JTD JOURNAL OF THORACIC DISEASE
A PEER-REVIEWED, OPEN ACCESS JOURNAL FOR HIGH-QUALITY RESEARCH IN THORACIC DISEASES
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Peer Review File

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Reviewer A

This report from the Okayama University Hospital in Japan is a retrospective analysis of the lung transplantation experience from a single center over 7 years with a focus on pulmonary hypertension status. Special focus is paid to the control of perihilar collateral vessels and prominent vaso vasora using hemoclips as a means of preventing intraoperative blood loss and decreasing blood product usage.

Major Criticisms

Comment 1. Although it is presented as the central element of the manuscript, relatively little explanation is provided for the exact technique of control of the collateral vessels described by the authors. Narrative comparisons are made between transplants performed before and after implementation of the hemoclip technique but the timing of this change and the number of transplants performed before/ after this change is unclear. Moreover, a retrospective radiographic quantification of the number of clips used is not a direct analog for careful and hemostatic surgical technique, it is difficult to accept this as a well-defined surgical intervention.

Reply 1: We would like to thank the constructive comments. We would appreciate if you could find the exact closure technique using hemoclips as described in Methods and Figure 2. This technique is very simple as follows, mechanical closure using hemoclips was carefully performed at the timing of tissue dissection for pneumonectomy and then, the peripheral tissue of the closure site was divided using electrocautery. Dissected tissue included the pulmonary ligament, perivascular area at the hilum of the lung, peribronchial tissue, fatty tissue around lymph nodes, and pleural adhesion sites as described in Methods. This technique with meticulous mechanical closure plus coagulation was performed to obtain more reliable hemostasis than coagulation. Timing of the change was the start of this study period in February 2008 as described in Methods. The number of LT performed before this change was 60 as described in Methods, “In addition, we compared intraoperative blood loss, blood transfusion volume and reoperation rate in the primary, secondary and non-PH groups of this study with that of historical data in 60 patients who underwent LT without preventive hemoclip closure, including patients with primary

PH (N = 19), patients with secondary PH (N = 15), and patients without PH (N=26), prior to the current study period." However, because this study was not a comparison study with historical control, we removed Figure 5 with legend and the following sentences in Methods and Discussion with regard to the historical control to avoid misunderstanding.

Changes in the text (see Page 7, line 6)
The following sentence was removed from Methods, "In addition, we compared intraoperative blood loss, blood transfusion volume and reoperation rate in the primary, secondary and non-PH groups of this study with that of historical data in 60 patients who underwent LT without preventive hemoclip closure, including patients with primary PH (N = 19), patients with secondary PH (N = 15), and patients without PH (N=26), prior to the current study period."

Changes in the text (see Page 8, line 12)
The following sentence was removed from Methods, "For comparison of intraoperative blood loss with historical data, Mann-Whitney U test was performed."

Changes in the text (see Page 14, line 4)
The following sentence was removed from limitations in Discussion, "Finally, although in the comparison with historical data, intraoperative blood loss was less in the patients with primary PH and without PH who received preventive hemoclip closure than those who did not, we could not directly compare the results of the current study with historical data due to different background, such as patient characteristics, perioperative management and immunosuppression protocol, etc."

A randomized controlled trial with or without the hemoclip would be ideal to check the effect of hemoclip closure, but we had not performed the clinical trial in the limited number of LT from an ethical standpoint, as described in the end of the limitations, "Fifth, this study compared the outcomes of preventive hemoclip closure between patients with and without PH undergoing LT, but not the outcomes in PH patients undergoing LT with and without preventive hemoclip use, due to ethical reasons."

As the reviewer pointed out, a retrospective radiographic quantification of the number of clips used might not be a direct analog for careful and hemostatic surgical technique. However, because we had only single transplant team and performed the limited number of lung transplantation, we kept performing the procedure in the same manner. Therefore, the procedure that we performed in each case was similar, and

counting the number of hemoclips could offer significant information what was performed during surgery to some extent. Off course, this technique was simple and primitive not well-defined surgical technique, but this study could revisit a importance of hemostasis with mechanical closure using hemoclips in LT. Nonetheless, the patient factor differed in each patient. We added the following sentence in the limitations of Discussion.

