DIAGNOSTIC YIELD AND SAFETY OF PLEURAL CRYOBIOPSY USING A FLEXIBLE BRONCHOSCOPE: A RETROSPECTIVE COHORT STUDY

Murugesu, T.[1*], Samsuddin, S.[2], Tawil, Z.[3], Wan, J.L.[1]

[1] Department of Medicine, Selayang Hospital, Selangor, Malaysia.
[2] Department of Medicine, UiTM Sungai Buloh, Selangor, Malaysia
[3] Department of Pathology, Selayang Hospital, Selangor, Malaysia.

***Corresponding author:**
Thamayanthy Murugesu, Department of Respiratory Medicine, Selayang Hospital, Lebuhraya, Selayang-Kepong, 68100 Batu Caves, Selangor, Malaysia.
Email: thama2710@gmail.com

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ABSTRACT:
Introduction: Pleuroscopy is a well-established diagnostic tool for undiagnosed exudative pleural effusion, conventionally performed with rigid or semi-rigid thoracoscopes when less invasive methods fail. Pleural cryobiopsy improves diagnostic yield and tissue quality, but data on its use with a flexible bronchoscope remain limited. In this study, we assessed the diagnostic yield, adequacy for molecular testing, safety, and feasibility of pleuroscopic cryobiopsy using a flexible bronchoscope.
Materials and Methods
We conducted a retrospective cohort study of consecutive adult patients with undiagnosed exudative pleural effusion despite thoracocentesis who underwent pleuroscopic cryobiopsy using a flexible bronchoscope at Selayang Hospital, Malaysia, between November 2023 and September 2025.
Results
Thirty patients underwent pleuroscopic cryobiopsy via a flexible bronchoscope. The diagnostic yield was 90.0% (95% confidence interval (CI): 73.5–97.9%), and malignancy was the most common diagnosis (56.7%), with lung adenocarcinoma being the most frequent. 88.9% of lung adenocarcinoma biopsy samples were sufficient for Epidermal Growth Factor Receptor (EGFR) mutation analysis. 30% of the specimens showed granulomatous inflammation, while 10% were non-diagnostic. Median cryoprobe activation time was 5 seconds (interquartile range (IQR) 3-5), procedure duration was 45 minutes (IQR 30 – 46), and biopsy size was 10 mm (IQR 8-16). Minor bleeding occurred in 23.3% (95% CI: 9.9–42.3%) of patients, and moderate bleeding in 6.7% (95% CI: 0.8–22.1%). No major bleeding events occurred. No significant associations were observed between procedural variables and clinical outcomes.
Conclusion
Pleural cryobiopsy performed via a flexible bronchoscope is an effective and feasible diagnostic modality for undiagnosed exudative pleural effusion, with a low bleeding risk, providing high diagnostic yield and adequacy for molecular testing.

Keywords: Pleural effusion; Cryobiopsy; Pleuroscopy; Flexible bronchoscope; Diagnostic yield

INTRODUCTION

Pleural effusion remains a common diagnostic challenge in clinical practice with a wide spectrum of aetiologies. In regions with a high prevalence of tuberculosis, such as Malaysia, the most frequent cause of pleural effusion is tuberculosis, followed by malignancy, both of which are associated with significant morbidity and mortality [1]. However, diagnosing exudative pleural effusions can be challenging, and studies report that around 20% of pleural effusions remain undiagnosed even after repeated thoracentesis and closed pleural biopsy [2].

In such cases, pleuroscopy, also known as medical thoracoscopy, has emerged as an important minimally invasive diagnostic tool. An incision is made in the chest wall, creating a pneumothorax, via which the scope is passed into the pleural space.
This allows direct inspection of the pleural cavity and targeted biopsy of suspicious lesions. [3,4]. Conventionally, biopsies are obtained using forceps introduced through rigid or semi-rigid thoracoscopes [4].

