THE PERIPHERAL PULMONARY LESION – BRONCHOSCOPIC TECHNIQUES TO IMPROVE DIAGNOSIS

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PUBLICATIONS AND PRESENTATIONS

Chapter One

1. Manuscript: Badiei A, Nguyen P, Jersmann H, Wong M. Radial Endobronchial Greyscale Texture Analysis Using Whole-Lesion Analysis Can Characterise Benign and Malignant Lesions without Region-of-Interest Selection Bias ***Respiration 2019;*** 97: 78-83

Chapter Two

2. Manuscript: Wong MX, Jersmann H, Holmes M, Nguyen P. Radial Endobronchial Ultrasound with Transbronchial Cryobiopsy versus Radial Endobronchial Ultrasound Alone for the Diagnosis of Peripheral Pulmonary Lesions ***Awaiting Submission***
3. Presentation: Wong MX, Jersmann H, Holmes M, Nguyen P. Radial Endobronchial Ultrasound with Transbronchial Cryobiopsy versus Radial Endobronchial Ultrasound Alone for the Diagnosis of Peripheral Pulmonary Lesions: A Prospective Randomised Trial ***Respirology 2019***

ABBREVIATIONS

ACCP	American College of Chest Physicians
BS	Bronchus sign
CB	Cryobiopsy
CBCT	Cone beam computed tomography
COLDICE	Cryobiopsy versus Open Lung Biopsy in the Diagnosis of Interstitial Lung Disease Study
CT	Computed tomography
EBUS	Endobronchial ultrasonography
EGFR	Epidermal growth factor receptor
FNA	Fine needle aspiration
ILD	Interstitial Lung Disease
IPF	Idiopathic Pulmonary Fibrosis
MDD	Multidisciplinary discussion
NELSON	Nederlands-Leuvens Longkanker Screenings Onderzoetrial
NLST	National Lung Screening Trial
NSCLC	Non small cell lung cancer
PD-L1	Programmed death-ligand 1
PPL	Peripheral pulmonary lesion
RP-EBUS	Radial endobronchial ultrasound
RES	Robotic Endoscopy System
ROI	Region of Interest
TBB	Transbronchial biopsy
TB-CB	Transbronchial Cryobiopsy
TB-FB	Transbronchial forceps biopsy
TBNA	Transbronchial needle aspiration
VBN	Virtual Bronchoscopic Navigation

ABSTRACT

Lung cancer is a leading cause of cancer-related deaths worldwide. This is no different in Australia where it is the main cause of cancer-related mortality, and the fifth most commonly cancer diagnosed in Australians.

The recent National Lung Screening Trial demonstrated an improvement in mortality when patients deemed high risk for lung cancer underwent annual screening with low dose computed tomography imaging. Nearly 25% of participants were shown to have imaging suspicious for lung cancer. In light of these results, and with the possibility of increased uptake of screening, it is very likely that the incidence of identified peripheral pulmonary lesions (PPL) will only continue to rise.

In evaluating PPLs, standard bronchoscopic investigation involves obtaining transbronchial forceps biopsies (TB-FB). However TBFB has variable diagnostic sensitivity, influenced by factors such as lesion size and position. The introduction of radial endobronchial ultrasound (RP-EBUS) has helped improve diagnostic yields further. Ultrasound images obtained by the miniprobe reflect the underlying structure of the peripheral lesion being examined and RP-EBUS is now a well-established technique in the evaluation of PPLs.

The overall aim of this thesis was to examine innovative bronchoscopic techniques which could further aid diagnostic yield in investigating PPLs.

Methods

(i) Radial Endobronchial Ultrasound Greyscale Texture Analysis Using Whole-Lesion Analysis Can Characterise Benign and Malignant Lesions without Region-of-Interest Selection Bias

Custom software was developed to analyse RP-EBUS images based on first and second order greyscale texture features. Unconstrained ROIs were mapped onto lesions. Features from expert and non-expert defined ROIs were compared, as were results of image analysis to tissue histology.

(ii) Radial Endobronchial Ultrasound with Transbronchial Cryobiopsy versus Radial EBUS alone for the Diagnosis of Peripheral Pulmonary Lesions

Prospective, single-centre randomised controlled trials of patients with PPLs. Patients were randomised to receive either one transbronchial cryobiopsy (TB-CB) sample, or 5 TB-FB samples.

Results

(i) Greyscale texture analysis of RP-EBUS images using unconstrained regions of interest (ROIs) demonstrated 5 features which were significantly different between benign and malignant lesions. Highest positive predictive values were associated with maximal and range of pixel intensities. No significant differences were seen between expert and non-expert-defined ROIs.

(ii) 28 lesions were evaluated with overall diagnostic yield 76.7%. Diagnostic yields of TB-CB and TB-FB were 91.7% and 68.8%

respectively (p=0.14). Median size of TB-CB was 7.0mm compared to 2.55mm (p<0.0001). There were no major complications with either technique.

Conclusion

Timely diagnosis of PPLs is critical to enable disease staging and to guide initiation of appropriate definitive treatment.

Greyscale image analysis and texture analysis using the whole RP-EBUS image as a ROI can assist in distinguishing between malignant and benign lesions. This is a potentially valuable additional clinical tool in the diagnosis of peripheral lesions. However further validation is required.

Cryotherapy has provided an alternative method of obtaining transbronchial biopsies (TBBs). Not only does it provide significantly larger biopsy sample, which is advantageous for further immunohistochemical and molecular analysis, but it also could be superior in diagnosing lesions which are not easily accessible by TB-FB.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Publications included in this thesis/Statement of Authorship

Chapter 2

Radial Endobronchial Ultrasound Greyscale Texture Analysis Using Whole-Lesion Analysis Can Characterise Benign and Malignant Lesions without Region-of-Interest Selection Bias

Badiei A, Nguyen P, Jersmann H, Wong M

Respiration. 2019; 97(1):78-83. Epub 2018 Oct 4. PMID: 30286457.

Certification: This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis.

Contributor	State of Contribution
Michelle Wong	Image Analysis 25% Statistical Analysis 25% Co-wrote Paper 25% Co-designed project 25% Executed Project 50%
Badiei, A	Wrote Paper 75% Image Analysis 75% Designed Project 50% Executed Project 50% Statistical Analysis 75%
Nguyen, P	Co-designed Project 25% Edited Paper 75% Image Analysis Supervisor 100% Supervised Project 50%
Jersmann, H	Edited Paper 25% Supervised Project 50%

Chapter 3

Radial Endobronchial Ultrasound with Transbronchial Cryobiopsy versus Radial Endobronchial Ultrasound Alone for the Diagnosis of Peripheral Pulmonary Lesions

Wong M, Jersmann H, Holmes M, Nguyen P

Unpublished – prepared in manuscript style for submission

Certification: This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis.

Contributor	State of Contribution
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Jersmann H	Edited Paper 25% Performed bronchoscopy procedures 20% Supervised Project 33% Patient Recruitment 10%
Holmes M	Edited Paper 25% Patient Recruitment 10% Supervised Project 33%
Nguyen, P	Designed Project 100% Edited Paper 25% Performed bronchoscopy procedures 80% Supervised Project 33% Patient Recruitment 10%

CHAPTER 1.1: PERIPHERAL PULMONARY LESIONS

Peripheral pulmonary lesions (PPLs) are focal parenchymal opacities typically recognised on chest imaging. These lesions can be further characterised by their size to be described in further detail as a pulmonary nodule (where the lesion is less than 3cm in diameter), or a mass (when larger than 3 centimetres) [1, 2]. By definition, peripheral lesions are completely surrounded by pulmonary parenchyma and cannot be visualised endobronchially during bronchoscopy [3]. There is no associated extrinsic compression on the bronchi, endobronchial lesions, narrowing, inflammation or bleeding of the bronchi when examined during bronchoscopy. The concern regarding these lesions are their potential to have an underlying malignant aetiology, with some high risk populations demonstrating prevalence of malignancy as high as 79% in PPLs [4]. It is now well recognised that that the larger the size of the PPL, the higher the probability that it may be malignant, regardless of whether it was detected incidentally or by screening [5-7].

The incidence of identified PPLs is increasing, particularly in light of increased usage of computed tomography (CT) imaging generally, and also for lung cancer screening [5, 8, 9]. Previous data from the United States in regards to solid pulmonary nodules was not able to accurately determine prevalence, but estimated a detection rate of 150,000 to 1 million new cases per year [10]. One factor for the expected increase in identification of these PPLs was the results of the recent National Lung Screening Trial (NLST) where participants were

randomised to receive annual screening with either low-dose CT scans or chest radiography for three years [8]. Results demonstrated a relative reduction in mortality from lung cancer of 20% when screening for lung cancer was performed with low-dose CT. This resulted in a B grade recommendation by the US preventative services task force to screen high risk individuals yearly. 24.2% of the low-dose CT screen group had imaging which was suspicious for malignancy, however it should also be noted that 96.4% of the detected nodules were subsequently found to be benign (i.e. false-positives). It was noted that as nodule size increased up to 30mm in diameter, so did the positive predictive value of a positive screen.

1.1.1: Guidelines for Management

To aid clinicians in the management of PPLs, several guidelines have been published over the last two decades, the most notable being those from the Fleischner Society [6, 11] and the American College of Chest Physicians (ACCP) [9]. Both of these recommend investigation and further management based on the patient's pre-test probability of cancer.

The Fleischner Society's guidelines were specifically designed to address management of small pulmonary nodules which had been identified incidentally on CT imaging which had been undertaken for a reason other than screening for lung cancer. Whilst serial follow up will allow early intervention of those nodules which declare themselves to be likely malignant, the authors recognised multiple disadvantages of serial imaging. These included morbidity

and mortality from surgery for benign lesions, increased health care costs and poor utilisation of limited resources, increased patient anxiety, and increased radiation exposure [11]. They referenced results from large screening programs which showed that nearly half of all smokers aged more than 50 would have at least one nodule incidentally discovered on screening examination whilst approximately 10% of participants screened would develop a new nodule over a 1 year period [12]. Detection of new nodules during follow up periods would result in increased length of screening, and potentially further detection of new nodules.

They therefore offered guidelines for management of smaller lesions, including which lesions to follow, and at which length of interval. Factors affecting pre-test probability of a nodule being malignant included smoking history, lesions size, whether they had a ground glass appearance versus being a solid lesion, and increasing age.

The guidelines were revised in 2017 in order to reduce the frequency of unnecessary repeat CT imaging [6] The authors took into account patient risk factors, in addition PPL features which were considered high risk such as large size, irregularity or spiculated appearance, and upper lobe location.

According to the ACCP, high-risk PPLs had at least 65% chance of having malignant aetiology, as opposed to low risk PPLs which had less than 5% chance [9].

