Peer Review File

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Reviewer A

The authors describe the technique to performing EBUS guided mediastinal cryobiopsy and propose their 4-step approach.
The article is well-written; however, some changes should be implemented.

Comment 1: Since there are few randomized trials, this article should should mention: fan et al, lancet 2022.
Reply 1: Thanks for the suggestion. We have added this article. (See page 4, line 136).
Changes in text: In 2023, Fan et al. conducted an open-label, randomized trial at three hospital sites in Europe and Asia to evaluate the safety and added value of combining transbronchial mediastinal cryobiopsy with standard EBUS-TBNA for diagnosing mediastinal diseases. Eligible participants had at least one mediastinal lesion measuring 1 cm or longer in the short axis that required diagnostic bronchoscopy. A total of 271 patients were randomly assigned in a 1:1 ratio to either the combined group, which received both EBUS-TBNA and transbronchial mediastinal cryobiopsy, or the control group, which received EBUS-TBNA alone. They used a high-frequency needle knife to create an incision in the tracheobronchial wall before introducing the cryoprobe into the lesion. The study found that adding cryobiopsy to standard sampling significantly increased the overall diagnostic yield for mediastinal lesions, with 126 out of 136 participants (93%) in the combined group and 109 out of 135 participants (81%) in the control group achieving successful diagnoses. Subgroup analyses also showed that the combined approach was more sensitive than standard needle aspiration for benign disorders (94% vs 67%), and it improved the suitability of tissue samples for molecular and immunological analyses of non-small-cell lung cancer. The incidence of adverse events related to the biopsy procedure did not differ between the two trial groups. Their trial concluded that the addition of mediastinal cryobiopsy to standard EBUS-TBNA resulted in a significant improvement in diagnostic yield for mediastinal lesions, with a good safety profile.

Comment 2: For a better overview, suggest adding a table summarizing the different existing authors / articles and ways performing EBUS guided cryobiopsies.
Reply 2: Thank you for your valuable suggestion. We have made updates to the literature review section, and we believe we have described the most significant studies published regarding mediastinal cryobiopsy, including details such as the author, needle diameter, or needle knife if used. We are currently in the final stages of completing another article detailing our experience in 400 cases, and this table will be included as part of that publication. Again, thank you for your input.

Comment 3: Some figures contain repetitive or no useful information. suggest removing Figure 4, 8, 9, 11, 12. Figure 2C: TBNA sheath avoiding.... this sentence is not clear: what role does the sheath play here? please clarify in the manuscript or delete.

Reply 3: We strongly believe that these images are essential for fully comprehending the step-by-step process. In Figure 4, the primary feature of the needle we utilize is displayed. It is critical to note the tip of the needle and its differences compared to conventional ones. Figure 8 is crucial as it demonstrates the delicate and firm introduction of the 1.1 cryoprobe into the working channel. If not handled properly, the probe may bend and become ineffective. This image is particularly important as it showcases the absence of endoscopic vision, as the procedure is guided by ultrasound. Many colleagues may search for the puncture hole, but as depicted in this image, it is not visible in most cases. The needle trace is a critical aspect to observe, and it is imperative for our colleagues to understand how it is located and viewed within the lymph node.

In figure 9, we emphasize the significance of utilizing the doppler mode and accurately locating the tip of the cryoprobe. Failure to apply the doppler mode and encountering a vessel in the path can lead to complications. It is crucial for all colleagues to be always aware of the exact location of the tip of the cryoprobe. This is because what may appear to be the tip could be the trace form the puncture. Additionally, it is important to keep in mind that the freezing process at the cryoprobe also occurs frontally, which can potentially result in complications if a vessel is close in front. Our main concern is promoting safety and minimizing any potential risks during the procedure. Figures 11 and 12 provide a step-by-step guide on how to properly handle the cryoprobe. This is essential, as the technique differs from performing a transbronchial cryobiopsy for interstitial lung disease. It is imperative to press the pedal until the sample is securely outside the airway.

