administration	Ht 41	Ht 40	Ht 20	Ht 22	Ht pre CPB ≤15; Ht during CPB ≤19 or symptomatic anemia; Ht after CPB/ICU ≤21.9

													or symptomatic anemia
Kochamba GS, 1996 [36]	Ann Thorac Surg	CABG on pump	100	10 mL/kg	869 mL	NR	NR	After CPB and Protamine administration	Ht 38.6	Ht 29.5	Ht 29.5	Ht 29.7	Ht<25
Spahn DR, 1996 [37]	Anesth Analg	CABG	90	12 mL/kg	NR	6% HES	1:1	NR	Hb 14.1	Hb 13.9	NR	NR	NO
Herregods L, 1997 [38]	J Cardiothorac Vasc Anest	CABG on pump	71	NR	750 mL	Succinyl-linked Gelatin	NR	NR	Hb 13.6 Ht 40.2	Hb 13.3 Ht 39	Hb 13.6 Ht 40.2	Hb 13.3 Ht 39	NO
Kahraman S, 1997 [39]	Acta Anaesthesiol Scand	CABG on pump	42	5-8 mL/kg or 12-15 mL/kg	741 mL	3.5% Haemaccel and Isolyte	1:1•	After Protamine administration	Ht 42.6	Ht 40.3	Ht 35.9	Ht 36.8	Ht<30 post CBP
Nuttall GA, 2000 [40]	Anesthesiology	CABG on pump and Valvular	75	12.5% of total blood volume	859 mL	NR	NR	After CPB and Protamine administration	NR	NR	NR	NR	Hb< 8 or Hb<7 during CPB
Zhang S, 2000 [41]	J Tongji Med Univ.	Valvular	24	Ht before surgery - Ht before CPB/ Ht before surgery	892 mL	NR	NR	30 min after CPB	Ht 45	Ht 47	Ht 42	Ht 33	NO
Casati V, 2002 [42]	Anesthesiology	All cardiac surgery	202	5–8 mL/kg	648 mL	Emagel	1:1	At the end of surgery, before transporting patients to the intensive care unit or during surgery if Ht<20 or Hb<6.5	Hb 14 Ht 42	Hb 13.6 Ht 41.6	Hb 11.7 Ht 34.5	Hb 11.6 Ht 34.2	Hb<6.5 and Ht<20% before CPB; Hb<6.5 or Ht<20 during CPB; Hb<8.5 or Ht<25 after CPB
Höhn L, 2002 [43]	Anesthesiology	All cardiac surgery	77	EBV (initial Ht- target Ht of 28%)/initial Ht	1,099 mL	6% HES	1:1	At last before leaving the operating room	Hb 14.1	Hb 14.2	Hb 9.9	Hb 10	Ht <28 before CPB; Ht <17 during CPB or 20% in increased mortality risk patients; Ht <25 after CPB
McGill N,	BMJ	CABG	168	10 mL/kg	843 mL	Gelofusine	1:1	After	Hb	Hb	Hb	Hb 10	Hb<9; Ht<27

2002 [44]		on pump						Protamine administration	14.5	14.2	10.8		
Durmus M, 2003 [45]	Anestezi Dergisi	CABG on pump	40	1 or 2 blood unit	612 mL	6% HES	1:1	NR	Ht 33.6	Ht 37.2	Ht 29	Ht 31.3	Ht<18 during CPB; Ht<29 after CPB
Casati V, 2004 [46]	Anesth Analg	CABG off pump	100	17% ± 2% of the circulating volume	850 mL	4% Succinylated Gelatin and Lactate Ringer solution	1:1	After Protamine administration	Hb 13.8 Ht 41.3	Hb 13.9 Ht 41.1	NR	NR	Hb<8 and Ht <24 after on-demand reinfusion of shed blood and, in the ANH group, after reinfusion of the harvested autologous blood
Licker M, 2005 [47]	Chest	CABG on pump	80	EBV (initial Ht- final Ht 28%)/initial Ht	NR	6% HES	1,15:1	During CPB o shortly after weaning of CPB	Ht 41	Ht 40	Ht 27	Ht 27	Ht<18 during CPB and <25 after CPB, or higher values (26 to 30) when accompanied by hemodynamic instability and/or ECG signs of myocardial ischemia
Licker M, 2007 [48]	Transfusion	Valvular	40	EBV (initial Ht- final Ht 28%)/initial Ht	NR	6% HES	1,15:1	During CPB o shortly after weaning of CPB	Hb 14.5	Hb 14.3	Hb 9	Hb 9.2	Ht<18 during CPB and <25 after CPB, or higher values (26 to 30) when accompanied by hemodynamic instability and/or ECG signs of myocardial ischemia
Jalali A, 2008 [49]	Acta Cardiol	CABG on pump	100	Up to a target of Hb	NR	0.9% Saline solution	3:1	Before leaving the operating	Ht 45.1	Ht 44.1	Ht 31.8	Ht 32.4	Hb<8

