Peer Review File
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Reviewer A

Comment 1: The benefits of BAR remain to be seen and most (i.e. 99.9%) of the LTx community members must not believe that the advantages are significant since essentially only 1 or 2 surgeons in the world are performing this procedure. That being said, theoretically, the physiologic benefits are intriguing and the question remains one of interest. Unfortunately, the number of cases in the world over the last 30 years are minimal and BAR adds to the duration and complexity of an already long and complex operation. Therefore, the advantages would need to be clear, relevant, and objectively measurable. Also, the limited numbers and the fact that they are all uncontrolled case series precludes the ability to do a proper meta-analysis.

Reply 1: It is unfortunate that more data do not exist for assessing the benefits of LTx with and without BAR, however, given its theoretical benefits and the enthusiasm of its proponents, it was though worthwhile to pool the existing knowledge on the topic. Since meta-analysis methods entail quantitative assessment of outcomes reported across studies whilst assuring quality standards, we have attempted the same here with the best available evidence. The quality assessment of each included study is included in the supplementary material as well. Overall, the studies were of moderate to good quality according to the Newcastle Ottawa scale.
Given our results, we would suggest that further investigation on the theoretical benefits (reduced BOS) of BAR is warranted.

Changes in the text: No new changes

Comment 2: In the introduction the authors talk about IR injury as an instigator of injury as if BAR might reduce that in some form or fashion, but in reality, BAR

doesn't impact IR injury that we know of. Rather it impacts chronic ischemia to the airways.

Reply 2: Thank you for highlighting this. The mention of IR was in the context of one of the causes of BOS; however, our main narrative remains that BAR may reduce airway ischemia, which may consequently reduce BOS. In an attempt to make this distinction clearer, we have rephrased the second paragraph in the introduction as follows (Lines 85-94):

Changes in the text: *The development of BOS has been hypothesized to occur due to many factors, such as acute rejection, cytomegalovirus infection, and ischemia-reperfusion injury. Airway ischemia, inflammation, and subsequent necrosis due to reduced oxygenated blood supply have also been implicated in the development of progressive inflammation and fibrosis potentially leading to BOS. This could be because following a typical lung transplantation; the lower airways are perfused via minimal retrograde flow from the pulmonary veins as the arterial flow from the bronchial arteries is sacrificed in the transplantation process. A permanent reduction in adequately oxygenated blood to the pulmonary airways could thus increase the risk of chronic ischemia and hypoxic damage*

Comment 3: The %'s in the text for various lung diseases appear wrong and add up to more than 100%?. Perhaps it's a typo for the A1A being 40% of the patients (line 154)

Reply 3: Thank you for the chance to clarify this. The lung diseases mentioned overlap among studies, which is why the percentages do not add up to 100%. Since this is cohort-level data, different cohorts report different incidences of these diseases. When pooled, these percentages do not add up to 100, given that pooled values do not always add up to 100%. For example, the 71% with emphysema and 40% with alpha 1-AT do not constitute mutually exclusive populations.

Changes in the text: No new changes

Comment 4: The authors indicate that only 87% of the tracheal anastomoses healed? That seems strange as it is much worse than the healing rate of a Bilateral sequential lung transplant with bronchial anastomosis.

Reply 4: Thank you for highlighting this. Regarding tracheal healing, 87% is the rate for complete healing. Five other patients had complicated/disturbed healing. In order to clarify this, we have made the following changes.

Changes in the text: "Healed tracheal anastomosis" in table 2 (page 26) has been changed to "Fully healed tracheal anastomosis".

Comment 5: Mean f/u was only 21 months. Did the authors prefer to use median perhaps as a non-normally distributed value? Also, that's less than 2 years and certainly not long enough f/u to adequately describe BOS development…which in theory is the main endpoint of interest.

Reply 5: Meta-analysis methods produce results as pooled mean with 95% CI, which is why we could not use medians. The upper limit of 95% CI for follow-up was a little over 3 years (38) months. Diagnosis of BOS starts as early as 3 months after transplantation with average time to diagnosis of 16-20 months, which is close to our mean follow-up time. Although longer follow-up (> 5years) would have been more informative, our current follow-up does cover enough patients to reasonably pool the available data.