Changes in the text (see Page 13, line 13)
"and other than preventive closure of collateral vessels with hemoclips, LT technique might have improved over the years during the study period. Although the single transplant team performed the limited number of LT in the same manner at our institution, the detailed technique could differ among the patients."

Comment 2. The authors' claims about the effectiveness of the hemoclip technique are largely based on decreased intraoperative blood loss when the technique is used. Rigorous quantification of intraoperative blood loss is extremely difficult and perioperative estimations of blood loss are notoriously inaccurate. Furthermore, the concept of estimated blood loss is almost meaningless in cardiopulmonary bypass cases because of the use of cardiotomy suction, red cell salvage, and other and similar techniques. Presumably some methodology could be implemented to quantify this more rigorously but none is reported by the authors and such prospectively-obtained values could not be meaningfully compared to historical data.
Comparison of postoperative chest tube output or reoperation for bleeding in patients managed with the hemoclip technique vs not may lend some more weight to the authors' argument. Rates of reoperation were bleeding were higher in the PH cohorts in this series, although this is based on small numbers that did not reach statistical significance.

Reply 2: We agree with the reviewer's point that the amount of intraoperative blood loss is not accurately determined due to factors such as cardiotomy suction and red cell salvage, although we did not generally use cardiotomy suction and red cell salvage to avoid bacterial contamination from the sputum in the bronchus and the lungs during LT. To compensate for this, we analyzed not only the amount of blood loss but also the amount of blood transfusion in this study. As the reviewer suggested, we added the following sentences to the limitations.

Changes in the text (see Page 13, line 20)
We added the following sentence in the limitations, "Third, the amount of

intraoperative blood loss might fluctuate according to cardiotomy suction and red blood cell salvage during CPB."

Because this study was not a comparison study with historical control, we removed the results with regard to the historical control to avoid misunderstanding as described above. Unfortunately, we did not have the data of postoperative tube output, and we apologize that we could not add these data in this study. As the reviewer pointed out, the rates of reoperation due to hemorrhage did not significantly differ among the groups based on the small numbers of this study. We added the following sentence in the limitation of Discussion.

Changes in the text (see Page 13, line 17)
"Second, because the number of patients was small, especially in the primary PH group, there is a possible risk of Type II statistical error."

Comment 3. The remainder of the manuscript serves as a retrospective single-center report of a relatively small lung transplant series, which probably lacks sufficient novelty to warrant publication on its own. The authors do not draw any comparisons between the central aspect of their paper, the meticulous control of perihilar collateral vessels, and any postoperative variables (chest tube output, ICU length of stay, survival). Some mention is made of blood product usage, but only to indicate that it was similar across transplant indications. No comparison of blood product usage between hemoclip and non-hemoclip patients is reported.

Reply 3: We would be grateful if you could find that we showed the primary outcome of overall survival and CLAD-free survival as postoperative variables among the three groups in Figure 4. Because ICU stay was too long in Japan and often influenced by the hospital condition as well as the patient's medical condition, we did not add the data of the length of ICU stay in this study. In addition, the chest tube output could not be evaluated due to the lack of data as described above. Because this study was not a comparison study with historical control, we removed the comparison data with the historical control to avoid misunderstanding as described above.

Overall Impression

The overall structure and tone of this manuscript are reasonable but its central feature, the assertion that diligent closure of perihilar collateral vessels, is not well-explored or

compared to any clinically meaningful outcome variables, making the exact purpose of the manuscript unclear. A repurposed and much more detailed description of the technique reported by the authors with high-quality photographs or other images may be interesting as technical/ operative technique report, but this manuscript does not warrant publication in its present form.

Reply: As the reviewer suggested, the manuscript type of "technical report" would be suitable for this study, but the form of technical report was too short to describe our data and we had to choose the current style. This technique was simple, and we think Figure 2 with the photos and the descriptive images was enough to explain this simple technique instead of high-quality photographs or other images. As we described in Introduction, the purpose of this study was to show that mechanical hemostasis using hemoclips is effective to control bleeding for LT even under the use of CPB. So, we removed the comparison data with historical control as described above. Also, we added the following sentence as our hypothesis of this study in Introduction as the other reviewer suggested.