Recently, cryobiopsy, a method of obtaining tissue biopsy using a cryoprobe, has gained more attention in interventional pulmonology. It has shown promise across various interventional pulmonology applications, including interstitial lung disease, peripheral pulmonary lesions, endobronchial and mediastinal lesions, pleural disease, and even surveillance for cellular rejection post-lung transplantation. This technique yields larger specimens with minimal crush artefact and better-preserved tissue architecture, enabling more reliable histopathological interpretation [5-11]. The larger samples obtained are not only crucial for establishing a histological diagnosis but also for performing further immunohistochemical staining (e.g., Programmed Death Ligand 1 (PD-L1)) and molecular testing (e.g., mutation assays such as Epidermal Growth Factor Receptor (EGFR)) in patients with malignancy [12-14].

Although findings on the risk of bleeding associated with transbronchial cryobiopsy compared to forceps biopsy range from statistically significant to non-significant, the existing literature evaluating pleural cryobiopsy supports its safety, reporting a low incidence of clinically significant bleeding complications [15-18]. While cryobiopsy in pleuroscopy has emerged as a promising diagnostic tool, it remains relatively novel compared to transbronchial cryobiopsy, and evidence regarding its diagnostic efficacy and safety profile remains limited. Most published studies on pleuroscopic cryobiopsy have utilised rigid or semi-rigid thoracoscopes. However, these instruments are not always available at every respiratory unit [19-20]. In the absence of such systems, a flexible bronchoscope can serve as a practical alternative, avoiding the need for thoracotomy and leading to shorter hospitalisation and earlier diagnosis of pleural disease [19]. While pleuroscopy with a flexible bronchoscope and forceps biopsy has been described, evidence on pleural cryobiopsy performed with a flexible bronchoscope remains scarce.

Given that the semi-rigid thoracoscope closely resembles the flexible bronchoscope in terms of flexibility and manoeuvrability, flexible bronchoscopes likely share similar limitations, namely reduced rigidity and mechanical leverage when attempting biopsy of thickened pleura, compared with rigid thoracoscopic systems [4,21]. The use of cryobiopsy helps overcome this limitation, as the cryoprobe adheres to the tissue during freezing, and the tissue is then extracted

under controlled conditions [4,22]. This combined approach may allow adequate tissue sampling while maintaining the flexibility of the bronchoscope.

Therefore, in this retrospective cohort study, we evaluated pleural cryobiopsy performed via a flexible bronchoscope. The primary objective was to determine the diagnostic yield of pleural cryobiopsy using this approach. Secondary objectives included assessing the molecular adequacy of the biopsy specimens, procedural feasibility, and the safety profile of the procedure.

METHODS

A retrospective cohort study was conducted on adult patients with undiagnosed exudative pleural effusion who underwent pleuroscopic cryobiopsy via a flexible bronchoscope at Selayang Hospital, a tertiary referral centre in Selangor, Malaysia, between November 2023 and September 2025. Thirty hospitalised patients aged 18 years or older with exudative pleural effusion of unknown aetiology despite thoracocentesis were included. All consecutive eligible patients who underwent the procedure during the study period were included in the analysis.

All procedures were performed by experienced pulmonologists trained in pleuroscopy. Pleuroscopy was performed using a flexible bronchoscope (1TH190, Olympus) after gaining access to the pleural cavity via a single port under local anaesthesia and mild conscious sedation with midazolam, as shown in Figure 1. Following inspection of the pleural cavity to identify abnormal pleural areas, cryobiopsies were obtained using a 1.7 mm flexible cryoprobe (Erbe Elektromedizin GmbH). The number of samples obtained per patient varied between procedures and was determined by the bronchoscopist based on visual assessment of the pleura.

Medical records were obtained from the hospital's electronic medical record system, the Backup Hospital Information System (BHIS), and histopathological examination reports. Data collected included patient demographics, side of pleural effusion, total procedure duration, cryobiopsy freeze time, sample size, histopathology, molecular adequacy, and bleeding complications. No missing data was identified for the variables included in the analysis.