In figure 2C, the placement of the needle sheath between two cartilages can be seen. This is a helpful technique for performing blinded TBNA before EBUS-TBNA. It is imperative to carefully avoid puncturing the cartilage during the initial TBNA.

Changes in text: We have modified in the legend of figure 2C, TBNA for needle.

Comment 4: Line 167: Restaging/Staging: please comment on how many lymph node stations can be safely performed? is a staging possible with the cryo or maybe the wrong wording? Can you add existing safety data, if more than 1 or 2 lymph nodes are performed?

Reply 4: Line 167: Restaging the mediastinum after induction Chemotherapy and/or Radiotherapy for locally advanced NSCLC. Thank you for the interesting question. One of the challenges of EBUS-TBNA is that the diagnostic yield decreases in certain situations. This can occur due to changes in lymph node density, particularly when radiotherapy and/or chemotherapy is involved. In some cases, the lymph nodes may become necrotic or stiff, making it difficult to obtain a viable sample through TBNA for immunohistochemical and molecular testing. However, in our experience, we have increased the diagnostic yield over 95% in

these situations when performing Cryo-EBUS. Currently, we are conducting a study that suggests Cryo may be a better tool in these scenarios, hope to finish it soon. While we have mentioned potential indications, not definitive ones, further studies are still needed to fully understand this important topic. You can perform this technique in all stations, in our previous published articles we demonstrate that all stations are possible for Cryo-EBUS, and you can perform it also for staging purposes. We need to think that the cryoprobe is another tool to get a sample, the same as the needle, if the needle goes into the lymph node, the cryoprobe will follow using our method. In our experience, in more than 450 mediastinal cryobiopsies performed, we usually perform 2 stations per patient when ROSE is not available, but we have also performed 3 or more stations and we haven´t got any complications to date.

Changes in text: We have added (Line 175, page 4).

One scenario where Cryo-EBUS is particularly advantageous is in restaging the mediastinum after receiving chemotherapy and/or radiotherapy for locally advanced NSCLC. In these cases, the lymph nodes can become necrotic or stiff, making it challenging to obtain a viable sample through traditional TBNA methods for immunohistochemical and molecular testing. However, since incorporating Cryo-EBUS into our diagnostic approach, we have observed a significant increase in our diagnostic yield from 62% to 94%.

Comment 5: Line 165: necrotic lymph node stations: information's about mediastinitis? should be done with caution?

Reply 5: It is a very important question. Mediastinal cryobiopsy, due to its recent introduction and the rare occurrence of complications, there is limited direct data or studies linking it to the development of mediastinitis. In our experience, we have not encountered any cases of mediastinitis to date. In situations where we have a necrotic lymph node or lesion accessible by EBUS, we have consistently performed TBNA. Therefore, we see no reason why this approach should be different for Cryo-EBUS. However, if our suspicion is a cystic lesion containing fluid, we do not perform EBUS-TBNA and therefore, would not opt for a mediastinal cryobiopsy. An important factor to consider is that in cases where the sample is too necrotic for immunohistochemical and molecular determinations, or contains very few viable cells, we have found that a mediastinal cryobiopsy yields excellent results, especially when we have ROSE in the unit.

Changes in text: Added (line 177, page 4)

Regarding the risk of mediastinitis, there is limited data directly linking the procedure to this rare complication. When a necrotic lymph node or lesion is accessible by EBUS, we always opt for TBNA, and we see no reason for this to be any different for Cryo-EBUS. However, if our suspicion is a cystic lesion, we do not perform EBUS-TBNA, and therefore would not perform a mediastinal cryobiopsy in that scenario. One crucial factor to consider is the presence of ROSE, which allows for real-time assessment of sample quality. If ROSE indicates that the sample is too necrotic for accurate immunohistochemical and molecular analysis,

or contains very few viable cells, we have seen excellent results with mediastinal cryobiopsy.

