Universitat Autònoma de Barcelona

ENDOBRONCHIAL ULTRASOUND GUIDED TRANSBRONCHIAL CRYOBIOPSY IN PERIPHERAL LUNG LESIONS

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DOCTORAL THESIS

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PhD Program in Medicine

Department of Medicine, Faculty of Medicine

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This thesis has been conducted at:

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Also, an abstract including data from this thesis was accepted to be presented at European respiratory congress (ERS) during September 2018 in Paris, France.

Abbreviations

AFB: Acid fast bacilli

BAC: Adenocarcinoma presenting lepidic growth

BAL: Bronchoalveolar lavage

BB: Bronchial brushing

BTPNA: Bronchoscopic trans-parenchymal nodule access

BW: Bronchial wash

COP: Cryptogenic organizing pneumonia

CP-EBUS: Convex probe Endobronchial Ultrasonography

CT: Computed tomography

CT-TTNA: CT guided transthoracic needle aspiration

DIP: Disquamative interstitial pneumonia

3D: Three dimensions

EBUS: Endobronchial ultrasonography

EBUS-TBNA: Endobronchial ultrasound guided trans-bronchial needle aspiration

EUS: Endoscopic ultrasonography

ENB: Electromagnetic navigation bronchoscopy

ETTNA: Electromagnetic transthoracic needle aspiration

FDG: Flurodeoxyglucose

FNA: Fine needle aspiration

GS: Guide sheath

HRCT: High resolution computed tomography

IPF: Idiopathic pulmonary fibrosis

NSIP: Non-specific interstitial pneumonia

PET: Positron emission tomography

PAP: Pulmonary alveolar proteinosis

RP-EBUS: Radial probe Endobronchial ultrasonography

RMSCT: Real time multi slice computed tomography

SCT: Spray cryotherapy

SCB: Suction catheter biopsy

SPN: Solitary pulmonary nodule

SUV: Standardized uptake value

TBB: Transbronchial biopsy

TBCB: Transbronchial cryobiopsy

TBNA: Transbronchial needle aspiration

TLC-ENB: Total lung capacity- electromagnetic bronchoscopy

TV-ENB: Tidal volume - electromagnetic navigation bronchoscopy

UIP: Usual interstitial pneumonia

UTB: Ultrathin bronchoscopy

VB: Virtual bronchoscopy

VBN: Virtual bronchoscopy navigation

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ABSTRACT

1. Abstract

Introducción: La Ultrasonografía Endobronquial con sonda radial (USEB-R) es una técnica moderna para el diagnóstico de lesiones pulmonares periféricas. La adición de la Criobiopsia Transbronquial (CBTB) incrementa en rendimiento diagnóstico de la USEB-R.

Objetivos: el objetivo del estudio es evaluar la eficacia y seguridad de la CBTB guiada por USEB-R en el diagnóstico de lesiones pulmonares periféricas. Objetivos secundarios son detectar los factores que pueden afectar a la eficacia de la biopsia guiada por USEB-R y evaluar la eficacia del procedimiento cuando la CBTB está contraindicada.

Métodos: Se incluyeron 60 pacientes con lesiones pulmonares periféricas y se dividieron en dos grupos. El grupo I incluyó 45 pacientes que fueron elegibles para CBTB y se sometieron a biopsia transbronquial con fórceps (BTB) y CBTB guiada por USEB-R. El grupo II incluyó 15 pacientes que no fueron elegibles para CBTB y sólo se sometieron a BTB con fórceps y/o procedimientos citológicos guiados por USEB-R. Se analizaron los resultados clínicos, incluido el soporte digital de las variables cualitativas y cuantitativas. Además, se registraron las complicaciones.

Resultados: En el grupo I, la BTB con fórceps tuvo una sensibilidad, especificidad, valor predictivo positivo (VPP), valor predictivo negativo (VPN) y precisión del 67.5%, 100%, q00%, 18.8% y 69.8% respectivamente. Mientras que la CBTB tuvo una sensibilidad, especificidad, valor predictivo positivo (VPP), valor predictivo negativo (VPN) y precisión del 75%, 100%, 100%, 32.1% y 76.7% respectivamente. La CBTB obtuvo un mayor rendimiento diagnóstico y mejor calidad de las muestras. La sensibilidad el grupo II fué del 80% y los resultados globales de ambos grupos demostraron una sensibilidad, especificidad, valor predictivo positivo (VPP), valor predictivo negativo (VPN) y precisión del 85.2%, 100%, 100%, 42.8% y 86.7% respectivamente. En cuanto a las complicaciones, 12 pacientes (20%) presentaron hemorragia, pero de ellos, 11 (18.3%) presentaron hemorragia moderada (grado II) y sólo 1 (1.7%) presentó hemorragia significativa (grado III). Un pacientes (1.7%) tuvo un neumotórax y otro (1.7%) sufrió de hipoxemia.

Conclusiones: La CBTB guiada por USEB-R es una técnica segura y efectiva para el diagnóstico de lesiones pulmonares periféricas. La CBTB puede obtener un mayor valor diagnóstico que la BTB con fórceps debido a una mayor calidad y cantidad de las muestras. La adecuada selección de los pacientes incluidos es esencial para evitar posibles complicaciones asociadas a la CBTB.

Introduction: Radial probe endobronchial ultrasound (RP-EBUS) is a modern technique for diagnosis of peripheral lung lesions. The addition of transbronchial cryobiopsy (TBCB) could increase the diagnostic value for RP-EBUS.

Objectives: The main objective is to evaluate the efficacy and safety of RP-EBUS guided TBCB for diagnosis of peripheral lung lesions. Secondary objectives are detecting factors that could affect the efficacy of RP-EBUS guided biopsy and evaluating the efficacy of the procedure when TBCB is contraindicated.

Methods: 60 patients with peripheral lung diseases were included and divided into two groups. Group I included 45 patients who were eligible for TBCB and they subjected to forceps transbronchial biopsy (forceps TBB) and TBCB guided by RP-EBUS. Group II included 15 patients who were not eligible for TBCB and they subjected only to forceps TBB and / or cytology retrieval procedures guided by RP-EBUS. The diagnostic outcomes including digital assessment for qualitative and quantitative measures of collected samples were detected. Also, the associated complications were recorded.

Results: In group I, forceps TBB had sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of; 67.5%, 100%, 100%, 18.8% and 69.8% respectively. While TBCB had sensitivity, specificity, PPV, NPV and accuracy of 75%, 100%, 100%, 23.1%, 76.7% respectively. TBCB has achieved higher diagnostic values and better quality of samples. The sensitivity in group II was 80% and the overall results including both groups were sensitivity, specificity, PPV, NPV and accuracy of 85.2%, 100%, 100%, 42.8% and 86.7% respectively. Regarding the complications, 12 patients (20%) had bleeding but 11 (18.3%) of them had moderate bleeding (grade II) and only one patient (1.7%) had significant bleeding (grade III). One patient (1.7%) had pneumothorax and another patient (1.7%) suffered from hypoxemia.

Conclusions: RP-EBUS guided TBCB is a safe and effective technique for diagnosis of peripheral lung lesions. TBCB could achieve higher diagnostic value than forceps TBB due to better quantity and quality of the samples. Proper selection of included patients is essential to avoid serious complications that could be associated with TBCB.

INTRODUCTION

2. Introduction

The diagnosis of peripheral lung lesions was considered as a challenge for pulmonologists. Peripheral lung lesions have a wide range of differential diagnosis including malignancy, so their management should be based on accurate evaluation. Some techniques are available for interpretation of peripheral lung lesions; however these techniques either associated with high rate of complications or low diagnostic value. Conventional bronchoscopy provided limited solutions for this challenge which is blind transbronchial biopsy (TBB) beonchoalveolar lavage (BAL) and bronchial brush (BB). The technique was described as blind due to absence of real time vision for sampling as the visual field of conventional flexible bronchoscopy is limited to the sub-segmental bronchial level. Although the technique is available nearly in all bronchoscopy centers, it is associated with low diagnostic value especially in small lesions "less than 2cm". [(1)]

Another available technique for sampling of peripheral lung lesions is transthoracic needle biopsy (TTNB) guided by ultrasound or more commonly computed tomography (CT). Even so this technique is accepted and described to be quite successful, but it is associated with a significant risk of pneumothorax ranged from 9-54%. [(2)]

Fluoroscopy guided TBB has been used to increase the diagnostic value of transbronchial biopsies and decrease the associated possibility for pneumothorax. The diagnostic yield of this technique is dependent on the site and size of peripheral lung lesions and was described to be between 48-80%. However, it carries a risk for exposure to radiation and its value decrease significantly in small lesion which may not be visible by fluoroscopy images. [(3)]

The recent technology has provided us with modern applications for increasing the diagnostic yield for transbronchial biopsy in peripheral lung lesions and to decrease the related risks. These applications are including; Electromagnetic Navigation

Bronchoscopy (ENB), Virtual Bronchoscopy (VB) and Radial Probe Endobronchial Ultrasound (RP-EBUS) which was selected to be the diagnostic tool for peripheral lung lesions in this study.

In last decade, Endobronchial Ultrasound (EBUS) has accepted a good reputation in the interventional pulmonology field. EBUS has two different subdivisions; Convex Probe EBUS (CP-EBUS) which is used mainly in sampling of mediastinal lymph nodes and (RP-EBUS). [4] Radial Probe EBUS utilizes a miniaturized ultrasound probe with radial side scanning properties, producing an ultrasound image of the surrounding lung parenchyma. These unique features qualified (RP-EBUS) to play an important role in evaluation of tracheobronchial wall features and mediastinal structures. [5]

As regards peripheral lung lesions, radial EBUS was proved to be a quite safe and accurate method for TBB of peripheral lung lesions specially those which are suspected to be malignant. [6] Owing to lack of real time image during biopsy, some factors determine the diagnostic value of RP-EBUS in peripheral lung lesions as experience of the working staff or factors related to the lesion itself including ; site , size, nature and relationship of the lesion to the bronchial tree. All these factors will be evaluated and discussed in this study. [7]

On the other hand, cryotherapy techniques have been used in airways mainly as palliative treatment of obstructing endobronchial tumors. However recently, cryoprobes have been used for obtaining lung tissue. A compressed gas is released at high flow, creating low temperature at the tip of probe, which enables collection of sufficient lung biopsies. A considerable advantage of cryobiopsy technique is the large samples which provide to the pathologist more tissue for making an accurate diagnosis. Also cryoprobe samples tend to have larger artifact-free areas in comparison with forceps samples. [8]

2.1 Peripheral Lung Lesions

A peripheral lung lesion is defined as a lesion that couldn't be seen within the bronchial tree using fibreoptic bronchoscopy. [9] This study is directed only towards the peripheral parenchymal lung lesions which couldn't be accessed by conventional fibreoptic bronchoscopy. Some of these lesions could be invisible by ordinary techniques as Chest X-ray. So, CT or more specifically High Resolution CT (HRCT) has become the standard radiological maneuver for diagnosis of peripheral lung lesions. [10]

Many configurations and differential diagnoses for peripheral lung lesions could be presented using HRCT, so the common patterns which could be included in our study will be discussed as the following;

2.1.1 Nodular pattern

In general, a pulmonary nodule is described as quite sharp, discrete and nearly circular lung opacity which measures less than 3 cm. while the micronodule is a radiological term for nodules which could be seen by HRCT and measure less than 3-7 mm. [11] Radiological interpretation of diffuse nodular diseases depends mainly on the site of nodules and their relation to the secondary pulmonary lobule which is divided to four pathological patterns; bronchiolocentric, angiocentric, perilymphatic and random. [12]

Specific radiological features for the centrilobular nodules are the roughly equal spacing between nodules, absence of contact between them and visceral pleura and the possibility of presence of "tree in bud" pattern which resembles the impaction of centrilobular bronchus with mucus, pus or fluid and usually seen in pulmonary infections.[13]

Perilymphatic nodules are seen radiologically in inter-lobular septa and in contact with visceral pleura. These nodules are related mainly to the diseases that could affect pulmonary lymphatics which are located in the previously mentioned

locations. They appear in HRCT as patchy septal or sub-pleural nodules. [14] Random nodules have no specific distribution; they may be presented in the center or periphery of secondary pulmonary nodules. Usually these types of nodules are not presented with patchy pattern, but usually distribute in random appearance bilaterally.

The previously mentioned classification of nodular patterns in relation to secondary pulmonary lobules could be helpful in the evaluation of their underlying pathology as the differential diagnosis is including; infection, hypersensitivity pneumonia, lymphocytic interstitial pneumonia (LIP), pulmonary edema, vasculitis and neoplasms. [15]

2.1.2 Solitary pulmonary nodule (SPN)

A solitary pulmonary nodule is described as a rounded opacity with well-defined edges that don't exceed 3cm in diameter. This type of lesions is usually detected by HRCT and could be missed by conventional radiological means. Solitary pulmonary nodules could appear as a solid or subsolid in nature. [16] The subsolid nodules have ground glass attenuations due to presence of vessels or other soft tissue structures. The mixture between solid and soft tissue within this type could be equal or not. [17]

There is a wide differential diagnosis for solitary pulmonary nodules, either for solid or subsolid types, and the accurate interpretation of the underlying pathology for these lesions should be obtained because lung cancer is suspected (Table 1). [18] According to the American Cancer Society 20%-30% of patients with definitive diagnosis of lung cancer in the United States were presented by solitary pulmonary nodules. [19]

Type of cause	Condition
Neoplastic	Primary lung malignancies (non–small cell, small cell, carcinoid, lymphoma),solitary metastasis
Benign	Hamartoma, arteriovenous malformation

Infection	Granuloma, round pneumonia, abscess, septic embolus
Non-infection	Amyloidoma, subpleural lymphnode, rheumatoid nodule, Wegener granulomatosis, focal scarring, infarction.
Congenital	Sequestration, bronchogenic cyst, bronchial atresia with mucoid impaction

Table 1. Differential diagnosis of SPNs.

Factors as; chronic smoking, family history of malignancy, exposure to radiations or pollution, may highly suggest malignancy. Also radiological features including; spiculated margins, absence of calcification and increasing size during follow up are suspicious. However, confirming malignancy could be achieved only by biopsy and pathological examination. [20] As regards radiological follow up for a nodule size, the doubling volume is defined as increase in diameter more than 26% (Fig 1). Usually the doubling time for malignant nodules is 20-400 days. However as previously mentioned, all these criteria can't certainly exclude malignancy as subsolid adenocarcinomas may take up more than 1000 days to reach the double volume. [21]

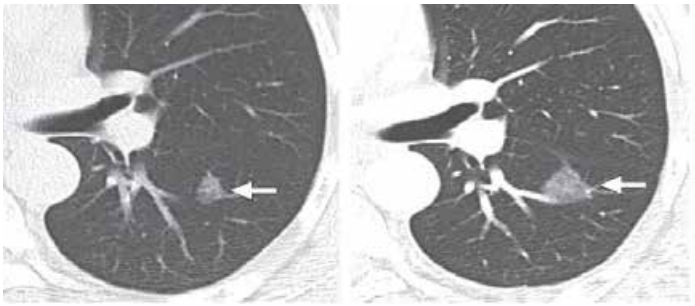

Figure 1. Doubling in size and spiculated margins of SPN, suggesting malignancy. *Infante M, et al. Slow-growing lung cancer as an emerging entity: from screening to clinical management. Eur Respir J 2013; 42:1706–1722.*

Positron Emission Tomography Scan (PET) is a commonly used technique for evaluation of SPN nature and could be integrated with CT which called PET/CT. It depends on measuring the glucose metabolism which is different between benign to malignant tissues. The widely used substance is labeled fluorodeoxyglucose (FDG). The standardized uptake value (SUV) is the value of FDG uptake within the tissue. The accepted cutoff value is 2.5, with benign lesions usually value below this value and the malignant lesions usually exceed it. [22]

Although the specificity and sensitivity of PET in diagnosis of SPNs is nearly 90%, these values may decrease significantly in subsolid SPNs with ground glass nature. Also false negative results of PET are reported in carcinoid tumors, adenocarcinomas and small SPNs. [23] On the other hand, some factors may be relied on false positive PET; these factors include injection artifact, inflammation, infection, sarcoidosis, and unhealed bony fractures. [24]

2.1.3 Ground glass opacity

Ground glass opacity is typically hazy with high attenuation that doesn't obscure the underlying vasculature. This abnormality could be pure alveolar, pure interstitial or mixed. [25] Due to the wide differential diagnosis of ground glass opacities, associated factors could be important including; onset of the symptoms, distribution of ground glass opacities in HRCT and presence of other radiological findings.

For example, presence of ground glass opacities associated with symptoms of infection in immunocompromised patients could suggest viral or Pneumocystis jiroveci pneumonia. The presence of these opacities in multifocal distribution with positive history of antigen exposure could suggest hypersensitivity pneumonitis. [26] In idiopathic pulmonary fibrosis (IPF) the ground glass opacities may be presented alone during the early stages of the disease and distributed mainly subpleural and basal. In late stages of IPF, the ground glass opacities are usually

associated with other radiological features as; honeycomb pattern, traction bronchiectasis and fibrosis. [27]

In general, existence of ground glass opacities alone without features of fibrosis indicates that the underlying pathology is active and reversible. If concomitant fibrosis is presented, the biopsy should be directed to the lung areas which show ground glass opacities. [28] The common causes of ground glass opacities could be infectious as in P jiroveci and viral pneumonia or non-infectious as; eosinophilic pneumonia, hypersensitivity pneumonia, alveolar hemorrhage, alveolar proteinosis, interstitial lung diseases, sarcoidosis and lymphoma. [29]

2.1.4 Consolidation

Consolidation is describing parenchymal opacities which obscure the underlying vasculature and usually associated with air bronchograms. Presence of consolidation reflects that the alveolar air is replaced by pathological substance like blood, pus, fluid or cells. [30] Many underlying pathologies are presented with consolidation, and the integration between clinical and radiological data is essential. Consolidation could be classified according to etiology into infectious, non-infectious inflammatory and others. Also it could be classified according to distribution into segmental and non-segmental. [31]

Segmental consolidation is usually presented as wedge-shaped opacity, few centimeters in size with apex directed toward the hilum. This usually indicates that the opacity is related to a segmental bronchus or artery. The underlying pathology is including; bronchial obstruction, pneumonia, focal aspiration and pulmonary embolism with infarction. [32] Non-segmental consolidation is distributed in more than one segment and could be related to variable differential diagnosis including infection or non-infectious inflammation as cryptogenic organizing pneumonia (COP), PAP and alveolar hemorrhage. [33]

2.1.5 Cavities

A pulmonary cavity can be described as a gas filled space within the lung parenchyma formed after expulsion of its necrotic content by bronchial tree. [34] Cavitary lesions of the lung could be caused by various etiologies and the differentiation between malignant and non-malignant cavities is essential. Radiological features of the cavity, presences of associated lesions and accurate clinical evaluation are required.