In the above described NLST [8], participants who were deemed “high risk” and appropriate for recruitment included those aged 55-74 years, with at least 30 pack years of smoking history. For those who were reformed smoker, they also must have quit within the previous 15 years.

The NELSON (Nederlands-Leuvens Longkander Screenings Onderzoek) trial [13] was a Dutch-Belgian lung-cancer screening trial which followed the NLST as the second largest randomised-controlled trial to demonstrate reduction in lung cancer mortality with CT screening. The main difference was the use of volume and volume-doubling time in NELSON to help direct management of PPLs. Participants underwent low dose screening CT at 1, 2 and 2.5 year intervals. Screens were considered negative, indeterminate or positive based on volume and volume-doubling times. They found that using this growth-rate assessment as an imaging biomarker resulted in fewer referral rates for additional assessments and markedly lower lung cancer mortality compared with no screening. The effects of screening on lung cancer mortality were also of higher benefit in women.

Tanner et al [2] performed a retrospective observational multicentre study of patients with pulmonary nodules for investigation and examined their management by community respiratory physicians. Inclusion criteria included patients aged between 40 and 89 years, referral to a respiratory physician and nodule size of 8-20 millimetres. Those patients who had previous diagnosis of cancer within 2 years of nodule detection were excluded. Definitive diagnosis was established from either tissue diagnosis or stability after two year

radiographic follow up. The authors noted that patients who were current smokers and those with larger nodules (16 to 20mm in size) were more likely to be referred for further invasive procedure such as biopsy or surgery.

In the above study, of the 377 patients who were included, 25% had nodules which were ultimately found to be malignant in nature. Not unexpectedly, of those who were diagnosed with cancer, they were more likely to be current or former smokers. However, although the patients with malignancy were found to have overall significantly higher smoking pack year history, 67% of patients with nodules (regardless of its underlying nature) were recorded as having smoked previously. The authors also noted that whilst malignant nodules were significantly larger than those which were eventually found to be benign, there was however no reliable measurement threshold over which malignancy could be consistently diagnosed on imaging size alone.

The paper noted the importance of the diagnostic investigation of solitary pulmonary nodules, given that 25% of the patients referred evaluation were found to be have cancer.

1.1.2: The Australian Situation

In Australia, lung cancer is the fifth most commonly diagnosed cancer in Australians, and also the leading cause for cancer-related deaths in both Australian men and women [14]. Higher mortality rates were seen for those people living within regional or remote parts of Australia, and for those with

lower socio-economic background. For Aboriginal and Torres Strait Islander people, lung cancer occurred more frequently, and at earlier age. This prompted an official enquiry into the efficacy, process and delivery of a national lung cancer screening program. The Report on the Lung Cancer Screening Enquiry [15] was subsequently released in October 2020. It recognised international evidence from the previously mentioned NLST [8] and NELSON trial [13] regarding the significant benefits of biennial low dose CT screening program. A two-step eligibility process was proposed using age, smoking history and risk assessment via the $PLCO_{m2012}$ risk model, which estimated 6-year lung cancer risk[16], and was derived from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening trial[17] which included data on over 80,000 participants who had ever smoked. It is the one risk prediction model which has been validated in an Australian population. Potential participants who meet eligibility criteria would be offered screening with low-dose CT. It was hoped that the Program could be fully implemented nationwide within a four-year timeframe. It was also estimated that more than 12,000 lung cancer-related deaths could ultimately be prevented in the first 10 year of the Program.

1.2: Bronchoscopy for Peripheral Pulmonary Lesions

Bronchoscopy has played a role in the investigation of peripheral lesions and pulmonary nodules for more than forty years [18]. Success with bronchoscopy however relies on the ability of the operator to firstly navigate the bronchial system to the PPL, and then to subsequently obtain a sample successfully from it [19].

There are multiple different diagnostic procedures which could be performed during bronchoscopic investigation of peripheral lesions including bronchial washings, bronchial brushings and transbronchial biopsies. The latter, with the assistance of fluoroscopic guidance, has become the most conventional bronchoscopic sampling method for obtaining tissue diagnosis from these lesions since the 1970's [3]. Diagnostic yield however for all these procedures, even when performed in combination, can be vary variable at 30-60% and sometimes even suboptimal [20, 21].

1.2.1: Bronchial Washings

In a retrospective study by Cortese et al [22] published by Chest in 1979, the authors reviewed 48 patients who had undergone flexible fibreoptic bronchoscopy for primary lung cancers which were endoscopically invisible. They found that bronchial washings added no further information to transbronchial biopsies and brushings. Of the 28 patients who had abnormal results from bronchoscopic procedure, the bronchial washings were only found to be abnormal when the biopsies and/or brushings were also abnormal.

Other studies noted that positive diagnostic yields from bronchial washings did not exceed 40%, when used in the investigation of solitary pulmonary nodules [20, 23]. In Baaklini et al's 4 year retrospective study of flexible fibreoptic bronchoscopy in evaluating pulmonary nodules [20], the yield from washings was 40%, however for 7 of these patients, the diagnosis would have been

missed but for the washings positive yield. These included 4 malignant and 3 benign conditions. Given that bronchial washings are not technically difficult to obtain, add very minimal time to procedure duration and very little extra cost, the authors argued that they were a valuable tool to utilise to increase diagnostic yield. It should be noted however, that even whilst positive washings might contribute towards diagnostic yield, they are typically insufficient for further ancillary tests such as molecular analysis and PDL-1 staining.

1.2.2: Bronchial Brushings and Biopsies

Performing the combination of both bronchial brushings in addition to transbronchial biopsies was found to optimise overall diagnostic yield with results as high as 79% and was recommended as early as the 1970s for investigating both central (visible) and peripheral lesions [24].

In the previously discussed study by Cortese et al [22], the authors showed that the two methods were complementary to each other. Results demonstrated an overall higher diagnostic yield with biopsies. However there was note of 7 cases where bronchial brushings were abnormal, but the corresponding biopsy sample was normal. Thus the additional use of brushings was seen to increase the overall rate of abnormal results by 14% for this particularly study.

In Kvale et al's study [24], bronchial brushings were shown to have superior diagnostic yield over forceps biopsies in central tumours which could be visualised endoscopically. The authors attributed this result to the fact that a

brush could access increased surface area of the tumour and therefore have increased chance of obtaining malignant cells. Conversely the biopsy forceps with its small tip could only sample one small portion of tissue, which could also be adversely affected by crush artefact.

The improvement in diagnostic accuracy with combination of bronchial brushings and biopsy was also seen in Yoshikawa et al's study where transbronchial biopsies obtained by endobronchial ultrasonography (EBUS) with a guide sheath (GS) as a guide were used to investigate peripheral lesions without the assistance of radiographic fluoroscopy [25]. A more recent retrospective study by Minami et al, again investigating transbronchial biopsies also showed that performing the combination of transbronchial biopsies, bronchial brushings and washings together provided greater diagnostic sensitivity than just biopsies alone [26].

Ost et al utilised results from the AQuIRE (ACCP Quality Improvement, Registry, Evaluation and Education) Registry to perform a multicentre study of patients undergoing transbronchial biopsies for investigation of peripheral lesions. They aimed to assess the diagnostic yields achieved by different types of bronchoscopy, typically performed in everyday practice [27]. Overall diagnostic yield by bronchoscopy was 53.7% (312/581 lesions). Transbronchial biopsies was successful in 43.2% (251/581 lesions), and was the sole diagnostic test in 11.1%.

1.2.3: Transbronchial Biopsies with X-ray Guidance

Fluoroscopic guidance during transbronchial biopsies is well known to help improve yield [28, 29] and transbronchial biopsies has long been the most common diagnostic bronchoscopic procedure for patients being evaluated for solitary pulmonary nodules [9]. Transbronchial biopsies were first introduced in the mid 1960's as a diagnostic procedure which could be applied clinically during the assessment of diffuse parenchymal lung diseases. Andersen et al al [30] noted that when flexible forceps were passed down rigid bronchoscopes, they were deflected towards the upper lobes and occasionally normal lung parenchymal tissue would be sampled without causing any subsequent adverse effects to the patient. Following the introduction of flexible bronchoscopes, transbronchial sampling became widely accepted as a safe and relatively non-invasive method of lung biopsy.

The procedure involves insertion of thin flexible forceps down the working channel of a bronchoscope. This is passed through the bronchoscope out into the region of interest. Because this area is typically beyond what can be visualised via the bronchoscope, fluoroscopic x-ray guidance is usually used to help guide the biopsies. A second operator assists the bronchoscopist to open and close the cup of the forceps in order to obtain the tissue.

The importance of having fluoroscopic guidance to assist in diagnosing lesions suspicious for lung cancer was established very early on. In 1975, Zavala [31] described outcomes of 600 patients who underwent flexible bronchoscopy for

investigation of radiology or clinical features which were suggestive of bronchogenic carcinoma or diffuse pulmonary disease. Of the 330 patients (85%) with primary lung cancer, 42% had lesions which were not visible endoscopically, emphasising the value of fluoroscopic guidance.

However, even with the assistance of imaging to guide biopsies, diagnostic yield for peripheral lesions is still highly variable. Previous studies have shown sensitivities for transbronchial biopsies to vary from as low as 14%, to higher than 70% [3, 22, 23, 28, 29, 32, 33]. Size and location of the lesion are both known the influence yield [9, 23, 34]. For nodules less than 2 centimetres in size, sensitivity with transbronchial biopsies can be as low as 14% [23, 33]. Conversely, in a meta-analysis by Wang Memoli et al [35], sensitivity was found to increase to 63% when nodules were larger than 2 centimetres, but also decrease as the location of the lesion from the hilum increased. Complication rate with transbronchial biopsies is generally low, but the use of fluoroscopy does result in radiation exposure to both the patient and the procedural staff [5, 9].

1.2.4: Peripheral Transbronchial Needle Aspiration

Transbronchial needle aspiration (TBNA) is another minimally invasive procedure, typically used with EBUS to sample mediastinal and hilar lymph nodes for diagnosis and staging. However conventional fluoroscopy-guided TBNA also has a role in diagnosing PPLs. Mondoni et al [36] recognised its importance, especially for centres which did not necessarily have the resources

or specific skills required for some of the more recent innovative bronchoscopic techniques. They conducted a systematic review and meta-analysis assessing the accuracy of conventional TBNA for PPLs, and to also identify factors which could positively affect diagnostic yield [36].

The authors identified 18 eligible studies, with overall pooled diagnostic yield for TBNA of 53%. When compared directly to TBB, TBNA showed higher diagnostic yield. Further sub-analyses also demonstrated improved yield with malignant lesions, lesions larger than 3 centimetres, lesions associated with a positive CT bronchus sign, and when there was presence of rapid on-site evaluation (ROSE).