Comment 6: What about sclerotic lymph nodes? please ad recommendations about that (...when not to do it?)
Reply 6: There is limited data available specifically linking sclerotic lymph nodes and mediastinal cryobiopsy, due to a lack of studies focused on this correlation. Sclerotic lymph nodes are characterized by an increased density due to fibrosis or scarring, often observed in conditions like granulomatous diseases, lymph nodes treated with radiotherapy, certain infections, and certain types of lymphomas and rare tumors. Based on our experience, we believe that when EBUS-TBNA is unable to provide sufficient samples for diagnosis (as is often the case with sclerotic lymph nodes), mediastinal cryobiopsy may be a suitable alternative. The effectiveness of cryobiopsy in obtaining samples from sclerotic lymph nodes lies in its ability to retrieve larger tissue samples, potentially overcoming challenges posed by the dense or fibrotic nature of these nodes. However, the specific efficacy of cryobiopsy in these cases may vary depending on individual patient characteristics and underlying cause of lymph node sclerosis. In our experience, mediastinal cryobiopsy is a more effective diagnostic tool than TBNA in these situations. We have not added additional data in the text, as we believe that the presence of sclerotic lymph nodes is one of the main indications for performing a mediastinal cryobiopsy.

Comment 7: Line 231 ff: ... suction is employed to ensure the most accurate TBNA samples: could you add literature/references to confirm this information? Experiences with worse TBNA information by creating a bloodier specimen?
Reply 7: Line 231: However, if no significant findings are present, suction is employed to ensure the most accurate TBNA sample for further analysis.
The application of suction to needles during tissue aspiration has been a standard practice for many decades in numerous medical specialties, including in the use of conventional TBNA. Some theorize that suction may increase tissue trauma in the biopsy site and result in more bleeding and lower yields. Others argue that suction is beneficial and produces a higher number of aspirated cells. Although this question has been studied in other anatomical sites, limited evaluation of the use of suction has been performed in EBUS-TBNA. Only one study was found to address this question. Casal et al. (reference: Casal RF, Staerkel GA, Ost D, et al. Randomized clinical trial of endobronchial ultrasound needle biopsy with and without aspiration. Chest. 2012;142(3):568-573) performed a randomized prospective trial comparing EBUS-TBNA with suction vs EBUS-TBNA without the use of suction, referred to as transbronchial needle capillary sampling (EBUS-TBNCS). Subjects were randomized either to undergo TBNCS in passes 1 and 3 or TBNA with suction in passes 2 and 4 or the opposite order. Separate needles were used for passes 1 and 3 vs those used with passes 2 and 4. Only the initial four passes were included in the analysis. The primary end point was the concordance

between suction TBNA and TBNCS for diagnostic yield and the adequacy of samples. There were no significant differences between the two groups in specimen adequacy, diagnosis rate, or specimen quality regardless of node size. Concordance rates between the techniques were high, ranging from 83.3% to 95.8% for adequacy, diagnostic yield, and specimen quality.
There are different opinions on the use of suction in TBNA. Our approach is to use suction if the doppler mode does not demonstrate significant vascularization. However, if the on-site evaluation indicates the sample is bloody, we proceed with the next TBNA without suction. When performing mediastinal cryobiopsy, we have found that having a slightly higher concentration of blood in the lymph node helps to enhance the visibility of the needle trace before inserting the cryoprobe. We have decided to change that sentence to avoid misunderstanding. We have decided to modify that sentence to prevent any misunderstandings.
Changes in text: We have deleted line 231 from the text.