Malignant cavities are not rare. In fact, up to 20% of lung cancers could be presented in CT with cavitary lesion, most commonly is squamous cell carcinoma followed by adenocarcinoma and large cell carcinoma. Usually primary malignant cavity is solitary; however, adenocarcinoma presenting lepidic growth could be presented with multiple cavities. [35] Some radiological features may be valuable in diagnosis of malignant cavities including; wall thickness and spiculated inner and outer margins (Fig 2). Measurement of wall thickness could be more specific in differentiation between benign and malignant cavities; with thickness less than 5mm usually refers to benign cavities, 5-15mm mixed and more than 15mm in malignant cavities. [36, 37]

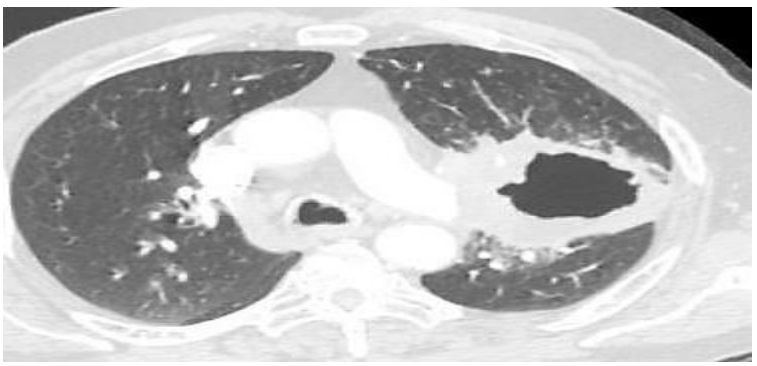

Figure 2. Malignant cavity. *Nin et al. Solitary lung cavities: CT findings in malignant and non-malignant disease. Clin Radiol 2016; 71:1132–6.*

Non- malignant lung cavities could be infectious or non-infectious. For example, Mycobacterium tuberculosis infection is

commonly associated with cavitation in the post primary phase. Tuberculous cavities are usually located in apical and posterior segments of upper lobes and superior segments of lower lobes. [38] Tuberculous cavities may have thick or thin walls and usually refers to high level of infectiousness due to high number of tuberculous bacilli. They could be differentiated from malignant cavities by positive acid fast bacilli (AFB) in sputum or BAL and presence of tree in bud sign or satellite nodules in radiology. [39]

Another common cause for infectious lung cavities is fungal infection. The fungal cavities may be primary in patients with immune deficiency or secondary to existing cavities as aspergilloma on top of tuberculous cavity. The "air-crescent sign" reflecting air separation between cavity wall and inner mass is common but not specific as it may be found in malignant cavities. [40] Other causes for infectious non-malignant lung cavities include; non-tuberculous mycobacterial infections, other bacterial pneumonias, pulmonary actinomycosis and septic emboli. [41]

2.1.6 Lung masses

A lung mass is defined as a rounded lung opacity, usually well-defined and measures more than 3cm. However, many underling pathologies could produce lung masses, but malignant lesions are the most suspected. [42] Lung cancer is highly suggested when the patient has a history of chronic smoking, positive family history of malignancy and occupational exposure to pollution. Also some radiological features could be suspicious as; absence of calcification, cavitation and irregular margins (Fig 3). [43]

Radiological maneuvers including CT and magnetic resonance imaging (MRI) could give accurate data about size, location, consistency and presence of associated lesions. Also PET scan could play an important role for inclusion or exclusion of malignant origin, however, biopsy and pathological examination is the only method for certain diagnosis. [44] Lung mass may be presented in other malignancies than bronchogenic

carcinoma as; sarcomas, lymphomas and metastases. Also it could be presented in benign tumors as hamartoma. Other possible etiologies are including; abscess, lipoid pneumonia, arteriovenous (AV) malformations and pulmonary artery aneurysms. [45]

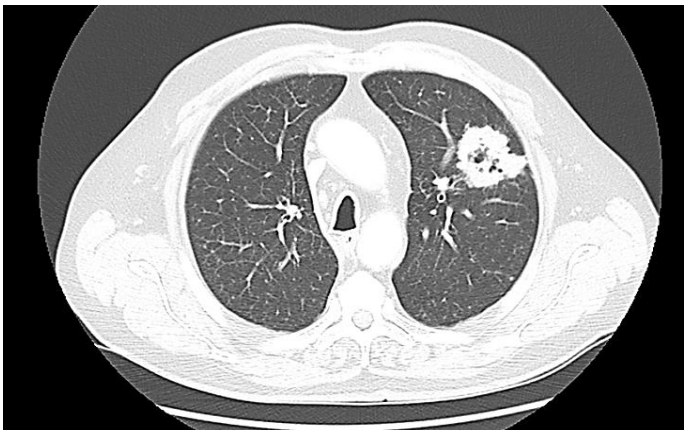

Figure 3 - Malignant lung mass.

2.1.7 Reticular opacities

Reticular abnormalities are interfering linear opacities forming net-like structures. These types of abnormalities are usually presented in lung diseases associated with fibrosis. Reticular opacities are early presented as interlobular interstitial thickening and in late stages it may be accompanied with other signs as traction bronchiectasis and honeycombs. [46] Many interstitial lung disorders could be presented with reticular opacities including; usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP), hypersensitivity pneumonitis (HP) and desquamative interstitial pneumonia (DIP). [47] Other etiologies may include; pneumoconiosis, sarcoidosis, collagen diseases, post infectious scarring, lymphoma, and lymphangitis carcinomatosis. [48]

2.2 Different procedures diagnosing peripheral pulmonary lesions

As previously mentioned, (RP-EBUS) is not the only method for diagnosis of peripheral lung lesions. Other procedures could be used and they can be illustrated as the following;

2.2.1 CT guided transthoracic needle aspiration (CT-TTNA)

CT-TTNA is reviewed as a highly accurate, sensitive and specific tool for diagnosis of peripheral lung lesions, especially malignant lesions. [49] Some factors could play a role in selection of the utilized needles for this procedure to be aspiration or cutting needles. These factors may be related to the lesion as; size, nature and depth, or related to the operator as; preference and personal experience.

Aspiration needles provide samples for only cytological examination, while core biopsy needles are designed to obtain tissue samples which allow histological analysis as well. [50] Aspiration samples are highly significant in diagnosis of malignant lesions. While adding core biopsy samples increase the diagnostic yield of benign lesions. Although core biopsy needles have higher risk for pneumothorax, they can be more accurate for diagnosis of small lesions (<10mm). [51]

CT guided percutaneous lung biopsy should be proceeded with chest CT or PET/CT and the patients should be carefully selected for minimizing the rate of complications. Coagulation profile should be performed and anticoagulants should be stopped before the procedure. Other contraindications include; positive pressure ventilation, respiratory failure and pulmonary hypertension. [52] CT fluoroscopy guidance is more favorable than conventional CT guidance as it is associated with shorter time of procedure and less complications. The superiority of CT fluoroscopy guidance is most probably due to better localization of the lesions which result in fewer needle passes. However, CT

fluoroscopy may be associated with higher exposure for radiations to both the patient and operators. [53].

As regards the efficacy of CT guided percutaneous lung biopsy, it is considered as previously mentioned, an accurate and minimally invasive tool for diagnosis of lung lesions. CT guided fine needle aspiration has low false positive value (0.2%); however it has variable rates of false negative values (6-54%). On the other side core biopsies seems to have higher rates of sensitivity, specificity and accuracy. [54-56]

Even so, possible associated complications should be mentioned for overall assessment of CT guided percutaneous lung biopsy. These complications are including:

- Pneumothorax: which was described as the most common complication following CT guided percutaneous lung biopsy. The rate of pneumothorax in general varies between 17-26.5%, while the percentage of pneumothorax which requires intercostal drainage is less (1-14.2%).[57-58]

- Pulmonary hemorrhage: it is the second most common complication of CT guided percutaneous lung biopsy (4-27%). Similar to pneumothorax, the incidence increase with factors like; small lesions (< 2 cm), long needle path, multiple punctures and type of needle (more with core biopsy needles).[59]

- Air embolism: it is a rare complication with CT guided percutaneous lung biopsy (0.061%). It occurs if pulmonary veins were penetrated during the procedure with leakage of air inside them through the needle. [60]

- Tumor seeding: spread of malignant cells after CT guided percutaneous lung biopsy to other lung sites, pleura or chest wall is an extremely rare complication with only (0.01-0.06%) rate. [61]

2.2.2. Ultrasound guided percutaneous lung biopsy

Efficacy of ultrasound guided percutaneous lung biopsy has been discussed in many researches. Some authors suggested that ultrasound is equal in effectiveness to CT in diagnosis of peripheral pulmonary lesions. They mentioned that ultrasound has advantages over CT in; real time monitoring, accuracy of lesion depth, no radiological exposure, mobility and cost-effectiveness (Fig 4).[62-63] Selection of included and excluded patients for ultrasound guided percutaneous lung biopsy is similar to that for CT guided percutaneous lung biopsy. Also precautions during maneuver and decision about using aspiration versus cutting needles could be equivalent. However, ultrasound biopsy could be less risky than CT biopsy even with use of core biopsy needles. [64]

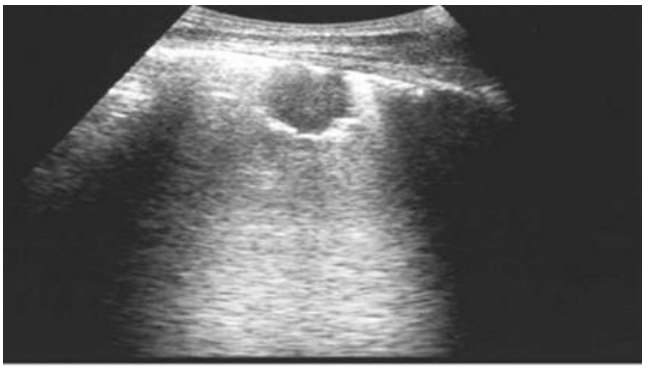

Figure 4. Sonographic image for a pulmonary mass.

The addition of contrast-enhanced sonography has improved the accuracy of ultrasound guided percutaneous lung biopsy. This modern technique has allowed better differentiation between viable and necrotic areas of pulmonary lesions to avoid sampling of necrotic sites which may lead to high false negative results. [65] Some studies reveled that false negative results using ultrasound guided percutaneous lung biopsy are more common among pulmonary lesions less than 1.5 cm in diameter due to insufficient samples and lesions more than 5cm in diameter due to

presence of necrotic areas. Using contrast –enhanced sonography could solve this problem due to improved visibility of lesions. [66]

Color Doppler ultrasound may give some information about the underlying pathology of pulmonary lesions. It was found that nearly 65% of malignant peripheral pulmonary masses have positive Color Doppler signals of low constant flow secondary to increased vascularity. This type of flow is distinct from the pulsatile flow which could be found in both benign and malignant lesions. [67]

Significance of ultrasound images in peripheral pulmonary lesions is also extended to diagnose benign lesions. For example, the vertical hyper-echoic artifacts called "B-Lines" which could appear on thoracic ultrasound images as multiple and bilateral lines are indicative for interstitial diseases. These lines usually tend to have non-homogenous distribution in diffuse parenchymal lesions as pulmonary fibrosis. [68] Ultrasound also could be remarkable in diagnosis of pulmonary embolism. Pulmonary infarction could be seen as a peripheral wedge shaped hypo-echoic lesion usually associated with pleural effusion. Although CT angiography remains the gold standard for diagnosis of pulmonary embolism, ultrasound could be helpful in some cases e.g. pregnancy and restricted mobility. [69]

Despite all the positive reviews about thoracic ultrasound, but it remains an operator dependent application. The results and rate of complications are mainly related to the applicant's experience and interpretation of ultrasound findings. Therefore, the Critical Care Network of the American College of Chest Physicians has recently recommended that chest physicians in general should learn the basic skills of thoracic ultrasound especially for critical care situations. [70] As regards complications which could be associated to ultrasound guided percutaneous lung biopsy, pneumothorax is the most common risk followed by pulmonary hemorrhage with or without hemoptysis. However, percentages of these complications are lower than in CT guided percutaneous lung biopsy as mentioned before. [71]

2.2.3 Fluoroscopy guided flexible bronchoscopy

The visual field of flexible bronchoscopy is restricted to bronchial tree, so combining it with fluoroscopy for guidance to peripheral pulmonary lesions could be useful. X-ray fluoroscopy can provide only two dimensional images for target areas. On the other side, CT fluoroscopy could provide cross-sectional images and application of Real Time Multi Slice CT (RMSCT) could allow real time manipulation of peripheral biopsies. [72] In both types of fluoroscopy guided flexible bronchoscopy, the usual pre-bronchoscopic procedures should be performed including; proper selection of the patients, exclusion of respiratory and coagulation disorders, also performing a high resolution CT for accurate localization of the lesion. During technique, the patient should be monitored continuously for oxygen saturation and ECG, and the operators should be protected by wearing Lead aprons. [73]

RMSCT fluoroscopy could achieve better results than X-ray fluoroscopy due to real time manipulation of biopsy. Also small peripheral lung lesions could be missed by X-ray fluoroscopy. The diagnostic yields for CT fluoroscopy compared with X-ray fluoroscopy were 88.9% for 75% in peripheral pulmonary lesions more than 2cm in diameter and 54.4% for 20% in lesions less than 2cm in diameter. [74] The associated complications including pneumothorax and hemorrhage are related mainly to the trans-bronchial sampling technique. These complications are higher in forceps or cryobiopsies than in brush or needle aspiration. However, the particular complication with fluoroscopy is exposure to radiations which depends on the duration of exposure and significantly increases with CT fluoroscopy. [75]

Despite the previously mentioned complications, fluoroscopy guided flexible bronchoscopy is considered as safe and effective procedure for sampling of peripheral parenchymal lesions. The sensitivity of fluoroscopy guided samples for malignant lesions could be improved, by adding ROSE (Rapid on Site Cyto-pathologic Examination), from 74.4% to 90.3%. This

improvement is owing to addition of other techniques for sampling as TBNA if the preliminary results were negative. [76]

2.2.4 Virtual Bronchoscopy Navigation (VBN)

Virtual bronchoscopy navigation is a relatively modern procedure used to improve the accuracy of flexible bronchoscopy in peripheral pulmonary lesions sampling. It is used to create three-dimensional images for bronchial tree and specific pathways for peripheral lung lesions based on helical CT images (Fig 5). This method could accurately guide the flexible bronchoscopy to its target. [77] Specific software programs were manufactured and continuously updated for creating virtual bronchoscopy images. These programs depend on threshold values to differentiate between bronchial lumens and walls and generate exact views for bifurcations. During procedure, bronchoscopic axis should be adjusted with the virtual images for accurate navigation. [78]

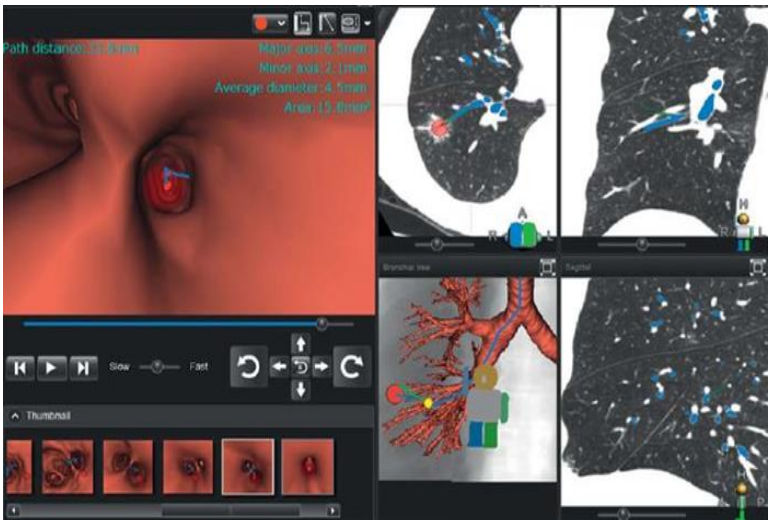

Figure 5. Virtual images for the airways where the target lesion is marked in red and bronchoscopy pathway is marked in blue. *Asano F, et al. Virtual bronchoscopic navigation for peripheral pulmonary lesions. Respiration. 2014; 88(5):430-40*

The process of virtual bronchoscopy navigation is consisted of two steps; planning and guidance. During planning stage, CT images are introduced to the system to create the three-dimensional images of airways and target lesion also for generating the ultimate route for bronchoscopy. The important note during this stage is utilizing suitable CT images with maximum slice thickness of 1mm or even thinner slices in more peripheral lesions. [79] During the guidance stage the real images collected during bronchoscopy are introduced to the system, and then the most identical virtual images are automatically selected and displayed. The data from virtual images as; names of bronchi, distance to target and surrounding vessels are superimposed on real images to create an integrated view. [80]

In general, virtual bronchoscopy navigation has good results in sampling peripheral pulmonary lesions. Based on previous studies, the overall diagnostic yield for VBN is 73.8% and for lesions smaller than 2cm in diameter is 67.4%. The diagnostic value may increase if VBN is combined with other techniques as fluoroscopy to 80.4%. VBN also could be combined with other techniques as radial probe EBUS and ultrathin bronchoscopy. [81-82] VBN guided trans-bronchial biopsy is considered as a safe procedure with minimal risks. The complication rate is ranged from 0-4% and not related to VBN itself but mainly to the trans-bronchial technique. Pneumothorax was reported as the most common risk followed by hemorrhage. [83]

2.2.5 Ultrathin Bronchoscope (UTB)

As regards the continuous researches to improve the diagnostic yield of peripheral pulmonary lesions, specialized companies produced a novel type of bronchoscopes which is ultrathin bronchoscope. This type characterized by thinner outer diameter which allows further passage to peripheral airways (Fig 6).

