Pleural Cryobiopsy and Frozen Section During Medical Thoracoscopy for Undiagnosed Pleural Effusion: A Prospective Observational Study

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Pleural effusion may remain undiagnosed after repeated pleural fluid evaluation, necessitating pleural tissue sampling. This prospective observational study evaluated the diagnostic yield of pleural cryobiopsy and the diagnostic performance of frozen section during medical thoracoscopy. Twenty consecutive adults with pleural effusion that remained undiagnosed after cytological and biochemical analysis were enrolled at a tertiary care center from February 2022 to February 2023. Medical thoracoscopy under general anesthesia was followed by targeted pleural cryobiopsy; specimens underwent frozen-section and final histopathological examination. Pleural mass lesions were the most frequent thoracoscopic finding (8/20, 40%), followed by a sago-grain appearance (5/20, 25%). Final histopathology yielded a definitive diagnosis in 18/20 (90%) patients; granulomatous pleuritis interpreted in the source study as tuberculosis and malignancy were each identified in 9/20 (45%). Adenocarcinoma was the most frequent malignant diagnosis (7/20, 35%). Frozen section yielded a diagnosis in 15/20 (75%) patients. Against final histopathology, frozen section showed a sensitivity of 83.3%, specificity of 100%, positive predictive value of 100%, negative predictive value of 40%, and overall accuracy of 85%. Pleural cryobiopsy during medical thoracoscopy achieved a high diagnostic yield in this small series, while frozen section provided rapid intra-procedural diagnostic information with high specificity. Larger comparative studies are needed to define the

incremental value of cryobiopsy and frozen section in undiagnosed pleural effusion.

Keywords: Pleural effusion; Medical thoracoscopy; Cryobiopsy; Frozen section; Diagnostic accuracy

INTRODUCTION

Pleural effusion is a frequently encountered clinical problem with varied etiologies, including tuberculosis, malignancy, infection, and systemic diseases.[1] Differentiation between transudative and exudative effusion through biochemical analysis forms the initial step in evaluation. However, despite thoracentesis and repeated pleural fluid analysis, approximately 20 to 25% of pleural effusions remain undiagnosed.[2]

Medical thoracoscopy enables direct visualization of the pleural cavity and targeted biopsy of abnormal areas, thereby significantly increasing diagnostic yield.[3,4] Several studies have reported diagnostic yields ranging from 75% to 96% in undiagnosed pleural effusion.[5–7]

Semirigid thoracoscopy has become widely accepted due to ease of use and safety profile. Nevertheless, conventional forceps biopsy may yield small and superficial samples, particularly in cases of pleural thickening or malignancy.[8,9] Inadequate sample size and crush artifact may limit diagnostic accuracy.

Cryobiopsy utilizes rapid freezing to adhere tissue to the probe tip, allowing retrieval of larger and structurally preserved specimens.[10–12] Its application in pleural disease remains relatively understudied.

Frozen section examination provides rapid histological assessment and may facilitate immediate therapeutic decisions, such as talc pleurodesis in malignant pleural effusion.[13] However, data evaluating the diagnostic performance of frozen section in pleural cryobiopsy specimens are limited.

This study aimed to assess the role of pleural cryobiopsy and evaluate the yield and diagnostic accuracy of frozen section during medical thoracoscopy in patients with undiagnosed pleural effusion.

MATERIAL AND METHODS

Study Design and Setting

This prospective observational study was conducted in the Department of Pulmonary Medicine at Yashoda Super Speciality Hospital, Malakpet, Hyderabad, India, from February 2022 to February 2023.

Participants and Eligibility

Twenty consecutive patients aged more than 18 years with pleural effusion that remained undiagnosed after initial pleural fluid cytological and biochemical evaluation were included. Inclusion criteria were undiagnosed pleural effusion after initial evaluation, recurrent effusion despite treatment, and age above 18 years. Exclusion criteria were uncorrected coagulopathy, hemodynamic instability, recent myocardial infarction, organized pleural effusion, and refusal to undergo thoracoscopy.

Initial Diagnostic Evaluation

All participants underwent clinical assessment and routine laboratory,

hematological, and radiological investigations. History included presenting symptoms, occupation, drug intake, smoking history, and relevant past medical history. Pleural fluid evaluation included microscopic examination and biochemical investigations comprising protein, albumin, glucose, and adenosine deaminase (ADA), together with cytology. Patients whose pleural effusion remained undiagnosed after initial and repeated biochemical and cytological analyses were enrolled.

Medical Thoracoscopy and Pleural Cryobiopsy

Patients were kept nil by mouth for 8 hours before the procedure. Intravenous access was secured in the upper limb opposite the side of thoracoscopy. Patients were positioned in the lateral decubitus position with the affected side upward. Electrocardiography, blood pressure, and oxygen saturation were monitored continuously.