The primary outcome was defined as the diagnostic yield of pleural cryobiopsy. A positive diagnostic outcome was defined as obtaining a specific histopathological diagnosis sufficient to guide clinical management. For cases with granulomatous inflammation, the final diagnosis was based on a comprehensive clinical assessment that incorporated histopathology, available microbiological data, radiological findings, and the response to therapy. Secondary outcomes included the adequacy of biopsy specimens for molecular testing, bleeding complication rates and procedural feasibility, with procedure duration and cryoprobe activation time used as surrogate measures of technical performance. Bleeding severity was classified using a study-specific grading system based on the level of intervention required to achieve haemostasis. Minor bleeding was defined as self-limiting bleeding or bleeding requiring adrenaline instillation, moderate bleeding as bleeding requiring argon plasma coagulation, and major bleeding as bleeding requiring blood transfusion or surgical intervention. This pragmatic classification was adopted to facilitate consistent assessment of bleeding severity based on documented interventions in this retrospective cohort. Non-bleeding adverse events were not consistently documented in the procedural or inpatient records and were therefore not analysed.

All statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) Statistics (Version 30). Normality of continuous variables was assessed using the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots. Age was found to be normally distributed and was reported as mean ± standard deviation (SD). Procedure duration, cryoprobe activation time, and

biopsy sample size were not normally distributed and were therefore reported as medians and interquartile ranges (IQRs). Where necessary for comparison with previously published studies that reported mean values, corresponding means are additionally presented in the Discussion. Categorical variables were reported as frequencies and percentages.

Associations between continuous variables were evaluated using Spearman's rank correlation coefficient, reported as the correlation coefficient (r's) and p value. Comparisons of continuous outcomes between two groups were performed using the Mann-Whitney U test, and comparisons across three or more groups were performed using the Kruskal-Wallis test. Statistical significance was set at $p < 0.05$.

RESULTS

A total of 30 patients underwent pleuroscopic cryobiopsy during the study period and were included in the analysis. The cohort comprised 18 males (60%) and 12 females (40%) with a mean age of 60.93 ± 10.71 years. 18 (60%) patients had right-sided effusion, whereas 12 (40%) had left-sided effusion. The majority of patients were Chinese (n=14, 46.7%), followed by Malay (n=12, 40%), Indian (n=3, 10%), and others (n=1, 3.3%).

A conclusive histopathologic diagnosis was obtained in 27 of the 30 cases, yielding a diagnostic rate of 90.0% (95% confidence interval (CI): 73.5–97.9%). Of these 17 (56.7%) were malignant and 10 (33.3%) were non-malignant. 3 (10%) of the 30 samples were non-diagnostic. Among malignant cases, adenocarcinoma was most frequent, and 88.9% of these specimens were adequate for EGFR analysis. Additionally, three cryobiopsy specimens underwent next-generation sequencing (NGS), and all were adequate for the requested analyses. A breakdown of histopathological diagnoses is given in Table 1. Representative histological images are shown in Figure 2.

Mycobacterial culture was performed on cryobiopsy specimens in 12 patients. One specimen (8.3%) yielded a positive culture for *Mycobacterium tuberculosis*, corresponding to a case with necrotising granulomatous inflammation on histopathology. Activation time for the cryoprobe ranged from 2 to 15 seconds, with a median of 5 seconds (Interquartile range (IQR) 3–5). The total procedure duration had a median of 45 minutes (IQR 30–46.25; range 25–80). Biopsy samples had a median size of 10 mm (IQR 8–16.25; range 4–25).

Procedure-related bleeding occurred in 9 (30.0%; 95% CI: 14.7–49.4%) patients. Minor bleeding occurred in 7 (23.3%; 95% CI: 9.9–42.3%) patients, of which most resolved spontaneously, and one required adrenaline instillation. Moderate bleeding occurred in 2 (6.7%; 95% CI: 0.8–22.1%) patients and required argon plasma coagulation for haemostasis. No major bleeding requiring transfusion or surgical intervention was observed (0%; 95% CI: 0.0–11.6%). Non-bleeding complications were not systematically captured due to the retrospective design and therefore were not analysed.

Exploratory correlation analyses revealed no statistically significant associations between procedural variables and clinical outcomes. Procedure duration ($p = 0.334$) and patient age ($p = 0.912$) were not significantly associated with complication rates. Likewise, neither biopsy sample size nor cryoprobe activation time demonstrated a significant relationship with complications ($p = 0.093$ and $p = 0.592$, respectively). Additionally, no significant correlations were identified between sample size and diagnostic yield ($p = 0.672$) or between cryoprobe activation time and sample size (Spearman's rho = 0.098, $p = 0.605$).

