Role Of Thoracoscopic Cryobiopsy In Comparison To Rigid Forceps Biopsy In Undiagnosed Exudative Pleural Effusion.

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Abstract

Background: Rigid thoracoscopy is the gold standard tool for diagnosing exudative pleural effusion, but sometimes it is difficult to obtain sufficient biopsies using the conventional rigid forceps biopsy (RFB). This study evaluated the efficacy, safety, and diagnostic value of a new technique using rigid cryoprobe to obtain pleural biopsies during rigid thoracoscopy.

Methods. In a single-center, interventional, prospective study, patients with exudative undiagnosed pleural effusion were evaluated with a rigid forceps biopsy pleuroscopy followed by a cryoprobe at the same setting between April 2022 to March 2023.All biopsies were processed for histopathology examination by an independent pathologist; any complications were recorded.

Results: A total of 22 patients (median age 63.95years) were included in the study, most of were men (54.5%). Both RFB and CPB established definitive diagnoses in 20/22 (90.9%) of cases. The predominant histopathological examination in patients was metastatic malignancy (45.45%): followed by mesothelioma (31.8%). The sample size (largest length in (mm) Mean ±SD) obtained by CPB (8.9±1.2mm) was significantly larger than RFB (5.1±1.02) ($P < 0.0001$). Crushed cells (artifacts) prevalence in biopsy there was a statistically significant decrease in CPB in comparison with RFB($P=0.006$). There was a significant decrease when comparing between two studied biopsies regarding bleeding at biopsy bed in favor to CPB ($P=0.031$). There were no significant complications or procedure-related deaths.

Conclusion: Cryobiopsies obtained during medical thoracoscopy are technically feasible and safe with high diagnostic value. Biopsies of cryoprobe were larger than that of rigid forceps, and with better preserved cellular architecture. These results will encourage the use of cryotechnique for diagnosis of undiagnosed exudative pleural effusion

Keywords: Thoracoscopy, CPB, RFB, Undiagnosed Exudative Pleural Effusion

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Abbreviations

RFB: Rigid forceps biopsy; CPB: Cryoprobe biopsy; LDH: Lactate dehydrogenase; INR: International normalized ratio IHC: Immunohistochemistry

Introduction

Recurrent persistent exudative pleural effusion is common in clinical practice. Thoracentesis or blind pleural biopsy may not provide definitive diagnosis.[1] Medical thoracoscopy is a minimally invasive technique used to examine the pleural space and obtain targeted biopsies of the parietal pleura.[2] Biopsy via cryoprobe, first used in 1968, has mainly been employed in the management of obstructive endobronchial tumors. Recently, cryotherapy in bronchoscopy has been used for several purposes including management of endobronchial

tuberculosis, endobronchial tumors, for diagnosis of interstitial lung disease, and peripheral lung lesion.[3] The tissue was well preserved, and the risk of hemorrhage seemed to be reduced.[4]

The interest in using thoracoscopy for diagnosis and therapy of pleural diseases gives the opportunity to combine cryo-techniques with thoracoscopy to take biopsies.[5]

Thoracoscopic forceps biopsy has some shortages e.g. small size and more crushed cells, so there is a need for new technique (cryobiopsy) for better diagnostic yield of exudative undiagnosed pleural effusion and minimize drawbacks of conventional forceps biopsy. This new technique was the 1st time to be applied at chest department at Zagazig university hospitals.

Patients and Methods

This prospective clinical trial study was conducted in the Department of Pulmonary Medicine at Zagazig University Hospitals during a period of 1 year. It included 22 consecutive patients with undiagnosed exudative pleural effusion, who underwent diagnostic medical rigid thoracoscopy. Written informed consent has been taken from all patients. The study was approved by the local institute ethics committee. **(ZU-IRB #9474-11-4-2022)**

All patients were subjected to the following: full medical history including occupational exposure to hazardous agents and history of any chronic disease; general and local chest examination; chest radiography and computed tomography chest with contrast; full laboratory investigations including bleeding profile, complete blood count, liver and kidney function tests, and other routine blood tests. Pleural fluid sampling was done for biochemical (glucose, protein, lactate dehydrogenase, and adenosine deaminase), bacteriological (culture and sensitivity and Ziehl–Neelsen staining), and cytological examination.

Chest ultrasonography was done by a **(Sonoscape ⁄ SSI- 4000, China color doppler ultrasonography machine)** for all patients to evaluate amount &nature of pleural fluid whether clear or turbid and pleural examination e.g., thickening, septations nodules and masses in real time assessment before thoracoscopy.