Changes in the text: No new changes

Comment 6: The authors interchange BOS and CLAD inappropriately as they are not one in the same.

Reply 6: Thank you for highlighting this. In the introduction (line 79), BOS is mentioned as a subtype of CLAD. As per ISHLT consensus statement, CLAD is the overarching pathology that includes other subtypes such as restrictive allograft syndrome (RAS), mixed CLAD, and undefined CLAD. However, in the discussion, we have made the following changes to make this distinction clearer:

Changes in the text: Line 202: *...lung transplantation has been hampered by the long-term development of CLAD.*

Line 208: … *improvements in surgical technique may limit its development later*

Line 246-248: *One of the many possible reasons for this difference could be CLAD which has a median onset of 2.3 years. It thus stands to reason that reduction in CLAD may contribute to improved long-term survival.*

Line 252-256: *In comparison, approximately 43% of patients develop CLAD (without subtype distinction) in a median time of 2.3 years. This difference may suggest a correlation between BAR, reduction of BOS development, and improved survival outcomes. Further long-term data is needed to determine if this difference is statistically significant.*

Comment 7: The authors use the term "harvest" in the discussion, which is not appropriate any more.

Reply 7: We appreciate the reviewer for highlighting this. Respect for organ donors must be shown in every way possible.

Changes in the text: We have changed "harvesting" to "procuring" (line 259).

Comment 8: A 30d mortality of 11% is completely unacceptable, especially considering that most of these patients were COPD/emphysema/CF as underlying disease.

Reply 8: Thank you for the opportunity to clarify this. Some of the patients died after 30 days while still admitted in the hospital. We reviewed each study in depth and have now combined 30-day morality and in-hospital mortality. We now report this as 30-day/in-hospital mortality, which is 6% (3-11).

Changes in the text: 30-day mortality has been changed to 30-day/in-hospital mortality as 6% (3-11) in the abstract (page 2), results (page 9), discussion (page 13), and tables (page 27).

Comment 9: Need the number at risk for each time point in the survival curve figure and Freedom from BOS.

Reply 9: We would like to clarify that the curves are not Kaplan-Meier analysis. They are only a graphical depiction of pooled survival and freedom from BOS at specific time points. These are not statistical time to event analysis. The red dots represent the pooled estimates while the shaded region represents the 95% confidence intervals. The dashed line connects these pooled estimates.

Changes in the text: No new changes

Reviewer B

Comment 1: The authors have looked at reviewing an area of considerable interest to lung transplant surgeons. It seems that a full review has not been performed before, and the authors are to be congratulated on the thoroughness of their approach
However, there do seem to be some misconceptions and, at least from a surgeon's point of view, the paper is disappointing.
From a historical standpoint, much of the early work on BAR was done to reduce the problem of airway healing and post-ischaemia airway healing. The emphasis was not on reduction of BOS, although it was thought, particularly by Petterson, that this might be a by-product. So the fact that in the combined heart-lung transplant there is very reliable airway healing, as a consequence of coronary to bronchial collaterals. But these transplants are equally prone to BOS
There is absolutely no doubt that early events after lung transplant can affect long term outcome. There is a link between PGD and BOS/CLAD. The deleterious early events have features of ischaemia/reperfusion, although ischaemia alone is probably a small component. It is incorrect to link airway ischaemia in a mechanistic way to early graft dysfunction and ischaemia/reperfusion injury; the two are different, but this does not come across in either the introduction or the discussion

Reply 1: We appreciate the reviewer's detailed comments. Heart-lung transplantation (HLTx) is relatively rare compared to isolated lung (LTx) or isolated heart transplant (HTx). There are reports suggesting lower incidence of BOS in HLTx recipients compared to LTx or HTx. There are also reports indicating reduced coronary allograft vasculopathy (CAV) incidence in combined heart-kidney and heart-liver transplantation. Perhaps the benefit of combined organ transplantation is an immuno-protective one, and in the HLTx population, that may be the reason for reduced BOS- and not collateral circulation. Further, the choice of transplant (LTx vs HLTx) is driven by pathology with little to no option for choosing one over the other. Thus, it is not a modifiable intervention like BAR, which the surgeon may choose to

perform.