The results of the AQuIRE Registry [27], as mentioned above, also recognised improved diagnostic yield with TBNA for PPLs. The authors also commented that it was an underutilised technique, with their data demonstrating usage in only approximately 16% cases. After adjusting for co-variates, they found that use of peripheral TBNA was the one factor amenable to physician control that improved yield. TBNA was diagnostic in 47.4% of their PPLs (45/95), and was also the sole positive test in 6.3% cases. The authors did note one particular limitation with TBNA which could explain its less frequent use. PPLs located within the upper lobes, or superior segments of the lowers lobes might not be as accessible with TBNA due to the more acute angling required with the needle.

1.2.5: EBUS as Alternative To Fluoroscopy

The development of a miniaturised ultrasound probe in the early 1990's has provided another tool in improving diagnostic yield in the investigation of peripheral lesions.

Herth et al were the first group to apply EBUS to guide transbronchial biopsies in the investigation of peripheral lesions [18]. They performed a prospective, crossover trial with 50 patients receiving both fluoroscopy-guided and EBUS-guided transbronchial biopsies. They noted distinct changes in ultrasound images when the probe was introduced into a solid lesion as opposed to when it was within normal air-filled alveoli. Typically, when surrounded by normal lung tissue, a snow-storm type appearance was produced representing the ultrasound being completely reflected by air within the lung [18, 37]. However, the difference in impedance between normal lung parenchyma and pulmonary lesions resulted in a change in ultrasound picture. Lesions which were solid in nature such as PPLs and masses were typically dark and hypoechoic in appearance, with a bright border (hyperechoic lines) which differentiated the lesion from the surrounding lung [5, 37]. Areas of atelectasis or inflammation typically demonstrate a more heterogeneous image on ultrasound representing the different structures within the lung. Fluid also appears dark on imaging**.** Essentially, EBUS allowed examination of the different layers of the bronchial wall and its surrounding structures, which has made it another valuable procedural tool in diagnosing peripheral lesions.

EBUS has also been shown that it can be utilised for image guidance in obtaining transbronchial biopsies, either as an alternative to fluoroscopy [18, 25, 38], or where lesions were fluoroscopically invisible [39]. Having acknowledged that size and location of PPLs significantly affected diagnostic sensitivity of transbronchial biopsies, and that nodules smaller than 3 centimetres were frequently not able to be seen with fluoroscopy, Herth et al's group [39] aimed to assess diagnostic yield in these fluoroscopically invisible lesions. Of the 54 patients with lesions meeting these criteria, the EBUS was able to localise the lesion in 48 patients (89%) and establish a definitive diagnosis in 38 patients (70%). Mean diameter of the lesions in this study was 2.2 +/- 0.7cm. The authors commented that the procedure was easily learned and added little extra procedural time. The efficacy of EBUS in guiding transbronchial biopsies meant that procedures need not be aborted simply for lack of image guidance, and also helped avert the need for surgical referrals.

The characteristics or pattern of the EBUS image can also help provide information in the underlying internal structure of the lesion, and even help in differentiating its nature. Kurimoto et al [40] was the first to examine the internal structure of PPLs as by their images obtained with EBUS. The images were compared to their eventual histopathological diagnosis, with the objective of creating a classification system which could be used to speculate on a lesion's underlying histology as based on their EBUS characteristics. The authors aimed to distinguish between benign and malignant disease, the subtype of cancer in the latter, and also the degree of differentiation. By focusing on the presence of internal echoes, the architecture of bronchi and vasculature, as

well as the morphology of hyperechoic areas (which reflected air, and the state of bronchi), three classes of lesions were identified. 92% of the type I lesions were benign, whilst 98 and 99% of types II and III were malignant. They concluded that this further information obtained during EBUS could further assist in suggesting the underlying nature of the lesion being investigated.

1.3: Alternative Diagnostic Methods for Investigating Peripheral Pulmonary Lesions

1.3.1: Surgical Resection

Surgical biopsy procedures such as video-assisted thoracoscopic surgery have been applied in some institutions to investigate smaller peripheral lesions. However, this is a much more invasive procedure compared to bronchoscopy, and would be less preferable in those patients with poor respiratory status, or those who are elderly [3].

In the ACCP's 2013 updated guidelines on the management pulmonary nodules, they recognised that the gold standard for both treatment and diagnosis of malignant nodules was surgery [9]. However the authors also recognised that this option should only be taken with full consideration of the benefits, compared with the surgical risks. Their recommendations (Grade 2C) for surgery included those patients with solid, indeterminate lesions greater than 8mm in diameter which either had high pre-test probability for cancer (>65%), were FDG-PET positive, had suspicious non-surgical biopsies for

cancer, or where the patient preferred a definitive procedure. Where surgical diagnosis was chosen, there was recommendation (Grade 1C) for thoracosopic wedge resection as the preferred approach, being less invasive, and with lower risk than open thoracotomy.

In Tanner et al's retrospective multicentre study of management patterns of solitary pulmonary nodules[2], 77 patients (20%) underwent a surgical procedure as their most invasive investigation. Of this subgroup, cancer was diagnosed in 50 patients (65%), meaning that 27 patients (35%) underwent surgery for benign lesions. The authors noted that whilst surgery was generally only recommended when pre-test probability of cancer was high (as a negative biopsy would still lead to surgery for definitive diagnosis, and positive biopsy result would also lead to surgery), and that the risk models evaluated in their study were generally good at excluding cancer, rates of surgical resection were still performed at rates as high as those with higher cancer risk. There was also no difference in surveillance patterns when stratified by pre-test risk, which suggested that physicians generally did not follow guidelines. However whilst pre-test risk did not appear to affect decisions regarding surveillance or surgery, there was a trend towards positive correlation between risk and biopsy ($p=0.07$). The authors acknowledged there was likely to be a multitude of factors, which in addition to pre-test probability, influenced their management decisions regarding pulmonary nodules however identifying these factors was outside the scope of this study.

1.3.2: Computed Tomography (CT) Guided Fine Needle Aspiration

Transthoracic fine needle aspiration (FNA) of pulmonary lesions is another well-established procedure, especially for lesions that are situated peripherally and closer to the pleura. Sensitivity with CT guided FNA has been as quoted as high as 96% [5]. However, this technique can be associated with a relatively high risk of post-procedural adverse effects. Peri-procedural pneumothorax was the most common complication with rates ranging from 15-44% of which at least 15% may require intercostal chest drain insertion [5, 9].

Fielding et al[23] performed a non-randomised prospective analysis of 140 RP-EBUS cases, and compared these to a retrospective review of 121 CT FNA cases. Overall diagnostic sensitivity for the two procedures were similar at 66% and 64% respectively. However, complication rate differed significantly with pneumothorax rate and need for chest drain insertion was 1% and 0% for RP-EBUS patients respectively, as compared to 28% and 5% for CT FNA patients ($p<0.001$ for both).

In a large retrospective study by Yeow et al, risk factors for complications such as pneumothorax and bleeding were identified by examining 660 consecutive cases of CT guided lung biopsy [41]. 155 patients developed pneumothorax (23%). On multivariate analysis, the factors associated risk of pneumothorax were lesion size <2cm (pneumothorax rate 33% compared to 17% for those >2cm), lesion depth (lesions touching the visceral pleura had a negligible pneumothorax complication rate, whereas the further away the lesion was

noted to be located from the pleura, the higher the incidence of pneumothorax) and radiologist experience. Bleeding complications occurred in 201 procedures (30%), however the majority of these were only mild). 4% (26/660) were of moderate severity where the patient presented with small-volume haemoptysis. Lesion size and depth were again seen to be significant factors in increasing risk of bleeding.

1.4: Radial Endobronchial Ultrasound

The application of ultrasound within an endobronchial setting was first described in the 1990s by Hurter and Hanrath [42, 43]. By using small sized catheters originally designed for vascular examination, the authors noted these were useful in the examination of patients with bronchial stenosis, or with peripheral lesions which were endoscopically invisible [43]. EBUS allowed differentiation of bronchial cancer which had poor echogenicity, to normal bronchial wall and lung parenchyma which was highly echogenic. They were among the first to suggest EBUS as a possible alternative to fluoroscopy for finding peripheral lesions as well as recognising its other advantage of being able to identify large vessels near lesions to avoid.

Whilst EBUS is still a relatively new bronchoscopic diagnostic technique, its value in identifying peripheral lesions and improving diagnostic yield with fibreoptic bronchoscopy has been well established [18, 40, 43, 44].

Fielding et al [23] were the first to formally compare the utility of radial EBUS (RP-EBUS) to CT guided lung biopsy. They recommended using the former, especially when the lesion was not in contact with the visceral pleura as RP-EBUS had significantly reduced pneumothorax rate, and higher diagnostic yield when considered independently.

This modality is particularly useful for small peripheral lesions, and even those which cannot be visualised on fluoroscopy [18, 39, 40]. Huang et al described RP-EBUS as the most effective method for investigating peripheral pulmonary lesions [45].

The procedure involves the insertion of a flexible ultrasound miniprobe within a transparent sheath through the working channel of a fibreoptic bronchoscope. Once the latter cannot be advanced any further, the miniprobe is passed into the more distal bronchi towards the lesion. When turned on, the ultrasound transducer rotates producing a 360 degree radial ultrasound image [46]. This image subsequently assists the proceduralist to confirm whether the scope is in the correct position, and ideally within the lesion of interest. The surrounding tissue has different ultrasound characteristics pending different densities of normal and abnormal lung tissue, allowing identification of the target lesion [37]. If this is the case, the ultrasound miniprobe can then be removed whilst the sheath is left in place, following which the sampling instruments such as forceps or brush can be inserted and passed down into the lesion for sampling. The advantage of this technique is that the location of bronchoscope can be confirmed with real-time visualisation and essentially, the guidesheath is

utilised as an extended working channel in order to maintain the location during biopsy[35].

Use of the guidesheath – a thin plastic tube, with a radio-opaque mark distally at the tip, was first reported by Kurimoto et al [47]. The guidesheath was developed in order to help maintain the position of the bronchoscope within the airways, and the authors noted several of the benefits conferred by it use. It allowed the repeated sampling into the same bronchial branch as identified by EBUS, thereby increasing the reliability and yield of the biopsies. There was also reduced bleeding risk, as the opposition of the guidesheath's outer surface "snug" against the bronchus wall would allow blood to drain into the sheath instead of the proximal airways post biopsy [47]. Also, without a guidesheath, repeated insertion and manipulation of a miniprobe or sampling device could cause increased friction against the bronchial mucosa with subsequent oedema which could hinder further attempts at correct insertion [47].