Changes in the text (see Page 5, line 4)
"We hypothesized that a higher number of hemoclips are required to attain meticulous hemostasis in the patients with primary PH as compared to those with secondary PH or non-PH."

Reviewer B

This is a study from a transplant center in Japan where they evaluated the role and impact of managing intraoperative bleeding with surgical hemoclips during the lung transplantation procedure for the patients with pulmonary hypertension on the outcomes.

The study includes a total of 80 patients for 7 years (2008-2015) and they report that better control for intraoperative bleeding with hemoclips was associated with less early postop complications.
I read the manuscript with great interest.
I have the following questions:

Comment 1. With regards to their surgical techniques, did they use hemoclips only for managing the collateral vessels in the hilum? Didn't they use them for other purposes such as managing the vascular adhesions between lungs and chest wall or dividing the lung ligament or dissecting hilar lymph nodes?

Reply 1: We would like to thank the constructive comment. This technique is used for all the sites the reviewer mentioned, including the collateral vessels in the hilum, pleural adhesions, the pulmonary ligament, and the hilar lymph nodes. The sentence of Methods was revised as below.

Changes in the text (see Page 7, line 13)
We added the phrase of "pleural adhesions" in the following sentence of Methods, "During the hilar dissection, collateral vessels in the perihilar mediastinal pleura containing the circumferential connective and adipose tissue around the hilar structures, including the pulmonary artery, the pulmonary vein and the main bronchus as well as the bronchial arteries, lymph nodes, pleural adhesions and the pulmonary ligament, were meticulously closed with hemoclips (Weck® horizon™ metal ligation system, Teleflex Medical, North Carolina, USA) (Figure 2)."

Comment 2. In line with the above question, how did they objectively and/or quantitatively assess the collateral vessels in the hilum in each case and determine the way of using hemoclips for them? If it was identified, all of them were clipped regardless of the size or the location? It seems that the authors assumed that the clips were only used for managing the collateral vessels, all the identified collateral vessels were clipped without any single vessel having been left unclipped, and that they contributed to complete control of surgical bleeding; however, it depends on the surgeons' decision in each case, to use surgical clips or not, which collaterals to be

clipped, how many clips to be used, or to confirm clipping being sufficient to control bleeding, etc.

Given the assessment was conducted so that the number of hemoclips was retrospectively counted on a single chest x ray image, the big question remains regarding the accuracy of their assessment. The authors should be advised to clear those questions associated with their bias.

Reply 2: We used preventive mechanical closure with hemoclips plus coagulation with electrocautery for hemostasis, and unclipped sites were coagulated with electrocautery for hemostasis. Because we had only single transplant team and performed the limited number of lung transplantation, we kept performing the procedure in the same manner. Therefore, the procedure that we performed in each case was similar, but the patient factor differed in each patient. We added the following sentence in the limitations of Discussion.

Changes in the text (see Page 13, line 15)

We added the following sentence in the limitations, "Although the single transplant team performed the limited number of LT in the same manner at our institution, the detailed technique could differ among the patients."

We adjusted the contrast of X-ray to enhance the hemoclip metal for accuracy in evaluation by two doctors. Three-dimensional computed tomography might be more precise for counting, but X-ray is simple and useful for the overview of the hemoclips, and we used X-ray for evaluation in this study. The following sentences were added to Methods and limitations in Discussion.

Changes in the text (see Page 7, line 20)

We added the following sentence in Methods, "The contrast of X-ray images was adjusted to enhance the hemoclip metal during the counting."

Changes in the text (see Page 13, line 23)

We added the following sentence in the limitations, "and three-dimensional computed tomography might be more precise for counting."

Comment 3. In Figure 5, how did they divide all the patients into those with and without hemoclips? Those who didn't receive hemoclips mean that they did not use any single clip for them during the procedure? The authors should clarify their stratification, which needs to be appropriately stated in the Methods. Also the total

number of each subgroup (NH vs H) is not consistent with the one stated in the Methods. Probably it will be better to add the subgrouping (NH vs H) to the current flow-diagram in Figure 1 so that the readers better understand the study design.