Both methods of biopsy taking were done in the same setting and same patient.

Fiberoptic bronchoscopy was performed in one case where central parenchymal lung mass was detected in enhanced computed tomography chest to investigate possible endobronchial lesions.

Inclusion Criteria:

Patients with undiagnosed exudative pleural effusion were considered for the study. A working definition of exudative pleural effusion was made based on:

1) Exudative pleural effusion according to Light's criteria: Pleural fluid is considered as an exudate if one or more of the following criteria are met.[6]

A. Pleural fluid protein/serum protein ≥0.5.
B. Pleural fluid LDH/serum LDH ≥ 0.6
C. Pleural fluid LDH is more than two-thirds of the upper limit of serum LDH.

2) Undiagnosed pleural effusion criteria.[7]

A. No evidence of lobar consolidation, mass lesion, or cavitary lesion in chest radiograph or CT chest
B. No evidence of pulmonary thromboembolism.
C. Pleural fluid for bacterial culture and TB (acid-fast bacilli smear are negative
D. Pleural fluid cytology and cell block for malignant cells negative on two separate pleural fluid samples.

Exclusion Criteria.[8]

A. A tendency for uncontrolled bleeding. At least, the prothrombin concentration should be greater than 60%, and the platelet count should be greater than 60,000/mm3.
B. Cannot tolerate lateral position.
C. Unstable cardiovascular status or severe heart failure, unstable hemodynamic status.
D. Respiratory insufficiency needs ventilatory support.
E. Potential pleural space size is less than 2 cm due to extensive pleural adhesions by using chest ultrasongraphy.

Sample size.

Assuming the frequency of crushed cell (-ve) was 70.8% vs 25% in cryoprobe vs rigid forceps. At 80% power and 95 % CI, the estimated sample was 22 cases in each method. Open epi

Technique

Medical thoracoscopy in the current study was performed under aseptic conditions in the endoscopy unit of the Chest Department, Zagazig University Hospital. Pulse, blood pressure, respiratory rate, ECG, and oxygen saturation using continuous pulse oximetry were monitored during the whole procedure. All patients received supplemental oxygen through the nasal cannula. Rigid thoracoscope **(TEKNO, Tuttlingen, Germany)** was the scope used in the current study. Instruments for rigid thoracoscopy included the following: The length of the thoracoscope was 200cm, outer diameter 7.5mm, inner one 3.5mm, and its working channel size was 4mm. A cryo-machine **(ERBE, Tübingen, Germany)** consisting of console, cryogen, and rigid cryoprobe (Erbokryo CA; ERBE) with a 2.8- mm CO2 cylinder was used for the procedure. The cooling agent was carbon dioxide to achieve a temperature of −79°C at the tip of the probe.

Patients were under conscious sedation, which was achieved by intravenous midazolam with a loading dose of 0.01–0.05mg/kg with or without fentanyl with an infusion rate of 0.7–10μg/ kg/h. During the procedure, the patients were kept in the lateral decubitus position with the affected side upwards. The portal of the entry was usually at the mid-axillary line, between fourth and sixth intercostal spaces. Local anesthesia (2% lignocaine) was administered to the skin, subcutaneous tissue, muscle, and parietal pleura. Skin incision about 1–2 cm was made with a scalpel, which was followed by blunt dissection of the intercostal muscles until the pleural space was reached. A rigid trocar 8mm in size and its length 100mm was inserted through the chest wall. Pleural fluid was then aspirated while air was allowed to enter the pleural space. A thorough examination of the pleural cavity was then undertaken by inserting the scope through the trocar. All patients underwent thoracoscopy whose biopsies were taken using the conventional forceps biopsy technique and the cryo-biopsy technique.

Forceps pleural biopsy samples were obtained from the parietal pleura with biopsy forceps, particularly where it appeared abnormal. They were always taken against a rib to minimize the risk of vessel or nerve injury. Those biopsies were taken using single-port single-instrument rigid technique through the scope working channel.

Tissue sampling by cryoprobe

The tip of the rigid cryoprobe (2.4 mm, Erbokryo CA, Erbe, Tübingen, Germany), cooled to −79°C with CO2, was attached to the selected part of the parietal pleura for 30 s by a foot-switch activation mechanism. The frozen tissue was extracted by gently pulling. The probe with the attached biopsy sample was removed through the thoracoscope. Each biopsy sample was released from the probe by thawing in saline and was then fixed in formalin. At least three biopsies were taken from each patient.