Concerning the mechanistic linking of ischemia to graft dysfunction, the intent was to show only a potential relationship based on existing evidence. Based on the reviewer's suggestions, we have softened our tone on this relationship in the introduction and discussion:

Changes in the text: Line 89-94*: This could be because following a typical lung transplantation; the lower airways are perfused via minimal retrograde flow from the pulmonary veins as the arterial flow from the bronchial arteries is sacrificed in the transplantation process. A permanent reduction in adequately oxygenated blood to the pulmonary airways could thus increase the risk of ischemia and hypoxic damage*

Line 215-218: *By restoring bronchial artery flow at the time of transplant, this ischemia time may be reduced, potentially resulting in reduced early ischemia to the bronchial anastomosis, improved overall survival, and reduced rates of BOS development.*

Line 219-221*: As a contributing factor to the development of BOS, understanding airway ischemia and its effects on the lung may be helpful in outlining the evolution of BOS*

Comment 2: The introduction is carelessly written, with meaningless sections such as" as median survival from 1990 to 2014 improved to 5.8 years from 4.2 years in 74 the 1990 to 1998 period"

Reply 2: We have attempted to make the introduction more concise and have made the following changes:

Changes in the text: Lines 74-77: *Survival after lung transplant has also been improving over the years. Despite this, when compared to other solid-organ*

transplants, lung transplant survival is substantially lower. The survival curve following lung transplant, shows a steady drop after the first-year of transplant

Lines 85-94: *The development of BOS has been hypothesized to occur due to many factors, such as acute rejection, cytomegalovirus infection, and ischemia-reperfusion injury. Airway ischemia, inflammation, and subsequent necrosis due to reduced oxygenated blood supply have also been implicated in the development of progressive inflammation and fibrosis potentially leading to BOS. This could be because following a typical lung transplantation; the lower airways are perfused via minimal retrograde flow from the pulmonary veins as the arterial flow from the bronchial arteries is sacrificed in the transplantation process. A permanent reduction in adequately oxygenated blood to the pulmonary airways could thus increase the risk of ischemia and hypoxic damage.*

Comment 3: In addition to not referring to the heart-lung transplant situation, the authors might reflect on the apparent increase in airway complications after lung transplant from DCD donors. In this setting, where there is an additional ischaemic insult, there does appear to be a higher airway healing problem but no difference (with the exception of a single series) in BOS.

Reply 3: We have added some text in the discussion relating to lung transplant and DCD donors.

Changes in the text: Line 323-330: *Donation after circulatory death (DCD) is another aspect that would be worth investigating. Lung transplant outcomes are generally comparable between DCD and Donation after Brainstem Death (DBD); however, a higher rate of BOS following DCD LTx has been reported. Given that, DCD is still relatively new and not widely accepted, and that the studies included in this analysis range from the 1990s to the last decade, not much information was available to analyze this. If more comparative studies are done on BAR, it would be*

interesting to have results stratified by donor type (DCD vs DBD).

Comment 4: Finally, the observations that there is a poor relation between airway anastomotic problems and late BOS should be in the introduction

Reply 4: Based on the reviewer's suggestion, we have added some text to the introduction (Line 95- 98)

Changes in the text: *Given this hypothesis, reducing lung ischemia in the early period is of paramount importance in order to reduce the chances for development of late BOS; however, it is still unclear how ischemia related airway anastomotic problems directly relate to late BOS.*

Comment 5: The Methods section is appropriate, with a good description of the rigorous approach adopted But the Results launch straight into the data. It would be much more useful (and actually conventional) to summarise the series of patients, the numbers involved in each series and the era from which they were transplanted. It is very frustrating for the reader that this information is only in table form

Reply 5: Based on the reviewer's suggestion, we have incorporated the following in the "Baseline study and patient characteristics" of results (Line 157-161)

Changes in the text: *Seven studies comprising 143 patients were included in this meta-analysis. Five of the studies comprising 105 patients were conducted from the year 1990-2000. Of the remaining two, one was conducted from 1993-2003 while the other was from 2007-2010. Additional details are presented in the supplementary tables.*

Comment 6: It is notable, that many of the reports are from over 20 years ago, and this perspective is important. We need to know if lung preservation techniques, donor

section and use of cardiopulmonary bypass (almost routine for most of these patients) is going to affect both overall lung injury and the likliehood of airway problems.