Medical thoracoscopy was performed under general anesthesia using an Olympus EVIS EXERA III CV 190 thoracoscope. The port was usually created in the fifth or sixth intercostal space in the midaxillary line. After a 1-2 cm incision and blunt dissection to the pleural cavity, a trocar and cannula were introduced. Pleural fluid was aspirated and the pleural cavity was systematically inspected.

Pleural biopsies were obtained from visually abnormal areas using a flexible cryoprobe. Typically, 8-10 cryobiopsy samples were obtained from suspicious lesions; when no gross parietal pleural abnormality was visible, multiple biopsies were taken from different pleural areas.

Specimen Processing and Reference Standard

Pleural cryobiopsy specimens were submitted for frozen-section examination and final histopathological examination. Gene Xpert testing was performed when tuberculosis was suspected according to the supplied methodology. Final histopathology was used as the reference standard for the diagnostic-accuracy analysis of frozen section.

At completion of thoracoscopy, a chest tube with an underwater seal was inserted through the thoracoscope port site after removal of the cannula. A chest radiograph was obtained approximately 2 hours later to assess lung re-expansion and identify post-procedural complications.

Outcomes and Statistical Analysis

Diagnostic yield was defined as the proportion of patients in whom a definitive diagnosis was established. The diagnostic yield of final cryobiopsy-based histopathology and frozen section was calculated. Using final histopathology as the reference standard, sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of frozen section were calculated from the supplied 2 x 2 classification table. Descriptive data were summarized as frequencies and percentages.

Ethical Considerations

The Institutional Ethics Committee approved the study before initiation (approval number DNBT-61/2022). The study was conducted in accordance with the Declaration of Helsinki. The procedure, its purpose, and associated risks were explained to participants and their relatives, and written informed consent

was obtained before enrollment and medical thoracoscopy. Participant confidentiality was maintained.

RESULTS

Twenty patients with undiagnosed pleural effusion underwent medical thoracoscopy with pleural cryobiopsy and were included in the analysis.

Demographic and Clinical Characteristics

Participants were 21-80 years old. Seven (35%) were aged 21-40 years, six (30%) were aged 41-60 years, and seven (35%) were aged 61-80 years. Twelve (60%) were male. Cough was reported by 10 (50%) patients; fever and chest pain by 7 (35%) each; and loss of appetite by 6 (30%). Eighteen (90%) were non-smokers, and 16 (80%) had no recorded comorbidity (Table 1).

Radiological and Thoracoscopic Findings

On chest radiography/ultrasonography, right moderate pleural effusion was reported in 8 (40%) patients, right gross effusion in 3 (15%), left moderate effusion in 6 (30%), and left gross effusion in 3 (15%) (Table 2). During thoracoscopy, pleural mass lesions were observed in 8 (40%) patients, a sago-grain appearance in 5 (25%), septations in 3 (15%), hyperemia in 2 (10%), and plaques in 2 (10%) (Table 3).

Microbiological, Biochemical, and Histopathological Findings

Gene Xpert detected Mycobacterium tuberculosis in 7 (35%) patients, while the supplied table recorded 13 (65%) as not detected. Pleural fluid ADA was greater than 40 IU/L in 3 (15%) and less than 40 IU/L in 17 (85%) patients (Table 4).

Frozen section showed granulomatous inflammation in 7 (35%) patients and adenocarcinoma/atypical malignant cells in 8 (40%); 5 (25%) specimens were reported as fibrocollagenous tissue. Final histopathology showed granulomatous pleuritis in 9 (45%), adenocarcinoma in 7 (35%), small-cell carcinoma in 1 (5%), mesothelioma in 1 (5%), and fibrocollagenous tissue in 2 (10%) patients (Table 5). Thus, malignancy accounted for 9 (45%) final histopathological diagnoses.

Diagnostic Yield and Accuracy of Frozen Section

Final cryobiopsy-based histopathology provided a definitive diagnosis in 18/20 (90%) patients, whereas frozen section provided a diagnosis in 15/20 (75%) (Table 6).

The supplied 2 x 2 comparison comprised 15 true-positive, 0 false-positive, 3 false-negative, and 2 true-negative classifications. Frozen section therefore had a sensitivity of 83.3%, specificity of 100%, positive predictive value of 100%, negative predictive value of 40%, and overall accuracy of 85% against final histopathology (Table 7).