Reply 3: We applied a new method of mechanical closure using hemoclips in February 2008. Prior to the application of this technique, we used few hemoclips in the very limited parts, such as the bronchial artery and bleeding sites, not in the perihilar mediastinal pleura. Because this study was not a comparison study with historical control, we removed the comparison data with the historical control to avoid misunderstanding, and we did not add the stratification in Methods. Therefore, we removed the total number of each subgroup (NH vs H) and did not add the subgrouping (NH vs H) to Figure 1 to avoid misunderstanding.

Changes in the text (see Page 8, line 12)
The following sentence was removed from Methods, "For comparison of intraoperative blood loss with historical data, Mann-Whitney U test was performed."

Changes in the text (see Page 14, line 4)
The following sentence was removed from the limitations, "Finally, although in the comparison with historical data, intraoperative blood loss was less in the patients with primary PH and without PH who received preventive hemoclip closure than those who did not, we could not directly compare the results of the current study with historical data due to different background, such as patient characteristics, perioperative management and immunosuppression protocol, etc."

Comment 4. The current outcomes for the patients with severe PH including IPAH continue to improve, which is consistent with the data in this study. As compared to earlier reports, they have been significantly improved thanks to the evolving management, surgical techniques in particular the utilization of the optimal mechanical circulatory support pre-, intra-, and post-operatively in lung transplantation. Indeed the recent series of reports from the Vienna group well reported their excellent outcomes by using elective and prophylactic utilization of VA-ECMO approach.
The weakness of this manuscript, however, is that the authors appear to fail to prove the strong association between their surgical techniques of using hemoclips and any of their transplant outcomes. There might be such association; however, due to lack of appropriate analysis through confounding variables that contributed to the outcomes following lung transplantation in the enrolled patients, all the current data

interpretation as well as the conclusion appear to be premature or substantially biased. The authors should be strongly advised to clear all of those questions.
Thank you for this privilege.

Reply 4: We totally agree with the points made by the reviewer. In order to clarify the significance of hemoclip, it would be desirable to directly randomize and compare the clipped and unclipped groups, but this could not be performed from an ethical perspective as described in the limitation, "this study compared the outcomes of preventive hemoclip closure between patients with and without PH undergoing LT, but not the outcomes in PH patients undergoing LT with and without preventive hemoclip use, due to ethical reasons." We fully understand that ECMO has become the global standard instead of CPB during this decade. However, this study period was 1998-2015, and we chose CPB as the first choice. We added the following sentences to Discussion.

Changes in the text (see Page 12, line 3)
"Recently, veno-arterial ECMO has been increasingly utilized as an alternative cardiopulmonary support with benefits for post-transplant outcomes (27, 28). ECMO is theoretically less invasive than conventional CPB and reduces the inflammatory response as well as the need for full heparinization. CPB use was associated with a significantly higher incidence of PGD; however, CPB provides safer, easier and more versatile surgical fields than ECMO (29). Moreover, meticulous management of graft reperfusion flow and low oxygen concentration ventilation with CPB contribute to comparable short- and long-term outcomes of LT using extended criteria donor lungs as compared to ECMO (29). For these reasons, we had used CPB as the first choice for LT at our institution during the study period."

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Reviewer C

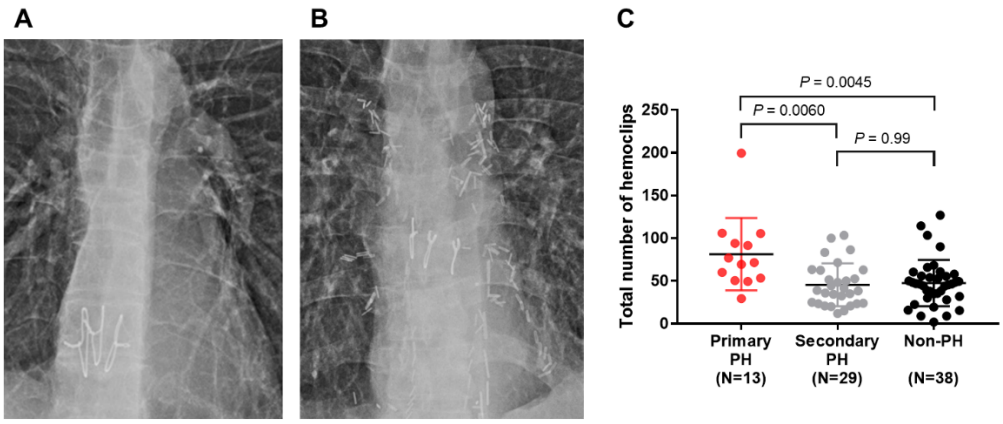
The authors are to be commended for their progress in achieving meticulous hemostasis in patients with primary pulmonary hypertension compared to historical controls, and this is perhaps the most remarkable finding of the present study. The authors clearly delineate many of the obvious limitations of the study in the discussion section, but several relevant and concerning points should be addressed.