DISCUSSION

In this retrospective cohort study, pleural cryobiopsy performed via a flexible bronchoscope achieved a diagnostic yield of 90% and provided adequate tissue for molecular analysis, supporting its feasibility as a diagnostic option for patients with undiagnosed exudative pleural effusion.

Although this is slightly lower than the pooled diagnostic rates reported in three prior meta-analyses, which exceeded 94%, it remains well within the range reported in the individual studies included in these reviews [17,18,23]. For instance, the systematic review by Giri et al., which analysed data from 15 studies conducted across diverse global settings, reported diagnostic yields ranging from 76.7% to 100% for cryobiopsy using rigid and semi-rigid thoracoscopes, and from 20% to 100% for forceps biopsy [17]. This suggests that substituting a rigid or semi-rigid thoracoscope with a flexible bronchoscope does not significantly compromise diagnostic performance when cryobiopsy is utilised.

The histopathological examinations identified a broad spectrum of diagnoses. Malignancy was the final diagnosis in most cases of undiagnosed pleural effusion, consistent with previous studies [24-26]. This is followed by granulomatous inflammation. In tuberculous endemic regions, including Malaysia, there is often significant clinical and radiological overlap between tuberculosis and malignancy. Hence, the ability to obtain an early biopsy and an accurate histopathological diagnosis is crucial for differentiating between the two conditions and initiating timely, appropriate treatment.

Among malignant histopathological diagnoses, adenocarcinoma was the most prevalent, and a substantial proportion of these samples were adequate for EGFR testing (88.9%). This aligns with observations from previous studies, which demonstrate that pleural cryobiopsy generally provides sufficient tissue with preserved architecture for molecular testing [27]. Given the increasing need for biomarker-guided therapy, ensuring adequacy at the time of diagnosis confers meaningful clinical value and may reduce the need for repeat procedures. Although additional molecular analyses, including Anaplastic Lymphoma Kinase (ALK) and Kirsten rat sarcoma viral oncogene homolog (KRAS) testing, and immunotherapy marker testing, such as PD-L1, are not routinely offered at our centre, three cryobiopsy specimens underwent next-generation sequencing (NGS) upon request by the treating pulmonologist or oncologists. All three specimens were adequate for the requested analyses. These findings further support the potential of pleural cryobiopsy to provide sufficient tissue for comprehensive molecular profiling, which is increasingly important in guiding personalised treatment strategies in thoracic oncology.

Of the nine patients with granulomatous inflammation, six were highly supportive of a diagnosis of tuberculosis, with either identification of acid-fast bacilli or necrotising or caseating granulomatous inflammation. Given Malaysia's tuberculosis endemicity, the remaining three patients with chronic granulomatous inflammation were also treated for tuberculosis based on integrated clinical judgement, including clinical history, biochemical parameters, and pleural-fluid findings consistent with routine practice in tuberculosis-endemic settings.

In our cohort, mycobacterial culture performed on cryobiopsy specimens yielded a positive *Mycobacterium tuberculosis* result in one case. Although based on a small number of samples, this suggests that cryobiopsy specimens may retain microbiological viability despite freezing, highlighting the potential role of cryobiopsy not only for histopathological diagnosis but also for microbiological assessment of the diseased pleura, especially in high tuberculosis prevalence settings.

A single case of acute-on-chronic inflammation was identified on pleural cryobiopsy. Although the initial thoracentesis yielded a pleural fluid culture positive for *Klebsiella pneumoniae*, clinical and

radiological findings remained highly suspicious for an underlying malignancy, warranting further tissue evaluation. Cryobiopsy demonstrated acute-on-chronic inflammation without histopathological evidence of malignancy, supporting an inflammatory aetiology consistent with the microbiological findings. The patient subsequently responded well to targeted antibiotic therapy. This case highlights the complementary role of pleural cryobiopsy in evaluating suspected coexisting pathology when initial pleural fluid investigations do not adequately explain the clinical presentation. Notably, two of the three non-diagnostic cases occurred early in the study period, whereas subsequent procedures nearly always yielded a conclusive diagnosis. This reflects a potential learning curve, with improved diagnostic performance after operator familiarisation.