The biopsy sites were assessed for bleeding each time before further biopsies were performed. the degree of bleeding at the biopsy site was assessed as follows: (nil) (slight), self-limited; (mild bleeding) requiring vasoactive drug (adrenaline) injection; and (moderate to severe bleeding) requiring electrocautery or argon plasma coagulation intervention.

Chest tube (32F) was introduced through the entry site and connected to an underwater seal at the end of the procedure. Post-procedure complications were recorded with emphasis on local site “wound” infection, pneumothorax/incomplete lung expansion, fever, pain, and bleeding. A chest radiograph was subsequently taken to monitor the re-expansion of the lung and check the position of chest tube, which was removed as soon as full lung expansion was achieved and the amount of pleural fluid drained less than 100 ml/day. The days till chest tube removal were calculated and recorded. Patients could be discharged on the same day when removing the chest tube.

Pathology and Diagnosis.

The biopsy tissue samples stained by hematoxylin and eosin stain, prepared on slide for histopathological evaluation. Biopsy samples were sent in two different containers to the same pathologist who was blinded to the biopsy technique in each container.

Histopathological grading of fat cell

Measurement of the quantity of fat cells is an indicator of depth of the taken pleural biopsy.[4]

We planned if there was no presence of fat cells in the obtained pleural biopsies, this was marked as negative "superficial sample", and classified into grades according to prevalence of fat cells ,if fat cells were present in less than 5% of the biopsy, if 5–25% of the biopsy, and if more than 25% .This was applied to compare biopsies obtained by both techniques (RFB and CPB) regarding biopsy depth.

Histopathological grading of crushed cells

Measurement of the quantity of crushed cells in the pleural biopsies was an indicator of tissue integrity or viability. If there was no presence of crushed cells in pleural biopsied, this was marked as negative "A well-integrated, viable biopsy"; and classified into grades according to prevalence of crushed cells. If crushed cells were found in less than 5% of the biopsy, if 5–25% of the biopsy; and if more than 25%. This was applied to compare the integrity and viability of pleural biopsies obtained by both techniques (RFB and CPB).

Statistical analysis

Data were statistically described in terms of mean ±SD, median, range, or frequencies and percentages when appropriate. Comparison of numerical variables between study groups was done using Student's t-test for independent samples in comparing two groups of normally distributed data and Mann–Whitney U-test for independent samples for comparing non-normal data. For categorical data, comparison was done using $\chi 2$-test. Exact test was used instead when the expected frequency is less than 5. P values less than 0.05 was considered statistically significant. All statistical calculations were done using the computer program IBM SPSS.

Results

Table (1): Demographic data of studied patients (n=22):

Characteristic	**Study group (N=22)**		
Age (years) Mean ±SD. Range	63.95±16.19 (30-85)		
Category		**No.**	**%**
Gender	Male	12	54.5
	Female	10	45.5
Special habits	Active smoker	9	40.9
	Passive smoker	1	4.5
	Ex-smoker	2	9
	Nonsmoker	10	45.5

Table (2): Thoracoscopic gross findings within the studied patients (n=22).

Study group (n=22)				
Characteristic		**No.**	**%**	**Total %**
Nodules	No nodules	4	18.2	81.81
	Multiple	9	40.9	
	Diffuse	6	27.3	
	Solitary	3	13.6	
Masses	No masses	15	68.2	31.81
	Multiple	5	22.7	
	Solitary	2	9.1	

Adhesions	Present	10	45.45	45.45
	No adhesions	*12*	54.54	
Plaques	Multiple	2	9	22.72
	Solitary	3	13.63	
	No plaques	*17*	77.27	
Ulcers	Present	1	4.5	4.5
	Absent	21	95.5	
Hyperemia	Present	10	45.45	45.45
	Absent	12	54.54	
Pleural thickening	Present	17	77.27	77.27
	Absent	5	22.7	

Table (3): Comparison between two studied groups regarding diagnostic yield and histopathological examination (n=22)

Variable		**Forceps Group (N=22)**		**Cryo Group (N=22)**		**Test**	**P value**
		N	**%**	**N**	**%**		
Diagnostic yield		20	90.9	20	90.9	0.000	1.000
Histopathology	Metastatic mucoid adenocarcinoma	6	27.3	6	27.3	0.000	1.000
	Metastatic small cell carcinoma	3	13.6	3	13.6	0.000	1.000
	Metastatic undifferentiated carcinoma	1	4.5	1	4.5	0.000	1.000
	Mesothelioma (epithelial type)	5	22.7	5	22.7	0.000	1.000
	Mesothelioma (mixed type)	2	9	2	9	0.000	1.000
	Round cell tumor	1	4.5	1	4.5	0.000	1.000

	Poorly differentiated adenocarcinoma	2	9	2	9	0.000	1.000
	Chronic nonspecific Pleurisy	2	9	2	9	0.000	1.000

Table (4): Comparison between two studied groups regarding biopsy size, integrity and quality.