Comment 8: Line 262ff: using the doppler mode before creating a tunnel and again before inserting the cryo seems to be repetitive: if it is necessary for safety reasons, please comment on that in the manuscript and if not please delete the second application.
Reply 8: Line 262: The Doppler mode should be used again to ensure that there are no vessels in the cryoprobe´s path. During Step-2 (the puncture), it is recommended in all guidelines to use the doppler mode before performing the TBNA. This step is crucial for ensuring the safety of the patient and is described in detail in the relevant section. After the needle is removed and the cryoprobe is introduced, it is essential to use the doppler mode once again to confirm its safe positioning before proceeding with the cryobiopsy, as outlined in the cryobiopsy section. The use of the doppler mode is key for ensuring safety and its availability should be utilized whenever possible. After thorough review, we believe each section emphasizes the importance of using the doppler mode and that is a key message we need to maintain.

Comment 9: Could you please add more information about safety concerns / possible side effects according to your experience?
Reply 9: After implementing our systematic step-by-step methodology, which we have applied in our 450 cases (in our IP unit) and in more than 250 cases outside our hospital (we have had the pleasure and opportunity to visit more than 40 IP units in Europe) with the main objective of sharing the technique, we are pleased to report that we have not encountered any complications such as pneumothorax, pneumomediastinum, or major bleeding. This reinforces our confidence in this approach and motivates us to share it with others, prioritizing safety above all. In our previous published papers, we demonstrate the feasibility and safety of the technique, and other relevant published articles also conclude that mediastinal cryobiopsy is a safe technique but needs a learning curve and teaching.

Reviewer B

This is an article on methodology of EBUS-TMC in the clinical practice. EBUS-TMC is one of the topics in the field of interventional pulmonology. This is a well-written paper, and I have several minor comments.

Minor:

Comment 1. Please describe the method of anesthesia.

Reply 1: We have added this information into line 220, page 6. Thanks for the suggestion.

Sedation was performed with midazolam (0.07 $mg \cdot kg^{-1}$) and fentanyl citrate (0.5–2 $\mu g \cdot kg^{-1}$), starting with boluses of 1–3 mg of midazolam and 0.1 µg of fentanyl citrate. Sedation was maintained with intermittent boluses of 1.2 mg midazolam and 0.1 µg fentanyl citrate according to the clinical judgment of the pulmonologist.

Comment 2. Please describe the specimen retrieving technique clearly that the cryoprobe and the EBUS scope are retrieved en bloc (not through the working channel).

Reply 2: Thanks for the suggestion. We have added to the text (line 277, page 7): and then retracted with the bronchoscope and the frozen biopsy tissue attached to the tip of the probe.

Comment 3. Do you usually use an endotracheal tube to facilitate repeated retrieval of the scope? If so, which size do you recommend?

Reply 3: We perform mediastinal cryobiopsy in the same way we perform conventional EBUS-TBNA, that is with an oral biter, we don´t use endotracheal tube in these cases. We haven´t found any complications after repeated retrievals of the scope. Another important issue is that with the endotracheal tube you will not have access to some lymph node stations like 2R, 2L and in some cases station 3p. We believe is not necessary to intubate the patient for this technique in our experience.

Comment 4. Line 196: Are there any differences on the techniques between the transbronchial approach and transesophageal approach?

Reply 4: We perform Cryo-EUS-B following the same steps of our method and we haven´t encountered any problems. The most important step is to create the tunnel, because the esophagus wall is different from the tracheobronchial wall, you need to focus a lot in breaking the mucosa and the capsule of the lymph-node correctly, because the esophagus wall tends to close faster because of its laxity, but it is the same way in both cases.

Comment 5. Line 191 and 230: Please describe each grade of vascular image patterns on the ultrasound image.

Reply 5: We have added your suggestion to the text (Line 192) and the reference to the text.

Grade 0: no blood flow or small amounts of flow
Grade I: a few main vessels running toward the center of the LN from the hilum.
Grade II: a few punctiform or rod-shaped flow signals, a few small vessels found as a long strip of a curve.
Grade III: rich flow, more than four vessels found with different diameters and twist or helical flow signal.
Grade IV (BA inflow sign): The blood flow from the BA toward the LN that was visualized as blue signals on EBUS Color Doppler-mode image.