The first meta-analysis which specifically assessed the efficacy and factors influencing performance of RP-EBUS in evaluating PPLs was Steinfort et al in 2011. They demonstrated a pooled sensitivity of 73% (range 49-88%), from a total of 1420 patients across 16 studies [46]

Multiple studies assessing diagnostic yield of RP-EBUS were subsequently published, and a much larger meta-analysis which included 7872 lesions across 57 retrospective and prospective studies was published by Ali et al in 2017 [48]. This remains the largest meta-analysis to date, and confirmed the high

performance of RP-EBUS in assessing PPLs with an overall diagnostic yield of 70.6% (range 49.4-92.3%). Subset analysis of the 4605 malignant lesions demonstrated overall yield of 72.4%

As such, RP-EBUS is recommended by the ACCP clinical guidelines [49] as an adjunct image modality for patients with peripheral nodules suspicious for lung cancer. The authors noted that the real time confirmation of bronchoscopic sampling increases diagnostic yield as opposed to conventional bronchoscopy [49].

1.4.1: Visualisation with Radial EBUS

Tay et al [44] retrospectively reviewed 196 patients who had undergone RP-EBUS procedure to determine what factors would influence visualisation with RP-EBUS. In this study, a definitive tissue diagnosis was obtained in 55.6% (109/196) patients using a combination of bronchial washings, brushings and transbronchial biopsies. Overall EBUS visualisation yield was found to be 79%. When looking at primary malignancy however, visualisation yield was increased at 83% (110/127 cases) with a diagnostic sensitivity of 69% (88/127). The authors noted that there were three factors on multivariate analysis which could significantly affect diagnostic yield: lesion size, malignancy and distance of the hilum from the lesion. Lesions which were larger than 2cm had significantly increased visualisation yield compared to smaller lesions (85% and 63% respectively, p=0.0022). EBUS visualisation of malignant lesions was also higher (85%) compared to the visualisation rate in benign lesions of 66%

(p=0.0025). As size of benign and malignant lesions in this study were fairly similar, particularly in the <2cm category, the authors argued malignant lesions typically had a higher EBUS visualisation yield due to more distinguishable appearance on ultrasound imaging. This was in comparison to benign lesions where the borders were often much more ill-defined. Ultrasound appearances of the latter were often more subtle, especially in inflammatory conditions, making it more difficult to distinguish from surrounding normal lung tissue.

Other literature confirmed that the visualisation yield from Tay et al's study of 79%, as well as its overall diagnostic yield of 56%, was very comparable to other studies. Earlier prospective study by Yoshikawa et al [25] of RP-EBUS investigation of PPLs without using fluoroscopic guidance, found that most of the PPLs in their study could be visualised clearly with EBUS (75.6%). The mean diameter of the 123 lesions included was 31mm. Kikuchi et al [3] also demonstrated that EBUS could detect 79.2% of peripheral lesions, all of which were <30mm in diameter (mean 18.4mm).

Visualisation with EBUS is relevant as there is a very strong association between visualisation of the lesion and subsequent diagnostic yield [3, 18, 25, 39, 44, 45, 47, 50]. If the lesion is not visible with EBUS, this makes the eventuality of a diagnosis being made minimal, with diagnostic yields quoted as low as 0-30% [3, 45, 47, 50]. In Tay et al's study, those lesions which were visibly identified with EBUS had significantly higher diagnostic yield (66%), compared to those lesions which were not seen with EBUS (diagnostic yield 20%) with p value of 0.00001 [44]. Steinfort et al [1] also agreed that location of

the lesion with RP-EBUS resulted in a significantly higher diagnostic yield in the first randomised controlled trial of EBUS guided transbronchial biopsies versus CT guided biopsy. Of the 32 patients randomised to undergo EBUS, diagnostic yield was 100% when the lesion was located by the ultrasound probe versus 50% when it was not (p=0.001). It was also demonstrated that the ability to locate the lesion was significantly associated with a subsequent diagnosis of primary lung cancer, and that RP-EBUS was more likely to be diagnostic in lung cancer compared to alternative diagnoses. Similarly, of the patients with primary cancer being investigated with EBUS, a positive correlation was noted between being able to locate the peripheral lesion with EBUS miniprobe and subsequent diagnosis (p=0.006).

1.4.2: Diagnostic Yield

The diagnostic sensitivity of RP-EBUS for detecting malignancy in a peripheral lesion has been shown to vary within individual studies. Generally however in randomised studies, the diagnostic yield of transbronchial biopsies taken with RP-EBUS for both malignant and benign lesions was between 70 and 85% [23]. Even without the use of fluoroscopic guidance, Yoshikawa et al were able to demonstrate a diagnostic yield of 61.8% with EBUS alone [25]. This was preceded by Herth et al's study [39] which demonstrated that even where lesions could not be visualised on fluoroscopy, a diagnostic yield of 70% could still be achieved with RP-EBUS.

1.4.2.1: Position

Position of the probe in relation to the lesion has also been shown to be important in influencing diagnostic yield. Of most particular relevance, was whether the miniprobe of the RP-EBUS could be positioned centrally within a lesion [47, 50, 51]. In Kurimoto et al's prospective study of RP-EBUS in 150 patients with peripheral lesions, highest yield was achieved when the probe could be advanced into the lesion (87%) as opposed to the situation where EBUS image showed the probe to be adjacent to lesion, resulting in yield of 42% [47]. In assessing diagnostic yield from transbronchial biopsies alone in the same scenarios, yield in the former was found to be very significantly increased (82% and 7% respectively, $p<0.0001$).

Yamada et al [50] noted similar results in their subsequent retrospective study of RP-EBUS in small PPLs, measuring <30mm in mean diameter. For lesions in which EBUS image demonstrated the probe positioned within the lesion, a high diagnostic yield of 83% was achieved. However diagnostic sensitivity of RP-EBUS fell when the probe was positioned adjacent to the lesion (61%), and was significantly worse in the cases where the probe was completely outside the lesion (4%, $p<0.001$). On univariate analysis, both lesion size (mean diameter less than 15 millimetres) and probe position were found to be significant in influencing diagnostic yield. However on subsequent multivariate analysis, only probe position was noted to be significant. Position within the lesion only had a trend towards significance onmultivariate analysis ($p=0.064$). Given this, the

authors recommended advancing the probe to either within or adjacent to the lesion to optimise yield of biopsy.

Yield was also affected, dependent upon whether the peripheral lesion was in contact with the lung's visceral pleural location as shown by Fielding [23]. Identifying whether there was any contact with the visceral pleura was found to be relevant, to guide which diagnostic procedure to choose (CT guided biopsy versus RP-EBUS) in order to maximise yield and minimise complications. In the RP-EBUS group, 117 of the lesions were not in contact with the pleura compared with 23 lesions which were on the pleura. These were associated with highly significant eventual diagnostic yields of 74% and 35% respectively ($p<0.001$).

Effect of lobar position was also assessed in several studies with differing opinions. Huang et al [45] did not find any effect of lobar position on diagnostic yield. In Yamada et al's study [50], the authors did document that the percentage of peripheral lesions diagnosed by EBUS was slightly higher when situated within the left upper lobe, right middle and lobe lobes however these findings were not significant ($p=0.66$). Similar findings were seen by Yoshikawa et al [25] with significantly higher yields obtained from lesions within the right middle lobe (90%) and lingula region (80%), but much lower yield from the right upper lobe and right lower lobe (<0.05). In the prospective study of RP-EBUS in nodules smaller than 20mm by Eberhardt et al [52], highest yield (83%) was seen from right middle lobe lesions ($p=0.02$) with lower yields from right upper and lower lesions. Right upper lobe lesions were also found to be associated

with lower diagnostic yield from studies by Herth [18] and Shirakawa [38]. Yoshikawa proposed that this location may be more difficult to access by the EBUS scope tip which was relatively long and stiff, and harder to manoeuvre through this part of the lung which generally had tortuous airways with many acute angles [25]. Their rationale for the lower yield obtained from lesions within the right lower lobe was the risk of the EBUS sheath being dislodged from the lesion with deep respiration. Conversely, as it was often easier to identify airways directly leading into the right middle lobe and lingula, this helped to account for the increased yield of lesions within these regions.

Whilst Steinfort et al's study also did not find lobar location to affect diagnostic sensitivity of RP-EBUS, they did observe note a trend towards significance for yield, depending on how far away the lesion was located from the pulmonary hilum [1]. Of lesions situated less than 6cm from the hilum, positive diagnosis was made in 20/21 cases, compared with 5/8 lesions located more than 6 cm from the hilum (p=0.058).

1.4.2.2: Presence of Air Bronchograms

The presence of an air bronchogram was also described in Fielding's study to be more likely associated with positive diagnosis [23]. Of the 24 patients in which an air bronchogram was identified on CT imaging, specimen positivity was seen in 20 (83%). The authors stated that the identification of an air bronchogram suggested that the pathologic process was peribronchial, in which case a transbronchial method of sampling the lesion was more likely to be

appropriate than a transthoracic approach. His study also showed that relatively more positive diagnoses were made in benign lesions compared to malignant. This was opposite to what was described earlier, whereby malignant lesions generally were easier to visualise with EBUS, and therefore found to have higher associated diagnostic yield. However the most common aetiologies of the benign lesions in Fielding's study were noted to be cryptogenic organising pneumonia, sarcoidosis, and focal collapse. All three of these pathologies are typically bronchocentric in nature and would therefore explain why they could be easily accessed via bronchoscopy.

In Yoshikawa's study of RP-EBUS without fluoroscopy [25], they also found significantly higher diagnostic yield if a bronchus leading to access of the peripheral lesion could be seen or presumed from the CT imaging. Of the 53 lesions where a bronchus providing access was identified, a positive diagnosis was subsequently made in 42 cases (79.2%). Conversely, for all the lesions where accessibility was not evident, no diagnosis was made.

Minezawa et al also assessed the relationship between target lesions and the nearest bronchus and identified 3 different types of CT bronchus signs [53]. A Type A CT bronchus sign (BS) was where the bronchus was seen to clearly extend into the peripheral lesion. A Type C CT BS was where no bronchus could be detected related to the lesion, and Type B encompassed all those lesions which could not categorised as Types A or C. They showed that the CT BS was the most important predictive factor for successful diagnosis of small PPLs, with diagnostic success rate for a Type A lesion 11.1 times higher than a

Type C. The authors noted that diagnostic yield was 88% for CT BS Type A lesions which were also visible on fluoroscopy, and this increased to 98.6% for malignant lesions. They concluded that thin-section CT and evaluation of CT BS could help determine which patients were most suitable for bronchoscopy.