Figure 6. Standard bronchoscope with a distal end diameter of 5.9 mm and a working channel of 2.0 mm (BF-240; Olympus, Tokyo, Japan). b) Thin bronchoscope with a distal end diameter of 3.5 mm and a working channel of 1.7 mm (XBF-3B40Y1; Olympus). *Oki M et al. Novel thin bronchoscope with a 1.7-mm working channel for peripheral pulmonary lesions. European Respiratory Journal 2008 32: 465-471.*

An early study suggested that ultrathin bronchoscope with outer diameter (3.3 mm) and working channel diameter (1.2mm) could deliver an accurate pathway to peripheral lung lesions and improve the diagnostic yield when it was added as an adjacent maneuver to standard bronchoscopy. (84) Despite that, the use of ultrathin bronchoscope remained restricted as the thin working channel (1.2mm) was an obstacle against collecting sufficient samples for accurate diagnosis of peripheral pulmonary lesions. (85)

Some researchers suggested using of bronchoscope with larger working channel diameter (1.7mm) and outer diameter (3.5mm). Results reveled that this type of bronchoscope was able to reach further two distal generations of bronchi in comparison with standard bronchoscope and increase diagnostic yields to 69% in peripheral lesions. (86) Recently, a novel ultrathin bronchoscope with outer diameter (3mm) and working channel diameter (1.7mm) was produced. Utilization of this bronchoscope combined with navigation techniques as; fluoroscopy, virtual bronchoscopy and radial EBUS achieved diagnostic value (74%) in small peripheral lung lesions (less than 3cm in diameter). (87)

Despite the advantages of ultrathin bronchoscopy, it has some limitations which include; cost of bronchoscope, requirement of experience, low suction power, possibility of obscuring transmitted video image even with minor hemorrhage and limitation of tools which could be manipulated by the narrow working channel. [88] Diagnostic value of trans-bronchial biopsies using ultrathin bronchoscope may increase if multiple maneuvers including; forceps, brushing and washing were combined. And complications from UTB trans-bronchial biopsy are similar to other techniques so it could be considered as safe and effective technique. [89]

2.2.6 Electromagnetic Navigation Bronchoscopy (ENB)

ENB is relatively a modern technique which allows navigation and guidance to peripheral pulmonary lesions. This technique was initially introduced in 2005. Since that time, many trials and studies were performed to evaluate it. [90] The initial phase of ENB is called the planning phase and it is similar to virtual bronchoscopy. The thin sliced CT images are introduced to the software then 3D images for the peripheral lesion are produced showing coronal, axial and sagittal views (Fig 7). Following that, seven anatomical points are marked bilaterally representing main and secondary carinas and they are called "registration points". [91]

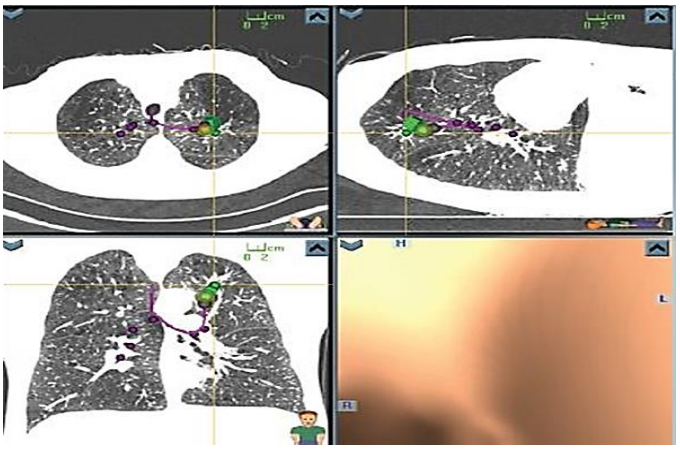

Figure 7. Planning phase of ENB. The green dot is the peripheral lesion and the purple dots are the registration points. *Leong S, et al. Electromagnetic navigation bronchoscopy: A descriptive analysis. J Thorac Dis. 2012 Apr 1; 4(2):173-85.*

During procedure, an extended working channel and locatable guide are introduced through the bronchoscopic working channel and matching is performed between real images and virtual images including 'registration points". The locatable guide is navigated live by the operator until it reach to the target lesion, then it should be removed to introduce the sampling tools. (92)

ENB is regarded as minimally invasive and accurate method for diagnosis of peripheral lung lesions. It allows accurate access to smaller and more peripheral lesions which could play an important role in early detection of lung cancer. The available results for ENB efficacy are mainly single- center researches; however the diagnostic yield values of these studies are promising and generally between 59-94%. (93) This technology is not restricted to diagnosis of peripheral lung lesions only and could be used in another applications including; diagnosis of mediastinal lymphadenopathy, video assisted thoracoscopic surgery, placing fiducial markers for stereotactic body radiation therapy. (94-96)

The complications rate with ENB is very low and it is considered as a safe procedure. Recent studies showed that the rate of pneumothorax accompanied with ENB technique is approximately 3% of which 1.6% required chest tube. Other complications including hemorrhage and infection are rarely mentioned. (97)

With the advanced studies on electromagnetic navigation bronchoscopy, some researchers noted that using total lung capacity CT images for navigation (TLC-mapped ENB) may not give perfect localization of small peripheral pulmonary nodules. They argued that using this technique in planning phase could be responsible for low diagnostic yield in some trials. (98, 99) The

possible hypothesis is that ;using CT images in TLC position with breath-holding technique could create virtual images for lesion's location that differ from intra-procedural location during respiratory movements. In some trials when fluoroscopy was combined with ENB for more accuracy, data showed that mean distance to some lesions centers could differ up to 23mm. [100,101]

A recent study suggested the use of a new type of ENB software which depend on tidal volume CT images (TV-EXP mapped ENB). The study showed that this modern technology combined inspiratory and expiratory CT images which allowed better localization for even small lung lesions during procedure and increased the diagnostic yield for peripheral nodules less than 3cm to 83.3%.[102] Recently, electromagnetic navigation was combined in the same technique with trans-thoracic needle aspiration and called (ETTNA). The procedure was used for diagnosis of solitary pulmonary nodules less than 3cm. The diagnostic yield was 87%; however the pneumothorax rate was 21%. [103]

Many factors may affect the results of ENB including factors related to the lesion site, size and nature. Another important point is the relation between the lesion and nearby bronchus. This factor is called "bronchus sign" where a bronchus could directly lead to the lesion which increases the diagnostic value. [104]

Although the previous data suggested that ENB technique has many advantages in diagnosis of peripheral lung lesions, some limitations should be considered including:

- Complexity of the procedure and requirement of well-trained operating team. As the outcome of ENB may depend significantly on the skills and experience of operators. Some studies suggested that at least 15 procedures are required to gain sufficient experience. [105]

- The high cost of ENB is a significant problem. This obstacle is leading to restriction of this technique to only large and highly specialized centers. More studies about the cost-benefit of the procedure are required. [106]

2.2.7Bronchoscopic trans-parenchymal nodule access (BTPNA)

BTPNA is a new technique for diagnosis of peripheral lung lesions, specifically solitary pulmonary nodules. The technique is based on creating a direct pathway "tunnel" from the bronchus to the target lesion without depending on the anatomy of airways. Theoretically, it has the advantages of trans-thoracic needle aspiration (direct access to lesion) and bronchoscopy (low rate of complications). [107]

The first research on BTPNA was performed on animals in 2014. Researchers have created 13 tunnels in 4 included canines and the average tunnels length was 32.3mm. The trial was successful as blood loss in the involved cases was minimal and pneumothorax was absent. BTPNA was considered as safe and effective technique for further studies on humans. [108] Later on, the first study of BTPNA on humans was released and the technique was described as the following (Fig 8); [109]

- First, the planning phase is done using modern virtual bronchoscopy software. The program transforms the high resolution thoracic CT images into 3D virtual images to create a point of entry "POE".
- The point of entry represents the point where the selected bronchus will be perforated to create a tunnel to the target lesion through the lung parenchyma. Virtual Doppler is used to ensure absence of vessels through the new tunnel.
- The data about bronchoscopic pathway to POE, tunnel diameter, tunnel length, lesion size and distance between lesion and pleura could be collected from the software.
- When POE is reached by bronchoscope, a coring needle perforates the bronchial wall and a radio-opaque guide

sheath with stylet is used to create the tunnel under dual observation of fluoroscopy and virtual bronchoscopy.

- When the target lesion is reached. The stylet is removed and forceps biopsy is introduced to collect specimens.

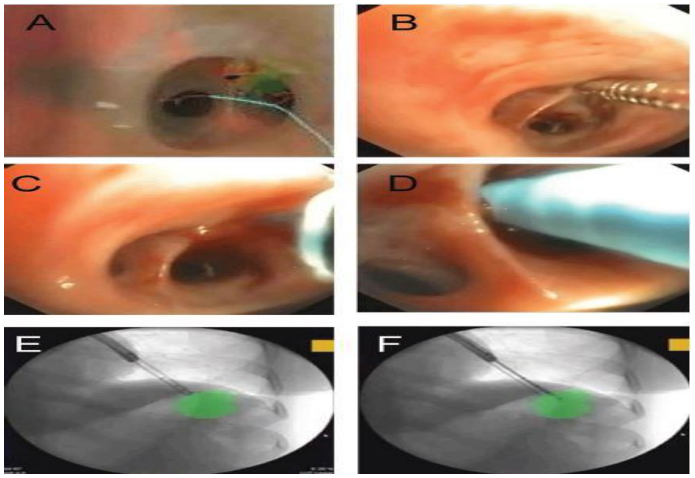

Figure 8. Steps of BTPNA. A) Virtual bronchoscopy image of POE, B) & C) Real images of creating POE, D) Introducing guide sheath, E) & F) Fluoroscopic images of sheath pathway to the lesion. *Herth F, et al. Bronchoscopic transparenchymal nodule access (BTPNA): first in human trial of a novel procedure for sampling solitary pulmonary nodules. Thorax.2015; 70: 326–332.*

In the previously mentioned study, operators avoided creating tunnels in patients with moderate to severe pulmonary hypertension, moderate to severe pulmonary fibrosis and moderate to severe emphysema. Successful tunnel creation and positive diagnostic biopsies for peripheral lesions less than 4cm in diameter were obtained in 83% of patients.

BTPNA could be a promising new technique especially for peripheral lung lesions with small size (less than 2cm) and absent access through bronchial airways (absent bronchus sign). The current trials are limited so; further studies should be performed for assessment of diagnostic value and safety of this new procedure. [110]

2.3 Radial Probe Endobronchial Ultrasound (RP-EBUS)

Radial EBUS was presented for first time in 1990. The first probe had frequency of 7.5MHz and the transducer was able to rotate for 360 degrees. However, the outer diameter was 3.4mm which was too large for any available flexible bronchoscope and it could only applied by rigid bronchoscopy. Another drawback was the limited contact with the walls of airways which was solved by addition of inflatable balloon which could be filled with saline and present good contact surface. [111] The first study using RP-EBUS in diagnosis of peripheral lung lesions was performed by *Hurther et al* in 1992. The researchers successfully located and sampled peripheral lesions in 19 patients out of 26. They recommended further studies in this promising field. [112]

With advanced radiological techniques for lung cancer screening, the rate of radiological findings for peripheral lung lesions with malignant susceptibility increased. Hence, the need for safe and accurate procedure for diagnosis of these lesions became necessary. Radial probe EBUS (RP-EBUS) could play an important role in this field. [113] Currently, Radial probes are available with frequency 20MHz which usually have penetration depth of 5cm. These probes are delicate and need special care during handling.

Normally, the alveoli are filled with air and give the characteristic image of “snow storm”. Any structure surrounding the radial probe and separated by air will not be visible due to reflection of ultrasound waves by air. So, contact surface is essential for images creation. [114] Ultrasound images are variable according to tissue conduction of ultrasound waves. For example, solid malignant masses usually appear as grey homogenous shadows with sharp bright borders separating them from normal tissue, while soft tissue as vessels and necrotic tissue usually appear black.

On the other hand, benign masses usually don’t have sharp edges and white spots could be noticed around their centers.

While inflammatory lesions have heterogeneous distribution on ultrasound images due to presence of different structures. A drawback in radial EBUS is the absence of Doppler mode. So when a black shadow resembling a vascular structure is seen, it could be difficult to differentiate between it and necrotic tissue for example. However, arterial vascular shadows usually pulsate concurrently with the heart beats.

Malignant cells of peripheral lung tumors invade the surrounding alveolar tissue; therefore lack of air bronchogram in ultrasound images of malignant lesions is logical. In contrast, air bronchogram could be a common finding in benign lesions as the alveolar morphology is less distorted. Air bronchograms usually appear by ultrasound as regular hyperechoic short lines with concentric alignment. [115] EBUS images depend on the arrangement of cells and distribution of fibrous stroma within the examined tissue. Heterogeneous echogenicity is suggestive for malignant lesions as the malignant cells tend to replace the normal cells in association with development of necrosis and hemorrhage which lead to the heterogeneous consistency in radiological finding. [116]

Lie et al, tried to collate between RP-EBUS images of peripheral lung lesions and their underlying pathologies. Authors generally classified the ultrasound images into 3 categories; hypoechoic, anechoic and luminant. The hyopechoic and luminant lesions failed to refer to certain pathology as it was found in both benign and malignant lesions equally. In contrast, anechoic lesions were commonly malignant. However, these findings couldn't replace pathological examination which is the absolute standard for diagnosis.[117] Another classification for RP-EBUS images was done by *Kuo et al* and described the peripheral lesions as; continuous margin, non-linear dotted air bronchogram and heterogeneous echogenicity. The researchers reported that the three characteristics suggesting malignancy are; presence of continuous margin, absence of non-linear dotted air bronchogram and heterogeneous opacity. [118]

Some studies reported addition of guide sheath (GS). The radial probe is introduced to the bronchoscopic working channel within a guide sheath. Then both are forwarded simultaneously while the transducer is navigating for peripheral lesions. Once a pathological image is sound, the probe is removed while the guide sheath remains in the working channel. Then, the sampling tool is introduced through the guide sheath to collect samples. [119] However, the use of guide sheath could also have drawbacks. Displacement of guide sheath could occur with vigorous insertion or removal of the probe or biopsy tools. Another important factor is the need of biopsy tools with small diameter to be fitted inside the guide sheath; these small tools could obtain small samples which aren't sufficient for histological or cytological analysis. Also, the common commercial cryoprobes are too large to be fitted in guide sheath.

2.3.1 Effectiveness and possible complications

RP-EBUS could combine the advantages of minimal invasion with low complications rate and high diagnostic value for diagnosis of peripheral lung lesions. The technique could be directed to peripheral lung lesions in general or specifically to certain pathologies as cavitary lung lesions, ground glass opacities or solitary pulmonary nodules. Associated risks as pneumothorax and bleeding are infrequent.[120] The diagnostic value for RP-EBUS is comparable to CT guided percutaneous needle biopsy (CT-PNB) for peripheral lung lesions; 73-83% for RP-EBUS versus ±90% for CT-PNB. However, the difference in complications rate is significant. Pneumothorax could be presented in 27% of patients following CT-PNB while the rate in RP-EBUS is 3%. [121]

Diagnostic value of RP-EBUS for peripheral lung lesions could be even higher if the procedure is combined with other techniques as; fluoroscopy, electromagnetic navigation and ultrathin bronchoscopy. These techniques could improve the navigation of RP-EBUS especially for small lesions, which achieve an accurate procedure with minimal risks. However, the

adjacent techniques could also increase the time and the cost of the procedure. [122] A particular challenge is accurate diagnosis of small peripheral lesions as solitary pulmonary nodules (SPNs). RP-EBUS could be valuable in diagnosis of these lesions specially if combined with other navigational techniques and the diagnostic yield could reach 91.7% for SPNs 2-3 cm in diameter.[123] Usually pure ground glass lesions are more difficult to be visualized by RP-EBUS than part-solid lesions. However, combining RP-EBUS with fluoroscopy to confirm the lesion site could allow diagnostic value of 79%. [124]

RP-EBUS could also be used in diagnosis of peripheral cavitary lesions. Different benign and malignant lesions could be presented with peripheral cavities. Radiological signs could estimate the underlying pathology however, biopsy and pathological examination are essential. Conventional bronchoscopy has low diagnostic value for peripheral cavitary lesions could reach 34% in small ones. [125]

Researchers tried to define the factors which could improve the diagnostic value of RP-EBUS and these factors could be illustrated as the following; [126]

- Intraoperative factors as identification of the target lesion by EBUS and placement of radial probe in the center of the lesion.
- Bronchus sign is considered the most important radiological index for high diagnostic yield. Bronchus sign means; the presence of bronchus leading directly to the lesion in CT. The sensitivity of RP-EBUS for lesions with positive bronchus sign could reach to 86.7%
- Other radiological factors could also be important as; lesion size, consistency, site and distance to pleura.

2.3.2 Other applications for RP-EBUS

RP-EBUS could be used in visualization of airway wall layers. This could be of high benefit for diagnosis of malignant

invasions to the airways walls which couldn't be predicted by CT. In management of bronchogenic carcinoma, it is important to reveal if the tumor is restricted to submucosal layers only or advanced to further cartilaginous layers. RP-EBUS could diagnose these data with specificity of 100%, sensitivity of 89% and accuracy of 94 % (Fig 9). [127] By using RP-EBUS, central airways could be examined and 7 layers could be visualized forming the walls. The inner 2 layers are for mucosa and submucosa, third to fifth layers are cartilaginous and the external two layers are loose and dense fibroelastic connective tissue. [128]

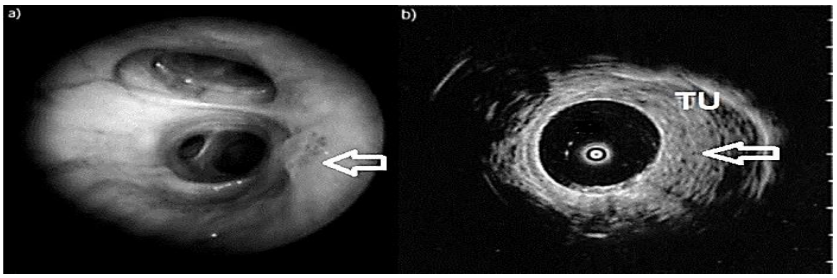

Figure 9. Radial EBUS in diagnosis of bronchial wall invasion. A) Bronchial malignant lesion by conventional bronchoscopic image which showed it as superficial lesion. B) RP-EBUS image show invasion of this tumor to bronchial wall which requires surgery. *Herth F, et al. Endobronchial ultrasound reliably differentiates between airway infiltration and compression by tumor. Chest. 2003; 123(2):458-62.*

RP-EBUS could also be useful in airway stenting procedure. The operators could correlate data from an airway examination by radial EBUS with radiological information for determining the proper size and diameter of airway stent. The radial probe could pass the site of stenosis when the bronchoscope couldn't, and allow measurement of an airway diameter. Also data from RP-EBUS could estimate the viability of distal airways and benefits from airway stent. [129]

Air way remodling could occur in asthma or COPD and associated with bronchial wall thickening. RP-EBUS was successfully applied in detection of bronchial wall changes even in mild to moderate cases. Airway remodling which is triggered

by uncontrolled inflammation could be monitored for evaluation of therapeutic response and prognosis of asthma or COPD cases. RP-EBUS could be more sensitive than HRCT in this field. [130]

Another application for RP-EBUS is preoperative evaluation of airways for localization of peripheral small lung lesions prior to surgical intervention. For thoracoscopic lobar or sub-lobar resection it could be difficult to locate small lung lesions specially those ≤ 1 cm and distant from pleura. RP-EBUS was used to locate these lesions and the affected segments were marked with methylene blue dye so it could be visualized and safely resected by the thoracoscope. [131]

2.4 Bronchoscopic sampling of peripheral lung lesions

Many tools could be used for sampling peripheral lung lesions. We will illustrate the commonly used tools and its evaluation.

2.4.1 Cryobiopsy

The first application for cold temperature in medicine was performed by ancient Egyptians. They used it for treatment of inflammations and trauma. After centuries, it was used for several purposes by the European scientists as treatment of headache and neuralgia, amputation surgeries and skin cancer. [132] Cryoprobes were used in bronchoscopy for first time in 1977. The initial purpose was therapeutic for airway stenosis. Later, debulking of malignant airway obstructions was introduced. Endo bronchial tumors were exposed to cold temperature causing necrosis of malignant tissue which could be expectorated by cough during the following days. [133]

With improvement of freezing power of the cryoprobes, the term "cryo-recanalization" was introduced. It referred to the ability of cryoprobes to restore the inner diameters of airways with large exophytic tumors even in absence of mechanical debulking effect of rigid bronchoscopy and by using flexible bronchoscopes. [134] With the continuous research, scientists were able to introduce a new non-contact technique for cryotherapy in airways. Endoscopic spray cryotherapy (SCT) was initially used for esophageal tumors. The technique delivers liquid nitrogen droplets in spray form to the endoluminal surface inducing visible frosting. Researchers considered this technique superior than hot procedures like laser and electrocautery as it is associated with less scares. [135]

As for cryobiopsy, it was initially used in endobronchial lesions. Studies proved that cryoprobes are able to sample larger tissue biopsies than forcipes. The presence of flexible cryoprobes which could fit in bronchoscopic working channels made the procedure more popular for chest physicians. [136] A multi-centric

study was performed to compare cryobiopsy versus forceps biopsy in endobronchial lesions. 600 patients with suspicious endobronchial lesions were involved and the histological analysis for specimens was performed blindly. A definitive diagnosis was reached in 85.1% of samples with forceps biopsy versus 95% in samples with cryobiopsy. [137]

Cryoprobe unit could be used for both cryotherapy and cryobiopsy. The structure and mechanism of action could be illustrated as the following; the unit is consisted mainly of two systems; 1) cryogen, which is the liquefied gas responsible for creating the low temperature and 2) the cryomachine. The most commonly used cryogen is Nitrous oxide (N_2O) owing to its ability to achieve temperature lower than -40^{o}C. Cryoprobes are available in many forms including rigid, semi-rigid and flexible. The flexible probes have usually outer diameter of 1.9mm or 2.4mm. For cryotherapy, operators perform recurrent cycles of freezing and thawing which lead to cellular damage and tissue necrosis as previously described. While for cryobiopsy, the operators usually contact the probe with the selected tissue for only 3 seconds to collect viable biopsies for histopathological examination. [138]

2.4.1.1 Transbronchial cryobiopsy

Transbronchial cryobiopsy was successfully applied in diagnosis of interstitial lung diseases. While CT features were not conclusive, the application of transbronchial cryobiopsy guided by RP-EBUS, fluoroscopy or other navigational applications allowed diagnosis of pathologies like; usual interstitial pneumonia, non-specific interstitial pneumonia, hypersensitivity pneumonia, eosinophilia pneumonia, organizing pneumonia and follicular bronchiolitis. [139]

The technique was also used for evaluation of lung transplant patients. The large size of cryobiopsy samples which involved sufficient alveolar tissue enabled early detection of acute lung rejection, pneumonitis and diffuse alveolar damage.

