Peer Review File

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Reviewer A

1. The explanation of how cryopreserved allografts are specifically collected and stored is insufficient.

Reply: We thank the reviewer for this valuable remark. We expanded the methods section to include specific information regarding donor harvesting procedures, cryopreservation protocols, storage conditions, and quality control measures in accordance with established tissue banking standards.

Change: Pages 6-7, lines 105-127

The The study institution has operated a tissue bank since 1988 . Allografts were harvested from brain dead organdonors. Donor serum samples were screened for hepatitis Bsurface antigen (HBsAg), antibody to hepatitis B surfaceantigen (HBsAb), hepatitis B core antibody (HBcAb), hepatitis C virus antibody (HCV Ab), human immunodeficiency virus 1/2 antibody (HIV 1/2 Ab), and VDRL for syphilis (RPR) (VDRL). Arterial grafts intended for cryopreservation undergo a stringent sterile processing workflow in a cleanroom environment (ISO7), using an ISO5 laminar flow hood to minimize microbial contamination. Upon opening each Cryokit containing the grafts, microbiological sampling is performed immediately. Specifically, two 5 mL aliquots of the transport solution are aseptically collected into aerobic and anaerobic blood culture bottles, and 1 mL is inoculated into a Schaedler enrichment broth. These samples, labeled with the graft batch number followed by the letter "A" (transport fluid), serve to assess microbial contamination prior to the introduction of the cryoprotectant. After the grafts are transferred into sterile freezing bags and filled with the cryoprotective solution, a second set of microbiological controls is performed: 5 mL of the final preservation fluid is inoculated into new aerobic and anaerobic blood culture bottles, and 1 mL is again placed in Schaedler broth. These are labeled with the same batch number followed by the letter "B" (preservation fluid). No antibiotics are added to either the transport or preservation solutions, ensuring that the results of microbiological testing remain unaffected by bacteriostatic agents. The cryoprotective solution is composed of 70% RPMI-1640 medium (Gibco), 20% human albumin 4% (LFB Vialebex, 40 mg/mL), and 10% dimethyl sulfoxide (DMSO, pure, density 1.1), resulting in a final concentration of 8 mg/mL albumin and 0.11 mg/mL DMSO in the 500 mL solution. This formulation ensures osmotic balance and cellular protection during the freezing process, while maintaining the structural integrity of the arterial tissue. The prepared solution is refrigerated between +2°C and +8°C and used within 6 hours of preparation.

2. It is necessary to describe in more detail the indications for cryopreserved allografts and the actual surgical technique. It would also be helpful to provide information on which part of the pulmonary artery was reconstructed.

Reply: Yes indeed

Change: Pages 7-8 , lines 137-155

Arterial involvement was attributed either to hilar tumors or lymph node invasion. All patients underwent lung lobectomy with radical mediastinal lymphadenectomy through a posterolateral thoracotomy, under general anesthesia and double-lumen endotracheal intubation. The feasibility of pulmonary artery (PA) sleeve resection was evaluated intraoperatively after achieving proximal and distal vascular control. Following intravenous administration of systemic heparin sodium (0.5 mg/kg), vascular clamps were applied to disease-free segments of the PA. Tumor resection was then carried out en bloc with the involved PA segment, and extended to adjacent structures when necessary. Frozen section analysis was systematically used to confirm negative resection margins. The method of PA reconstruction was chosen after resection, based on the extent of arterial involvement. Three techniques were used: (1) patch angioplasty using either prosthetic (HemaCarotid®, Maquet® Gentige Group, Rastatt, Germany) or bovine pericardial patches (XenoSure®, LeMaitre Vascular, Burlington); (2) end-to-end anastomosis; and (3) bypass reconstruction using cryopreserved allografts (PA or thoracic aorta) when direct reconstruction was not feasible. Vascular suturing was performed using 5-0 or 6-0 polypropylene. At the end of the procedure, the distal clamp was released before finalizing the suture to ensure adequate flushing and proper alignment of the reconstructed vessel (without kinking or torsion). In cases requiring a double-sleeve resection, the bronchial anastomosis was completed first using interrupted 4-0 PDS sutures. Postoperative anticoagulation included subcutaneous administration of 6000 IU of low-molecular-weight heparin for 15 days.

3. It is unclear how the efficacy of cryopreserved allografts is evaluated. While postoperative outcomes are presented, I believe the association with cryopreserved allografts is limited.

Reply 3:

We agree with you, we cannot assert this is directly associated with the arterial allograft; however, the study does confirm the non-inferiority of the allograft.