The correlation between BS and increased diagnostic yield of RP-EBUS for PPLs has been further supported in other studies [25, 54, 55]

1.4.2.3: Size

Lesion size is well recognised to correlate with diagnostic yield with transbronchial biopsies in general, and this is also the case with RP-EBUS, with sensitivity typically being lower for smaller peripheral lesions [50]. Diagnostic yield has been shown to vary depending on whether the lesion in interest is larger or smaller than 2 centimetres. Yang et al [56] looked at EBUS guided transbronchial biopsies in peripheral lung cancers. They found that for lesions greater than 2 centimetres, diagnostic yield was 66%; however this was reduced to 55% when the lesions were smaller. In Yoshikawa's study [25], similar findings was also shown was significantly increased yield ($p<0.01$) for lesions greater than 2 centimetres in diameter. Kikuchi et al [3] also showed that smaller lesions with mean diameter less than 2 centimetres had a lower diagnostic yield. Even in restricting yield to lesions less than 2 centimetres in mean diameter alone, the authors were still able to show diagnostic sensitivity for RP-EBUS as high as 53.3% (8/15 lesions), with 6 lesions (40%) being diagnosed on histopathology. Multivariate analysis in study by Tay et al

showed that lesions greater than 2 centimetres which also had shorter distance from the hilum (less than 5 centimetres) were associated with improved diagnostic yield [44]

Interestingly, in Steinfort et al's study comparing the evaluation of peripheral lesions by RP-EBUS versus CT guided biopsy, lesion size was not shown to influence the diagnostic performance of EBUS [1]. In Schuhmann's 2013 review of RP-EBUS for peripheral lesions, range of yield was quoted between 61 and 80% regardless of lesion size [5].

1.5: Operator Experience

Yamada's study [50] assessed factors which would influence diagnostic yield with RP-EBUS, included evaluating training and experience by the proceduralists. They demonstrated that there was no significant difference to diagnostic yield amongst operators who had performed more than 11 examinations, and with more than 4 years of general bronchoscopic experience and training. The RP-EBUS procedure requires two operators – the bronchoscopist who holds the sheath in place after the correct area for biopsy is identified with the miniprobe, and another operator who manages the biopsy forceps and brush. The authors explained that as long as there was coordination between the two, and at least one person was familiar with the biopsy process with RP-EBUS, the procedure was relatively safe with good chance of achieving diagnostic yield.

1.6: Number of Biopsies

Optimal number of biopsy samples in conventional forceps transbronchial biopsies was assessed by Descombes et al [57] who assessed diagnostic yield by retrospectively reviewing 530 consecutive transbronchial biopsies from 510 immunocompetent patients performed over an 8 year period. They found direct positive correlation between number of transbronchial biopsies obtained per procedure and the overall diagnostic yield. They recommended that a minimum 5-6 transbronchial biopsies be obtained per procedure in order to improve overall sensitivity of transbronchial biopsies. For investigation of localised lesions and stage I sarcoidosis, increased number (7-10 biopsies) was recommended, however the authors recognised this was then associated with increased risk of complications.

Other authors assessed the use of transbronchial biopsies in the immunocompromised population, also making similar general recommendation that given the small surface area of samples obtained by forceps, that at least 6-8 samples be taken to ensure adequate material for diagnosis [58-62]

The same issue has also been addressed for RP-EBUS. In the retrospective study by Yamada et al, they assessed the cumulative diagnostic yield following each consecutive biopsy, to determine the ideal number of specimens to obtain [50]. Whilst they observed that absolute yield obtained varied dependent upon the underlying disease, the overall size of the lesions studied, position of probe, and stepwise increment in cumulative diagnostic yield was similar amongst the

cases. The authors subsequently showed that after 10 sequential biopsies were achieved, diagnostic yield of RP-EBUS for peripheral lesions increased to 100%. By taking at least 5 sequential specimens, this would correlate with achieving more than 95% diagnostic yield. As such, the authors made the recommendation that a minimum of 5 specimens be taken during RP-EBUS.

Their rationale behind obtaining needing multiple biopsies for smaller peripheral lesions was that the guidesheath may not always remain well seated within the lesion, in which case several biopsies are required. In the situation where the probe was positioned adjacent to the lesion, repeated biopsy was required. This was to help break through the benign bronchial mucosa in between the probe and the lesion, before the lesion could be accessed. Also in patients with patients with benign disease, not all tissue samples may be truly representative of underlying diagnosis, so having more samples is ideal.

1.7: Complications

One of the largest benefits of the RP-EBUS to obtain transbronchial biopsies is its well-recognised safety profile [44]. The safety and efficacy of RP-EBUS has been well established [38, 39].The complication rate associated with RP-EBUS has been found to be the lowest compared with other modalities for lung biopsy [46].

Complication rates for both pneumothorax and bleeding post transbronchial biopsies with RP-EBUS have been shown to be both approximately 0.5-1% [39,

46, 47].The low bleeding rate can be attributed to the ability to tamponade the bronchus following biopsy with the guidesheath which is left in place after the biopsy has been taken. In Kikuchi et al's study of RP-EBUS for peripheral lesions [3], they noted there was occasionally coagulated bloods and less than 1ml of bleeding caught within the guide sheath post biopsy, however there was minimal blood outside the guide sheath even after its removal from the bronchus. This ability to wedge the guide sheath against the bronchus for bleeding control would be the main reason bleeding complication rates are minimal.

The largest study to date assessing complications was a retrospective review of 965 patients who underwent RP-EBUSRP-EBUS for investigations of PPL over a 2 year period [63]. The authors defined a major complication as an event which either resulted in the procedure being ceased prematurely, or the development of symptomatic post-procedural sequelae. Only 13 patients (1.3%) were documented as having major complications. Of these, there were 8 instances of pneumothorax (0.8%) of which 3 patients required chest drain insertion. 5 patients developed pulmonary infection (0.5%) and there were no cases of significant bleeding. All patients with complications improved with treatment. There were 4 probes which broke during procedure, but none of these were associated with any adverse effects. These results reinforced the tolerability and safety of RP-EBUS as a diagnostic procedure for PPLs.

1.8: TRANSBRONCHIAL CRYOBIOPSY

Cryoprobes have had a role within bronchoscopic procedures as early as 1976 [64] where their primary use was a debulking tool. The extreme cold properties of cryotherapy were initially utilised for its devitalising effects. After application of the cryoprobe to endobronchial tissue, necrosis would develop after several days, following which further procedures could be carried out to remove the dead tissue.

Subsequent modifications in cryotherapy technology resulted in increased freezing power and increased tensile strength of the cryoprobe [64]. This allowed another form of debulking technique, utilising the frozen probe's adhesive effects. In this context, the proceduralist was able to freeze endobronchial tumour directly onto the cryoprobe, allowing for the tissue to be subsequently extracted and removed. This further type of debulking technique was termed cryorecanalisation.

More recently, the observation was made that the endobronchial tumours extracted using this cryorecanalisation technique was of remarkably good size and quality, despite being frozen and unfrozen. Hetzel et al were the first [64] to question whether tissue removed using this technique could be utilised for further histological analysis. Since then, cryotherapy has been further investigated as an additional new diagnostic tool for use during bronchoscopy.

1.8.1: Larger Biopsies

One very useful advantage of cryotherapy is the ability to obtain generally larger specimens than those taken with conventional forceps biopsy. In the above mentioned study by Hetzel et al [64], the authors retrospectively analysed 12 biopsy samples which were harvested from prior cryorecanalisation procedures of endobronchial lesions. Quantitative digital assessment was performed on the specimens, revealing median sample area size of 3.5mm^2 (range 1.4-14.1mm^2) and median diameter of 6.7mm (range 4.2-13mm). In their discussion, the authors commented that the size of the tissue samples achieved with this procedure exceeded that from any other bronchoscopic technique and that a freezing process of approximately 5 seconds did not significantly prolong procedure time.

Schumann et al [65] were one of the first groups to prospectively evaluate the safety and efficacy of cryobiopsy versus forceps biopsy in a large cohort of patients with endobronchial lesions. 296 patients were recruited in total over the 6 year study period. The first 55 received both cryobiopsy and forceps biopsy, with the sequence of biopsy procedure randomised, and the subsequent patients received cryotherapy alone. Biopsy specimens from the patients receiving both procedures were assessed using quantitative image analysis. Sections from the biopsy samples with the least amount of damage and with the best preservation were chosen to evaluate both size and quality. They demonstrated that significantly larger mean total tissue areas were obtained from the cryobiopsy (10.4mm^2 vs 5.2mm^2) with p value <0.0001.

In a subsequent retrospective review assessing the safety and utility of cryotherapy in the investigation of all types of endobronchial lesions (both exophytic and flat), similar results were seen when assessing volume of biopsy material [66]. Rubio et al showed that the mean volume of tissue obtained with cryobiopsy, was significantly larger than those taken with forceps. Of the 31 patients included in this study, 22 received both cryobiopsy as well as conventional forceps biopsy, with the mean volume of material obtained in the former (0.696cm^3) found to be significantly larger than those achieved with traditional forceps (0.0373cm^3) with p value 0.0014.

The authors recognised that other studies tended to report on size of tissue area, but they chose to analyse volume. Whilst they could not assess effect of biopsy depth on overall diagnostic yield from their results, they appreciated there would be circumstances where the smaller conventional forceps would not achieve adequate depth to biopsy deeper located malignant cells. For one of their cases, an exophytic tumour was nearly completely removed from the first cryobiopsy taken, with very high volume of biopsy material (1.25cm^3).

The efficacy of cryotherapy in transbronchial cryobiopsy was most apparent earliest in the evaluation of patients with diffuse parenchymal lung disease. Similar to its results obtained from analysis of endobronchial biopsies, transbronchial cryobiopsy samples were shown to have mean diameters 2-5 times larger than those by conventional forceps biopsy [67, 68] and with 3-4 times larger mean specimen areas [67, 69, 70].

Babiak et al [69] were one of the first groups to examine feasibility and safety of transbronchial cryobiopsy during flexible bronchoscopy. They retrospectively reviewed data from 41 patients who received both transbronchial forceps biopsies as well as cryobiopsies within the same procedure. They observed significantly larger median sample area from the cryoprobe, compared to the forceps (15.11mm2 vs 5.82mm^2, p<0.01).

In a subsequent randomised clinical trial of 77 patients being investigated for interstitial lung disease, Pajares et al [67] demonstrated significantly larger mean diameter, sample area, and number of alveoli sampled for cryotherapy, compared with the conventional forceps groups (p<0.001).

Griff et al [70] also reported on a prospective case series of 15 patients who underwent transbronchial cryobiopsy. These were compared to a control group of 18 patients who received traditional forceps transbronchial biopsies. The authors commented that the cryoprobe produced samples which were much more representative of real lung structure. Similar to the other studies, specimen size in the cryobiopsies was significantly larger (mean 17.11mm^2) than those which were taken with forceps (mean 3.8mm^2) with p <0.001. There was also a trend towards more alveolar tissue being obtained with cryobiopsy where it was found in 73% samples, compared to 56% of the forceps samples (0.290)

Schumann et al [71] were later the first group to apply cryotherapy to the investigation of peripheral lesions, using RP-EBUS to guide transbronchial cryobiopsies. 39 patients were recruited; however one patient was subsequently excluded because of endobronchial disease. The remaining 38 patients were scheduled to receive both cryobiopsies and forceps biopsies via RP-EBUS in a randomised order. Similar to results seen from the ILD studies, size of the tissue samples obtained with cryobiopsy were nearly 3 times larger than those taken with traditional forceps with mean sample areas of 11.18mm and 4.69 mm respectively.