DISCUSSION

This prospective study evaluated the role of pleural cryobiopsy and the yield of frozen section during medical thoracoscopy in patients with undiagnosed pleural effusion. Medical thoracoscopy remains an important minimally invasive diagnostic procedure that allows direct visualization of the pleural cavity and targeted biopsy of abnormal pleural areas,

thereby improving diagnostic accuracy when conventional pleural fluid analysis fails to establish a diagnosis.

Demographic characteristics

In the present study, males constituted 60% of the study population and females 40%, with a male to female ratio of 1.5:1. Similar male predominance has been reported in previous studies by Chetan Basavaraj Patil et al. (71.3% males), Khurshid Ahmad Dar et al. (64% males), and Pujan Parikh et al. (68% males).[14–16] The higher proportion of male patients may reflect greater healthcare-seeking behavior among men compared with women in many clinical settings.[17–19]

The age distribution in the present study showed two peak groups: 21–40 years and 61–80 years, each accounting for 35% of patients. These findings are comparable to those reported by Rajesh Swarnakar et al., in which 35.55% of patients were in the 21–40-year age group and 31.11% in the 61–80-year age group.[20] However, Pujan Parikh et al. reported that most patients were in the 31–40-year age group, which differs slightly from our findings.[16]

Clinical presentation

Cough was the most common presenting symptom in the present study, observed in 50% of patients, followed by fever and chest pain, each observed in 35% of patients. Similar findings were reported by Chetan Basavaraj Patil et al., where cough was present in 53.4% of cases.[14] In contrast, Zay Soe et al. reported higher frequencies of fever (52.4%) and chest pain (72.6%), whereas Prabhu and Narasimhan et al. reported breathlessness and cough as the predominant symptoms.[21,22] These variations in symptom distribution may reflect differences in the underlying etiologies of pleural effusion across study populations.

Comorbid conditions

In the present study, hypertension was the most common comorbidity (10%), followed by diabetes mellitus (5%). Both hypertension and diabetes were present in 5% of patients. These findings are comparable with the results reported by Keith Sigel et al., where 18.19% of patients had hypertension and 12.16% had diabetes, and Rajesh Swarnakar et al., who reported hypertension and diabetes in 11.11% of patients each.[20,23] The coexistence of metabolic comorbidities may influence the clinical course and management of pleural disease.

Laterality of pleural effusion

In our study, right-sided pleural effusion was more common (55%) than left-sided effusion (45%). Similar findings were reported in studies by Chetan Basavaraj Patil et al. (52.7% right sided effusion) and Zay Soe et al. (61.6% right sided effusion).[14,21] The predominance of right-sided effusion has been consistently observed in several pleural disease studies.

Histopathological findings

Histopathological examination revealed tuberculous pleuritis as the most common etiology of pleural effusion in the present study (45%). This finding is consistent with the results reported by Rajesh Swarnakar et al., who observed tuberculous pleuritis in 44.44% of patients.[20]

Malignancy was the second most common cause of pleural effusion, with adenocarcinoma accounting for 35% of cases. Similar findings have been reported

by Rajesh Swarnakar et al., in which adenocarcinoma constituted 26.66% of cases.[20] Small cell carcinoma was identified in 5% of patients in our study, which is slightly lower than the 11.11% reported by Wang et al.[8] Mesothelioma was diagnosed in 5% of patients, which is comparable with the 5.55% prevalence reported by Thangakunam et al.[24] Additionally, 10% of patients in our study experienced acute inflammatory reactions, consistent with findings reported by Wang et al.[8]

The high proportion of tuberculosis observed in the present study likely reflects the continued high burden of tuberculosis in developing countries, where tuberculous pleuritis remains a major cause of exudative pleural effusion.

Diagnostic yield of thoracoscopy

The overall diagnostic yield of medical thoracoscopy in the present study was 90%. This finding is comparable with previously reported diagnostic yields. Lee et al. and Tscheikuna et al. reported diagnostic yields of 96.07% and 95.34%, respectively.[25,26] Slightly lower diagnostic yields were reported in studies by Law et al. (78.57%), Mehta et al. (76%), and Mootha et al. (74.28%).[27–29]

The high diagnostic yield observed in our study may be attributed to the use of pleural cryobiopsy, which allows retrieval of larger tissue samples with preserved architecture and minimal crush artifact. This improves histopathological interpretation and diagnostic accuracy.

Strengths, limitations, and future prospects of the study

A major strength of the present study is that it highlights the practical value of combining pleural cryobiopsy with frozen section analysis during medical thoracoscopy. Cryobiopsy enabled the acquisition of larger and better-preserved tissue samples, thereby improving histopathological interpretation, while frozen section provided rapid intraoperative assessment and helped guide the appropriate allocation of specimens for pathological and microbiological evaluation. In addition, early frozen-section interpretation may reduce the number of biopsy samples required and shorten procedural time, which may be especially useful in elderly patients and those at increased risk of bleeding.