Reviewer B

Thank you for the opportunity to review the manuscript "Pulmonary Artery Reconstruction Using Cyropreserved Allograft for Lung Cancer: A Bi-Centric Comparative Study" for Journal of Thoracic Disease.

They spend a lot of effort in preparation of this manuscript and are according to the corresponding checklist. The manuscript deals with a very interesting topic and the authors present excellent results.

I got one annotation:

- I do not know the expression "oncological sound". Probably this should be checked. Otherwise the text is comprehensive and clear.

Reply:

Thank you for your comment. The expression "oncological sound" can indeed be confusing, we clarified this term in the text to provide better understanding for readers.

Change: Highlight box Page 4 ,line 65

Pulmonary artery reconstruction during lung cancer lobectomy is now widely accepted as an effective parenchyma-preserving technique from an oncological perspective. However, the use of cryopreserved homograft remains limited

Reviewer C

1-Thank you for submitting this study to the JTD. The study is very linear and easy to read. It does not contribute any new features to the existing literature on the subject, although the series of cases in which the cryopreserved graft was used is certainly one of the largest in existence. Since this is essentially a surgical technique paper, I would have expected some useful technical details for the readers of the JTD. Please add any technical details you consider relevant.

Reply: Thank you for your comment, we truly appreciate your thoughtful feedback and encouragement throughout the review process. Technical details are added to the text

Change: Pages 7-8 , lines 137-155

Arterial involvement was attributed either to hilar tumors or lymph node invasion. All patients underwent lung lobectomy with radical mediastinal lymphadenectomy through a posterolateral thoracotomy, under general anesthesia and double-lumen endotracheal intubation. The feasibility of pulmonary artery (PA) sleeve resection was evaluated intraoperatively after achieving proximal and distal vascular control. Following intravenous administration of systemic heparin sodium (0.5 mg/kg), vascular clamps were applied to disease-free segments of the PA. Tumor resection was then carried out en bloc with the involved PA segment, and extended to adjacent structures when necessary. Frozen section analysis was systematically used to confirm negative resection margins. The method of PA reconstruction was chosen after resection, based on the extent of arterial involvement. Three techniques were used: (1) patch angioplasty using either prosthetic (HemaCarotid®, Maquet® Gentige Group, Rastatt, Germany) or bovine pericardial patches (XenoSure®, LeMaitre Vascular, Burlington); (2) end-to-end anastomosis; and (3) bypass reconstruction using cryopreserved allografts (PA or thoracic aorta) when direct reconstruction was not feasible. Vascular suturing was performed using 5-0 or 6-0 polypropylene. At the end of the procedure, the distal clamp was released before finalizing the suture to ensure adequate flushing and proper alignment of the reconstructed vessel (without kinking or torsion). In cases requiring a double-sleeve resection, the bronchial anastomosis was completed first using interrupted 4-0 PDS sutures. Postoperative anticoagulation included subcutaneous administration of 6000 IU of low-molecular-weight heparin for 15 days.

2-Another important aspect to consider is the fact that the case history concerns 20 years of activity in which there has been an important evolution in both intra- and post-operative management, just think of the advent until its complete adoption of the minimally invasive surgical approach. However, we do not have details on this.

Reply: Indeed, the development of minimally invasive surgery over the past 30 years has significantly transformed postoperative outcomes in thoracic surgery. In our center, we have been performing VATS since the late 1990s. However, we do not perform sleeve resections via VATS. As you know, this is a technically challenging surgical scenario for which we have consistently favored an open surgical approach

3-The percentage of recurrent nerve palsy was quite consistent, (6.7, 8.3, 13% in the 3 groups respectively), although it is obviously linked to the fact that most of the lung resections consisted of left upper lobectomy. Have you developed some tips and tricks over time to reduce this very annoying complication as much as possible?

Good luck with the publication of this paper.

Reply: All cases of recurrent nerve palsy in our series were associated with left-sided pulmonary resections. These were tumors invading the hilum and/or the aortopulmonary region, most often in patients who had undergone induction therapy. Such treatment frequently leads to dense fibrosis and a significant inflammatory reaction, which makes surgical dissection particularly challenging. Even in the absence of direct nerve transection, recurrent nerve paresis is often unavoidable due to thermal injury or neural stunning from coagulation in close proximity. We are fully aware of this risk and take every possible precaution, but in some cases, despite careful dissection, the complication remains difficult to prevent.