Researchers found that cryoprobes could achieve larger samples and higher diagnostic value than conventional forceps biopsies. [140] A study demonstrated the role of transbronchial cryobiopsy in immune-compromised patients with lung infiltrates. Patients with underlying malignant diseases who showed clinical and radiological evidence of pulmonary infiltrates were included. Diagnosis of underlying pathology was achieved in 80% of them with no major complications. [141]

Transbronchial cryobiopsy guided by RP-EBUS was used in diagnosis of peripheral lung lesions suspicious for malignancy as solitary pulmonary nodules. The large samples of cryobiopsies comparing with forceps biopsies enabled histopathological diagnosis of malignancy also immunohistochemistry and molecular studies which are used recently for tumors classification for the ideal management (Fig 10). [142] Generally, transbronchial cryobiopsy is guided by a navigational tool as RP-EBUS, fluoroscopy or others. The aim is accurate localization of peripheral pulmonary lesions, specially the small lesions as solitary pulmonary nodules which increase the diagnostic value. Another role is maintaining sufficient space between the tip of cryoprobe and the pleura to avoid pneumothorax. [143]

Figure 10. Transbronchial biopsies with significant difference in size. The left is a conventional forceps biopsy and the right is a cryobiopsy. *Dhooria S, et al. Transbronchial lung biopsy with a flexible cryoprobe: First case report from India. Lung India; 2016; 33:64-8.*

As for associated hazards, the most common complication was pneumothorax which had a variable range between different reports (0%-20%). However, only half of these cases required chest tube. Bleeding was usually mild to moderate; no cases of massive hemorrhage causing vital instability or respiratory insufficiency were frequently recorded. [144]

Using inflatable balloons during transbronchial cryobiopsy technique could play an important role in preventing severe bleeding. Fogarty catheter which was originally indicated for embolectomy is an example for these types of balloons; it is passed usually through double luminal endotracheal tube to the entry of selected lobe and inflated immediately after biopsy. The improper location or insufficient inflation of the balloon could lead to severe hemorrhage and blockage of visual field. Many factors could affect the rate of complications in transbronchial cryobiopsy technique including; experience of operating team, nature of the lesion, distance between lesion and pleura and comorbidities as COPD, pulmonary hypertension and bleeding tendency. [145]

2.4.2 Forceps biopsy

Transbronchial forceps biopsy was first presented for diagnosis of interstitial lung diseases in 1965. It was noticed that using flexible forceps biopsy could allow parenchymal tissue biopsy without major complications. [146] With the spread of using flexible bronchoscopy, transbronchial forceps biopsy became more popular. Radiological features like; nodular lesions, peripheral lesion with positive bronchus sign, alveolar opacities and reticular opacities became indications for this technique where the high diagnostic yield is predicted. [147]

Transbronchial forceps biopsy was usually performed with guidance of fluoroscopy or other navigational techniques to ensure suitable location of forceps and avoid pleural injury. The exact number of sufficient specimens for high diagnostic yield wasn’t specified; however several studies suggest that 4-5

biopsies are the minimal acceptable number. [148] Variable sizes and types of forcipes are available including; cutting or serrated edge, fenestrated and elliptical or spherical shaped cups. No conclusive data are available about difference in diagnostic value between these different types. However, studies reported that the larger sizes of forcipes could provide larger and more sufficient samples for histopathological analysis. [149]

In general, transbronchial forceps biopsy is considered as a safe technique. The most frequent complication is pneumothorax which had variable reports according to different studies and ranged between 2%-10%. The percentage of significant hemorrhage is usually less than 2% and it is higher in cases with pulmonary hypertension. The major complications including; pulmonary edema, arrhythmia and death are rarely reported. [150]

Transbronchial forceps biopsies could be confirmatory for malignancy or infectious conditions like TB when microbiological analysis is positive or inflammatory conditions as alveolar proteinosis and histocytosis. In other conditions biopsies could give characteristic but not specific findings which should be combined with the radiological data to reach a diagnosis as in hypersensitivity or organizing pneumonia. While in some cases biopsies couldn't be sufficient for diagnosis and considered negative. [151]

Some artefacts could affect transbronchial forceps biopsies and affect its diagnostic value including; crushing, cribriform stripes simulating carcinoma, fresh hemorrhage and bubble artefacts. These drawbacks made transbronchial forceps biopsy reported with low sensitivity and specificity in diagnosis of interstitial lung diseases and suspected malignant peripheral lung lesions in some studies. [152]

Researchers realized that obtaining larger biopsies could increase the diagnostic value. So, studies utilizing larger forceps or "jumbo" forceps for transbronchial biopsy were performed. This forceps couldn't be fitted in flexible bronchoscope so rigid

bronchoscope was used. The jumbo forceps was able to provide samples nearly double in size if compared with conventional flexible forceps which increased the diagnostic value. However, experience with rigid bronchoscopy technique was required and the forceps size was limited to large airways. [153]

Transbronchial forceps biopsy was also applied successfully for diagnosis of solitary pulmonary nodules guided with different navigational techniques. The average percentage for forceps samples considered as sufficient and achieved final diagnosis was around 73% for lesions less than 3 cm and higher in larger lesions. [154]

2.4.3 Bronchoalveolar lavage (BAL)

Bronchoalveolar lavage is considered as a conventional procedure for diagnosis of peripheral lung lesions. The process is usually done by wedging the flexible bronchoscope into the selected lung segment based on the radiological data. Usually 150-200ml of sterile saline is injected and the collected fluid is applied for cytological and microbiological tests. [155] Bronchoalveolar lavage outcome could be affected by several factors including; underlying pathology, mechanical obstruction of small airways, collapsibility of small airways due to asthma or COPD and smoking which could increase the macrophages presentation. In diffuse lung diseases, the best lobes for collecting sufficient BAL are right middle lobe and lingual. [156]

Many technical aspects were recommended for proper BAL collection. Wedging is essential to prevent contamination of samples by larger proximal airways. Some researchers considered the first aliquot of BAL as representation of airway cells and secretions which should be separated for microbiological analysis while the remaining BAL should be used for cytological analysis. [157] The accepted volume of retrieved BAL is $\geq$ 30% while the normal composition of BAL is usually as the following; 80-90% alveolar macrophages, 5-15% lymphocytes $\leq$ 3% neutrophils and

≤ 1% esinophils. As previously mentioned, the volume and composition of BAL may be affected by several factors. [158]

In 2006, researches suggested new modifications of BAL technique. They reported that attachment of a plastic tube between syringe and working channel could enable recovery of more fluid. The newly developed technique allowed recovery of 8% more fluid with 17.4% improvement in diagnostic yield and 6.9% fewer complications. [159]

The diagnostic values of BAL for diagnosis of peripheral lung lesions are variable according to the underlying pathologies. BAL could be significant in infectious diseases when microbiological tests are positive for specific microorganisms. For example, BAL Zheil-Nelsen staining could be positive in 82.2% of previously sputum smear negative patients for TB while BAL cultures could achieve positive diagnostic value of 90.9% if compared with sputum cultures for TB 26.4%. [160] BAL also could play an important role in diagnosis of radiological infiltrates suspected to be pneumonia especially in immune-compromised patients. Patients with mechanical ventilation, steroid therapy, HIV and chemotherapy are liable for infections with variable typical or atypical microorganisms which may develop serious complications including Acute Respiratory Distress Syndrome (ARDS). BAL could identify the underlying microorganisms in up to 56% of these patients. [161]

In interstitial lung diseases, BAL was also used as minimally invasive procedure for diagnosis. However, in these diseases BAL may not give conclusive diagnosis and could be used in combination with radiological findings to narrow the differential diagnosis. Differential cell count and CD4/CD8 ratio could be useful for diagnosis of many interstitial lesions including sarcoidosis. However, the role of BAL in diagnosis of IPF, COP and connective tissue related disorders could be limited and depends mainly on exclusion of other pathologies. [162]

Diagnostic value of BAL in suspicious malignant masses or nodules is very low with conventional bronchoscopy without adding navigational techniques. The process is called non-guided bronchoscopy technique and the sensitivity of BAL combined with bronchial wash could reach 11-14%. The diagnostic value could be increased by using guidance techniques like fluoroscopy or RP-EBUS however; BAL couldn't replace biopsy as the main diagnostic procedure. (163)

As regards possible associated complications, bronchoscopic BAL is considered a safe procedure. The reported complications included; hypoxemia, post procedure fever, transient radiological infiltrates, hemorrhage, pneumothorax and acute exacerbation of IPF. However, the rate of these complications is very low < 1%. (164)

2.4.4 Bronchial brushing (BB)

Cytology brushes are available in different sizes and models. Brushes are covered by sheath to protect it from contamination and protect bronchoscopic working channels from injury. At the target location, brush is pushed out the guide sheath and friction between it and bronchial wall is performed to obtain samples for cytological or microbiological analysis.(165) Several factors may affect the diagnostic value of brushing technique. The diameter of used brushes usually doesn't affect its accuracy, but model of brush could play a role; for example, long and wide brushes could retrieve more cells. It was reported that acceptable number of brushings is 4-5 times and the diagnostic value could be 72% for central malignant lesions versus 45% in peripheral malignant lesions. (166)

Bronchoscopic brushing is a safe technique. Minimal bleeding could occur specially if the bronchial mucosa is not healthy however, this bleeding usually doesn't need further procedures for management. The brush contents could be applied directly on a glass slide and fixed by 95% alcohol to be stained or

placed in sterile saline and shacked powerfully to dislodge its content and send for microbiological examination. [167]

With introduction of molecular assessment of lung cancer, bronchial brushing has gained more importance. Studies revealed that brush tip washing could retrieve sufficient cellular amoles called cell blocks. By applying these cell blocks to immunohistochemical analysis, diagnostic value of peripheral malignant lung lesions could be increased to 77%. [168]

2.4.5 Transbronchial needle aspiration (TBNA)

Bronchoscopic transbronchial needle aspiration could play important role in diagnosis of peripheral lung lesions. However, peripheral lung lesions are difficult to be detected by conventional bronchoscopy so TBNA should be guided with a navigational technique as RP-EBUS for accurate sampling. Before modern navigational procedures, conventional TBNA with no guidance was a popular maneuver combined with other conventional techniques as blind forceps biopsy and bronchial wash for diagnosis of peripheral lung lesions. The diagnostic value of each technique was reported and blind TBNA had low diagnostic yield of 35%. The procedure was described as relatively safe with few complications. [169]

With the available navigational techniques, diagnostic value of guided TBNA is increased to 60-70%. Studies showed that several factors could determine the accuracy of TBNA including; size of the lesions as TBNA is more diagnostic in lesions > 3cm, using rapid on site cytological examination (ROSE) and nature of disease as TBNA value increase in malignant lesions. [170]

The diagnostic value for TBNA in malignant lesions could be increased by applying the cell blocks collected by TBNA to molecular and genetic analysis. These techniques could increase diagnostic accuracy of TBNA by nearly 7%. [171]

2.4.6 Bronchial wash (BW)

Bronchial wash is a widely used procedure during bronchoscopy. Usually the cytological presentation in bronchial wash is less than other conventional cytological procedures as BAL, TBNA and bronchial brush. Some researchers considered that bronchial wash could be used as a secondary cytological method when the target areas are beyond reach by TBNA and bronchial brush. [172] Bronchial wash is different than bronchoalveolar lavage as no wedging is performed. If bronchial secretions are present, bronchial aspiration is done for microbiological and cytological analysis. If no secretions, bronchial wash could be done with approximate 20cc of sterile saline. The procedure has low accuracy for peripheral lung lesions specially if no guidance is applied and diagnostic value could reach 29%. [173]

In general, bronchial wash should be combined with other procedures for diagnosis of malignancy. The integration of biopsy with cytological methods is recommended to increase the diagnostic value.

RESEARCH OBJECTIVES

3. Research objectives

3.1 Justification

Failure of obtaining an accurate diagnosis for peripheral lung lesions using only the conventional bronchoscopy is a common problem. As many of these lesions are suspected to be malignant, the demand for more advanced diagnostic tools was raised. According to previous researches, radial EBUS was recommended as an accurate and safe technique for detection of peripheral lung lesions. Furthermore, TBCB has proved more efficacy than other transbronchial biopsy techniques. The combination of RP-EBUS guided TBCB is assumed to achieve high diagnostic value with low rate of complications.

3.2 Main objective

The main objective of this study is to evaluate the diagnostic value and safety of RP-EBUS guided TBCB in diagnosing peripheral lung lesions.

3.3 Secondary objectives

Secondary objectives of this study are:

- Evaluating the diagnostic value of RP-EBUS guided biopsy when TBCB is contraindicated.
- Analyzing factors that could affect the diagnostic value of RP-EBUS guided biopsy including: size of lesion, nature of lesion, positive bronchus sign and location of radial probe within the target lesion.

METHODS

4. Methods

4.1. Protocol

The study was carried out at Bronchoscopy Unit of Hospital Universetari Germans Trias i Pujol, Badalona, Barcelona. It was performed in the period from June 2016 to June 2018. The study protocol was accepted by Germans Trias i Pujol hospital ethical committee. It included 60 patients with peripheral parenchymal lesions detected by chest CT.

In order to be involved in the study, the selected patients had to embrace the following inclusion criteria:

- A peripheral parenchymal lesion with diameter larger than 1cm detected by thoracic CT and has no visualized endobronchial extension during conventional flexible bronchoscopy.
- Failure of flexible bronchoscopy to diagnose the underlying pathology through the conventional techniques as; endobronchial biopsies, bronchial wash and bronco-alveolar lavage.
- Capability of the patient to undergo anesthesia after satisfactory results of arterial blood gases, pulmonary function tests, electrocardiogram, liver & renal function tests and physical examination.
- Capability of the patient to undergo trans-bronchial biopsy after exclusion of coagulation disorders by laboratory tests as platelets count, prothrombin time & INR, activated partial thromboplastin time (APTT) and fibrinogen levels.

All the included patients have accepted to participate in the study by signing written consents which explained the technique and its possible side effects and ensured the privacy of patients' personal data.

While the exclusion criteria were:

- Detection of endobronchial extension for the parenchymal lesion by conventional flexible bronchoscopy.
- Respiratory failure with Pao_2 less than 60 mmHg and $Paco_2$ more than 50 mmHg.
- Severe chronic obstructive lung disease with FEV1 < 35 % of predicted.
- Cardio vascular instability e.g. unstable angina, recent myocardial infarction, arrhythmia or uncontrolled severe hypertension.
- Bleeding disorders with INR > 1.3 or platelets count less than 50,000/ mm^3
- Refusal of the patient to undergo the study after explaining the procedure and possible accompanied complications.
- Failure of RP-EBUS to detect the peripheral lesion after 20 minutes of scanning.

Following the previous criteria, the study initially included 63 cases and then three cases were excluded. Two of the excluded cases had endobronchial lesions detected by standard bronchoscopy. In the third case, we failed to detect the peripheral lesion by RP-EBUS after 20 minutes of screening.

Prior to the procedure, the included patients had to obtain a recent high resolution thoracic CT (HRCT) with contrast. This type of CT had a great significance in revealing the segmental and sub-segmental anatomy of the target lesions, selecting the ideal access and location of biopsy sites and visualizing the vascular structures surrounding the lesions. [(174)]

Within included patients, further criteria were required for eligibility to transbronchial cryobiopsy (TBCB) as the following:

- Fitness for general anesthesia; as TBCB required endotracheal intubation. This was decided by professional

anesthesiologists according to the patient's physical condition and investigations.

- Low risk of bleeding; severe bleeding was highly suspected if the patient had history of recent anticoagulant intake or proximity of the lung lesion to vessels >3mm in diameter identified by HRCT or radial probe EBUS during the procedures.

On the light of previous criteria, the included patients were furthermore divided into:

- Group I (TBCB eligible patients) which included 45 patients. Within this group; TBCB were performed in addition to forceps transbronchial biopsies (forceps TBB).
- Group II (TBCB non-eligible patients) which included 15 patients; only forceps TBB were performed and/or samples for cytological examination as brush samples, bronchial wash and bronchoalveolar lavage (Fig 11).

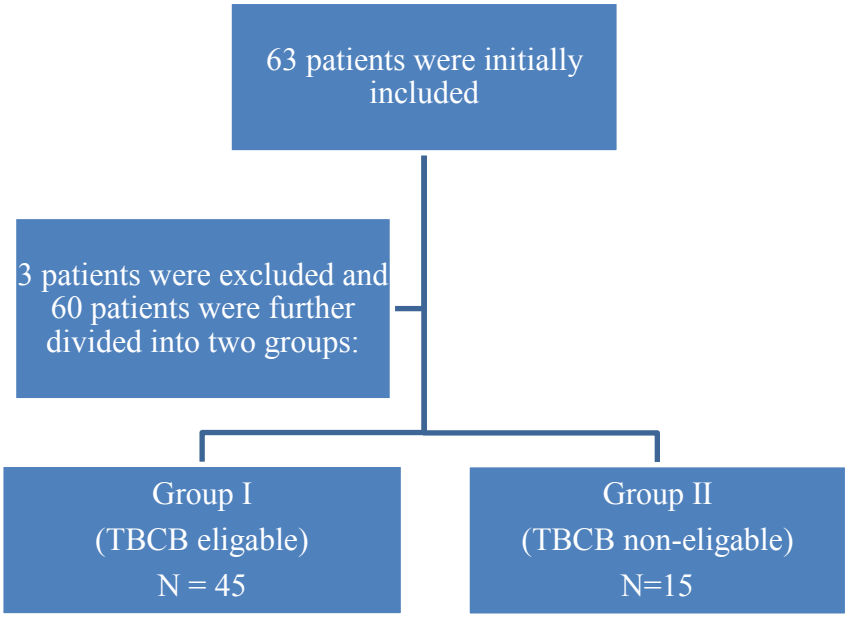

Figure 11. Algorithm of the study.