				9				room					
Ela Y, 2009 [50]	Heart Surg Forum	CABG off pump	57	EBV (initial Ht- final Ht 28%)/initial Ht	NR	6% HES	1.10:1	Operating room	Hb 15 Ht 46.3	Hb 14.7 Ht 44.1	NR	NR	Hb<8 or Ht<25
Mahoori A, 2009 [51]	Mid East J Anaesthesiol	CABG on pump	101	10% of patients' blood volume	490 mL	Gelatin solution	1:1	After CPB when Hb<10 or Ht<30	Hb 13.7 Ht 40	Hb 13.2 Ht 39	Hb 10	Hb 11	Hb<10; Ht<30
Zisman E, 2010 [52]	Eur J Anaestesiol.	CABG on pump and Valvular	62	10 mL/kg (15% of EBV)	600 mL	6% HES	1:1	After CPB	Hb 14.4	Hb 14.2	Hb 9.5	Hb 9.8	Hb <7 during CPB or <9 after surgery
Virmani S, 2010 [53]	Ann Card Anaesth	Valvular	188	10% of EBV in patients with Hb>12g% and 7% when the Hb was <12g%	270 mL	HES	1:1	After CPB and Protamine administration	Hb 12.4	Hb 12.1	Hb 10.6	Hb 10.3	Hb≤6 during CPB; Hb≤8 after CPB
Momeni M, 2012 [54]	J Cardiothorac Vasc Anest	CABG on pump	61	NR	NR	6% Volulyte	1:1	If Ht < 20 during CPB or Ht < 25 after CPB	NR	NR	NR	NR	Ht≤20 during CPB Ht≤25 after CPB
Soltanzadeh M, 2012 [55]	Life Sci J	CABG on pump	100	500 mL	500 mL	Ringer lactate solution	3:1	After Protamine administration	NR	NR	NR	NR	NO

ANH, acute normovolemic hemodilution; CPB, cardiopulmonary bypass; ECG, electrocardiogram; EBV, estimated blood volume; final Ht, lowest acceptable Ht; Ht, hematocrit (values expressed in percentage); Hb, hemoglobin (values expressed in grams/deciliter); min, minutes; NR, not reported; PPF, plasma protein fraction; •50% crystalloids and 50% colloids

Table 2. Baseline characteristics

Baseline Characteristics	**ANH group** (N 1252)	**Control group** (N 1187)	**M-H pooled RR/I-V pooled MD**		**I^2(%) for heterogeneity**
			RR/MD (95%CI)	**p**	
Age (years)	58.4±8.7	58.1±8.5	0.50 (-1.62 , 2.62)	0.65	97
Weight (kg)	74.1±10.5	72.3±10.6	1.31 (-0.31 , 3.78)	0.1	75
Height (cm)	168.2±26.7	166.3±23.3	1.97 (-0.38 , 4.33)	0.1	86
Female (%)	21.6	24.4	0.90 (0.77 , 1.05)	0.19	0.0
PT (sec)	11.7±1.8	11.4±1.4	0.40 (-0.05 , 0.84)	0.08	51
PTT (sec)	39.9±15.3	40.6±12.6	-1.33 (-3.00 , 0.35)	0.12	91
PLT (10^3/mm^3)	210.1±47.4	202.7±46	10.82 (-2.89 , 24.53)	0.12	94
Hb (g/dl)	13.9	13.6	-7.05 (-19.90 , 5.80)	0.28	99
Ht (%)	40.9	39.5	1.12 (-0.64 , 2.89)	0.21	99
Creatinine (mg/dl)	1.07±0.2	1.05±0.2	0.02 (-0.01, 0.06)	0.20	11
LVEF (%)	50.3	50.2	0.24 (-0.26 , 0.74)	0.34	87

Baseline values are showed as weighted means or as percentage. No statistically significant differences between the groups. ANH, acute normovolemic hemodilution; Hb, hemoglobin; Ht, hematocrit; LVEF, left ventricular ejection fraction; MD, mean difference; N, number of patients; PLT, platelets; PT, prothrombin time; PTT, partial thromboplastin time; RR, risk ratio

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04/16/2016

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Manuscript Title: **Acute Normovolemic Hemodilution reduces Allogeneic Red Blood Cell Transfusion in Cardiac Surgery: A Systematic Review and Meta-Analysis of Randomized Trials**

Author: Alberto Zangrillo

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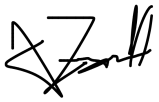

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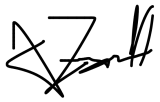

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