Variable		**Forceps Group (N=22)**		**Cryo Group (N=22)**		**Test**	**P value**
Biopsy largest length in (mm) Mean ±SD		**5.1±1.02**		**8.9±1.2**		**-11.390**	**0.000****
Interpretability (difficulty in reading of slides)	**Slight**	16	72.7	17	85	0.864	0.649
	Mild	5	22.7	3	13.6		
	High	1	4.5	2	9.1		
Crushed cells (artifacts) % of biopsy	**less than 5%**	6	27.3	17	77.3	10.1	**0.006****
	5–25%	11	50	5	22.7		
	more than 25%	5	22.7	0	0		
Fat cells % of biopsy (depth)	**less than 5%**	14	63.6	11	50	2.618	0.454
	5–25%	7	31.8	7	31.8		
	more than 25%	1	4.5	4	18.2		

Table (5): Comparison between two studied groups according to bleeding at biopsy bed.

Variable		**Forceps Group (N=22)**		**Cryo Group (N=22)**		**Test**	**P value**
Bleeding at biopsy bed		**N**	**%**	**N**	**%**	4.659	**0.031****
	Slight	**14**	63.6	20	90.9		
	Mild	**8**	36.4	2	9.1		

	Severe	**0**	0	0	0		

Table (6): Frequency of complications in the studied patients (n=22).

Characteristic	**Study group (N=22)**	
No.		**%**
Pain	15	68.1
Fever	5	22.7
Prolonged air leak	1	4.5
Surgical emphysema	2	9
Trapped lung	1	4.5
Wound infection	3	13.6
Dislodged drain	1	4.5

Discussion

In many cases of exudative pleural effusion, the results were not conclusive even after (repeated) thoracocentesis; that mostly need confirmatory pleural tissue biopsy. The pleural biopsy obtaining techniques are variable starting from blind pleural biopsy to the newer techniques such as image guided pleural biopsy and thoracoscopy guided pleural biopsy.[9] The conventional forceps rigid thoracoscope has good sensitivity (91%) and specificity (100%) in the diagnosis of exudative pleural effusions.[10]

The growing need for larger histological samples for the molecular characterization of tumors has promoted the development of new pleural biopsy techniques such as cryobiopsy.[2] Cryobiopsy during medical thoracoscopy involves freezing a piece of pleural tissue and removing it en bloc instead of taking a "bite" of tissue using forceps. This enables the gaining of larger and better-preserved pieces of pleural tissue.[4]

In our study **(Table 3)** there was a non-significant difference in the diagnostic yield between forceps biopsy and cryobiopsy. A definitive diagnosis was reached in *(*20/22*)*cases, with a diagnostic yield of 90.9%.

Our results were in line with **Elhadidy et al., *(*2020*)***The diagnostic yield of cryobiopsy in their study was 100% and was not significantly different from conventional biopsy (P=0.772 and our study agrees with **Tousheed et al., (2018)** were confident diagnosis was reached in 99% (86/87 patients) in cryoprobe samples and 96% (50/52 patients) in conventional biopsy.

Also, **Refaat and Tealeb (2020)** and **Baess et al., (2021)** demonstrated in their study the diagnostic yield reached 100%, regarding both rigid forceps biopsies and cryoprobe biopsies.

In an Egyptian study by **Ahmed et al., (2019)** they reported that there was no difference in diagnostic yield (76.7%) in both techniques and CPB showed better performance in thin highly vascular pleura where obtaining biopsies can be performed with minimal risk of bleeding.

Regarding **(Table 3)** the predominated histopathological examination in the studied patients was metastatic malignant pleural effusion (45.45%): Metastatic mucoid adenocarcinoma (27.3%), metastatic small cell carcinoma (13.6%) and metastatic undifferentiated carcinoma (4.5%). The second most common histopathological finding was mesothelioma (31.8%): Mesothelioma (epithelial type) (22.7%), Mesothelioma (mixed type) (9%) followed by poorly differentiated adenocarcinoma (9%), chronic non-specific pleurisy (9%) and round cell tumor (4.5%).