4.2 Anesthesia and ventilation

Anesthesia was mediated by intravenous midazolam, propofol and/ or fentanyl in accordance with the standard recommendations for anesthesia during bronchoscopic procedures. [175] Patients who were selected for TBCB were subjected to general anesthesia with endotracheal intubation (Armored tracheal tube size 8.5mm, Bronchoflex set, RUSH, Germany) (Fig 12).

The endotracheal tube had a side pathway which allowed the insertion of Fogarty balloon that was essential for mechanical compression at the site of biopsy to prevent massive bleeding as will be discussed later. During general anesthesia, the patients were connected to double Swivel adapter with fiberoptic cap (Fig 13). The adapter connected the endotracheal tube with the mechanical ventilator and allowed bronchoscopic passage through a third opening.

Patients who were not eligible for TBCB were subjected to deep sedation with spontaneous breathing and oxygen supply through an oronasal mask. In both types of anesthesia, the vital data of the patients including respiratory rate, oxygen saturation, ECG and blood pressure were closely monitored during the procedure.

Figure 12. Endotracheal tube.

Figure 13. Double Swivel adapter.

4.3. Bronchoscopic detection of the lesions

Before starting the procedure, the video bronchoscope (OLYMPUS BF-P190, slim 4.2mm outer diameter and 2.0mm instrument channel) was connected to the video processor (EVIS EXERA III Video System Center CV-190, OLYMPUS) while the radial ultrasound probe (OLYMPUS UM-S20-17S with outer diameter 1.4mm and frequency 20MHz) was connected to the probe driving unit (OLYMPUS MAJ-935) (Fig 14).

After speculating the proper bronchoscopic pathway to the lesion using the HRCT images, the flexible bronchoscopy was introduced through the oral route to the target bronchial segment. At the bifurcation of the target bronchial segment, the radial ultrasound probe was advanced through the bronchoscopic working channel to the lumen of the target segment.

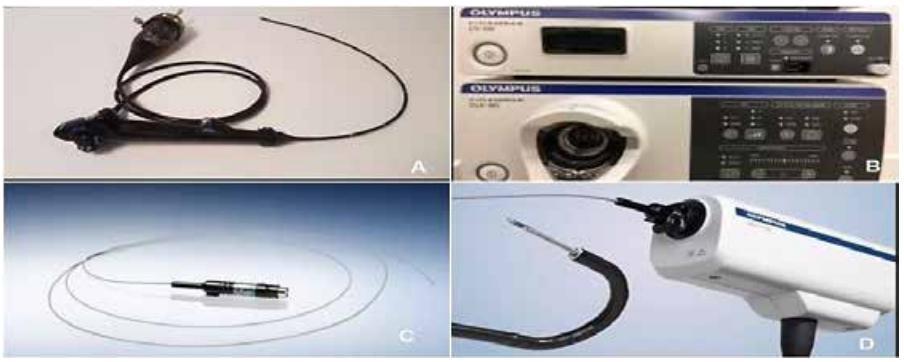

Figure 14. A) Video bronchoscope. B) Video processor. C) Radial EBUS probe. D) Probe driving unit.

On contact with the bronchial walls, the radial probe was operated to rotate for 360 degrees and screening the parenchymal structures surrounding the target bronchial segment. The generated ultrasound images were monitored by the operating team. The characteristic “snow storm” appearance of the air-filled alveoli was considered as normal ultrasound finding of the surrounding parenchyma and radial probe was further advanced for screening of the parenchymal pathology.

If RP-EBUS failed to obtain any pathological ultrasound images from the target bronchial segment or the nearby segments after 20 minutes of screening, then the patient was excluded from the study. On the other hand, the radial probe rotation was stopped once the ultrasound images show the criteria of parenchymal pathology e.g. grey homogenous shadows with sharp bright borders for solid masses or heterogeneous shadows with no specific borders in inflammatory lesions.

Using the video processor, the pathological images were captured and the diameters of suspicious lesions were measured (Fig 15). The ultrasound images were also examined for vascular structures surrounding the lesion which appeared as black homogenous spaces. The patient was considered non-eligible for transbronchial cryobiopsy if the diameter of nearby vessels exceeded 3mm due to high risk of severe bleeding.

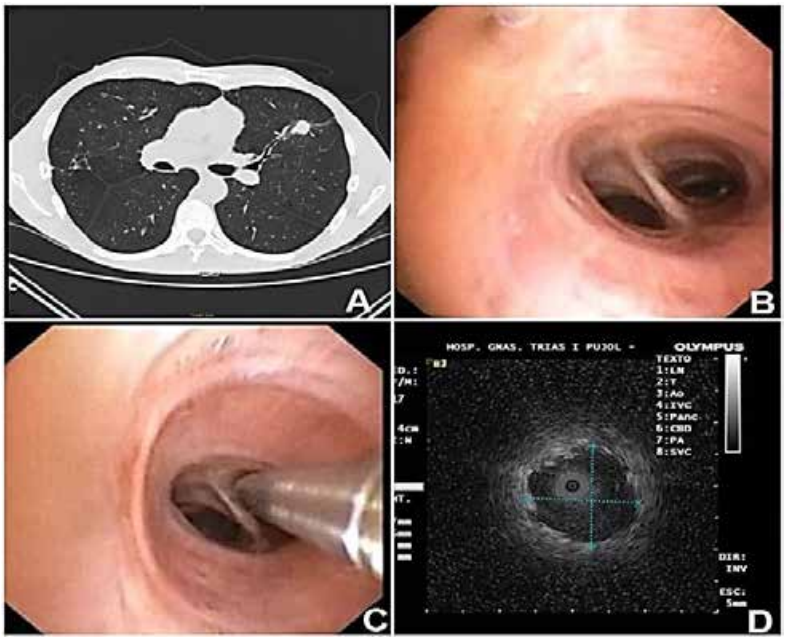

Figure 15 (Case 1): A) Thoracic CT showing a solitary nodule in the left upper lobe. B) White light image for the airways by bronchoscope. C) Radial probe EBUS rotating in the target segment in contact with the bronchial walls. D) Ultrasound image of the nodule captured and measured.

4.4. Transbronchial biopsy procedure

Once the lesion was visible on ultrasound monitor, the distance to the nearest segmental bifurcation was calculated in centimeters. For more accuracy, three measurements were taken 1) distance to the beginning of lesion where the pathological ultrasound images started to be visualized, 2) distance to the center of lesion where the pathological ultrasound images were most clear "biopsy site" and 3) distance to the distal end of the lesion where the normal snow storm appearance was re-visualized. Then, the radial probe was withdrawn from the bronchoscopic working channel.

Before starting the transbronchial biopsy maneuvers, a Fogarty catheter sized 5 or 6 F was introduced through the side channel of the endotracheal tube to reach the site of biopsy (Fig 16). The importance of this ballooned catheter was to apply compression on the biopsy site to prevent massive endobronchial

hemorrhage also to prevent bleeding from obstructing other lung segments. The size of the used catheter was decided by the operators before starting the procedure according to the segment that had to be occluded. [176]

Furthermore, a flexible forceps (HEYINOVO WF-1810BS, diameter 1.8mm) was advanced through the working channel until reaching the biopsy point then 4-5 transbronchial forceps biopsies were taken. After evaluating the collected samples to ensure their sufficiency for pathological examination, the forceps was withdrawn from the bronchoscopic working channel. Then, a flexible cryoprobe (ERBE cryoprobe 1.9mm) was connected to a cryo-unit (ERBOKRYO-C, 230V) with attached nitrous oxide (N_2o) tank and a footswitch to control the cryobiopsy time (Fig 17). After testing the cryo-system by generating an ice ball at the tip of the cryoprobe, it was advanced through the bronchoscopic working channel to the biopsy site. The operator then switched on the cryo-system by pressing the footswitch for 3-4 seconds to achieve cryobiopsy. After 4-5 sufficient cryobiopsies, the cryobrope was withdrawn.

It should be noted that the forceps biopsy were suitable to be withdrawn through the bronchoscopic working channel, while the cryobiopsies were too large so the operator had to remove the whole bronchoscope outside the endotracheal tube after each cryobiopsy which has consumed more time. Also, the operator assistant had to inflate the Fogarty balloon following each cryobiopsy to achieve hemostasis. Before starting the following cryobiopsy, Fogarty balloon was deflated and any active bleeding was managed by installing cold saline or local adrenaline. The severity of bleeding following forceps or cryobiopsy was estimated by the time needed by Fogarty balloon to achieve hemostasis.

In patients who were not eligible for TBCB, only 4-5 transbronchial forceps biopsies were made. Within this group, cytological means as transbronchial needle aspiration, bronchoalveolar lavage or protected brush sampling at the biopsy

site were done to increase the diagnostic value. When the risk for bleeding was very high, even the transbronchial forceps biopsies were avoided and cytological means were only done (Fig 18).

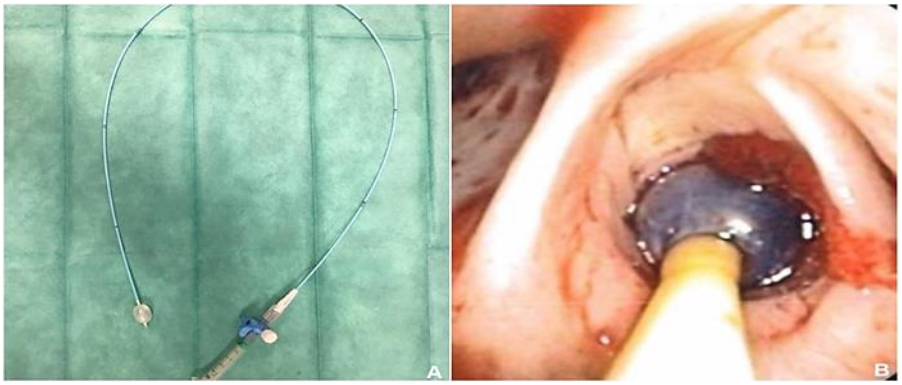

Figure 16. Fogarty catheter.

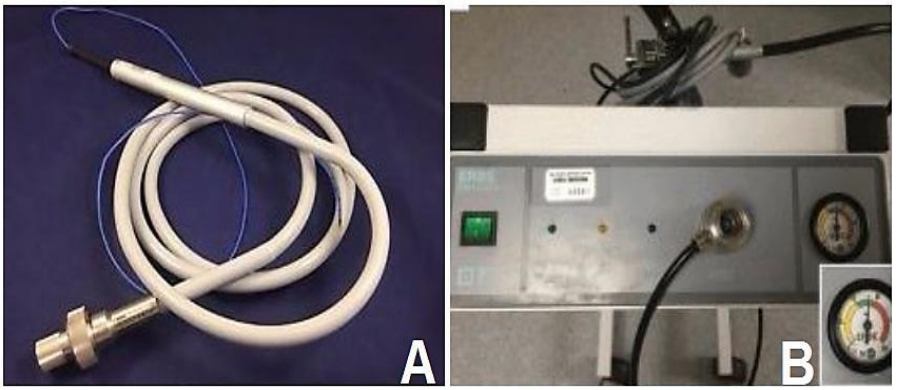

Figure 17. A) Flexible cryoprobe. B) Cryo-unit connected to N_2o tank.

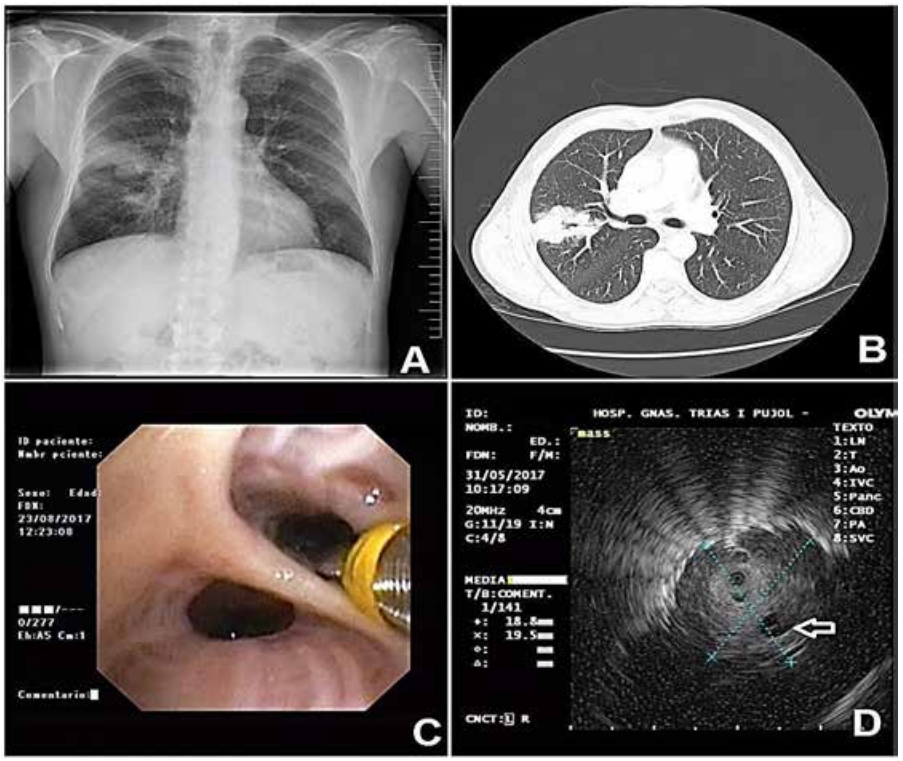

Figure 18 (Case 2): A) Chest X-ray showing a mass in right lung. B) Thoracic CT showing the mass in the right middle lobe. C) Radial probe EBUS operating to visualize the mass. D) Ultrasound image of the mass showing a nearby vessel (arrow) with high risk of severe bleeding if TBCB was performed.

4.5. Virtual bronchoscopy

In 12 patients, virtual bronchoscopy system (Lung Quest software/Bronchus Lung Point) was used to give further information about the locations of target lesions. HRCT images were introduced to the system to create 3-dimensional images for the airways. The VB role was restricted only to the planning phase; which is a prebronchoscopic phase to create theoretical routes to the target lesions based on the HRCT images.

Selected cases for VB planning had obtained thoracic HRCT with ≤ 1mm slice thickness devoid of respiratory artifacts to ensure accurate VB images. Following that VB system created axial, sagittal and coronal cross sectional images for the lesions

and their possible bronchial routes. Further information were obtained about; the distance between the lesion and the last accessible bronchial bifurcation; distance to the biopsy site and distal end of the lesion; number of bronchial bifurcations from trachea to the lesion and diameter of the bronchus where the lesion is located to evaluate the accessibility of the bronchoscope and biopsy tools.

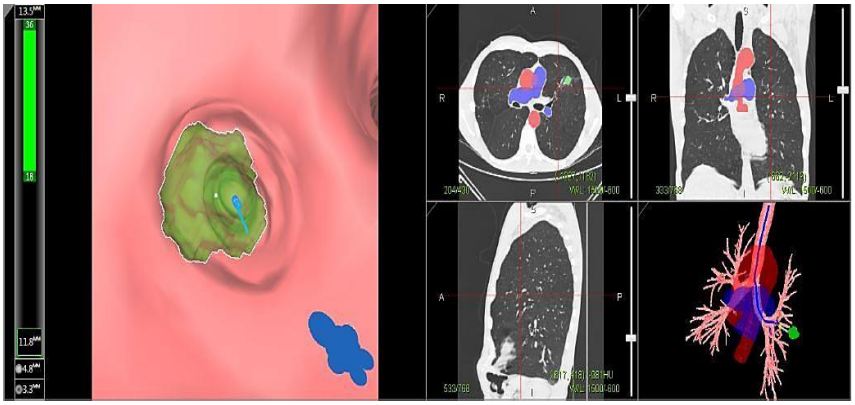

Figure 19. VBN navigation to (case 1) that was illustrated previously.

4.6. Samples collection and pathological analysis

Histopathological samples of forceps and cryobiopsies were handled carefully and fixed separately with formalin (Fig 20). Later, the samples were applied for haematoxylin and eosin and periodic acid–Schiff stainings and examined by professional pathologists at pathology department of Hospital Universetari Germans Trias i Pujol, Badalona, Barcelona. In some cases, immunohistochemical markers were used to establish a definitive diagnosis of malignancy.

Cytological samples were applied for Papanicolau stain to reveal malignant cells. If both cryobiopsy and forceps biopsy were decided to be avoided for high risk of bleeding, the pathologist had to attend the procedure for rapid on site cytopathological examination (ROSE) to decide the viability of the cytological samples. In some cases with high possibility of infectious underlying pathology, BAL and brush samples were

applied for microbiological examinations e.g. smear stain, Ziehl–Neelsen stain and bacterial cultures.

The procedure was considered diagnostic when a definitive histopathological or cytological diagnosis was established. At least one of the four collected samples by forceps or cryobiopsy had to be positive to describe the procedure as diagnostic. Otherwise, the procedure was considered non-diagnostic when the histopathological and cytological examination failed to obtain a definitive diagnosis. In this case, further procedures were decided to obtain a definitive diagnosis e.g. CT guided transthoracic biopsy, surgical biopsy or radiological follow up for two years to exclude malignancy.

The data including the patients' characteristics, biopsy techniques, associated complications and histological findings were recorded.

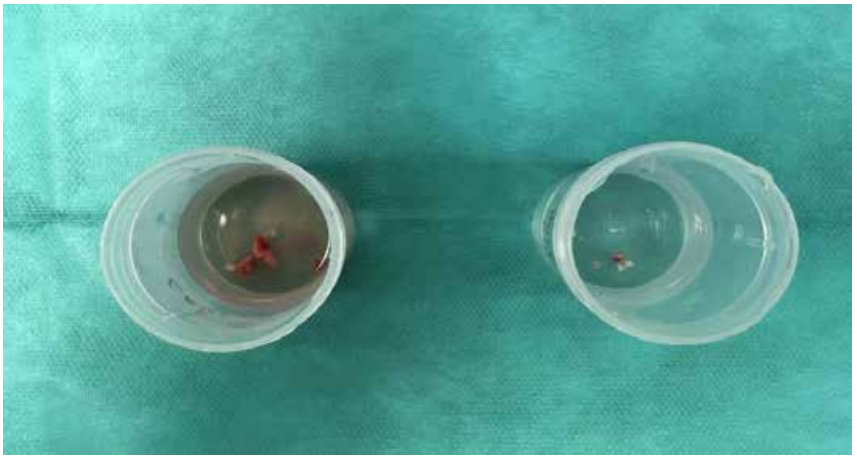

Figure 20. Collected samples of cryobiopsy (left) and forceps biopsy (right).

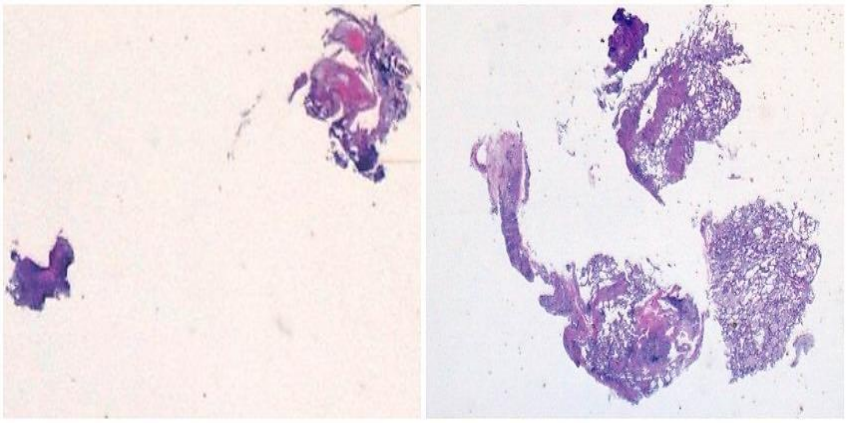

Figure 21. Microscopic images of forceps TBB (left) and TBCB (right) samples for the same patients. The images showing significant difference in the samples sizes in favor of TBCB (1x1.5 mm versus 5 x7 mm).