Reviewer D

I congratulate the authors to their wonderful work. This retrospective, bi-centric study evaluates the safety and long-term outcomes of three pulmonary artery reconstruction techniques patch closure, end-to-end anastomosis, and cryopreserved allograft grafting in patients undergoing lobectomy for centrally located non-small cell lung cancer. The study spans a 20-year period and includes 107 patients.

The topic is clinically significant, addressing a technically challenging surgical scenario. The inclusion of cryopreserved allografts provides a novel comparative perspective. Data collected from two tertiary centers enhances external validity. The study assesses perioperative morbidity, patency, perfusion, survival, and recurrence, which are appropriate and meaningful endpoints for vascular reconstruction techniques. Inclusion of multivariate Cox regression to adjust for confounders in recurrence analysis is methodologically sound.

1-Study Design:

The retrospective nature introduces selection bias; however, the authors acknowledge this. The grouping by surgical technique (n=60, 24, and 23) is moderately imbalanced, particularly limiting power in subgroup analysis. The inclusion criteria were appropriate, though excluding patients undergoing bronchial sleeve resection alone or prosthetic grafting should have been better justified or clarified.

Reply: Thank you for your comment

Only 7 patients had undergone prosthetic bypass surgery, which is why we excluded them

2-Statistical Analysis:

Descriptive statistics are correctly used for baseline characteristics. P-values are provided throughout, but confidence intervals (CIs) for means are missing, which could offer more insight. Kaplan–Meier survival curves and hazard ratios from multivariate Cox regression are well-suited for the survival and recurrence analysis. The selection of variables for multivariable regression ($p < 0.2$ threshold in univariate analysis) and use of Akaike Information Criterion (AIC) for model optimization is appropriate.

The patency comparison reports a non-significant difference ($p = 0.776$), but statistical power is likely insufficient to detect differences given the small sample in

Group 3.

One concern is the overinterpretation of negative findings—e.g., stating no difference in survival/patency despite small sample sizes and possible Type II error.

Reply :

While the comparison of patency rates across the three groups did not yield a statistically significant difference ($p = 0.776$, Fisher's exact test), we acknowledge that this finding does not imply equivalence between the groups. The post hoc power analysis, comparing: The "Biologic bypass" group ($n = 23$) Versus the combined "Anastomosis" and "Angioplasty" groups ($n = 83$) using a two-sided test for comparing independent proportions (z-test approximation), the estimated statistical power was only 43.5% for patency and 33.4 % for OS far below the conventional 80% threshold. This low power indicates indeed a substantial risk of Type II error, meaning that a true difference might not have been detected due to limited sample size. We have accordingly acknowledge this in the discussion section

Changes

Page 9, lines 185-186 : A post hoc power analysis using two-proportion Z-tests was performed in order to detect a substantial risk of Type II error in non-significant comparisons.

Page 12, lines 252-253: The absence of conduit thrombosis in our study bears consideration although these results should be interpreted with caution due to the small sample size.

3-Data Reporting

Some standard deviations are quite large (e.g., time to recurrence), indicating high variability, yet this is not discussed. Tables are comprehensive, though the legends and explanations could be clearer. For instance, Table 2 uses abbreviations without defining them again.

Reply :

We thank the reviewer for this observation. Indeed, variables such as time to recurrence displayed high variability, as reflected by large standard deviations. This heterogeneity likely reflects the diversity of tumor biology, treatment responses, and postoperative follow-up durations among patients.

Regarding table clarity, however we would like to clarify that Tables 2, 3, and 4 do not contain any abbreviations requiring definition.

4-Minor typos:

"perfused lobe perfusion" → redundant phrasing. Suggest: "preserved lobe perfusion."

“folow-up protocole” → “follow-up protocol”
“aerostasis failure” → should clarify this term; "persistent air leak" or "surgical air leak" might be more appropriate.
Inconsistent use of hyphens (e.g., "end to end" vs. "end-to-end"; "p value" vs. "p-value").
“Cryopreserved homograft have been used…” → should be “homografts have been used.”
Several minor subject–verb agreement errors (e.g., “data was collected” should be “data were collected”).
“Cryopreserved allografts as a viable option…” → missing verb; suggest: “This study supports cryopreserved allografts as a viable option…”

Syntax/Clarity Suggestions:
“The feasibility of arterial sleeve lobectomy was assessed after proximal and distal control of the PA.”
Suggest rephrasing: “Arterial sleeve lobectomy feasibility was assessed following proximal and distal PA control.”
“However, a significant difference was observed in the frequency of associated bronchial reconstruction…”
Suggest: “However, bronchial reconstruction was significantly more frequent in Group 3 than in Group 1.”