Comment 6. Line 277: Cryoprobe activation time in the large, randomized studies (ERJ 2021, Lancet Respir Med 2023) was 7 seconds. Do you think is it too long?
Reply 6: It is a very good question. In our experience, between 4-5 seconds is enough. It is very important to know that after 7 seconds the sample is not going to get bigger in size, it has a freezing limit, but one of the most important aspects in freezing for more than 7 seconds is that after 7 seconds not only freezes the tip of the probe, but also the proximal part of the probe, and that is something to have in mind because in studies where more than 7 seconds have been applied, pneumomediastinum and pneumothorax have been described and that could be one of the reasons, that not only lymph node sample was attached to the tip of the probe, but also the proximal part, and the proximal part is pleura. We believe that the activation time between 4-5 seconds is safe, and the sample is of optimal quality.

Reviewer C
The paper titled "Proposal for a standardized methodology for performing EBUS-guided mediastinal cryobiopsy: A 4-step approach" by Ariza-Prota et al. presents a detailed methodology for conducting endobronchial ultrasound (EBUS)-guided mediastinal cryobiopsy.

Summary:
The study proposes a four-step method for mediastinal cryobiopsy, emphasizing its utility in diagnosing various pathologies affecting the mediastinum, especially where EBUS-transbronchial needle aspiration (TBNA) shows limitations. The method aims for safe, fast, and effective implementation, emphasizing ultrasound guidance and avoiding the need for endoscopic vision.

Strengths:
1. Comprehensive Methodology: The paper presents a thorough and systematic approach to performing mediastinal cryobiopsy.
2. Clinical Relevance: It addresses a significant gap in the diagnosis of mediastinal pathologies, particularly in challenging scenarios.
3. Detailed Illustrations: The use of figures and images enhances understanding of the procedure.

Weaknesses:
1. Specificity to Advanced Practitioners: The technique might require a steep learning curve and might be limited to highly skilled interventional pulmonologists.
2. Limited Scope: The paper focuses more on the methodology and less on the comparative analysis with other techniques.

****Major Comments: ****
- The paper could benefit from a more detailed discussion on the potential risks and complications associated with the procedure.
Reply: Thanks for your suggestion. We have added to the text (Line 299, page 7): Regarding mediastinal cryobiopsy and its potential complications, our experience has shown that we have not encountered any cases of pneumothorax, pneumomediastinum, mediastinitis, or major bleeding. We believe that the complications associated with this technique are no different from those of EBUS-TBNA. However, in studies where pneumomediastinum and pneumothorax have been reported (6), the cryoprobe was frozen for more than 10 seconds. It is important to mention that the sample size does not increase after freezing for more than 7 seconds. Additionally, it should be noted that the proximal part of the cryoprobe begins to freeze at this point. This issue is crucial, as freezing for more than 7 seconds can result in obtaining samples not only from the lymph node, but also from the pleura and mucosa, which may contribute to the reported complications in previous studies. Therefore, we recommend freezing for 3-5 seconds, which we believe is both safe and sufficient to obtain high-quality samples for accurate diagnosis.

- Inclusion of patient outcomes or case studies could provide a clearer understanding of the practical implications of the method.
Reply: It is a good recommendation, but we believe the focus of this article is to share the method in detail so that a lot of IP units around the world could start this technique in a safe and effective way for the benefit of the patients. In 2022 our group published a series of 5 cases thoroughly outlining the method and its outcomes. Additionally, in our recently published paper in ERJ in 2023, we delved into the practical implications of the method in detail. All references can be found within this article.