1.8.2: Higher quality specimens

Because cryobiopsies are generally larger than those obtained with conventional forceps as described earlier, this provides the pathologist with more tissue in order to perform histopathological analysis, and potentially also further molecular studies. This is particularly relevant, especially in today's era of personalised lung cancer treatment [64, 66].

However the other advantage is the quality of the specimens achieved with cryotherapy. Cryobiopsy samples are typically not exposed to crush artefacts which is common with forceps-obtained biopsies. Forceps crush artefacts occur secondary to mechanical compression of the lung parenchyma from the tip of the forceps. This results in alteration and poor preservation of cellular architecture within the tissue sample which can adversely affect histopathologic analysis [64, 67, 69, 72].

Cryobiopsy samples tend to have larger artefact-free tissue areas or, in some studies, have been found to be completely artefact-free [66, 68-70, 73]. They have generally good preservation of tissue architecture and cellular structures making them overall histologically superior, and of better quality than those achieved with forceps.

Hetzel et al [64] demonstrated this in their review of exophytic endobronchial malignancies extracted with cryorecanalisation. They found that all 12 specimens showed well preserved cellular structures, and that the integrity of the different tissue layers were all maintained. 9 of the 12 samples examined had more than 75% biopsy area devoid of any artefact, with the remaining 3 samples showing 50-75% unaltered tissue area. The authors commented that the high quality of the specimens obtained allowed easy and detailed subsequent histopathological assessment. The authors attributed these findings to the lack of mechanical and compressing which typically cause crush artefact with forceps. As the lung tissue was frozen before being removed , there was no artefacts secondary to tearing during the extraction process. They also did not observe any freezing artefacts.

In a prospective study by Pourabdollah et al [73] comparing conventional forceps transbronchial lung biopsies to cryobiopsies in patients with diffuse parenchymal lung disease, specimen adequacy was determined by either the assessment of a minimum 50 alveolar spaces within the sample, or positive diagnosis being obtained. In considering these criterion, a significant difference

was seen, with 97% of samples achieved with cryobiopsy found to be adequate compared to just 63.4% specimens with conventional forceps. Pathologists who were blinded to the study also graded the percentage of artefact-free alveolar spaces in 25% intervals. A higher percentage of cryobiopsy specimens (55%) were found to have more than 75% artefact free biopsy area, which was comparable to Hetzel et al's study [64].

Pourabdollah did however make note of a new artefact which seemed unique to cryobiopsies. They described a finding of ciliated columnar epithelial cells within alveolar spaces which had no connection to the lining cells [73]. This was observed in 7 out of the 40 specimens. The authors emphasised that pathologists would need to be familiar with this particular artefact, otherwise misdiagnosis with other pathologist such as adenomatous metaplasia could occur.

In the first prospective study of cryotherapy in lung transplant recipients, Yarmus et al [74] compared transbronchial biopsies by conventional forceps to cryoprobe for assessment of transplant surveillance. Prior to their report, there had been no data assessing the safety of cryobiopsy in lung transplant recipients. The mean specimen size was significantly larger with cryoprobe, with significantly increased percentage of open alveoli. Additionally, all the specimens obtained with cryobiopsy were free of crush artefact whilst conversely, all samples taken with forceps biopsy showed significant amount of crush artefact when examined. There was no freezing artefact on any of the cryobiopsies. The authors stressed the importance of achieving higher quality

specimens, especially in this particular population of patients, where accurate diagnosis and early treatment of acute cellular rejection can potentially help reduce further risk of chronic rejection and increased mortality.

1.8.3: Diagnostic Yield

In Rubio's retrospective review of cryobiopsy versus forceps biopsy for endobronchial disease [66], analysis of the 22 patients who received biopsies with both techniques showed a 100% diagnostic yield with cryobiopsy, and 95.4% yield with forceps. Overall diagnostic yield for all cryobiopsy specimens was 96.77%. One sample only revealed evidence of necrotic debris resulting in no diagnosis.

Similarly in Schumanns's prospective study evaluating feasibility and safety of cryobiopsy for endobronchial tumours [65], cryobiopsy achieved a diagnosis in 89.1% patients, compared to 65.5% with forceps in the group of patients who received both techniques ($p<0.05$). In evaluating all 296 patients who received cryobiopsy, overall diagnostic sensitivity was found to be 89.5%.

Comparable findings were seen in Hetzel's et al randomised multicentre trial [75]. In looking at the subgroup of patients with proven malignant disease, cryotherapy showed a diagnostic yield of 95% (268 of 282 patients) compared to 85.1% (239 of 281 patients) with conventional forceps ($p<0.001$). For the 56 patients in whom a definitive diagnosis was not made at bronchoscopy, alternative procedures (including surgery) were performed. Upon evaluating

diagnostic yield of the different techniques for all patients, cryobiopsy was still significantly higher than forceps (95.2% vs 82.2%, p=0.0001). However, when rigid bronchoscopy was used under general anaesthetic, there was only a trend towards increased yield with cryobiopsy. The authors suggested that this was likely the case as the reduced breathing amplitude under general anaesthetic allowed easier positioning and use of the forceps, subsequently reducing the advantages achieved with cryobiopsy. However, the larger size, and better quality of the cryobiopsies still attributed towards their overall superiority. Additionally, the authors noted that there were less non-diagnostic results in patients with lung cancer following cryobiopsy then compared to after forceps biopsies. This observation seemed to suggest that cryobiopsy was more sensitive and more reliable in regards to diagnostic yield.

In Schuhmann et al's [71] more recent study of RP-EBUS and cryobiopsy for peripheral lesions, 4 of the cases which were non-diagnostic with forceps biopsy had positive diagnosis on cryobiopsy. This resulted in a yield of 74.2% for cryotherapy, compared to 61.3% for conventional forceps with RP-EBUS. The authors explained that as the cryoprobe was able to freeze all tissue surrounding its tip, this allowed biopsies to be obtained even in lateral directions. This was of particular advantage, especially in the situations where the EBUS probe cannot be advanced directly into the lesion. Of the 4 lesions in Schuhmann's study where diagnosis was only achieved with cryobiopsy, the cryoprobe was located either within or adjacent to the lesion.

More recently, Nasu et al performed retrospective review of peripheral lung cancer cases with the aim of comparing efficacy of cryobiopsy using RP-EBUS to conventional forceps biopsy. Forceps biopsies were performed first, followed by cryobiopsy with RP-EBUS [76]. 53 patients were evaluated with median lesion size 32 mm and positive BS present in 92% cases. There was no significant difference found between diagnostic yields of forceps biopsies was 86.6% compared to cryobiopsies with 81.1% ($p = 0.60$). Mean sample size obtained by cryobiopsy was significantly larger at 14.1mm2 compared to 2.62mm2 ($p<0.001$). Univariate analysis showed that cryobiopsy with guide sheath had significantly higher yield than without guide sheath (95.8% and 69%, $p=0.15$). On multivariate analysis, a positive BS was associated with increased diagnostic yield of cryobiopsy with RP-EBUS.

1.8.4: Lesion Position

Kho et al specifically evaluated the performance of transbronchial cryobiopsy vs forceps biopsy in eccentrically and adjacently orientated RP-EBUS lesions [77]. A target lesion was described as concentric where the probe was completely located within the lesion, whilst a lesion was classified as eccentric when the probe was still located within the lesion, but more biased towards one side. Only 43% (49/114) lesions had concentric orientation whereas the other 57% (65/114) were seen as eccentric or adjacent to the radial probe. Cryobiopsy did not increase the diagnostic yield significantly for concentric lesions compared to the forceps group (85.7% vs 77.1%, $p=0.501$). For the 48 eccentric lesions, diagnostic yield was 80% for cryobiopsy vs 56.3% with forceps biopsy ($p = 0.114$). Of the 18 adjacently orientated lesions, cryobiopsy increased diagnostic

yield to 66.7% (6/9 cases) from 22% (2/9) with forceps (p=0.058). In combining eccentrically and adjacently orientated lesions together, there was a significant increase with cryobiopsy from 48.8% (20/41) to 75% (18/24) with $p<0.05$. However, these results needed to be considered in light of the fact that this was a non-randomised study with study design compounded by selection bias. In a subgroup of 24 cases where patients underwent both conventional forceps and cryobiopsy, diagnosis was only obtained by cryobiopsy in 43.8% cases.

The advantage of the cryoprobe with adjacent lesions, with its ability to obtain a sphere of tissue from its frozen tip and therefore take lateral biopsies as opposed to just sampling in a forward direction has been previously described [71, 78] In Kho's study, 57.2% of inconclusive forceps biopsies from eccentrically and adjacently orientated lesions were found to be bronchial epithelium [77] and the authors commented this was likely to due to too superficial and inadequate depth of lateral biopsies with the forceps.

1.8.5: Fluoroscopy

The feasibility of RP-EBUS-guided cryobiopsy without fluoroscopy was assessed in a small retrospective review by Chang et al [79]. 11 patients were included, with 6 of these undergoing bronchoscopy for diffuse parenchymal lung disease, and the other 5 being investigated for PPLs. There were no major complications recorded. Pathological diagnosis was able to be obtained in 9 patients. Of the 2 patients who had pathology negative for malignancy, one was later confirmed to have non-small cell lung cancer from CT guided lung

biopsy, whilst the other patient did not return for follow up and remain undiagnosed. Whilst this study only had very small sample size, with limited generalisability of results, it did suggest that select PPL cases could be sampled with EBUS-guided cryobiopsy at centres without fluoroscopy equipment, however further study into this was required.

1.8.6: Cryobiopsy without Guide Sheath

The importance of guide sheath had been described above in Nasu et al's study [76] where it was associated with increased diagnostic yield. A more recent study assessed the safety and efficacy of cryobiopsy with RP-EBUS, without the use of guide sheath [80] . The authors noted that the most significant limitation of the cryobiopsy technique was the rigidity of the cryoprobe which could hinder access to PPLs. This was especially the case in areas such as the lung apices where the bronchi are highly curved. A cryoprobe bending technique which relied on the probe's shape bending property was utilised instead of the guide sheath, to help manoeuvrer the probe. An endobronchial blocker was used to limit bleeding. Diagnostic yield from histology for cryobiopsies was 86.1% (31/36). Grade 1 bleeding occurred in 25 patients with grade 2 bleeding occurring in 1 patient only.