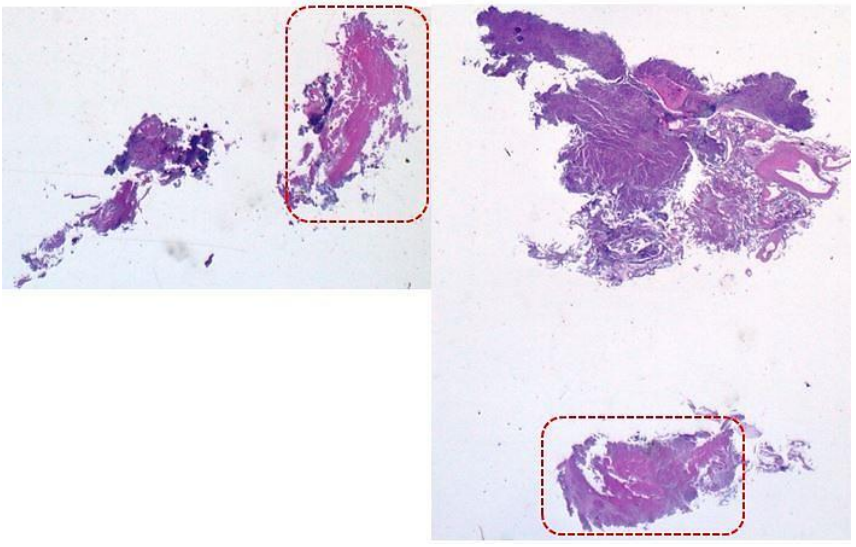

Figure 22. Microscopic images of necrotic areas (marked in red) in forceps TBB (left) and TBCB (right) samples for the same patient. The images showing that necrotic area percentage in TBCB (25%) is much lesser than forceps TBB (80%).

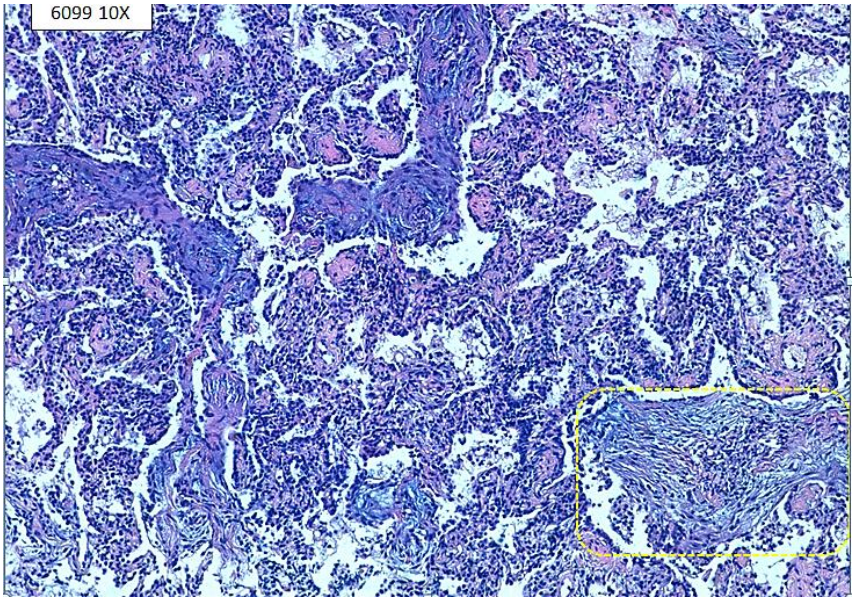

Figure 23. A microscopic image of organizing pneumonia, in which there are much thickened alveolar spaces - in comparison with the normal alveolar walls, and type 1 and 2 pneumocytes are seen and are full of air-, together with areas of fibrosis (yellow frame).

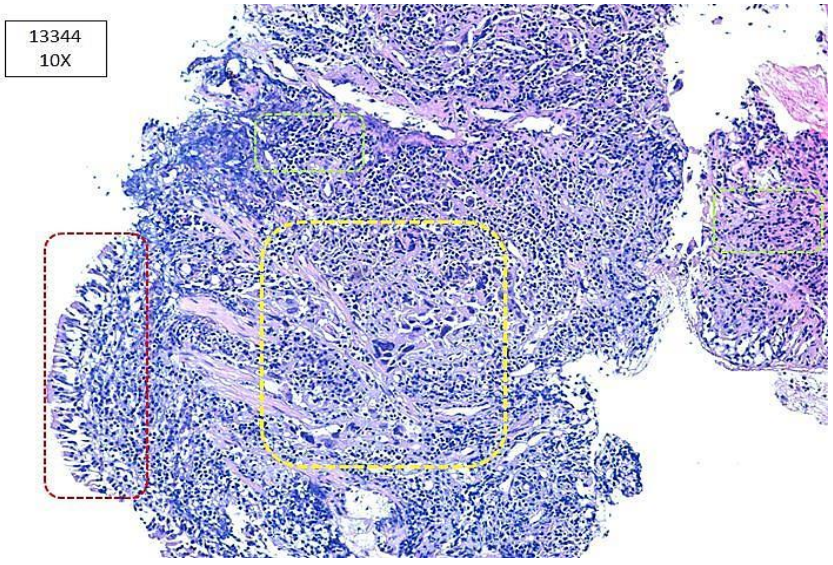

Figure 24 (A). A microscopic image showing markedly atypical cells (yellow frame) compared to the surrounding cellularity constituted by normal bronchial epithelium (red frame) and an infiltrate chronic inflammatory with predominance of lymphocytes (green frame).

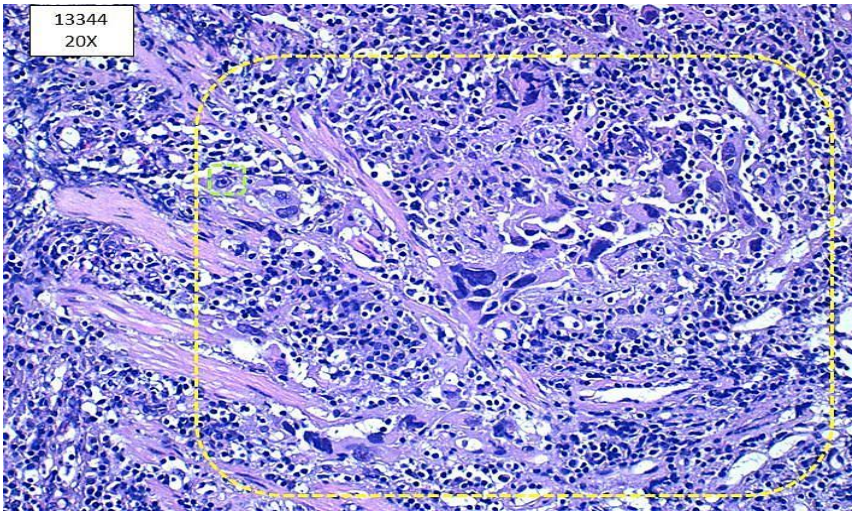

Figure 24 (B). At higher magnification of the previous image, the cells corresponding to the lesion-poorly differentiated adenocarcinoma- are seen in greater detail (yellow frame) showing atypical nuclei, irregular contours, alteration of the nucleus-cytoplasm ratio, with a reduction of the cytoplasm and nuclear pleomorphism. The Immunohistochemical techniques and the positivity of TTF-1 confirmed the diagnosis of a poor differentiated adenocarcinoma.

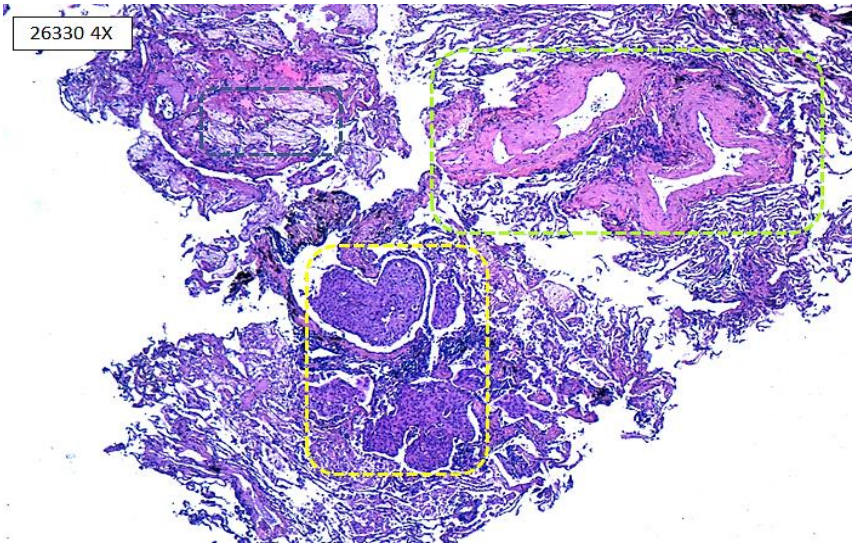

Figure 25 (A). A microscopic image with thick-walled vascular structures (green frame), some edema (black color), and the interior of the alveolar spaces is covered by a few cell nests (yellow frame)

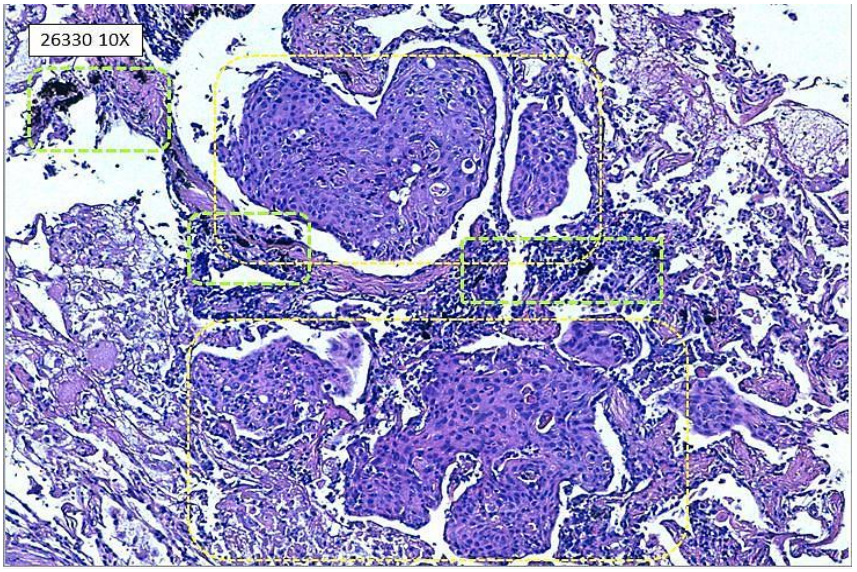

Figure 25 (B). At higher magnification we see anthracosis (green frame), along with markedly atypical cell nests, with a cohesive growth pattern, inside the alveoli (yellow frame) with the diagnosis of squamous cell carcinoma.

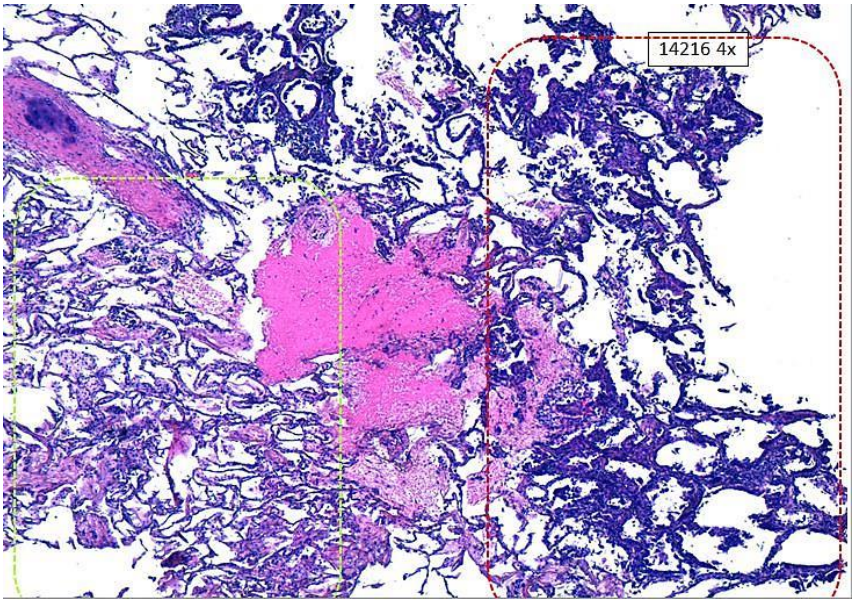

Figure 26 (A). A microscopic image showing thickening of the alveolar septa (red frame) observed on the right side of the image, compared to the left side, where we see normal alveolar spaces, with thin walls (green frame).

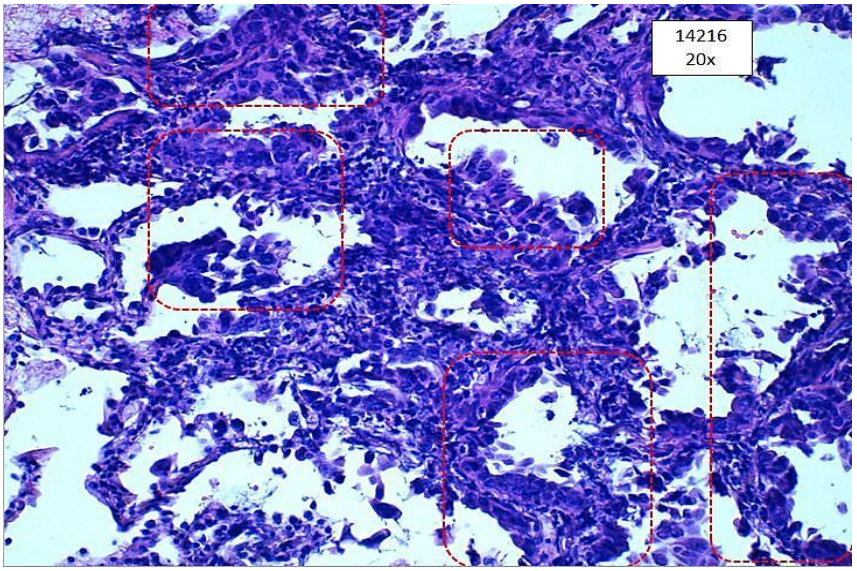

Figure 26 (B). At higher magnification, it could be seen that these alveolar septa are lined by markedly atypical cells (lepid pattern), with nuclei have irregular membranes, scarce cytoplasm, and prominent nucleolus, with a markedly disordered disposition (red frames). The diagnosis was Adenocarcinoma with lepidic pattern.

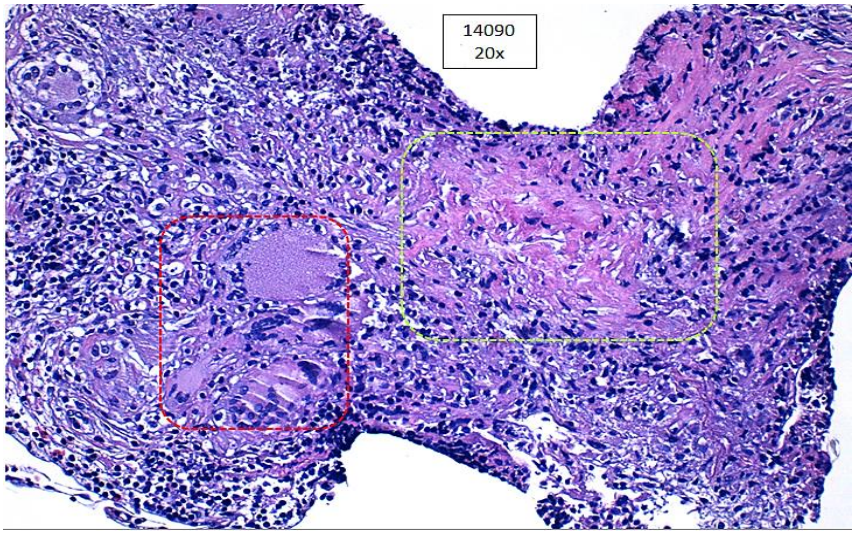

Figure 27. A microscopic image showing proliferation of granulomatous habit. Also, numerous multinucleated giant cells (red frame) and epithelioid cells with elongated nuclei and abundant cytoplasm (green frame). Diagnosis was TB.

RESULTS

5. RESULTS

5.1 Characteristics of included patients

The study included 60 patients with peripheral lung lesions that couldn't be visualized by standard bronchoscopy. The statistical analysis of this study was performed using the data analysis and software STATA (version 15). The patients were 45 males (75%) and 15 females (25%). The mean age for included patients was 66.6 ± 12.4 years (range 35.5 - 87.5). Most of patients were active smokers (68.3 %) while (3.3%) were ex-smokers and (28.3%) were never smokers. 12 patients (20%) had history of previous malignancy. The mean diameter for the included peripheral lung lesions on CT scan was 38.9 ± 16.8 mm, and the mean distance between the lesions and pleura was 12.8 ± 16.9 mm, range (0-64mm) (Table 2).

The most frequent radiological pattern on CT was mass in 22 cases (36.7%) followed by; solitary nodule in 19 cases (31.7%), consolidation in 13 cases (21.7%), reticular/ reticulonodular pattern in 4 cases (6.7%) and cavity in 2 cases (3.3%) (Table 3). Regarding the location of the lesions, 19 lesions were located in left upper lobe (31.7%), 12 in right upper lobe (20%), 12 in right lower lobe (20%), 10 in right middle lobe (16.7%) and 7 in left lower lobe (11.7%) (Table 4).

Table 2. Characteristics of included patients (n=60).

Variable	Subsets		Number	Percentage
Age (years)	Mean ± SD	66.6 ± 12.4		
	Range	35.5-87.5		
Gender	Male		45	75%
	Female		15	25%
Smoking	Chronic active		41	68.3%
	Ex-smoker		2	3.3%
	Never smoker		17	28.3%
Diameter of lesion on CT (mm)	Mean ± SD	38.9 ± 16.8		
	Range	11-90		
Distance to pleura (mm)	Mean ± SD	12.8 ± 16.9		
	Range	0-64		

Table 3. Radiological patterns of the lesions on CT (n=60).

Radiological pattern	Number	Percentage
Mass	22	36.7%
Solitary nodule	19	31.7%
Consolidation	13	21.7%
Reticular/ reticule-nodular	4	6.7%
Cavity	2	3.3%

Table 4. Location of the lesions on CT (n=60).

Location	Number	Percentage
Left upper lobe	19	31.7%
Right upper lobe	12	20%
Right lower lobe	12	20%
Right middle lobe	10	16.7%
Left lower lobe	7	11.7%

5.2 Outcomes of forceps TBB and TBCB in group I

Group I included 45 patients who were eligible for TBCB. The patients were subjected for forceps TBB and TBCB. With forceps TBB the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were; 67.5%, 100%, 100%, 18.8% and 69.8% respectively. While TBCB had sensitivity, specificity, PPV, NPV and accuracy of 75%, 100%, 100%, 23.1%, 76.7% respectively. When both techniques were combined for the same lesions, the diagnostic yield was improved with sensitivity, specificity, PPV, NPV and accuracy of 82.5%, 100%, 100%, 30% and 83.7% respectively (Table 5).