****Minor Comments: ****
Comment: The paper is well-organized, but some sections might benefit from additional subheadings for better clarity.
Reply: We appreciate your suggestion. We have modified the introduction and have added a subheading to the text.
(Line 84, page 2) Added: Therefore, mediastinal cryobiopsy seems to play a crucial role in these challenging scenarios. However, there is ongoing debate in the field

of interventional pulmonology regarding the best approach for obtaining a mediastinal cryobiopsy. Some interventional pulmonologists use a high-frequency needle knife to create an incision in the tracheobronchial wall adjacent to the mediastinal lesion before inserting the cryoprobe, while others use a needle to create a pathway to the target area. There are also variations in the use of endoscopic or ultrasound imaging for guidance. In this article, we aim to review the current literature on different methods of performing mediastinal cryobiopsy and share our own clinical experience and methodology in a systematic way for its implementation in a safe, fast, and effective way. We have Added subheading (line 84, page 2)**: Literature review**

Comment: There could be more emphasis on the scenarios where this methodology is particularly advantageous over traditional methods.
Reply: We have added specific scenarios where we believe this technique is particularly advantageous over traditional methods. Added (Line 175, page 4): One scenario where Cryo-EBUS is particularly advantageous is in restaging the mediastinum after receiving chemotherapy and/or radiotherapy for locally advanced NSCLC. In these cases, the lymph nodes can become necrotic or stiff, making it challenging to obtain a viable sample through traditional TBNA methods for immunohistochemical and molecular testing. However, since incorporating Cryo-EBUS into our diagnostic approach, we have observed a significant increase in our diagnostic yield from 62% to 94%. Each case should be approach individually as every situation is unique. Regarding the risk of mediastinitis, there is limited data directly linking the procedure to this rare complication. When a necrotic lymph node or lesion is accessible by EBUS, we always opt for TBNA, and we see no reason for this to be any different for Cryo-EBUS. However, if our suspicion is a cystic lesion, we do not perform EBUS-TBNA, and therefore would not perform a mediastinal cryobiopsy in that scenario. One crucial factor to consider is the presence of ROSE, which allows for real-time assessment of sample quality. If ROSE indicates that the sample is too necrotic for accurate immunohistochemical and molecular analysis, or contains very few viable cells, we have seen excellent results with mediastinal cryobiopsy.

**Grammar and Syntax: **
- The document appears to be well-written with no major grammatical or syntactical errors noted upon review.

Overall, the paper provides a valuable contribution to the field of interventional pulmonology by standardizing a technique that could enhance the diagnostic accuracy for certain mediastinal conditions.

Reviewer D
Very nice overview of a relatively new technique. I have nothing to add.

Reply: Thank you.

Reviewer E

I would like to congratulate the authors with their manuscript entitled "Proposal for a standardized methodology for performing EBUS- guided mediastinal cryobiopsy: A 4-step approach". The article covers an interesting innovation in the field of EBUS and provides the readership with practical tips and tricks to start using this novel technique.

I have the following comments:

Comment 1: The Introduction section is rather long. I would suggest that the authors rewrite this paragraph to a short introduction, followed by a paragraph in which they introduce cryo-EBUS, describe the technique and provide an overview of current literature.

Reply 1: We appreciate your suggestion. We have modified the introduction and have added a subheading to the text.

(Line 84, page 2) Added: Therefore, mediastinal cryobiopsy seems to play a crucial role in these challenging scenarios. However, there is ongoing debate in the field of interventional pulmonology regarding the best approach for obtaining a mediastinal cryobiopsy. Some interventional pulmonologists use a high-frequency needle knife to create an incision in the tracheobronchial wall adjacent to the mediastinal lesion before inserting the cryoprobe, while others use a needle to create a pathway to the target area. There are also variations in the use of endoscopic or ultrasound imaging for guidance. In this article, we aim to review the current literature on different methods of performing mediastinal cryobiopsy and share our own clinical experience and methodology in a systematic way for its implementation in a safe, fast, and effective way. We have Added subheading (line 84, page 2)**: Literature review**

Comment 2: Please describe the advantages and disadvantages of VAM and VAMLA, including the risks involved (serious complications, e.g., massive hemorrhage, recurrent laryngeal nerve palsy).