Major criticisms

Comment 1. The "Primary PH" group is actually a combination of three separate pathophysiologic primary groups/diagnoses: true primary PH, CTEPH, and PVOD. This is a concerning leap and possibly explains the great variability in the number of Hemoclips used (Figure 3) and the amount of bleeding (Figure 5) in the PPH group. It is entirely possible that just a few extreme outliers account for the statistical significance between the PPH group and the other groups with respect to the number of Hemoclips and the amount of bleeding. Perhaps clarifying the exact number of patients with PPH, CTEPH and PVOD in the PPH group would clarify the significant variability in this group and perhaps using dots/points for each subject (as opposed to bars) might better portray elucidate and portray the granularity of the data.

Reply 1: We would like to thank the constructive comment. According to the reviewer's suggestion, we revised Figure 3 with plot diagrams. Because this study was not a comparison study with historical control, we removed Figure 5 to avoid misunderstanding. According to the reviewer's suggestion, we additionally examined the amount of blood loss by the PH subclassification (PAH, PVOD, CTEPH). We have added description in Methods and Results.

Changes in the figures (see Figure 3)



Changes in the text (see Page 7, line 2)
We added the following sentences in Methods, "In addition, subgroup analysis for total amount of blood loss was performed according to primary PH subcategories: PAH, PVOD, and CTEPH."

Changes in the text (see Page 8, line 12)
We added the following sentences in Methods, "Subgroup analysis for total amount of blood loss in primary PH subcategories was tested by the Kruskal–Wallis test and Bonferroni correction was employed for multiple comparisons (PAH vs. PVOD, PH vs. CTEPH, and PVOD vs. CEPH)."

Changes in the text (see Page 9, line 14)
We added the following sentences in Results, "There was no significant difference in the amount of intraoperative blood loss in the subclassification of primary PH (P = 0.19; PAH vs PVOD, P = 0.99; PAH vs CTEPH, P = 0.53; PVOD vs CTEPH, P = 0.99)."

Changes in the text (see Page 13, line 20)
The following sentence was removed from the limitation, "Third, the primary PH group included not only patients with primary PAH, but also those with PVOD and CTEPH, and further subgroup analysis will be required."

Comment 2. The study is at great risk of a Type II statistical error due to the low number of subjects, especially in the PPH group. Perhaps this actually explains the similar outcomes in PGD and survival, and with a larger study, there in fact might be a difference between the groups. This is always a risk when the null hypothesis is not rejected, and so the small number of subjects is especially problematic.

Reply 2: We would like to thank the constructive comment. We have added the following sentence to the limitation according to the reviewer's suggestion.

Changes in the text (see Page 13, line 17)
We added the following sentence in Discussion, "Second, because the number of patients was small, especially in the primary PH group, there is a possible risk of Type II statistical error."

Comment 3. The actual hypothesis of the paper, in my opinion, should be restated as something to the effect that: A higher number of hemoclips are required to attain

meticulous hemostasis in patients with PPH compared to LT recipients with secondary PH or no PH.

Reply 3: We would like to thank the reviewer's suggestion. We added the following sentence as the hypothesis of this study in Introduction.

Changes in the text (see Page 5, line 4)
"We hypothesized that a higher number of hemoclips are required to attain meticulous hemostasis in the patients with primary PH as compared to those with secondary PH or non-PH."