The explanation of previous results is the most common cause of malignancy which cause exudative pleural effusion was metastasis to the pleura from primaries most commonly lung cancer as it is the most common malignancy to invade the pleura and produce malignant and para-malignant effusions as proved by

Thompson et al.,(2023) Also, this result agrees with **Mootha et al., (2011)** and **Prabhu and Narasimhan (2012) Abd El Rehim et al., (2016)** who reported that the most common pleural malignancy among their studied patients was metastatic adenocarcinoma.

Refaat and Tealeb (2020) reported that nonspecific inflammation was found in (8%) of cases. **Venekamp et al., (2005) & Davies (2010), Patil et al., (2016) and Ji et al., (2020)** mentioned that the histological finding of 'nonspecific pleuritis' is common in thoracoscopic forceps biopsies, and their false-negative rate for the detection of pleural malignancy has been determined to be around 5%, with the most frequent false-negative diagnosis being mesothelioma. **Chen et al., (2018)** study showed that CPB reduces the histological finding of "nonspecific pleuritic," which presents a management dilemma for clinicians.

As regards the biopsy size **(Table 4)** in the current study, The sizes of specimens obtained by CPB were significantly larger than those obtained by forceps biopsy (8.9 mm ± 1.2mm) versus RFB (5.1mm ± 1.02 mm) ($P < 0.0001$) which might be explained by the longer time for freezing effect (around 30 s). Compared with **Thomas et al., (2015) and Rozman et al., (2016)** studies which allowed more tissue freezing and larger area for biopsy.

Chen et al., (2018) reported in their study that CPB was significantly larger ($P < 0.0001$) when compared with RFB (9.1 ± 4.5mm versus 4.0 ± 2.1 mm). In **Muhammad et al., 2019** study, the mean surface area of rigid forceps biopsies was 8.193 mm2, whereas the mean surface area of cryoprobe biopsies was 3.377mm2. This was less than the mean surface area of rigid forceps and cryoprobe biopsies obtained by **Wurps et al., (2016)** which was 22.6 and 14.4 mm2, respectively.

Baess et al., (2021) demonstrated that when comparing the two main groups, there was statistically significant difference regarding biopsy surface area ($p = 0.007$), CPB were larger (mean = 4.1 ± 2.4 cm2), and RFB (mean = 2.7 ± 1 cm2), but in regard of biopsy largest diameter there was no difference.

Elhadidy et al., (2020) found that there was a significant difference between the cryobiopsy samples and that of the forceps biopsy (mean ± SD length of the biopsy in mm), 14±4.29 and 5.04±0.53, respectively ($P \leq 0.001$).

However, **Ahmed et al., (2019)** reported in their study that there was no difference between both techniques regarding biopsy size in cases of thickened pleura.

This difference may be attributed to the way of measurement of biopsy size and the number of biopsies was not standardized in all studies.

Regarding comparison between two studied groups according to **biopsy integrity and quality. (Table 4)** there was a statistically significant difference when comparing between two studied groups regarding crushed cells. Crushed cells were less in biopsies taken by cryo probe There was a non-significant difference when comparing between two studied groups regarding histopathological interpretation and depth of biopsy.

In the study mentioned before, **Rozman et al., (2016)** agreed with the current study findings regarding the quality of the biopsy and stated that the majority of the cryoprobe samples were assessed as "easily interpretable" or "interpretable with some difficulty" which reflected minimal amount of the artifacts observed by the pathologists. **Rozman et al., (2016)** found that cryobiopsy samples were bigger and significantly easier for interpretation than flexible forceps biopsy samples. Also, **Hetzel et al., (2008)** confirmed in their study that CPB has proven to have excellent tissue quality for immunohistochemistry (IHC).

Cryobiopsy is easily interpretable in the **Elhadidy et al., (2020)** study as focal and diffuse hemorrhage and crush artifact/atelectasis is less in biopsy, while rigid forceps biopsies are interpretable with some difficulty in 60% of forceps cases. So cryoprobe preserves tissue integrity and important molecular markers, and this agreed with **Hetzel et al., (2008)** who concluded that while tissue was frozen without any mechanical force and detached while frozen, no tearing artifacts due to the withdrawal process were seen. So, the tissue construction and lining were conserved and agree with **Rozman et al. (2016),** who also concluded that the cryobiopsy samples showed a low level of tissue damage.