Table 5. Diagnostic yields for forceps TBB versus TBCB in group I (n=45).

Variable	Forceps TBB	TBCB	Combined
Sensitivity	67.5%	75%	82.5%
Specificity	100%	100%	100%
PPV	100%	100%	100%
NPV	18.8%	23.1%	30%
Accuracy	69.8%	76.7%	83.7%

Illustration of the diagnostic yields could be achieved by graphic plots where accuracy is measured by the area under the curve in the receiver operating characteristic curve (ROC curve). An area of 1 represents a perfect test and an area of 0.5 represents a worthless test. A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system: [177]

- 0.90-1 = excellent (A)
- 0.80-0.90 = good (B)
- 0.70-0.80 = fair (C)
- 0.60-0.70 = poor (D)
- 0.50-.060 = fail (F)

TBCB has achieved higher accuracy than forceps TBB (ROC area of 0.88 versus 0.84 respectively). Based on the previous point system, both techniques had good accuracy with no statistical difference between their values (p = 0.32). The combination between both techniques has achieved excellent accuracy (ROC area 0.91). Comparing each single technique with the combined accuracy (forceps TBB + TBCB), there was a significant difference in the statistical analysis between forceps TBB alone versus the combined accuracy (ROC are of 0.84 versus 0.91 with p = 0.008) while this statistical difference was not significant between TBCB and combined accuracy (ROC 0.88 versus 0.91 with p = 0.075) (Figures 28-30).

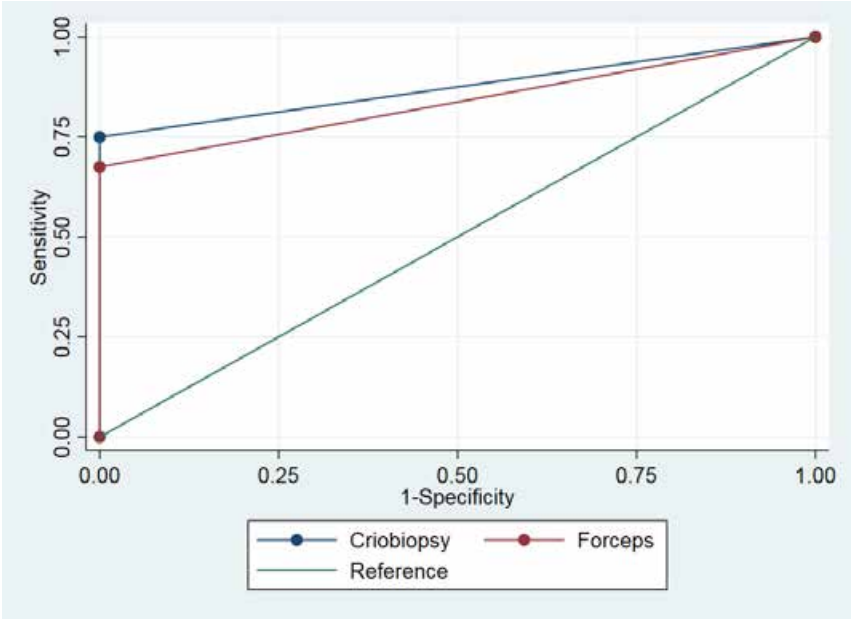

Figure 28. Forceps TBB versus TBCB accuracy.

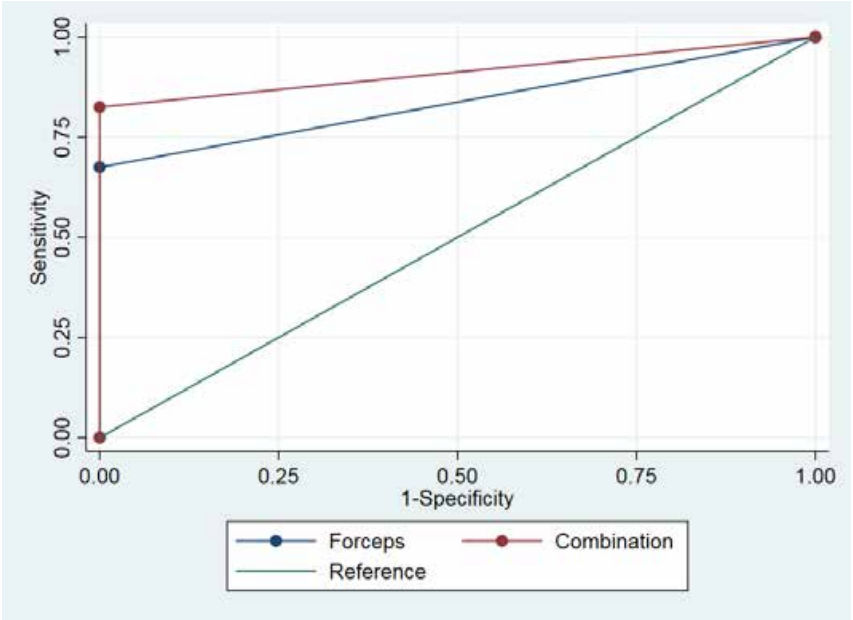

Figure 29. Forceps TBB versus combined techniques accuracy.

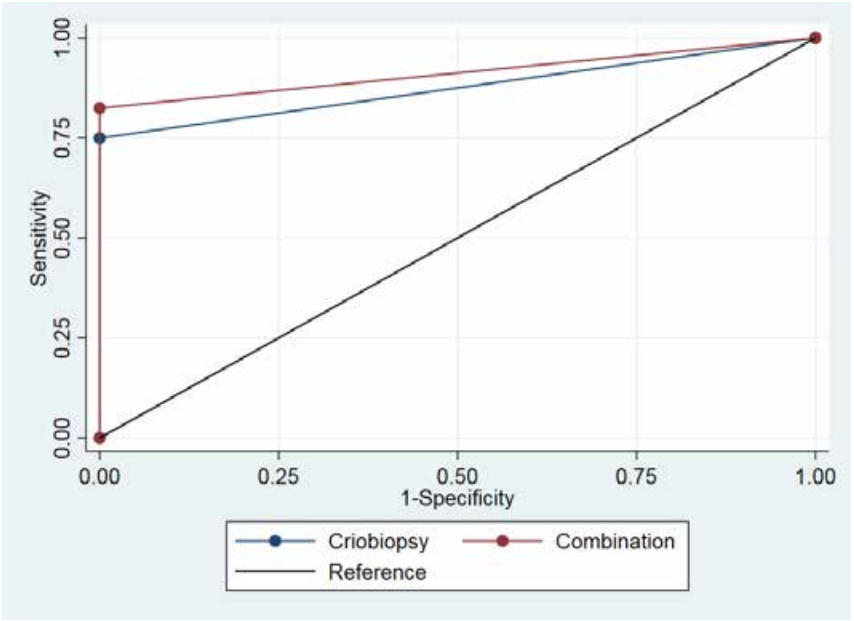

Figure 30. TBCB versus combined techniques accuracy.

As for quantitative and qualitative comparison between the two biopsy techniques, samples from 9 cases were considered inadequate and discarded by the pathologists. In the remaining 36 cases TBCB was found to have larger samples with better quality than forceps TBB. In forceps TBB the mean biopsy number was 4.1 ± 0.6 and the biopsies had the following outcome; mean diameter of 4 ± 1.6 mm, mean surface area of 12.7 ± 1.7 mm2, mean necrotic tissue percentage of 6.8 ± 15.9 %, mean artifact percentage of 10.6 ± 15.8%, mean viable tissue percentage of 81.1 ± 27.3 % and mean viable tissue surface area of 12.3 ± 16.5 mm2. For TBCB, the mean number of biopsies was 3.97 ± 0.6 and the biopsies had the following outcome; mean diameter of 7.3 ± 2.1 mm, mean surface area of 38.6 ± 20.4 mm2, mean necrotic tissue percentage of 1.3 ± 4.3 %, mean artifact percentage of 15.3 ± 24%, mean viable tissue percentage of 89.8 ± 18% and mean viable tissue surface area of 34.9 ± 17.9 mm2.

There were significant statistical differences ($p \leq 0.05$) in favor of TBCB when compared to forceps TBB regarding; mean

biopsy diameter, mean biopsy surface area, mean biopsy necrosis percentage and mean biopsy viable tissue area (Table 6).

Table 6. Quantitative and qualitative assessment of forceps TBB versus TBCB biopsies (n=36).

Variable	Forceps TBB	TBCB	P value
Mean biopsy number ± SD	4.1 ± 0.6	3.97 ± 0.6	0.98
Mean biopsy diameter (mm) ± SD	3.9 ± 1.6	7.3 ± 2.1	< 0.01*
Mean biopsy surface area (mm2) ± SD	12.7 ± 1.7	38.6 ± 20.4	< 0.01*
Mean biopsy necrotic area % ± SD	6.8 ± 15.9	1.3 ± 4.3	0.008*
Mean biopsy artifact % ± SD	10.6 ± 15.8	15.3 ± 24%	0.21
Mean biopsy viable tissue % ± SD	81.1 ± 27.3	89.8 ± 18	0.053
Mean biopsy viable tissue surface area (mm2) ± SD	12.3 ± 16.5	34.9 ± 17.9	< 0.01*

5.3 Diagnostic outcomes in group II and overall the study

Group II included 15 patients, who are non-eligible for TBCB. Those patients were applied for cytological retrieving means as BAL, TBNA and/or bronchial brushing with or without forceps TBB under guidance of RP-EBUS which achieved sensitivity of 80 %.

Regarding the overall results including both groups, generally RP-EBUS achieved sensitivity, specificity, PPV, NPV and accuracy of 85.2%, 100%, 100%, 42.8% and 86.7% respectively. In 14 cases (23.3%) further investigations had to be performed to confirm the underlying pathologies including; CT guided TTNA, surgical biopsy and radiological follow up. Out of those 14 cases, 5 patients (8.3%) had adenocarcinoma, 2 patients (3.3%) had metastatic lesions, 3 patients (5%) had non-specific inflammations, 3 patients (5%) had benign neoplastic lesions and 1 patient (1.7) had sarcoidosis. The final histopathological

outcome of included patients either achieved by RP-EBUS or other means is shown in table 7.

Table 7. Histopathological diagnosis of the included patients (n = 60).

Histopathological diagnosis	Diagnostic RP-EBUS. N (%)		Non diagnostic RP-EBUS. N (%)	
Malignant lesions				
• Adenocarcinoma	28	46.7%	5	8.3%
• Squamous cell carcinoma	5	8.3%	-	-
• Undifferentiated NSCLC	4	6.7%	-	-
• Neuroendocrine tumors	2	3.3%	-	-
• Metastasis	-	-	2	3.3%
Benign lesions				
• NSI	-	-	3	5%
• Typical TB	2	3.3%	-	-
• Atypical TB	1	1.7%	-	-
• Other bacterial infections	2	3.3%	-	-
• Sarcoidosis	-	-	1	1.7%
• UIP	1	1.7%	-	-
• BOOP	1	1.7%	-	-
• Benign neoplastic	-	-	3	5%

NSCLC = non-small cell lung cancer. NSI = non-specific inflammation. UIP = usual interstitial pneumonia. BOOP = bronchiolitis obliterans organizing pneumonia.

5.4 Factors affecting the diagnostic value of RP-EBUS.

Size of the lesion, nature of the lesion, positive bronchus sign and position of the radial probe within the lesion are four factors that were analyzed to determine their effect on the diagnostic value of RP-EBUS. 41 cases had lesions with diameter of ≥ 3cm on CT, within these cases RP-EBUS sensitivity was 87.8% while 19 cases had lesions with diameter < 3cm on CT, where RP-EBUS sensitivity was 52.6%. There was a significant statistical difference between sensitivity of RP-EBUS between the two groups (p = 0.003).

Regarding the nature of lesions, 46 cases (76.7%) had final diagnosis of malignancy, where RP-EBUS sensitivity was 84.8%,

while 14 cases (23.3%) had final diagnosis of benign lesions where RP-EBUS sensitivity was 70%.

44 cases (73.3%) had positive bronchus sign on CT; among them RP-EBUS was diagnostic in 34 cases (77.3%) and non-diagnostic in 10 cases (22.7%). The probability of RP-EBUS to be diagnostic among patients with positive bronchus sign was significantly higher than being non-diagnostic ($p < 0.01$). The same findings were noticed when the radial probe was placed within the target lesions among 43 cases (71.7%). 36 cases were diagnosed by RP-EBUS (83.7%) while 7 cases (16.3%) had negative RP-EBUS ($p < 0.01$).

5.5 Associated complications

All the complications during or after the procedure were recorded. Overall, 12 patients (20%) had bleeding but 11 (18.3%) of them had moderate bleeding (grade II) and only one patient (1.7%) had significant bleeding (grade III). In TBCB eligible group of patients (45 patients), 8 patients had grade II bleeding following TBCB (17.8%) and 3 patients following forceps TBB (6.7%). The bleeding was more frequent with TBCB than forceps TBB. The only patient who suffered significant bleeding (grade III) was among the TBCB non-eligible group of patients and the bleeding followed forceps TBB.

One patient (1.7%) had pneumothorax which was detected by the routine X-ray after the procedure. The patient did not require intercostal tube drainage and the management was mainly conservative. Another patient (1.7%) suffered from hypoxemia ($SpO2 < 90\%$ and $PaO2 < 60$ mmHg) following the procedure and was managed by oxygen supply. No life threatening complications were recorded.

Table 8. Complications recorded in the study.

Variable	Number (n=60)	Percentage
Bleeding • Grade II • Grade III	 11 1	 18.3% 1.7%
Pneumothorax	1	1.7%
Respiratory failure	1	1.7%

DISCUSSION

6. DISCUSSION

Diagnosis of peripheral lung lesions is a challenging task for pulmonologists. The absence of endobronchial extension for peripheral lesions makes the diagnostic role of conventional bronchoscopy limited. With high probability of underlying neoplastic etiology, early and accurate diagnosis of peripheral lung lesions turns out to be demanding.

Lung cancer is one of the most common malignancies in both sexes worldwide. Prognosis is mainly affected by the stage of disease at the time of diagnosis. Unfortunately, lung cancer is firstly diagnosed during advanced stages in most of patients which results in low curative rate with the available therapies. On the contrary, diagnosis of lung cancer in early stages may lead to definitive cure through surgical resection or other means. Screening of lung cancer in high risk population using low-dose CT has been able to decrease lung cancer related death rates. [178]

Differential diagnosis of peripheral lung lesions is very wide, including infectious, inflammatory, vascular, granulomatous and neoplastic etiologies. For example, 50 % of solitary pulmonary nodules larger than 2cm in diameter are likely to be malignant lesions. Although presence of radiological features suggesting malignancy could be helpful, pathological diagnosis is required for confirmation and assessment of most suitable methods of treatment. [179] Non-neoplastic etiologies are also important to be accurately diagnosed. Pneumonia, tuberculosis, sarcoidosis and interstitial lung diseases could be presented as peripheral lung lesions in radiology. Definitive diagnosis of such diseases is essential to decide the appropriate lines of treatment that could decrease the associated morbidity and mortality. [180]

Some peripheral lung lesions may show low probability for lung cancer as; lesions that improve clinically and radiologically on therapy, infiltrations with radiological features of interstitial lung diseases and nodules without malignant growth pattern in

radiological follow up. On contrary, other lesions may suggest high susceptibility of lung cancer especially when associated with history of chronic smoking, other malignancies or positive family history of cancer. [181]

Diagnostic interventions for peripheral lung lesions could be generally classified into surgical and non-surgical methods. Surgical biopsy or resection is selected for lesions with high probability of malignancy without conclusive diagnosis by other means. However, many patients could be considered not candidates for surgery due to associated comorbidities or extended disease. Also, many professionals are convinced that less invasive diagnostic techniques should be utilized initially before referring the patients to surgery, especially those without high risk of malignancy. [182] Non-surgical methods or so called minimally invasive techniques include transthoracic biopsy and bronchoscopic transbronchial biopsy. These maneuvers should be considered when a definitive diagnosis of the underlying lung pathology is required, mainly if malignancy is suspected. TTNA is proposed for more peripheral lung lesions especially with pleural contact. [183]

Accuracy of transthoracic needle biopsy under CT is dependent on many factors as; the size of lesion and distance from pleura. It could achieve high value of sensitivity ranging from 74% to 96% in malignant lesions while specificity is nearly 100%. However, a major drawback in this technique is the high incidence of associated pneumothorax. The risk of pneumothorax increases with COPD patients and lesions distal to pleura and could reach 15-20% according to different studies. [184,185]

As for bronchoscopic maneuvers, conventional bronchoscopy has limited role in diagnosing peripheral lung lesions. Non-guided conventional bronchoscopic TBB has achieved diagnostic sensitivity of 57% and 43% for BAL. [186] Therefore using bronchoscopic guided TBB was assumed to achieve higher diagnostic values. Many techniques were developed to guide bronchoscopic TBB and improve the

diagnostic value as: fluoroscopy, RP-EBUS, VBN and ENB while more recent techniques are still under trials as BTPNA.

The present study was performed in a high risk population for lung cancer as 68.3% of the included patients had positive history of chronic smoking and 20% had history of other malignancies. The high possibility of neoplastic origin besides comorbidities in many patients made the accurate and definitive diagnosis of peripheral lung lesions, through a minimal invasive procedure, essential. The most frequent radiological pattern within included patients was lung mass in 22 patients (36.7%) and the least pattern was lung cavity in 2 patients (3.3%). The principal utilized technique for guidance of bronchoscopic TBB in all patients was RP-EBUS; however VBN was used as an adjuvant technique in 12 patients.

Regarding the diagnostic yields, the overall sensitivity for RP-EBUS guided TBB for 60 patients was 85.2%. In group I, TBCB achieved higher diagnostic values than forceps TBB as a biopsy tool among 45 patients. TBCB had sensitivity, specificity, PPV, NPV and accuracy of 75%, 100%, 100%, 23.1%, and 76.7% versus 67.5%, 100%, 100%, 18.8% and 69.8% for forceps TBB respectively. When the diagnostic yields of both biopsy tools were combined for the same patients, they achieved higher results with sensitivity, specificity, PPV, NPV and accuracy of 82.5%, 100%, 100%, 30% and 83.7% respectively. Although there was no significant statistical difference between accuracy of TBCB and forceps TBB, when the result of combined techniques was compared with each of them individually, a significant difference was noticed between forceps TBB and the combination accuracy.