Reply 7: Some reports are indeed from more than twenty years ago; however, given the lack of research on BAR, this is an in inherent limitation that can only be overcome with more studies. We have included the below text in the discussion and added two references:

Changes in the text: Line 291- 295: *It is possible that advancement in lung transplantation techniques and policies may have resulted in the improved overall survival after lung transplantation. By extension, it can be argued that BOS rates may also have improved. However, our results showed no effect of time on the 30-day/in-hospital mortality.*

a. Heng D, Sharples LD, McNeil K, Stewart S, Wreghitt T, Wallwork J. Bronchiolitis obliterans syndrome: incidence, natural history, prognosis, and risk factors. J Heart Lung Transplant. 1998;17(12):1255-1263.
b. Kulkarni HS, Cherikh WS, Chambers DC, et al. Bronchiolitis obliterans syndrome-free survival after lung transplantation: An International Society for Heart and Lung Transplantation Thoracic Transplant Registry analysis. J Heart Lung Transplant. 2019;38(1):5-16. doi:10.1016/j.healun.2018.09.016

Comment 7: It is good that the re-opening and bleeding rate associated with BAR is pulled out. There is some interesting data, but it is difficult to pick put, and needs greater highlighting in both the Results and the Discussion.

Reply 7: Thank you for the suggestion. To make these findings more prominent, we have divided the results into the following subsections

Changes in the text:

Baseline study and patient characteristics

Perioperative characteristics

Postoperative outcomes and complications

Comment 8: Do the authors feel that a proper comparative study, with only the absence or presences of BAR as a difference, in a group of patients treated in an otherwise high standard, at a major institution, is warranted?

Reply 8: We think such a study is warranted and we have mentioned this in the discussion (lines 298-300) and conclusion as well (lines 335- 339). Since BAR is a procedural intervention, we suggest that proper techniques be learned from institutions and surgeons with extensive experience in it prior to initiating any study. Otherwise, inexperience with the technique may mask true outcomes.

Changes in the text: No new change

Reviewer C

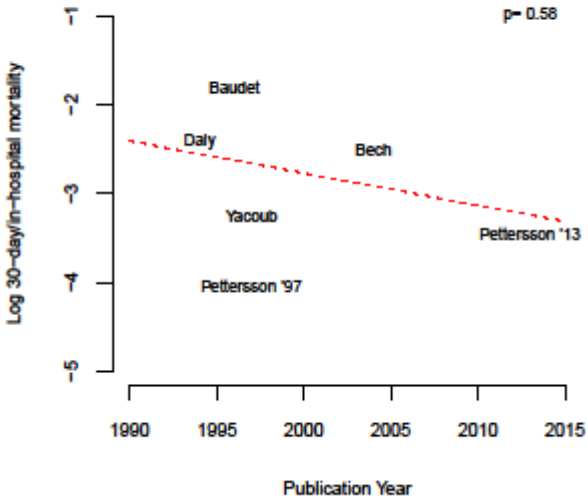
In their manuscript, "Bronchial Artery Revascularization in Lung Transplantation: A systematic review and meta-analysis", Ahmad et al describe and summarize outcomes after BAR following lung transplantation. Overall, the search is well-designed and conducted. Below are my feedback, which I hope the authors find constructive and helpful in improving and informing their work.

Comment 1: I worry about the validity of the meta-analysis for several key reasons. The majority of included studies (5/7) occur not only pre-LAS, but in the early to mid-90s which introduces significant variability as immunosuppression strategies and management strategies varied compared to the later studies in the late 2000s to early 2010s. Given the significant variation, not only in demographic and management but

also in the measured outcomes themselves (survival) including their definitions, studies before and after this period are not necessarily comparable, particularly when evaluating survival and CLAD (especially BOS) outcomes. There is significant heterogeneity in key variables relevant to the outcomes of survival and BOS including nearly every variable in Table 1. Given the significant heterogeneity, a random-effects meta-analysis might be a more appropriate approach.