The median activation time in our cohort was 5 seconds (IQR 3-5). The activation times appeared lower in the later cases, with values of 2-3 seconds observed toward the end of the study, without compromising sample size or diagnostic yield. These later specimens remained of adequate size (8-20 mm), and all ten cases yielded a positive histopathological diagnosis. The activation times used in our study are broadly consistent with published literature. Botana Rial et al., in their systematic review of pleural cryobiopsy, reported that most studies used an activation time of 3 seconds, whereas Thousheed et al. reported a longer average freeze time of 8 seconds [23].

As for total procedure duration, our study recorded an average of approximately 42 minutes, with most procedures completed between 30 and 45 minutes. Compared with a published Japanese series by Kamatani et al., analysing cases performed exclusively with cryobiopsy using semi-rigid thoracoscopes, our procedural times fall within a similar range [28]. This reinforces the technical feasibility and reproducibility of flexible-scope-guided cryobiopsy.

The bleeding complications in our cohort were infrequent and predominantly mild, with no major bleeding events requiring transfusion or surgical intervention. Previous studies have also reported pleural cryobiopsy as a safe procedure with a low incidence of clinically significant bleeding [17-18]. Our study demonstrated no significant association between the total procedural time or cryobiopsy activation time and bleeding rates. However, a study evaluating the optimal freeze time of the cryoprobe in transbronchial cryobiopsy found that, although there was no significant difference in bleeding rates across cryobiopsy freezing times, there was a trend toward increased bleeding with freezing times greater than 10 seconds [29]. This suggests that a shorter activation time may reduce the risk of procedure-related bleeding complications without compromising diagnostic efficacy. Although our study did not find a correlation between freezing time and bleeding complications, more definitive studies are needed to determine the impact of activation time on bleeding rates in pleural cryobiopsy.

The mean sample size of 12.33 mm in our study closely aligns with those reported across Asian centres such as India, Japan, Taiwan, and China (7.67 mm - 17.97 mm), which are significantly larger than those typically achieved with forceps biopsy (3.75 mm - 6.8 mm) [17,30]. Although no significant correlations were observed between activation time and sample size, or between sample size and diagnostic yield, factors beyond tissue dimension, such as biopsy site selection, may contribute to diagnostic outcomes. Future head-to-head comparisons with forceps biopsy in similar patient populations would provide more valuable insights into the local context.

The key strength of this study is its demonstration that pleural cryobiopsy can be performed effectively using a flexible bronchoscope, an instrument widely available in healthcare settings with pulmonology services. Hence, this technique may be particularly applicable in settings where

rigid and semi-rigid thoracoscopes are not readily available.

However, this study has several limitations. Its retrospective design introduces potential for selection and documentation bias. Several clinical and biochemical variables, such as symptom duration, pleural fluid investigation results, and the number of cryobiopsy samples taken per patient, could not be analysed due to incomplete documentation. The relatively small sample size also limits the study's statistical power, and the absence of a direct comparison group with forceps biopsy limits our ability to assess the added benefit of cryobiopsy. Hence, larger multicentre, prospective studies comparing cryobiopsy and forceps biopsies are needed to validate these findings, further optimise procedural techniques, and standardise protocols.

CONCLUSION

In summary, our study supports the use of pleuroscopic cryobiopsy via a flexible bronchoscope as a practical, feasible diagnostic method with high diagnostic yield, adequate molecular sampling, and manageable bleeding risk in patients with undiagnosed exudative pleural effusion, with performance comparable to that reported with conventional rigid and semi-rigid thoracoscopes.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the conduct of this study or the preparation of this manuscript.

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ETHICAL APPROVAL

This retrospective cohort study was approved by the Medical Research and Ethics committee (MREC), Ministry of Health Malaysia (NMRR ID-25-01832-DZR). All methods were carried out in accordance with relevant guidelines and regulations, including Declaration of Helsinki.