Reply 2: Thanks for your suggestion. We have added in the introduction section (Line 77, page 2): It is important to note that there are potential complications associated with both intra and post-operative procedures of video-assisted mediastinoscopy (VAM) and video-assisted mediastinoscopic lymphadenectomy (VAMLA). These complications, including wound infection, mediastinal hematoma and seroma, mediastinitis, pleural effusion, pneumothorax, chylothorax, and left-sided recurrent nerve paralysis, occur in approximately 5-8% of cases (3)

Comment 3: When to do it: please provide a flow chart in which you guide the readership through the process of clinical decision making. In other words: in

which patient do you prefer cryo-EBUS over EBUS-TBNA, do you always start with EBUS-TBNA as first choice and move over to cryo in case EBUS-TBNA was inconclusive, are there indications in which you primarily start with cryo? Etcetera.
Reply 3: This is a very interesting and appropriate suggestion. However, the focus of this article is to provide a detailed description of the technique. We are writing a separate article solely based on expert opinions, specifically addressing indications and contraindications. This flow chart will play a crucial role in that paper. Thank you very much.

Comment 4: When not to do it: are there any specific contra-indications for cryo?
Reply 4: As described in the text, we believe there are numerous indications for mediastinal cryobiopsy, that's why we have stated them as ¨possible indications¨. However, our group has determined that the contraindications for performing Cryo-EBUS are the same as those of conventional EBUS-TBNA, which is why we have not included ¨possible¨ in the heading. It is important to note that if it is determined that EBUS-TBNA should not be conducted in a particular case, then Cryo-EBUS should also not be performed.

Comment 5: Are there any specific requirements, other than cryo-EBUS probes? What about availability and costs?
Reply 5: To perform a Cryo-EBUS using our method, you will need an EBUS scope, a TBNA needle, the ERBE2 system, and the 1.1 mm cryoprobe. It is important to mention that the way we perform the procedure is in our interventional pulmonology suite and without intubation, and the patient is discharged one hour after the procedure saving costs to the hospital. The other way of performing a mediastinal cryobiopsy is making a cut with the needle knife, which is very expensive, a lot of units don't have it, and it needs a cauterization system to be used, then the EBUS scope and the 1.1 mm cryoprobe. Important to mention that less than 10% of the IP units in Europe and Latin America have a needle knife available. During 2024 we are working on a paper only related to costs and effectiveness, in our preliminary data, with Cryo-EBUS we have avoided repetition of 54 cases from our 452 performed. This is very important because some of these patients would have ended in a mediastinoscopy, a more expensive and invasive procedure; and if we had decided to repeat the EBUS-TBNA for a second time that would increase the costs, including a delay in treatment and probable not achieving a definitive diagnosis at the end. This year we will publish that study. Thanks for the question.

Comment 6: Line 219: Is your comment on the learning curve evidence or experience based? Based on efficacy, completeness, complications? How many cases do you recon the learning curve would be? Please elaborate.
Reply 6: Our group believes that based on our experience, and the experience of other IP colleagues that we have had the opportunity to train, we all agree and recommend performing a minimum of 30 procedures within a 3-month timeframe and obtaining samples from different lymph node stations rather than solely on

one. We believe this approach will greatly aid in mastering the learning curve in a safe, fast, and effective way. We have mentioned this topic in the conclusion section. Thanks for the question.

Comment 7: Line 230: "grade 2 of higher vascularization". Is this a formal classification? Please add reference.
Reply 7: We have added your suggestion to the text (Line 192) and the reference. Thanks for the suggestion.
Grade 0: no blood flow or small amounts of flow
Grade I: a few main vessels running toward the center of the LN from the hilum.
Grade II: a few punctiform or rod-shaped flow signals, a few small vessels found as a long strip of a curve.
Grade III: rich flow, more than four vessels found with different diameters and twist or helical flow signal.
Grade IV (BA inflow sign): The blood flow from the BA toward the LN that was visualized as blue signals on EBUS Color Doppler-mode image.