Reply 1: We appreciate the reviewer's concerns. It is indeed the case that 5/7 studies are from the 1990s and that management strategies have been evolving. In a niche topic such as BAR, we are limited by the available data. There is also lack of enough data for pre vs post- LAS analysis of outcomes. This inherent limitation can only be overcome by newer studies. We would also add that broad analysis of registries such as UNOS also includes patients from the 1980s and 1990s. As such, our study aims to give as general a view of BAR as possible.
Concerning heterogeneity, only three of the nine variables in table 1 had significant statistical heterogeneity, or I^2. In the outcomes, only follow-up months (table 3) had significant heterogeneity. However, we understand that statistical heterogeneity does not fully describe clinical heterogeneity. Random effects model in the package for R Software did not handle the outliers well and inverse variance method was adopted. Given the reviewer's feedback, to further characterize the effect of "era" over the outcomes, we performed meta-regression of 30-day/in-hospital mortality over time and found no statistically significant effect.

Changes in the text: Line 195-197: *A meta-regression analysis of Log 30-day/in-hospital mortality over time (publication year), shown in Figure 4, suggests no significant effect of time on the mortality outcome (p=0.58).*



Comment 2: For many variables assessed, it appears only 3-4 of the studies were used. Were these the same three studies which reported most of the data and, if so, does the addition of the other studies improve the quality of the analysis. This might be particularly pressing if the three studies with the most data were the later studies, and therefore more comparable.

Reply 2: Studies were not the same for variables reported by 3-4 studies. Different studies reported similar and different variables. Of note, six of the seven studies had overlapping study periods extending from1990-2003. Only one study was conducted from 2007-2010. As such, no stratification for comparison was found to be feasible.

Changes in the text: No new change

Comment 3: There is a known and significant survival difference among patients undergoing single vs. double lung transplantation.

Reply 3: Thank you for highlighting this issue. In our experience, the literature is equivocal on the advantages of single vs double lung transplantation (LTx). This may

be more nuanced than it seems.

Changes in the text: We have added the following to the discussion (line 321-323) along with two references

However, it should be noted that the literature on survival difference between single vs. double lung transplantation is still conflicting

a. Ranganath NK, Malas J, Phillips KG, et al. Single and Double Lung Transplantation Have Equivalent Survival for Idiopathic Pulmonary Fibrosis. Ann Thorac Surg. 2020;109(1):211-217. doi:10.1016/j.athoracsur.2019.06.090
b. Schaffer JM, Singh SK, Reitz BA, Zamanian RT, Mallidi HR. Single- vs double-lung transplantation in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis since the implementation of lung allocation based on medical need. JAMA. 2015;313(9):936-948. doi:10.1001/jama.2015.1175

Comment 4: For figure 1, to better understand the selections, summary/categories of reasons for exclusion should be included at minimum from the full text stage on and ideally from the records screened stage on.

Reply 4: Thank you for highlighting this. In our study, the five articles excluded after full text review were on the basis of lack of adequate extractable data, as such, there were no categories of reasons to exclude that could be added on the PRISMA chart. The detailed exclusion criteria is mentioned in the methods. We have added the following to it

Changes in the text: Line 135-136: *…and studies without adequate extractable data were also excluded.*

Comment 5: A more detailed explanation of the approach to solving discrepancies in the data extracted from the studies is warranted.

Reply 5: Discussion and consensus were used to resolve discrepancies in the data. Most of these discussions centered around combining of variables as studies do not always use the same name for similar variables. This was especially necessary as more than one person was carrying out data extraction simultaneously from the included studies. We have added the following in the methods section:

Changes in the text: Line 141-143: *Data were extracted from article texts, tables, and figures with discrepancies and disagreements being resolved by discussion and consensus. Disagreement between two coauthors were resolved via adjudication by a senior coauthor.*

Comment 6: The abstract reports that 93% of all grafts were successful by angiography, however per line 162, only 89% of patients underwent angiography and 93% of those were found to be successful. The abstract should be updated to reflect this (i.e. 83% of all patients demonstrated successful conduit on angiography.