Comment 4. One of the major problems of the study is related to impact--William Stewart Halsted established meticulous hemostasis as one of the fundamental tenants of surgery over a century ago, and still we as surgeons struggle to achieve this in difficult cases such as lung transplant. The observations of the group in the present study may simply represent a learning curve, which every lung transplant surgeon and group must scale.

Reply 4: According to the reviewer's suggestion, we have added the following sentence to the limitation.

Changes in the text (see Page 13, line 13)
We added the following sentence in Discussion, "and other than preventive closure of collateral vessels with hemoclips, LT technique might have improved over the years during the study period."

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Reviewer D

This manuscript deals with an interesting retrospective analysis by authors from Okayama University Hospital in Japan on a lung transplant cohort with 80 patients looking at the impact of "pre-emptive" clipping of perihilar collateral vessels in patients undergoing lung transplantation for primary or secondary pulmonary hypertension (PH) and for other, non-PH pulmonary diseases.

The authors have found: 1) that more clips for vessel closure were used in patients with primary PH compared to secondary PH and non-PH (Figure 4); 2) that intra-operative blood loss was higher in patients with primary PH compared to secondary PH and non-PH (Figure 5); 3) that blood loss was significantly less in patients with hemoclips compared to a historical control without the use of hemoclips (Figure 5).

I thank the authors for the opportunity to review their results and data.

Major comments:

Comment 1. Use of CPB versus ECMO versus off-pump:
The important lesson from this paper and conclusion by the reviewer is that "blood loss is higher whenever cardiopulmonary bypass (CPB) is being used almost routinely during lung transplantation" see data on CPB use in Table 1: 100% in primary PH; 93.1% in secondary PH; 76.3% in non-PH!
In the reviewer's opinion, not all lung transplant procedures will need the use of CPB! 1) Many transplants can be done off-pump in non-PH patients and often in patients with secondary PH while adequately ventilated to remove carbon dioxide thereby reducing PA pressures; 2) in patients still needing intra-operative right-to left cardiopulmonary support for hemodynamics, the use of ECMO is less invasive with less heparine needed and less bleeding!
- the authors should explain in the discussion why they have used CPB in nearly all patients, especially in the non-PH group.
- the authors should elaborate in the discussion on the risks of using CPB versus ECMO.

Reply 1: We would like to thank the constructive comment. We fully understand that ECMO has become the global standard instead of CPB during this decade. However, this study period was 1998-2015, and we chose CPB as the first choice. We added the following sentences to Discussion.

Changes in the text (see Page 12, line 3)

"Recently, veno-arterial ECMO has been increasingly utilized as an alternative cardiopulmonary support with benefits for post-transplant outcomes (27, 28). ECMO is theoretically less invasive than conventional CPB and reduces the inflammatory response as well as the need for full heparinization. CPB use was associated with a significantly higher incidence of PGD; however, CPB provides safer, easier and more versatile surgical fields than ECMO (29). Moreover, meticulous management of graft reperfusion flow and low oxygen concentration ventilation with CPB contribute to comparable short- and long-term outcomes of LT using extended criteria donor lungs as compared to ECMO (29). For these reasons, we had used CPB as the first choice for LT at our institution during the study period."

Comment 2. Hemostasis while on CPB:
The authors are correct in stating (in the discussion) that in a patient on CPB, small hilar vessels initially look harmless and can be easily divided with electrocautery. However, because of the full anti-coagulation with heparine, these vessels will continue oozing during the transplant procedure and some (bronchial) vessels will continue to bleed after the transplant with higher systemic blood pressure. Meticulous hemostasis is technically more difficult at the end of the procedure because the new ventilating donor lung is obstructing the view on the (posterior) hilum.
- the authors should elaborate in the discussion on the use of heparine when doing transplants on CPB and on the difficulties of obtaining hemostasis on the (posterior) hilum after the transplant once the patient is disconnected from CPB.
- do the authors use a double-lumen tube routinely in patients being transplanted on CPB? This will certainly help to go on one lung ventilation at the end of the transplant to check for hemostasis in the (posterior) hilum. Please discuss further.

Reply 2: We would like to thank the constructive comment. As the reviewer pointed out, hemostasis on the posterior hilum is a key to success of LT. We routinely use a double-lumen tube, and added the following sentences to Discussion.