DATA AVAILABILITY STATEMENT

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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TABLE LEGENDS:

Table 1: Histological diagnoses of pleural cryobiopsy (n = 30)

Diagnosis		**N**	**(%)**
Malignancy	Lung adenocarcinoma	9	30.0
	Metastatic squamous cell carcinoma	1	3.3
	Small cell carcinoma	1	3.3
	Mesothelioma	1	3.3
	Metastatic breast carcinoma	1	3.3
	Metastatic neuroendocrine tumour	1	3.3
	Metastatic thyroid carcinoma	1	3.3
	Malignant spindle cell neoplasm	1	3.3
	Metastatic tumour of gynaecological origin	1	3.3
	Subtotal	17	56.7
Non-malignancy	Granulomatous inflammation	3	10.0
	Chronic granulomatous inflammation with acid-fast bacilli	1	3.3
	Necrotising granulomatous inflammation (with caseation)	3	10.0
	Necrotising granulomatous inflammation (without caseation)	2	6.7
	Acute on chronic inflammation	1	3.3
	Subtotal	10	33.3
Non-diagnostic	-	3	10.0
Total		30	100.0

FIGURE LEGENDS:

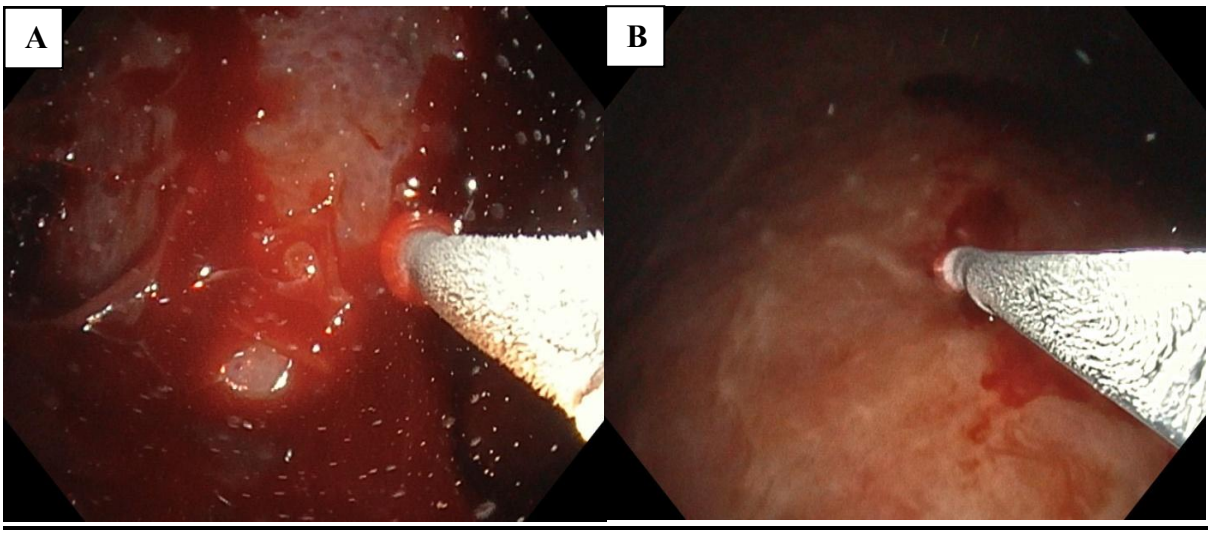


Figure 1 (A,B): Pleuroscopic cryobiopsy using a flexible bronchoscope. A 1.7 mm flexible cryoprobe was applied to the parietal pleura under direct visualisation, demonstrating frosting at the probe tip after cryoactivation, indicating adequate tissue freezing before biopsy retrieval (1A,1B).