However, the study has certain limitations, including a small sample size, a single-center design, and a lack of direct comparison with conventional forceps biopsy, which may limit the generalizability of the findings.

Future multicenter studies with larger sample sizes are needed to validate these observations. Comparative studies between cryobiopsy and conventional pleural biopsy techniques may further clarify the optimal sampling method, while incorporating molecular diagnostic methods into thoracoscopic biopsy evaluation may enhance diagnostic accuracy and support more individualized management of pleural malignancies.

CONCLUSION

In this 20-patient prospective observational study, pleural cryobiopsy obtained during medical thoracoscopy yielded a definitive final histopathological diagnosis in 18 (90%) patients. Frozen section yielded an intra-procedural diagnosis in 15 (75%) and showed 83.3%

sensitivity, 100% specificity, and 85% overall accuracy against final histopathology. Granulomatous pleuritis and malignancy were each identified in 45% of participants. The findings support the potential diagnostic utility of combining pleural cryobiopsy with frozen section in undiagnosed pleural effusion, but the small single-center sample, incomplete technical reporting, and absence of a forceps-biopsy comparator require cautious interpretation and larger comparative validation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Ethics Committee (approval number DNBT-61/2022). Written informed consent was obtained from all participants before enrollment and medical thoracoscopy.

AVAILABILITY OF DATA AND MATERIAL

The data supporting the findings of this study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

No funding was received for this study.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the study. Data collection, analysis, and interpretation were performed by the authors. All authors participated in drafting or critically revising the manuscript for important intellectual content, and all authors read and approved the final manuscript.

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TABLES

Variable	Category	n (%)
Age group (years)	21-40	7 (35)
	41-60	6 (30)
	61-80	7 (35)
Sex	Male	12 (60)
	Female	8 (40)
Symptoms	Cough	10 (50)
	Fever	7 (35)
	Chest pain	7 (35)
	Loss of appetite	6 (30)
Smoking status	Smoker	2 (10)
	Non-smoker	18 (90)
Comorbidities	None	16 (80)
	Hypertension	2 (10)
	Diabetes mellitus	1 (5)
	Hypertension + diabetes mellitus	1 (5)

Table 1. Demographic and clinical characteristics of study participants (n=20)

Note: Symptoms were not mutually exclusive.

Imaging modality	Finding	n (%)
Chest radiography/ultrasonography	Right moderate pleural effusion	8 (40)
	Right gross pleural effusion	3 (15)
	Left moderate pleural effusion	6 (30)
	Left gross pleural effusion	3 (15)
Computed tomography chest	Right moderate pleural effusion with mass	8 (40)
	Right gross pleural effusion with mass	3 (15)
	Left moderate pleural effusion	6 (30)
	Left gross pleural effusion	3 (15)
	Pleural mass	3 (15)

Table 2. Radiological findings of pleural effusion (n=20)

Finding	n (%)
Mass lesion	8 (40)
Sago-grain appearance	5 (25)
Septations	3 (15)
Hyperemia	2 (10)
Plaques	2 (10)

Table 3. Thoracoscopic findings (n=20)

Investigation	Result	n (%)
Gene Xpert	Mycobacterium tuberculosis detected	7 (35)
	Mycobacterium tuberculosis not detected	13 (65)
Adenosine deaminase	<40 IU/L	17 (85)
	>40 IU/L	3 (15)

Table 4. Microbiological and biochemical findings (n=20)

Diagnosis	Frozen section n (%)	Final histopathology n (%)
Granulomatous inflammation/pleuritis	7 (35)	9 (45)
Adenocarcinoma	8 (40)	7 (35)
Small-cell carcinoma	-	1 (5)
Mesothelioma	-	1 (5)
Fibrocollagenous tissue	5 (25)	2 (10)

Table 5. Frozen-section and final histopathological findings (n=20)

Method	Diagnosed n (%)	Undiagnosed n (%)
Cryobiopsy-based final histopathology	18 (90)	2 (10)
Frozen section	15 (75)	5 (25)

Table 6. Diagnostic yield of pleural cryobiopsy and frozen section (n=20)

Frozen-section result	Histopathology positive	Histopathology negative	Total
Positive	15	0	15
Negative	3	2	5
Total	18	2	20

Frozen-section result	Histopathology positive	Histopathology negative	Total
Diagnostic index	Value		
Sensitivity	83.3%		
Specificity	100%		
Positive predictive value	100%		
Negative predictive value	40%		
Overall accuracy	85%		

Table 7. Diagnostic performance of frozen section compared with final histopathology (n=20)