Churg et al.et al., (2012) suggested that the presence of tumor cell invasion into the adipose tissue is the most reliable feature for diagnosing malignant pleural mesothelioma accurately, as some benign conditions

(i.e., reactive mesothelial hyperplasia and fibrous pleuritis) often mimic malignant pleural mesothelioma in histopathological findings.

In **Dhooria et al., (2019)** study the depth of tissue was defined as the presence of fat or muscle tissue, a factor that is particularly relevant for determining tumor invasion, was greater in cryoprobe biopsies 65% of all samples versus 40% of FBF, depending on the series.

Muhammad et al., (2019) illustrated that, deep biopsies containing fatty tissue were significantly obtained in 70% of cryoprobe biopsies and in 40% of rigid forceps biopsies. Obtaining deeper tissue may be of much importance in establishing a histological diagnosis of mesothelioma where the pleura is extremely tough and thick.

In contrast, **Wurps et al. (2015)** showed that a deep biopsy containing fatty tissue was obtained in 63% of the rigid forceps biopsies, 39.5% of flexible forceps biopsies and in 49.5% of the samples harvested using cryoprobe.

The difference between studies regarding tissue depth and percentage of fat cells depends on freezing material cryogen (liquid nitrogen or liquid carbon dioxide). Cryo technique using liquid nitrogen allows better freezing area and deeper tissue penetration. In **Baess et al., (2021)** study, it has been found that when comparing the cryoprobe biopsy with the rigid forceps' biopsy regarding the presence of fat cells, there was no difference between both groups regarding the quantity of fat cells and hence the depth of the biopsies taken.

Regarding **(Table 5)** there was a significant difference when comparing between two studied patients regarding bleeding at biopsy bed being less cryo forceps group.

In our study bleeding was mainly slightly self-limited in 90.9% of cryobiopsy cases and in 63.6% of rigid forceps biopsy cases that is not necessitating intervention in both cryobiopsy and forceps biopsy. Mild bleeding was seen in 9.1% in cryobiopsy and 36.4% of forceps biopsies and no patients develop severe bleeding in both groups.

Thomas et al., (2015) reported that CPB was safe in their cohort study. There were no significant complications reported in any patient following cryobiopsy. No moderate or severe bleeding occurred. Mild, self-limiting, and localized bleeding at biopsy site, of no clinical importance, was observed in both groups (4/22 patients after FFB and5/22 patients after CPB).

Also, **Rial et al., (2020)** found that a total of 67% (95% CI 62-72) of patients undergoing CPB had mild bleeding compared with 85% (95% CI 79-90) of those in whom the sample was obtained with RFB, with statistically significant differences ($p < 0.001$).

Chen et al., (2018) observed in their study that the incidence of mild bleeding was similar between the CPB group (8/92) and FB group (6/92). No moderate to severe bleeding. They did not observe any other complications.

However, **Rozman et al., (2016) and Shafiq et al., (2019)** suggested taking all due precautions when pulling the probe during the cryobiopsy process. The force should be minimal to avoid deep tearing and severe hemorrhage.

Conclusions

CPB during medical thoracoscopy is a safe and feasible technique and it is comparable to conventional pleural biopsy using RFB in cases of undiagnosed exudative pleural effusion. CPB was also easier to interpret by the pathologist because of the larger tissue samples with better preserved cellular architecture.

Limitations

Despite our careful study design, we acknowledge a number of limitations in our study.

1. Only 22 patients were enrolled in our study in a single center -Zagazig Faculty of Medicine- thus underpowered to detect any small difference that may exist between the diagnostic yield of CPB and RFB.
2. The CPB was taken after conventional FFB. Bleeding may not be fairly evaluated when samples from the same lesion were taken by CPB and FFB.
3. There were multiple operators performing the procedure. Although all were skilled thoracoscopists, they had variable experience of cryobiopsy techniques.

4. Formal volumetric analysis of the sample was not done, and the volume was calculated based on the length, breadth, and height of the sample.

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Authors' contributions
Mohamed Hassan Farouk conceived the idea and performed thoracoscopy to the studied cases. **Reda M. El Gamry** collected data and assisted in performing the procedure. **Amany Fawzy Morsy** substantially contributed to interpretation and analysis of data. **Mohamed Fawzy M. Ismail** substantially contributed to writing the manuscript. The authors revised and approved the final manuscript.

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