1.8.7: Cryobiopsy and Ancillary Testing

Current management and treatment of non-small cell lung cancer (NSCLC) has becoming increasingly personalised, depending on identified

immunohistochemical and molecular features [81] Having large enough biopsy specimens in order to perform these tests and establish appropriate and optimal treatment is critical [76, 82]. The ability to perform ancillary testing with adequate specimens is particularly relevant with NSCLC which not only represents up to 85% of all lung cancer, but typically presents with non-curative disease requiring systemic treatment.

The most significant molecular genetic alteration is the EGFR mutation [83]. Haentschel et al evaluated that rate of EGFR mutations detected in cryobiopsy compared to other biopsy techniques, in pathologically proven NSCLC. 414 NSCLC patients were included, with cryobiopsy used in 125 (30.2%) cases. They confirmed that cryobiopsy increased detection rate with 21.6% (27/125) showing EGFR activation mutations, as compared to 13.8% (40/289) in the non-cryobiopsy group ($p<0.05$). The 289 cases in the non-cryobiopsy group were sampled by other bronchoscopic techniques (forceps biopsy, FNA cytology), radiology guided transthoracic techniques and surgical procedures.

Whilst there was no overall significant difference in detection of these activating EGFR mutations from cryobiopsy compared to conventional forceps, cryobiopsy was able to detect more EGFR activating mutations in central lung tumours compared to conventional forceps (19.6% vs 6.5%, $p<-0.05$). This was a retrospective study, but the first analysis of biopsy technique on EGFR mutation detection in NSCLC patients.

Arimura et al assessed tumour cell numbers and PD-L1 expression in a prospective study of cryobiopsy and transbronchial forceps biopsies in 16 NSCLC patients [84]. Their findings demonstrated significantly more tumour cells from a single cryobiopsy at 1321 +/- 303.7, compared to forceps biopsies at 208.8 +/- 38.24 ($p<0.0001$). The average number of tumour cells obtained with cryobiopsy was also significantly larger (1406 +/- 310.3) than that obtained with forceps (208.8 +/-37.81), $p=0.0006$. Their results suggested that performing 1-2 cryobiopsies would provide more DNA for subsequent analyses of lung cancers compared to 5 conventional forceps specimens. This could also have implications for treatment in patients who had initially been deemed PD-L1 negative by transbronchial forceps biopsies.

1.8.8: Limitations with Cryotherapy

One recognised limitation with cryobiopsy is the need for the whole cryoprobe, with the tissue sample frozen to its tip, to be removed en-bloc with the bronchoscope as a single unit. This differs to the conventional method with forceps biopsies, where the latter can be removed through the working channel of the bronchoscope whilst leaving the bronchoscope in place. Previous studies have acknowledged that all patients undergoing cryobiopsy should be intubated either with a flexible endotracheal tube or through a rigid bronchoscope [69, 74]. Obtaining a secure airway means that in the case of any potential bleeding, the proceduralist would be able to quickly reinsert the bronchoscope. In Rubio et al's study [66] however, the investigators were able to safely perform bronchoscopy with cryobiopsy using a supraglottic airway mask in place of an

endotracheal tube in order to obtain a secure airway. They noted it served the same purpose as the endotracheal tube during bronchoscopy, but also had the added benefit of causing less risk of vocal cord or tracheal damage.

1.8.9: Adverse Effects and Complications

Despite previous concerns that extraction of the cryoprobe with tissue frozen to its tip would result in tears to the airways and bleeding, cryobiopsy has been shown to be comparable and a safe alternative to biopsy with forceps [64, 66, 68, 69, 73].

In Rubio et al's [66] retrospective review of patients being investigated for endobronchial disease with either cryobiopsy alone, or in combination with forceps biopsy, only one patient was recorded as experiencing minor bleeding. In Hetzel et al's [75] multicentre controlled trial, bleeding events were classified depending upon the level of intervention which was required to control it. There was, overall, more number of bleeding events which occurred following cryobiopsy compared to those who received biopsies with conventional forceps ($p=0.009$). However this was secondary to the increased number of mild bleeds. In examining the subset of events where further control was required (either argon plasma coagulation laser, tamponade, iced normal saline or vasconstrictive medication) there was no difference between the two biopsy methods. There was also no difference between the number of patients who were on aspirin or clopidogrel. There was no need for surgical intervention and no deaths.

The authors attributed the increased mild bleeding seen with cryobiopsy to the removal of the cryoprobe and bronchoscope for retrieval of the sample. This caused a longer duration for blood to pool at the biopsy site before any control could be commenced. This would differ when using conventional forceps biopsies where any minor bleeding occurring post biopsy could be suctioned, or tamponade to the biopsy site with the scope could be applied immediately, as the scope remains in place.

The other major complication concern associated with cryotherapy, in addition to bleeding, has been that of pneumothorax. The incidence of this in studies assessing cryobiopsy in diffuse parenchymal lung diseases has been quite varied, ranging from 0-33% [68-70, 85-87]. In Babiak et al's retrospective study, only 2 patients (4.8%) developed pneumothorax, with the authors finding this complication rate quite comparable to that associated with conventional forceps transbronchial biopsies [69]. Higher rates occurred in two prospective studies by Casoni [86] and Tomassetti [87], both of which investigated cryobiopsy in idiopathic pulmonary fibrosis. Casoni et al [86] found that the risk of pneumothorax did not correlate either with size of the specimen, or the number of samples taken, but did increase if pleura was sampled during the biopsy process. They attributed their higher complication rate to the fact that they were purposely aiming for biopsy tissue located in a subpleural location. Biopsy at proximately 1cm from the chest wall was considered appropriate, for this particular population with fibrotic lung disease, in order to achieve maximise diagnostic yield. Their group had also previously noted that risk of

pneumothorax in fibrotic lung disease, exceeded that in other diffuse parenchymal lung diseases [88]. Casoni's study was also the first to report on a death following cryobiopsy. This occurred in an HIV-positive patient who required post-procedural chest drain insertion and high flow oxygen for a large pneumothorax. The authors felt that this triggered the patient's acute exacerbation, who subsequently died 7 days following the procedure.

In Schuhmann et al's [71] feasibility study of cryobiopsy for the investigation of peripheral lesions, there was only one patient who developed moderate-severity bleeding. This required only suctioning alone, in order to achieve control. There was also no post procedural pneumothoraces observed in this study. The authors felt that there were a combination of factors which helped reduce the risk of damage to the pleura – use of fluoroscopic guidance, the radial sheath assisting to maintain biopsy position, and the fact that only lesions visually identified with EBUS underwent biopsy. It does need to be noted however, that given that the patients in this study received a combination of both different biopsy techniques, results from the safety data could not be differentiated as having occurred due to one particular biopsy technique.

1.9: Cryobiopsy versus Surgical Lung Biopsy

Hagmeyer et al [85] were the first to directly compare cryobiopsy and surgical lung biopsy in suspected diffuse parenchymal lung disease. Whilst the latter technique still remained the gold standard for tissue diagnosis in these conditions, the authors wanted to assess situations where patients could not be

referred, either due to non-consent, or because of high perioperative risk. A retrospective review was performed of 32 patients in whom surgery was not appropriate for the above reasons, who underwent cryobiopsy instead. For those whose diagnosis remained equivocal after cryobiopsy, and who had declined surgical referral initially, surgery was again re-offered as a step-up procedure. Subsequent correlation between histology and other clinical and radiological findings were assessed in a multidisciplinary setting. 23/32 cases (72%) showed strong congruence. 9 cases had equivocal diagnoses following multidisciplinary evaluation with 8 patients subsequently consenting to surgical referral. Of these patients, definitive histological diagnosis was ultimately achieved in 6 patients (75%), and a probable congruence in 1. 2 of the surgical patients died within 30 days secondary to acute exacerbations. Overall, the pathological findings from cryobiopsy were thought to be highly congruent with surgical biopsies. They did recognise however, because of the latter's larger size, surgery did lead to more definitive diagnosis. Given their findings, it was suggested that transbronchial cryobiopsies were a safe diagnostic tool, especially where surgical may not be appropriate. For some patients, it may even help remove the need for surgery altogether.

More recently, results of the first prospective study comparing diagnostic agreement between cryobiopsy to surgical lung biopsy for the investigation of interstitial lung disease (ILD) was published. The COLDICE study [89] is the largest study to date, and had co-primary end points of (1) agreement of histopathological interpretation between transbronchial cryobiopsy and surgical lung biopsy, and (2) agreement between matched cryobiopsy and surgical

specimens in determining final consensus clinico-radiological-pathological diagnosis at ILD multidisciplinary discussion (MDD). 65 patients with low-confidence diagnosis or unclassifiable ILD were recruited from 9 Australian tertiary hospitals with expertise in both interventional pulmonology and ILD. Patients underwent both transbronchial cryobiopsy and surgical lung biopsy sequentially, with biopsy information deemed helpful if it changed the degree of confidence in diagnosis, or provided an unanticipated diagnosis.

Results from COLDICE confirmed good concordance between surgery and transbronchial cryobiopsy in interpretation of histopathology, as well as for diagnosis at MDD. In cases where the cryobiopsy diagnosis was deemed definite or of high confidence, surgical biopsies provided minimal additional diagnostic value. Conversely, for the cryobiopsy cases deemed unclassifiable or low confidence diagnosis at MDD, there were only a minority of cases (6/26) where addition of surgery subsequently provided a definite or high-confidence diagnosis. The study suggested that transbronchial cryobiopsy could therefore provide significant diagnostic information, especially in cases deemed to have high-confidence ILD pattern and supported its use as a first-line minimally invasive option for ILD patients who would otherwise require surgical investigation.

Given the study design in COLDICE involved patients undergoing both procedures sequentially, it was therefore not possible to directly compare safety. Of the 25 adverse events which occurred, only some could be directly attributed to cryobiopsy – with mild-moderate bleeding occurring in 14 patients

(22%) as well as one incidence of pneumothorax occurring immediately prior to surgery. Overall, there were 2 acute exacerbations of IPF, with overall 90-day mortality 2%.

1.10: Emerging Bronchoscopic Technologies

1.10.1: Robotic Bronchoscopy

Rojas-Solano et al documented their initial experiences with robotic bronchoscopy in a pilot study which assessed technical feasibility using a Robotic Endoscopy System (RES)[90]. 15 patients with suspicious lesions underwent the procedure under general anaesthesia. The sterile robotic bronchoscope was manually inserted into the endotracheal tube, and then further advanced to the target lesion using an endoscopy controller. Once the lesion was reached, biopsy instruments were passed down the bronchoscope's working channel. There were no events of pneumothorax or significant bleeding requiring further intervention. There were three minor complications which were not related to the RES. Biopsy was obtained under direct visualisation with the RES in 93% patients (14/15). The authors' primary aim was to assess technical feasibility, and the study was therefore not designed to address diagnostic yield. As a result, the patients were selected on the basis of a positive BS on CT. The absence of significant adverse effects was thought to be multifactorial including atraumatic bronchoscope tip, the ability to have precise control of the bronchoscope and to lock the scope into a desired position, and thorough training of the RES beforehand [90]. The robotic bronchoscope was thought to

have improved ability to access more distally located lesions, as opposed to conventional bronchoscope given its better column strength and telescoping design.