Reply:
We thank the reviewer for their careful reading and helpful suggestions regarding grammar, syntax, and clarity. The manuscript was revised accordingly to address all the points raised

5-Discussion:
The discussion is balanced and references key studies, including Berthet et al. (2013), aligning current findings with historical outcomes. The claim of “no conduit thrombosis” in Group 3 is compelling but should be tempered given the short average follow-up for vascular degeneration (32 months). More critical discussion is needed on the cost, logistical considerations, and availability of cryopreserved grafts. The conclusion that cryopreserved allografts “should not be limited in use” is optimistic; the sample size does not support generalizable conclusions.
Reply
You are absolutely right to point out the relatively short mean follow-up in our study. However, given the rarity of this condition, we believe that the sample size is reasonable. Studies involving the use of arterial allografts in cardiac surgery have

reported longer-term outcomes with satisfactory results, and we have drawn parallels with those findings to support our conclusions. Regarding the economic and logistical challenges, this study was not designed to assess the economic impact of the technique. However, we acknowledge that the high cost, logistical complexity of harvesting and preservation, and limited availability of cryopreserved allografts remain important considerations for broader implementation. We have added a specific clarification in the limitation section and conclusion to address them accordingly.

Change:

Page 13, lines 260-262: However, their high cost, the logistical resources required for harvesting and preservation, and their limited availability in tissue banks remain important limiting factors to the broader use of cryopreserved allografts.

Page 14, lines 303-305 : The cost and availability of cryopreserved allografts represent the primary constraints limiting their broader use . Late graft-related complications should not represent an additional concern; however, long-term follow-up remains essential.

6-Limitations:

Short Follow-Up: Though 32 months is reasonable, longer-term durability data is needed to truly assess allograft degeneration and survival benefits.

Lack of Randomization or Propensity Matching: Without adjustment, the choice of technique may reflect surgeon preference or patient condition, confounding results.

Missing Patient-Reported Outcomes: No quality-of-life or functional status outcomes were reported, which are important for evaluating lung-sparing procedures.

No Cost Analysis: Given the high cost and limited availability of cryopreserved allografts, a brief economic evaluation would strengthen the practical implications.

Reply

Thank you for your thoughtful and pertinent comments, which will be addressed in the limitations section of our manuscript. We agree that longer-term follow-up is essential to fully assess allograft durability and potential survival benefits. Regarding the lack of randomization, it is important to note that conducting a randomized trial in this setting is nearly impossible due to the rarity of the condition and ethical considerations . As for patient-reported outcomes such as satisfaction and quality of life, our study is retrospective and observational in nature, and such data were unfortunately not available. Additionally, there was no comparative group (e.g., pneumonectomy) in our cohort. Nevertheless, previous studies have already demonstrated the superiority of parenchyma-sparing procedures such as arterial sleeve resections over pneumonectomy in terms of both morbidity, mortality, and quality of life.

Change: Page 14, lines 283-298

Our study has several important limitations. First, its retrospective design introduces a potential risk of selection bias, primarily influenced by surgeon preference and experience. Second, the relatively small sample size limited our ability to conduct more detailed stratified analyses, particularly by tumor stage. Third, although the median follow-up of 32 months is reasonable, it remains insufficient to fully assess the long-term durability of cryopreserved allografts and their potential impact on survival. Fourth, neoadjuvant treatment may result in pathological downstaging, this could potentially confound the interpretation of recurrence risk. Although neoadjuvant therapy was not significantly associated with recurrence in univariate analysis and was therefore excluded from the final multivariate model, this effect should be considered when interpreting pathological staging as a prognostic factor. Fifth, patient-reported outcomes—such as quality of life and functional status—were not assessed . Furthermore , no cost analysis was included, as this study was not designed to assess the economic impact of the technique. However, we acknowledge that the high cost, logistical complexity of harvesting and preservation, and limited availability of cryopreserved allografts remain important considerations for broader implementation . Despite these limitations, our study provides valuable insight into the feasibility and safety of using cryopreserved allografts for pulmonary artery reconstruction and highlights the need for further studies with longer follow-up.

7-Recommendations for Improvement:

Include 95% confidence intervals for means and medians where appropriate.

Add a sample size/power calculation, even post hoc, to inform interpretation of non-significant findings.