Changes in the text (see Page 12, line 19)
"Furthermore, single lung ventilation using double-lumen endotracheal tube can provide working space in the surgical field and contribute to hemostasis especially of the dorsal portion of the hilum, which could be difficult after reperfusion and reventilation of the grafts."

Comment 3. Bronchial collateral vessels:

a) the collateral vessels in the hilum are in fact bronchial vessels connected to the systemic circulation (originating from the aorta). The words "bronchial vessels", "systemic circulation", or "high-pressure circulation", however, are never being used in the paper? For non-surgeons reading the paper this is not always obvious; these readers may believe that these vessels originate from the pulmonary arterial circulation, which is obviously not correct. The reason for these collaterals in PH patients is a mechanism to compensate the hypoxia in lung tissues.
- please mention in the discussion/introduction that these collaterals are bronchial vessels coming from the systemic (high-pressure) circulation responsible for the intraoperative and postoperative bleeding when not clipped (especially during CPB when fully heparinized).

Reply 3a): As the reviewer pointed out, bleeding from bronchial arteries originating from the aorta can cause critical bleeding, and to secure hemostasis of the bronchial arteries is important. Other than bronchial arteries, with the advancement of pulmonary hypertension, collateral vessels around the hilum increase from the pulmonary circulation and pulmonary arteries as shown in Reference #15 (Davie NJ, Gerasimovskaya EV, Hofmeister SE, et al. Pulmonary artery adventitial fibroblasts cooperate with vasa vasorum endothelial cells to regulate vasa vasorum neovascularization: a process mediated by hypoxia and endothelin-1. Am J Pathol. 2006;168:1793-807.) We believe that it is necessary to secure hemostasis of these small collateral vessels around the hilum.

b) patients with suppurative lung diseases (cystic fibrosis/bronchiectasis) may also have impressive bronchial vessels and collaterals and are also at risk for intra-op and post-op bleeding; Five patients in the secondary PH group suffered from end-stage bronchiectasis.
- did these 5 patients needed more clips and did they experience more blood loss compared to the other patients with secondary PH?
- please discuss the risk of collateral bronchial arteries in patients with suppurative disease.

Reply 3b): We performed additional subgroup analysis for the total number of hemoclip and total amount of blood loss in the patients of secondary PH with and without bronchiectasis. The following sentences were added to Methods and Results.

Changes in the text (see Page 7, line 3)
"Also, subgroup analysis for total number of hemoclips and total amount of blood loss was performed in the patients of secondary PH with and without bronchiectasis."

Changes in the text (see Page 8, line 12)
"Subgroup analysis for total number of hemoclips and total amount of blood loss in the patients of secondary PH with and without bronchiectasis was performed by Mann-Whitney U test."

Changes in the text (see Page 9, line 16)
"Although the patients of secondary PH with bronchiectasis had significantly higher amount of intraoperative blood loss (P = 0.038) than those without bronchiectasis, the number of hemoclips did not differ (P = 0.23)."

Comment 4. Chest x-ray:
Small clips were counted on chest X-ray. These small clips are barely seen on chest X-ray.
- can the authors better explain in the methods how the chest x ray was taken to be able to count these hemoclips? Was there a special x-ray protocol to better visualise mediastinal structures? Have the authors compared the number of clips on X-ray versus CT scan?

Reply 4: We adjusted the contrast of X-ray to enhance the hemoclip metal for accuracy in evaluation by two doctors. Because X-ray is simple and useful for the overview of the hemoclips, we used X-ray for evaluation in this study. We did not count clips on CT scan because it is difficult to count clips on CT as compared to X-ray. Three-dimensional computed tomography might be more precise for counting. The following sentences were added to Methods and limitations in Discussion.

Changes in the text (see Page 7, line 20)
"The contrast of X-ray images was adjusted to enhance the hemoclip metal during the counting."

Changes in the text (see Page 13, line 23)
", and three-dimensional computed tomography might be more precise for counting."