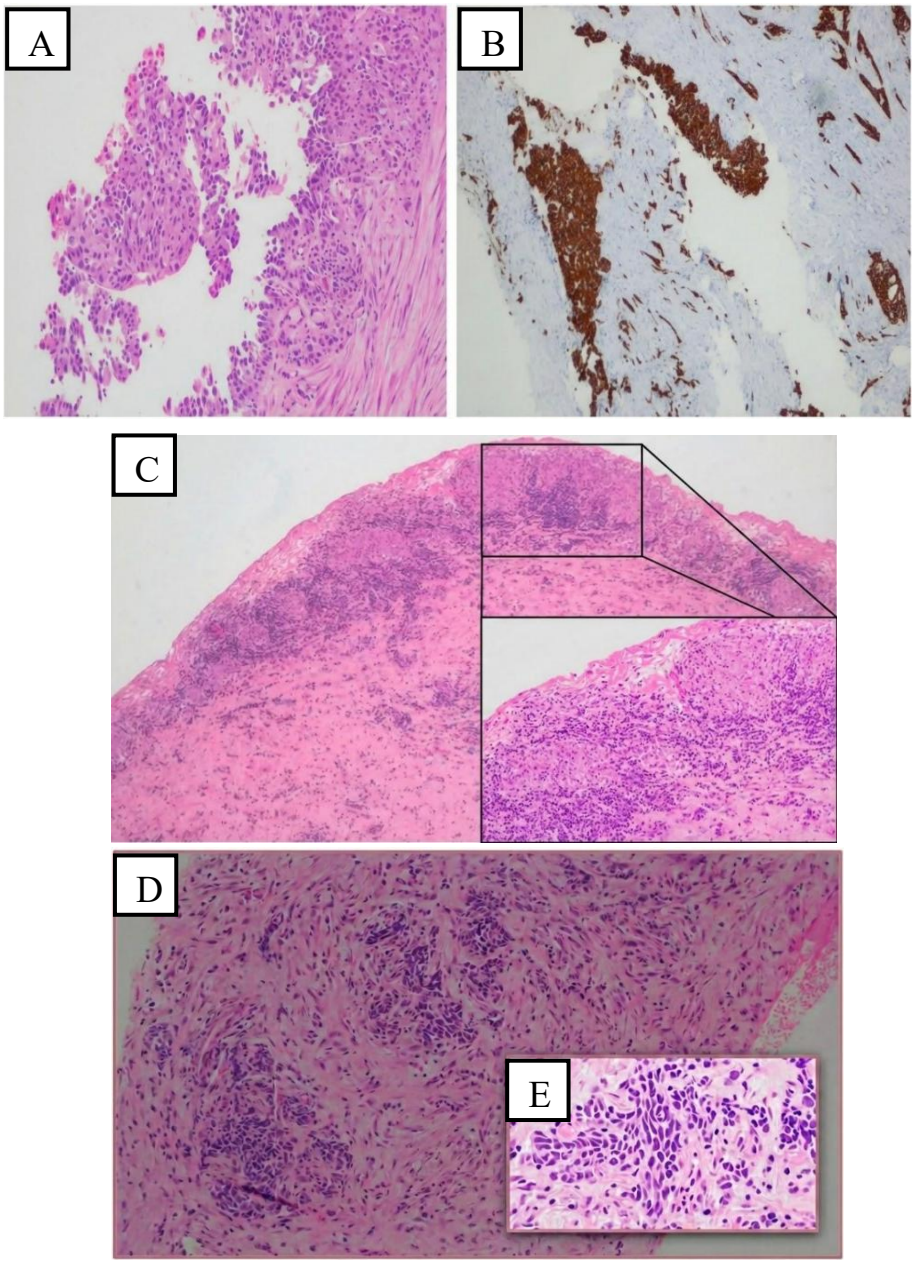


Figure 2 (A-E): Histopathological findings from pleural cryobiopsy. Lung adenocarcinoma showing glandular structures and pleomorphic tumour cells (Haemotoxylin and Eosin (H&E) ×200) (2A). Tumour cells showing positivity for Cytokeratin 7 (CK7) on immunohistochemistry (×200) (2B). Granulomatous inflammation with epithelioid histiocytes (H&E ×40) with higher magnification showing granuloma formation (H&E ×100) (2C). Mesothelioma composed of epithelioid and spindle cells (H&E ×400) (2D). Tumour cells showing cytoplasmic positivity for calretinin (×200) (2E).