The initial reports for utilizing RP-EBUS in diagnosis of peripheral lung lesions were in 2002. *Herth et al* reported a diagnostic value of 80 % for RP-EBUS compared with 76 % for fluoroscopy among included patients. The authors concluded that RP-EBUS is comparable with fluoroscopy for diagnosis of peripheral lung lesions with no associated risk of radiation. [187]

From that point, other studies have described RP-EBUS guided TBB as a feasible and effective technique for diagnosis of peripheral lung lesions. *Huang et al* reported a diagnostic value of 76% for RP-EBUS guided TBB. Furthermore, repeated RP-EBUS guided TBB was diagnostic in 53 % of cases with previously negative outcome. [188] *Boonsarngsuk et al* reported a diagnostic value of 79.9% for combined TBB and brush smear guided by RP-EBUS [189] while *Gnass et al* achieved sensitivity, specificity, accuracy, PPV and NPV of 75%, 97%, 83%, 97% and 70%, respectively.[190]

The largest meta-analysis for the value of RP-EBUS in diagnosing peripheral lung lesions was reported in 2017. *Ali et al* included 57 previous studies with total number that exceeded 7000 peripheral lung lesions. [191] The mean diagnostic value was 70.6 % which is much higher than conventional forceps and comparable with other technique with much more cost as ENB. The diagnostic value of RP-EBUS was higher (77%) when other guidance techniques as fluoroscopy were added. The authors reported that the factors impacting the diagnostic yield were; size of peripheral lung lesions, nature of the lesion (benign or malignant), positive bronchus sign on CT and location of the RP-RBUS probe within the lesion. Even though, other individual reports have published higher diagnostic values that could reach 92.3%. [192]

Our results were comparable with the previous studies regarding the diagnostic values of RP-EBUS guided forceps TBB. However, we achieved higher results when TBCB alone or combined with forceps TBB were evaluated. Several factors are contributed for the higher outcome of the current study. As mentioned, TBCB achieved superior diagnostic outcomes versus forceps TBB which were increased further when both biopsy tools were combined for the same patients. Additionally, many previous studies included peripheral lesions irrespective to their detection by RP-EBUS while we excluded lesions that were not visualized by EBUS. Also, many authors focused in evaluating

RP-EBUS for diagnosing solitary pulmonary nodules alone while we included all peripheral lesions on condition that they do not have endobronchial extension.

Schuhmann et al compared the diagnostic value of TBCB with forceps TBB when both were guided with RP-EBUS in 31 patients and reported a diagnostic value of 74.2% for TBCB versus 61.3% with forceps when 3 samples were taken by each technique. The study has reported a significant difference between TBCB and forceps biopsy only regarding the quantitative digital assessment (diameter and surface area) of the biopsies and did not evaluate the qualitative differences. [(174)] We assume that the difference of diagnostic values in *Schuhmann's* study in comparison with ours was owing to their inclusion of smaller lesions (29.7±7.3 versus 38.9 ± 16.8 mm) and performing less number of biopsies (6 versus 8 total biopsies). Other advantages in our study are the larger number of lesions to compare TBCB with forceps TBB (45 versus 31) and comparing the samples collected from both biopsy tools regarding both quantitative and qualitative measures as will be illustrated.

As mentioned TBCB had better diagnostic values, without significant statistical differences when compared with forceps TBB. However when each individual technique was compared to the combined results, there was a significant difference between forceps TBB and the overall accuracy while this difference was not found with TBCB. Furthermore, within similar number of biopsies (mean 4.1 biopsies for forceps TBB and 3.97 biopsies for TBCB) we noticed clear superiority for TBCB regarding quantitative and qualitative findings of the collected samples. There were significant statistical differences in favor of TBCB regarding mean biopsy diameter, mean biopsy surface area, mean biopsy necrotic percentage and mean biopsy viable tissue surface area.

TBCB has proved to be effective transbronchial biopsy technique. Several studies reported that TBCB could be utilized safely in diagnosis of diseases with peripheral lung involvment

and could provide a high diagnostic value. A large meta-analysis that included 14 studies and more than 1000 patients, who were subjected for TBCB for diagnosis of diffuse peripheral lung diseases, demonstrated a diagnostic value range of (76.9- 85.9%) for TBCB with subsequent pneumothorax in (6.8%) and severe bleeding in (0.3%). [193] The introduction of TBCB has reduced the demand for surgical lung biopsy to diagnose diffuse parenchymal lung diseases as it provided a comparable diagnostic value with fewer risks. When compared, TBCB achieved diagnostic value of 82.8% versus 98.7% for surgical lung biopsy with lower rate of complications including fever, persistent air leak and prolonged hospital admission. [194]

TBCB proved higher efficiency in comparison with other bronchoscopic transbronchial biopsy techniques. *Pajares et al* compared TBCB with forceps TBB for diagnosis of interstitial lung diseases guided by fluoroscopy. Authors reported a diagnostic value of 74.4 % for TBCB versus 34.1% for forceps biopsy. There was a significant difference in favor of TBCB regarding biopsy diameter, viable tissue and artifact percentage among total number of 266 lung tissue samples. Also, there was higher tendency for moderate bleeding following TBCB (56.4%) when compared to forceps TBB (34.2%) [195]

Even though, excluding *Schuhmann's* study, few other case reports have compared TBCB with other RP-EBUS guided biopsy tools or mentioned a detailed qualitative and quantitative outcomes of the collected samples. *Herath et al* (2017) compared RP-EBUS guided TBCB and forceps TBB in a case series that included only 6 patients using guide sheath in both techniques with a bronchoscopic working channel of (2.8 mm) diameter. The overall diagnostic value was (83%) and the authors compared the samples obtained by both techniques in only 4 patients with mean diameter of TBCB samples (6.4 mm) and forceps samples (3.4 mm). The authors didn't compare the quality of collected samples between both techniques. [196] *Chang et al* (2017) applied RP-EBUS guided TBCB alone in only 11 patients with peripheral

lung lesions after exclusion of 90 patients with endobronchial lesions. A definitive diagnosis was obtained in 9 patients (81.8%) without reported complications. The authors didn't compare between TBCB and other techniques. [197]

We believe that our study could be a leading one to compare RP-EBUS guided TBCB with transbronchial forceps biopsy including adequate number of patients and broad spectrum of quantitative and qualitative histological variants for collected samples. Also, in our study we used a thin bronchoscope (4.2mm) for better access to peripheral lesions, commercial cryoprobe (1.9 mm) and assistance of VBN in some cases.

We defined the patients who are non-eligible for TBCB as those who could suffer significant bleeding if subjected to TBCB. Certain criteria were established to consider the patients fit for TBCB including absence of large surrounding vessels on CT images or during RP-EBUS screening. Visualization of surrounding vascular structure to the lesions is an advantage of RP-EBUS over other guidance techniques as fluoroscopy, while obtaining this benefit with other techniques as ENB could be associated with higher cost. [198]

In group II that included 15 patients (20%) who were not eligible for TBCB, RP-EBUS guided forceps TBB or cytology retrieving tools as TBNA, bronchial brush and BAL achieved sensitivity of 80%. This is further proof for the efficiency of RP-EBUS as a guiding technique for diagnosis of peripheral lung lesions. Even when TBCB is avoided in patients with high risk for complications, RP-EBUS using cytology retrieving tools could be highly diagnostic. For this group, a pathologist attended the procedure to perform rapid on site cytological examination (ROSE) and evaluate the quality of collected samples. ROSE technique has proved to increase the diagnostic yield of cytological samples collected with EBUS guidance particularly regarding diagnosis of lung cancer. [199]

The overall sensitivity for malignant lesions and for benign lesions was 84.8% and 70% respectively. In 14 patients (23.3%) the diagnosis was not confirmed by RP-EBUS TBB alone and needed further investigations as CT guided TTNA, surgical biopsy or radiological follow up. Malignant lesions represented 76.7% of the included lesions while benign lesions represented 23.3%. There was no significant statistical difference for diagnostic values according to the underlying pathologies of the lesions. Microbiological analysis of the collected samples was able to diagnose five cases (8.3%) of bacterial infections (2 cases of bacterial infections other than TB, 2 cases of typical TB and 1 case of atypical TB).

The efficacy of RP-EBUS guided biopsy in detecting both malignant and benign peripheral lung lesions was proved by several studies. Through collecting adequate samples for evident histopathological diagnosis with low complications rate, RP-EBUS has become more favorable technique for diagnosis of lung cancer than other procedures as CT guided TTNA in many professional centers. [200] Furthermore, cytological and molecular analyses of samples collected with RP-EBUS guidance could be highly valuable in diagnosing NSCLC and epidermal growth factor receptor (EGFR) mutation. [201] *Guisier et al* successfully used samples collected by RP-EBUS guided forceps TBB for multi – gene molecular analysis in 111 cases with non-squamous NSCLC. [202]

Regarding lesions of benign etiology, *Chan et al* performed RP-EBUS guided TBB for peripheral lung lesions within a high risk population for tuberculosis. The authors reported a diagnostic value of 77.3% for tuberculous lesions when microbiological investigations were performed on guided TBB and BAL samples. They stated that RP-EBUS guided technique has achieved a comparable diagnostic value with CT guided TTNA with lower incidence of complications. [203] Another study by *Hayama et al* utilized RP-EBUS guided TBB only for peripheral cavitary lung lesions. The study showed diagnostic value of 74.1% for

malignant lesions and 69.6% for benign lesions. The most common benign etiology was non-tuberculous mycobacteria followed by pyogenic lung abscess and mycobacterium tuberculosis. [204]

Factors as; the lesion size, positive bronchus sign and location of the radial probe within the target lesion had remarkable impacts on the diagnostic value. Sensitivity of the procedure among lesions with diameter ≥ 3 cm on CT (87.8%) was significantly higher than in lesions smaller than 3 cm in diameter (52.6%). Furthermore, detecting positive bronchus sign on CT and locating the probe within the target lesions during the procedure were key predictors for a successful technique. Among cases with positive bronchus sign, RP-EBUS guided TBB was diagnostic in 77.3%. Similar findings were noticed when the radial probe was located within the lesions as RP-EBUS was diagnostic in 83.7%.

Paone et al reported RP-EBUS guided TBB sensitivity of 75% for lesions smaller than 3 cm in diameter versus 82% for lesions larger than 3 cm in diameter while *Georgiou et al* stated that the diagnostic value was significantly affected by the lesion size. The overall yield was 63% while in lesions measured 2 cm or less it was 46%. [205,206] Besides avoiding hazardous irradiation, RP-EBUS has another advantage over fluoroscopy as a navigational technique for TBB. Peripheral lesions with outer diameter less than 2cm could be missed by fluoroscopy but usually are detected by RP-EBUS. In the current study, six patients (10%) had peripheral lesions smaller than 2 cm in diameter. RP-EBUS had sensitivity of 50% among these patients. Similarly, *Eberhardt et al* reported a diagnostic value of 46% for RP-EBUS TBB in solitary pulmonary nodules less than 2 cm in diameter. [207]

Positive bronchus sign on CT indicates the presence of direct bronchial access to the lesion. A study that included 760 patients has described positive bronchus sign as the most influential radiological finding that could increase the visibility

field of RP-EBUS for the target lesions and consequently increase the accuracy of guided TBB. [208] *Oki et al* reported that finding a direct bronchial path to peripheral lung lesions by combining RP-EBUS with VBN and ultrathin bronchoscope has increased the diagnostic value for small lesions (3cm or less) to 74%. [87]

Several studies reported using guide sheath to maintain the biopsy tool situated within the target lesion during sampling. Using RP-EBUS guided TBB with guide sheath for peripheral lung lesions, *Sánchez-Font et al* had a diagnostic value of 78 % in 60 patients, *Ishida et al* had a diagnostic value of 64.6 % in 65 patients while *Menami et al* had a diagnostic value of 83.3% in 60 patients. [209-211] One of the limitations in our study is inability to use guide sheath for the biopsy tools. The used bronchoscope (OLYMPUS BF-P190) had a working channel of 2 mm; while the commercially available cryo-probe had an outer diameter of 1.9 mm. Using guide sheath for TBCB was impossible, while using it only for forceps TBB would give it an extra advantage and affect the reliability of the study. Even though, in a similar number of included patients we achieved higher overall diagnostic value than the previous studies. Again, we should refer that in our study we utilized TBCB and included all the peripheral lung lesions irrespective to their size which supposedly increased our diagnostic yields.

As for associated complications, bleeding severity was classified into 4 grades; grade 0 – no bleeding; grade I – estimated volume of aspirated blood <50 ml; grade II – estimated volume of aspirated blood of between 50 and 100 ml and requiring endoscopic procedures as instillation of topical adrenaline and/or ice-cold saline; grade III – estimated volume of aspirated blood >100 ml and requiring endoscopic procedures as instillation of topical adrenaline and/or ice-cold saline and/or hemostatic tamponade therapy; grade IV – any life-threatening bleeding requiring transfusion or escalation of care such as admission to the intensive care unit or surgery consultation. [212]

Based on the mentioned classification, 11 (18.3%) of our patients had grade II bleeding. In group I; 8/45 patients (17.8%) had grade II bleeding following TBCB and 3/45 patients (6.7%) had grade II bleeding following forceps TBB. Bleeding tendency was obviously higher following TBCB. Interestingly, the only patient with grade III bleeding (1.7%) was among group II that included non-eligible patients for TBCB which highlights the importance of patients' selection for TBCB to avoid serious complications. This patient was excluded from TBCB due to presence of a vessel with diameter larger than 3cm nearby to the lesion that was noticed by RP-EBUS during the screening process. Other complications included one patient (1.7%) with pneumothorax that did not require intercostal tube drainage and one patient (1.7%) with hypoxemia that was managed only by non-invasive ventilation. In both cases, the complications were noticed following the whole procedure after TBCB and forceps TBB were used, so we didn't conclude which biopsy technique was responsible. No life threatening complications were recorded.

Several reports have described RP-EBUS guided TBB as a safe maneuver for diagnosis of peripheral lung lesions. *Zhang et al* compared RP-EBUS TBB with CT guided TTNA for diagnostic yield and associated complications among 513 patients with peripheral lung lesions. The authors stated that CT guided TTNA achieved higher diagnostic value only for small lesions (< 2cm) while in larger lesions there was no significant difference in diagnostic value between both techniques. However, the post procedure complications were 3.1% in RP-EBUS TBB versus 22.7% in CT-TTNA. In the RP-EBUS TBB group, the most common complication was grade II bleeding and no life threatening complications were reported. [213]

The complications rates were variable according to previous studies that utilized RP-EBUS guided TBB. Most authors have described the associated bleeding as mild, moderate and severe without specific grading. Furthermore, some authors didn't report the associated complications in their reviews. *Fukusumi et al*

reported no associated complications among 27 peripheral lesions. *Ushimura et al* and *Tang et al* reported diagnostic value of 71% and 72.5% among 76 and 40 lesions respectively. However, both studies didn't describe the associated complications. Variously, *Nakahodo et al* reported a high pneumothorax rate (11.1%) when utilized RP-EBUS guided TBCB among 36 patients. [214-217] However, *Ali et al* study, that was previously mentioned as the largest meta-analysis for RP-EBUS performance, reported a complications rate of 2.8% among more than 7000 patients. [191]

Despite the evidences which support using TBCB over conventional TBB, a major concern for the health professionals was the possible complications associated with TBCB. [218] Studies that compared both techniques within diffuse peripheral lung lesions concluded that TBCB could obtain larger samples with better diagnostic yield, however, associated with higher risk of bleeding. [219] *Dibardino et al* reported severe complications following introduction of TBCB into an academic medical center. The authors stated that three patients (12%) suffered from life threatening bleeding, two patients (8%) had iatrogenic pneumothorax and one patient experienced hypercapnic respiratory failure. [220] *Linhas et al* reported an exceptional high rate of pneumothorax in a study where TBCB was guided by fluoroscopy for diagnosis of ILDs. 22 patients out of included 90 (24.4%) had pneumothorax and 18 of them (81.8%) required chest tube. [221]

To avoid life threatening complications and improve the quality of technique, some recommendations were reported for utilizing TBCB. Applying the cryoprobe in contact with pleura or in distance less than 1 cm could increase the risk for pneumothorax into comparable rates with CT guided TTNA. Some reports recommended guidance with fluoroscopy to view the distance between pleura and the tip of cryoprobe. [222] As for bleeding risk, *Kronborg et al* recommended intravenous administration of tranexamic acid prior to the cryobiopsy

procedure and experienced moderate bleeding in 15.7 % of patients [223] while other reports suggested that applying endobronchial blocker could significantly control the bleeding at the biopsy site. [224] In this context, several studies emphasized the importance of using endobronchial blocker while utilizing TBCB and different blockers have been utilized successfully as Fogarty catheter and Arndt blocker. [225]

In the current study, no severe or life threatening bleeding has been recorded. We justify this to the significant role of RP-EBUS in excluding lesions with proximal large vessels and using an endobronchial blocker (Fogarty catheter) to control bleeding. [226] Selecting suitable candidates for TBCB was a major concern in this study. Also, low rate of pneumothorax was recorded (1.7%) despite not using fluoroscopic guidance during the procedure due to maintain a safety distance between the cryobrope and the point of terminal airway resistance representing pleura, general anesthesia of the patients and using length marked biopsy tools. Inability to utilize guide sheath assisted biopsies, performance of the procedures in a single center, utilizing a slim bronchoscope for the procedure and using VBN in limited number of patients were the main limitations of this study.

CONCLUSIONS

7. CONCLUSIONS

7.1. RP-EBUS as a diagnostic technique for peripheral lung lesions.

This study supports the use of RP-EBUS as a safe and effective technique for diagnosis of peripheral lung lesions. RP-EBUS could achieve comparable diagnostic values to other techniques as CT guided TTNA and ENB, in lesions with diameter ≥ 3 cm, with less associated complications and better cost-benefit.

7.2. Addition of TBCB to RP-EBUS procedure.

We found that adding TBCB could improve the efficacy of RP-EBUS procedure. When the result of combined techniques was compared with each of them individually, a significant difference was noticed between forceps TBB and the combination accuracy. Consequently, we can consider TBCB for peripheral pulmonary lesions when TBB fails. Also, TBCB has obtained better samples regarding the quality and quantity measures when compared to forceps TBB.

7.3. Selection of eligible patients for TBCB

TBCB could be utilized without increasing the associated complications if proper selection of the included patients is done. Lesions surrounded by large vessels should be excluded and a safe distance should be maintained from the pleura during biopsy. Visualization of the vessels nearby to the lesions is an advantage of RP-EBUS over other guiding techniques as fluoroscopy.

FUTURE RESEARCH LINES

8. FUTURE RESEARCH LINES

RP-EBUS is an established technique for diagnosis of peripheral lung lesions. Along with previous reports, this study has found RP-EBUS guided TBB as a safe and effective maneuver. Consequently, training programs should offer the chance for interventional pulmonologists to be familiar with this technique as the efficacy of this procedure could be significantly affected by the experience of the operating team.

For example, a study was published from Robley Rex Veteran Medical Center in USA and described the results of using RP-EBUS for the first time in the hospital between 2013 and 2015. The researchers included 42 patients with peripheral lesions mean diameter of 4.4cm. The sensitivity of RP-EBUS was only 38.5% which was much lower than expected yield for specialized centers. [227] With increased awareness about RP-EBUS importance, number of learning programs and workshops was increased. Also number of interested researchers and affiliated studies was improved. Even so, the percentage of bronchoscopy programs which afford convenient training on RP-EBUS is still low and should be increased. [228]

In this frame work, the bronchoscopy unit at Hospital Universitari Germans Trias i Pujol, has provided number of workshops to tutor Spanish and external pulmonologists about EBUS techniques, including RP-EBUS. We recommend increasing the use of RP-EBUS in diagnosing peripheral lung lesions in the Spanish medical centers and perform more number of researches on this procedure.

Several future research lines could be performed in this field including: adding further navigational modifications to RP-EBUS that allow real time biopsy with the advantage of using Doppler mode to increase safety and diagnostic yield, assessment of RP-EBUS guided biopsies for molecular analysis and evaluating safety and efficacy of RP-EBUS guided TBCB including larger number of patients in multi-center studies.

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9. REFERENCES

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