Reply 6: Thank you for highlighting this. We have qualified the statement in the abstract (Lines 43- 45) to reflect that 93% successful BAR is only for those patients that underwent angiography:

Changes in the text*: In patients with postoperative angiography, successful BAR was demonstrated in 93% [82-97] of all assessed conduits.*

Comment 7: Given the reported follow up time of an average of 21 months and 95% CI of 2-38 months, it is unclear how a 5-year survival could be reported, let alone be 71%. Additionally, the methods section should explain the statistical approach to the pooled survival analysis.

Reply 7: Thank you for the opportunity to clarify this. (A) One reason for the apparent discrepancy between pooled follow-up time and pooled 5-year survival is because only 3 articles reported a 5 year survival and fewer articles mentioned a specific follow up time period as well. Pooling at least three studies is considered acceptable for meta-analysis. (B) Further, when individual studies present Kaplan-Meier survival data, the median patient follow-up time in these studies is always shorter than the extent of the X axis on Kaplan-Meier curve. (C) We would also like to clarify that in meta-analysis, medians from various studies are converted to pooled mean, and its 95% confidence interval indicates 95% certainty where the true mean is really located as opposed to describing its spread (in other words, there is no pooled standard deviation, and 95% CI for SD). We have added a brief explanation in the methods of how pooled survival was obtained. Additional clarification is also provided in response to comment 1.9 above.

Changes in the text: Line 146-148: *Survival data from each study were collected and pooled to retrieve a weighted mean and 95% confidence interval at specific time points. Such data were then graphically displayed to visualize survival over time.*

Comment 8: The authors report freedom from airway ischemia, but how this was determined (i.e. bronchoscopic visualization) should be clearly defined, or if studies used a range of determining factors that should be stated as well.

Reply 8: In order to assess airway ischemia, studies used bronchoscopic evaluation of the airway at various time points. We have added this method of assessment (in parenthesis) in the results:

Changes in the text: Line 191: *83% [95% CI 29-98] of patients were free from signs of airway ischemia (assessed via bronchoscopy) at three and six months*

Comment 9: The reference used to report UNOS overall survival is 7 years old. A

more recent reference should be undertaken and given that international cohorts are included in this meta-analysis, an international database, such as the ISHLT report, should be included in addition to or instead of UNOS.

Reply 9: We appreciate the reviewer for highlighting this. We have included an updated OPTN/SRTR reference for overall survival. Since, we have been using UNOS survival rates throughout the discussion; we chose to continue this for the sake of consistency. The updated survival and the new reference are given below. We have also referenced ISHLT survival in the discussion (Line 294).

Changes in the text: Line 238- 240: *The United Network for Organ Sharing (UNOS) reports overall survival percentages for lung transplantation at 1-year, 3-year, and 5-year as 89.4%, 74.8%, and 61.2%, respectively.*

Valapour M, Lehr CJ, Skeans MA, et al. OPTN/SRTR 2020 Annual Data Report: Lung. Am J Transplant. 2022;22 Suppl 2:438-518. doi:10.1111/ajt.16991

Comment 10: Again, the authors reference 5-year survival for their meta-analysis but the reported follow ups simply do not support those conclusions.

Reply 10: Please refer to our reply in 3.7. Briefly, Kaplan-Meier curves extend further than the median follow-up times for the patients. If a KM curve extends to 5 years, median f/u time for these patients will be much shorter. Since meta-analysis simply pools these values together, pooled mean follow-up time will also be shorter than 5-years.

Changes in the text: No new changes

Comment 11: In the discussion, the authors should add, if available, data on how widespread the current use of BAR (particularly since most of the included studies are

historical) and their thoughts for how it could be adopted/incorporated into cardiothoracic transplant/organ retrieval training and practice, particularly since, as they note, it denotes an additional technical aspect to those interventions.

Reply 11: The few studies reporting on BAR all mention single center experiences. Thus, it can be assumed that BAR utilization is not widespread in present day lung transplantation.
Before incorporating into practice, clear benefits of BAR have to be reported. This can only be done through a multi-center comparative analysis. We have highlighted the need for such an analysis in the discussion and conclusion as well. We have added the following to the discussion:

Changes in the text: Line 330-333: *Further, due to the technical requirements, all surgeons participating in a comparative study should be adequately trained at centers with extensive experience in BAR, otherwise, true results may be masked by imperfect technique.*