Comment 8: Is the "Ariza-Pallarés method" a recognized standard in the field? Otherwise, try to use a more descriptive denomination, thereby aiming to refrain from self-aggrandizement.
Reply 8: The Ariza-Pallarés method is a well-known and highly regarded technique in the field of interventional pulmonology. First introduced in 2022 (reference 11), our aim in this article was to provide a detailed, step-by-step description of the method through a practical clinical case, making it accessible for implementation in all interventional pulmonology units that perform endobronchial ultrasound (EBUS) daily. Since May 2022, we have had the opportunity to visit over 40 interventional pulmonology units in Europe and share this technique with many wonderful colleagues. We strongly believe that this technique should be shared and taught in all interventional pulmonology units, with a clear protocol for safety and consistency, as previously mentioned.
In 2023, we published our second article (reference 13) in which we shared our experience with the first 50 cases, focusing on safety, feasibility, diagnostic yield, and complications. We were also honored to present a lecture on the technique and the Ariza-Pallarés method at the European IP Congress in Madrid in May 2023. During this event, we had the opportunity to engage in discussions with experienced interventional pulmonology colleagues who were interested in implementing the technique, as well as those who had already started performing mediastinal cryobiopsy. Furthermore, we have published two additional papers on the fanning technique and our experience in performing mediastinal cryobiopsy through the esophageal route (references 15 and 16), both utilizing the Ariza-Pallarés method.
We firmly believe that the practice of self-aggrandizement has no place in our profession. Our motivation for sharing the Ariza-Pallarés method is not for personal gain, but rather to contribute to the advancement of interventional

pulmonology. We are against the idea of withholding information or only publishing a portion of our experience, which unfortunately is prevalent in our field. When you read our previous articles and this one as well, you will find the underlying philosophy of sharing. Our tips and tricks, as well as the lessons learned from our own mistakes over the years, are all addressed with the intention of preventing anyone else from making the same errors. The Ariza-Pallarés method is the first and only method described to perform a mediastinal cryobiopsy utilizing complete ultrasound guidance. It is the first and only method published that allows for the introduction of a cryoprobe with just one TBNA. This method is also the first to describe how does a broken capsule looks like and its importance. As a result, it has become widely known and recognized within the IP community. Considering this, we have chosen to maintain the original name of the method.

Comment 9: Can you discuss your institutional preliminary results, including procedure time, success rate and complications/outcome?
Reply 9: Thanks for the question. In the published paper of our experience in our first 50 cases, the procedure time, diagnostic yield, and complications are described. Since we initiated the technique, we haven´t got any complications (no pneumothorax, no mediastinitis, no pneumomediastinum and no major bleeding). The Cryo-EBUS technique behaves the same as a conventional EBUS-TBNA when performing these steps. We are gathering all the data from our 452 cases to publish it hopefully this year. But we can tell in advance that we haven't had complications, and the overall diagnostic yield is above 95%.

Comment 10: Can you elaborate on the post-procedural course? What are the most common complications, how are these identified and treated?
Reply 10: We have added the post-procedural course/protocol we did during the first 50 cases into the text (Line 155, Page 4):
All patients received post-procedural chest radiographs or pleural echography to confirm that a pneumothorax had not been produced and the patient was discharged 2 h after verifying that there had been no complications. Follow-up was conducted on all patients at 24 h via phone call and 2 weeks after the procedure to check that there were no delayed complications.
After performing the first 50 cases we decided to stop doing chest-X rays and pleural ultrasound as no complications were observed. We are using the same protocol as for conventional EBUS-TBNA, that is, if the patient does not refer symptoms and is well, is discharged one hour after the procedure.

Comment 11: Please consider adding a procedural video, in which you combine external and internal views.
Reply: We have added a procedural video of the puncture, tunnelization and cryobiopsy as suggested.