Comment 5. Limitations:
in the limitation section, the authors mention that the comparison was done with an historical control group. So results (including less blood loss) may have improved with more experience built up over the years with technical improvements other than

the routine use of hemoclips.
- the retrospective nature of the study and the potential of "historical bias" should be more stressed in the limitation section.

Reply 5: We have added the following sentence to the limitation according to the reviewer's suggestion.

Changes in the text (see Page 13, line 13)
"and other than preventive closure of collateral vessels with hemoclips, LT technique might have improved over the years during the study period.

Minor Comments
Comment 6. Abstract (page 2):
Purpose (line 2): "massive haemorrhage"; the risk for PGD is not related to the bleeding, but to blood transfusion".
- please correct this sentence by stating "Massive blood transfusion to compensate haemorrhage…"

Reply 6: We revised the following sentence in the abstract according to the reviewer's suggestion.

Changes in the text (see Page 3, line 2)
"Massive hemorrhage during lung transplantation (LT) results in primary graft dysfunction (PGD) and worse outcomes after LT."
↓
Massive blood transfusion compensating hemorrhage during lung transplantation (LT) results in primary graft dysfunction (PGD) and worse outcomes after LT.

Comment 7. Introduction (page 3):
lines 10-11: "cardiopulmonary support …..in patients that cannot tolerate respiratory management with single-lung ventilation";
- please correct sentence into: "…. in patients that cannot tolerate hemodynamically during single-lung perfusion after hilar clamping" (ventilation is usually not the initial problem!)

Reply 7: We modified the following sentence in the introduction according to the reviewer's suggestion.

Changes in the text (see Page 4, line 11)

"Most patients with PH require cardiopulmonary support, such as with veno-arterial extracorporeal membrane oxygenation (ECMO) and cardiopulmonary bypass (CPB), during LT, as they cannot tolerate respiratory management with single lung ventilation. (8)"

↓

"Most patients with PH require cardiopulmonary support, such as with veno-arterial extracorporeal membrane oxygenation (ECMO) and cardiopulmonary bypass (CPB), during LT, as they cannot tolerate hemodynamically during single-lung perfusion after hilar clamping. (8)"

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Reviewer E

Comment 1. This article is timely and addresses one of the most common associated issues encountered in lung transplantation, that of both primary and secondary pulmonary hypertension This is a well written article which addresses a rather common surgical technique to reduce bleeding from the pleural tissues that we as lung transplant surgeons frequently encounter when our patients have pulmonary hypertension. The authors describe a technique of pleural hemoclip application which reduces blood loss, primary pulmonary graft dysfunction, and mortality and decreases coagulopathy. The prospects of doing a study like this even crossed my mind a couple of decades ago. The authors counted the number of hemoclips used to effect hemostasis in their various patent populations and observed a benefit with the use of more clips. This is not a real scientific observation and conclusion. Actually, the effectiveness of pleural clipping in these patients depends on the effective application of the clips and not the number of clips as described here. certainly this technique is superior to that of suture closure of the pleural collateral which is attended with significant and troublesome intraoperaoperative hemorrhage, to which I can attest.

Reply 1: We would like to thank the constructive comments. We revised the following sentences in the limitation section according to the reviewer's suggestion.

Changes in the text (see Page 13, line 12)
"First, this was a retrospective study conducted at a single transplant center"
↓
"First, this was a retrospective study conducted at a single transplant center, and other than preventive closure of collateral vessels with hemoclips, LT technique might have improved over the years during the study period. Although the single transplant team performed the limited number of LT in the same manner at our institution, the detailed technique could differ among the patients."

Comment 2. The next issue that I find troublesome is the authors statement That the Institutional Review Board (IRB) approved the study in the presence of a "Waiver of an informed and signed personal consent. These lung recipients were alive and cognitive. Can the authors explain the actions of their Institutional Review Board in the decision Waive Informed Consent for their patients? This appears very irregular for their patient population and this study.

Reply 2: At the timing of transplantation, patients had given prior consent for the research use of the patient's data, and for this study, consent was obtained in an opt-

out format rather than in writing. We revised the following sentence in Methods.

Changes in the text (see Page 6, line 4)

“The need for obtaining written informed consent from each patient was waived.”

↓

“Informed consent was obtained in the form of opt-out on the website.”