Correct grammatical/spelling inconsistencies.

Add clarity on terminology (e.g., “aerostasis,” “conduit degeneration”).

Discuss logistical considerations (availability, cost, regulation) of using cryopreserved allografts.

Reply:

We confirm that all the points raised have been addressed throughout our detailed responses above.

Reviewer E

Congrats for this study! Although the rate of this situation, the sample is good.

With the development of surgical techniques and new treatments in the area of thoracic oncology, more complex surgeries will be performed more and more. Situations requiring bronchovascular reconstruction will be more frequent. Therefore, the development of new techniques is essential for progress in technical development.

Reply:

We sincerely thank the reviewer for these encouraging comment, and we are pleased that our study can contribute, even modestly, to this important field of technical and oncological progress

Reviewer F

I commend the authors for conducting a bi-centric retrospective study assessing the outcomes of different pulmonary artery (PA) reconstruction techniques in lung cancer surgery. The topic is relevant, and the data presented are valuable. However, I have several concerns that should be addressed to strengthen the validity and interpretation of the findings:

1- Temporal Distribution of Surgical Groups

The study spans over 20 years (2001–2021), yet there is no description of the temporal distribution of cases in each group. It is likely that cryopreserved allografts (Group 3) were introduced more recently, which could confer advantages due to improvements in surgical technique, perioperative care, and adjuvant treatment. Please clarify the chronological breakdown of each group or perform a sensitivity analysis adjusting for surgical era to minimize potential time-related bias.

Reply:

We thank the reviewer for raising this important point. Over the 20-year study period (2001–2021), the distribution of cases across the surgical groups was not uniform. Group 1 (end-to-end anastomosis) and Group 2 (patch reconstruction) were performed throughout the period, both before and after 2015. In contrast, Group 3 (cryopreserved allografts) was almost exclusively used in more recent years, with only 2 cases before 2015 and 28 cases afterward. This reflects the progressive adoption of allograft techniques at our institution.

To address potential time-related bias, we performed a sensitivity analysis comparing recurrence-free survival between the pre-2015 and post-2015 eras. The analysis showed no statistically significant difference in RFS between the two periods (median RFS: 367.5 vs. 332.0 days, $p = 0.81$), suggesting that surgical era did not significantly influence recurrence outcomes.

Change: Page 10, lines 196-197

Table S1 (Supplementary Material) summarizes the temporal distribution of surgical groups across the study period

2-Association Between Reconstruction Technique and Recurrence

The multivariable analysis revealed that the type of PA reconstruction was not an independent predictor of recurrence. While this supports the non-inferiority of cryopreserved allografts, it does not justify any oncological superiority. Please temper the wording in the discussion and conclusion accordingly.

Reply:

We agree that our results support the oncological safety and non-inferiority of cryopreserved allografts, but do not demonstrate oncological superiority. Our aim was to highlight the feasibility, safety and analyze the performance of this technique without overstating its oncological benefit. We adressed these in the limitation section

Change : Page 14 , lines 286-287

Third, although the median follow-up of 32 months is reasonable, it remains insufficient to fully assess the long-term durability of cryopreserved allografts and their potential impact on survival

3- Heterogeneity in Pathological Staging Due to Neoadjuvant Therapy

Patients who received neoadjuvant therapy and those who did not were included in the same pathologic staging analysis. Given that neoadjuvant therapy can result in pathological downstaging, interpreting recurrence risk solely based on pathological stage without accounting for treatment response may be misleading. I recommend either stratifying outcomes by neoadjuvant therapy or including it as a covariate in the recurrence analysis.

Addressing these issues would enhance the scientific rigor and clinical applicability of this important study.

Reply:

As suggested, we evaluated the impact of neoadjuvant therapy on recurrence risk. In the univariate logistic regression analysis, neoadjuvant therapy was not significantly associated with recurrence (OR = 1.25, 95% CI: 0.56–2.80, p = 0.58). Following our predefined methodology, only variables with a p-value < 0.20 in univariate analysis were included in the multivariate model. Therefore, neoadjuvant therapy was excluded from the final model we have now explicitly mentioned this limitation in the revised manuscript to improve interpretability.

Change: Page 14, lines 288-291

Fourth, neoadjuvant treatment may result in pathological downstaging, this could potentially confound the interpretation of recurrence risk. Although neoadjuvant therapy was not significantly associated with recurrence in univariate analysis and was therefore excluded from the final multivariate model, this effect should be considered when interpreting pathological staging as a prognostic factor