A further study by Fielding et al relayed the first in-human evaluation of another novel robotic-assisted bronchoscopic system (Intuitive Robotic Bronchoscope System) for patients with PPLs. Pre-procedural CT scans allowed virtual planning, and the proceduralist was able to manoeuvre a robotically controlled catheter to the lesion of interest whilst viewing a virtual pathway, in addition to live direct airway visualisation from a video probe. The catheter could be advanced in all planes of direction, and could also be held still in a specific position without slippage and whilst maintaining its angulation. 29 patients were included and the primary feasibility endpoint of accessing the PPL and subsequent positive histopathological diagnosis from biopsy was met in 29 patients (96.6%). Similar to the earlier study [90], there were no observed pneumothoraces or significant bleeding. This was attributed to the continuous visualisation of catheter, its ability to hold position, and the ability of the catheter to tamponade the airway, similar to a guide sheath. RP-EBUS was used following navigation to confirm lesion location. In comparison to Rojas-Solano's study [90], there only 17 cases in this series (58.6%) who had positive BS on CT, with 13 cases (44.8%) having an eccentric image. Mean nodule size was also 14.8mm (10- 26.6mm) in the latter study, compared to 26mm (range 10-63mm).

These early studies suggested robotic bronchoscopic systems had favourable safety profiles, and good capability of accessing PPLs due to their ease of manipulation and maintenance of position for sampling.

1.10.2: Cone Beam Computed Tomography (CBCT)

CBCT is an emerging imaging modality typically utilised by interventional radiologists [91]. The CBCT system is mounted upon a moving C-arm fluoroscopy system which can be orbited around a stationary target (the patient). With one single orbit around the anatomic region of interest, an entire volumetric dataset can be obtained, and this is used to generate images similar to CT-images in real time [92, 93]

A 2018 pilot study combined CBCT technology with thin/ultra-thin bronchoscopy to investigate PPLs [92]. The study's primary aim was to assess radiation exposure associated with CBCT; however their secondary aim was to also evaluate the utility of CBCT as a navigational and diagnostic tool. "Navigational yield" described the proportion of patients where RP-EBUS identified the lesion as positive/inconclusive, and CBCT confirmed the radial probe to be in contact with lesion. A positive RP-EBUS image was one where the target lesion was identified. Aerated lung was considered a negative image, and those images not meeting the criteria for positive or negative were considered to be inconclusive. Of the 20 patients enrolled, 12 lesions (60%) were fluoroscopically invisible. The other 8 were all identified with RP-EBUS, and 6 were confirmed with CBCT. Pre-CBCT navigation and diagnostic yields were 50% (10/20). In

the remaining 10 patients, further manoeuvring as guided by CBCT to reach the lesion that was not identified beforehand increased the navigational yield to 75% (p=0.02), and the post-CBCT navigational yield was increased to 70% (p=0.04).

A subsequent study by Ali et al [93] combined CBCT with VBN and ultrathin bronchoscopy for the diagnosis of small PPLs. CBCT findings were categorised according to the CBCT target-forceps sign, which was assessed by the position of lesion relative to the forceps. Patients were classified as Type A when the forceps were seen to reach inside the lesion and Type C when the forceps did not access the lesion. Type B encompassed those patients who did not fall under Types A or C. Biopsy forceps position was readjusted until there was correspondence between the CBCT target forceps sign, and the CT bronchus sign. Of the 40 patients enrolled, there were 29 lesions (72.5%) which were fluoroscopically invisible, but visible with CBCT. 32 (80%) patients had Type A CT BS, and 8 (20%) were classified as Type B. Overall diagnostic yield was 90%. Yields for CT BS types A and B were 96.9% and 62.5% respectively. Diagnostic yields for CBCT target forceps signs Type A, B and C were 100%, 75% and 0% respectively. In 95% patients, the forceps were able to be maneuverered into or adjacent to the target lesion with CBCT, and in this regard CBCT was thought to be superior to VBN, or VBN with fluoroscopy. The authors concluded that CBCT images contributed not only as a navigational tool, but to also help clarify reliability of bronchoscopy biopsy diagnosis.

1.11: EBUS IMAGE ANALYSIS

Investigation of peripheral pulmonary lesions with RP-EBUS is a well-recognised bronchoscopic technique. Its ability to provide information as to the position of the miniprobe relative to the lesion, in order to guide biopsy, has made it a valued additional diagnostic tool. However the image produced by ultrasound has also been studied as to how its features can provide further information and potentially clinical data about the lesion's underlying nature. Most of the previous studies addressing analysis of EBUS images have been descriptive in nature [94].

Image analysis of EBUS images was used by Kurimoto et al [95] to assess whether depth of invasion by tracheobronchial cancers could be determined. This knowledge would be of significance, given that level of invasion was an important factor in deciding subsequent treatment modality. Kurimoto was not the first to report on EBUS analysis of bronchial structure - previous authors [43] had also done so, however they did not confirm accuracy by correlating their results with histology. Kurimoto recognized that this was imperative, as an accurate understanding of the bronchial structure would then assist in correctly determining depth of tumour disease. Needle puncture was performed on 45 surgically resected normal tracheal and bronchi specimens, and 24 specimens from patients with lung cancer. High frequency ultrasonographic examination was also performed on the specimens. They were able to demonstrate 5 distinct layers within both cartilaginous and membranous regions of the

tracheobronchial tree, as identified by different imaging features on EBUS. Correlation between EBUS image and histopathological findings occurred in 95.8% (23 of 24 lesions). There was only one case where EBUS overestimated depth of invasion.

A subsequent study, also assessing accuracy of EBUS to image the tracheobronchial wall and to determine depth of invasion, was reported by Baba et al [96]. Their results also supporting those found earlier by Kurimoto [95]. They studied 61 patients – 21 of which received EBUS during bronchoscopic examination in vivo. The other 40 with peripheral tumours or no malignancy had resected specimens on which EBUS was performed ex vivo postoperatively. Ultrasonographic examination of the bronchi demonstrated 6 different layers consisting of epithelium, lamina propropia and submucosa, cartilage layers and adventitia. Surrounding structures such as vasculature and lymph nodes could also be visualized. The authors noted that depth of malignant invasion could be determined based on disruption of the cartilage layer, but width and size could not be determined due to the nature of the radial scanning image. Histologic subtype of the tumour could also not be determined by the EBUS.

The first study to report on the internal structure of a peripheral pulmonary lesion, in order to improve criteria for distinguishing between benign and malignant disease were Kurimoto et al [40]. They performed a retrospective review of 124 patients with PPLs who had undergone EBUS, with a subsequent definitive histopathological diagnosis obtained. In 69 patients, surgically resected specimens were available for correlation. Upon analysing the internal

structure of the lesion, Kurimoto was able to identify 3 different classes (or 6 subtypes) of lesions based on their EBUS pattern for the presence of internal echoes, vascular patency, and the morphology of hyperechoic echoes which reflected presence of air and the state of the bronchi. 23 of the 25 Type I lesions were benign (92%), whilst 98 of 99 Type II and III lesions were malignant (99%). Majority of Type II lesions were well differentiated adenocarcinomas (21 of 24, 87.5%). The authors noted that the presence of hyperechoic dots, when distributed irregularly within the lesions, was characteristic of well-differentiated adenocarcinoma, and represented residual air in the alveoli which grows to replace the alveolar epithelium. All Type IIIb lesions were malignant, of which 18 cases were poorly differentiated adenocarcinomas (81.8%) and were seen to be avascular on EBUS image with scant mottled or linear hyperechoic areas. The authors concluded that this information gained from analysis of the EBUS image of PPLs would be useful in speculating its underlying histology.

The issues raised, however, with Kurimoto's [40] technique was that it relied on well-trained operators, and was time-consuming to distinguish between different vascular and neoplastic patterns [97].

Subsequently, a later study by Chao et al [97] also investigated the use of EBUS in peripheral lesions, but aimed to do so using a more simplified method of analysing the EBUS images to speculate on the lesion's underlying nature. 151 patients with peripheral lesions were included. EBUS characteristics from the first 20 were compared to their tissue diagnosis, and used to train 6

experienced bronchoscopists in identifying distinct image characteristics and patterns. For the subsequent 131 patients recruited, 2 of the bronchoscopists would type the EBUS patterns during the procedure. Where the patterns identified were congruent, hard copies of the EBUS image were then sent to an independent radiologist reviewer who was blinded to the previous results. Only the 126 images with agreeance between all 3 reviewers were included for analysis. The authors identified that were generally 4 distinct patterns which were easy to identify and type – continuous hyperechoic margin outside the lesion, homogenous or heterogenous internal echoes, hyperechoic dots in the lesion, and concentric circles along the probe. Images demonstrated homogenous internal echoes or concentric circles were more likely to be benign lesion by univariate analysis (p=0.001), but only the concentric circles remained significant on multivariate analysis. 13 of 16 lesions (81.3%) with continuous hyperechoic margins were malignant however there was only a trend towards significance with this observation.(p=0.090)

A further study by Kuo et al [98] also aimed to simplify and reclassify the EBUS image characteristics earlier described by Kurimoto [40] into 3 distinct patterns: (1) continuous margin; (2) nonlinear, dotted or mottled air bronchogram; and (3) heterogenous echogenicity. 224 patients with peripheral lesions were recruited, of which 123 had malignant disease, and 101 had benign lesions. Lesions with a continuous margin were noted in 27.6% malignant lesions, and 6.9% benign (p=0.0001). The air bronchogram pattern observed in 91.9% malignant lesions, and 37.6% benign lesions (p=0.0001). Finally, the heterogeneous echogenicity pattern was observed in 65% malignant lesions, and 9.9% benign lesions with

p=0.0001. The combination of all three patterns was found in 8.9% malignant lesions, but not in any of the benign lesions. Conversely, the absence of all three features within the lesion was only found in 3.3% malignant lesions, and in 59.4% of the benign lesions. They demonstrated a positive predictive value for malignancy in a lesion with all three patterns of 100%, whilst the negative predictive value for malignancy when no features were present was 93.7%. The authors did note that the sensitivity of identified continuous margins in predicating malignancy varied depending upon lesion size and probe position. The pattern was less accurate in detecting malignancy where the lesion was larger than 3cm in diameter or where the probe was adjacent to the lesion, as opposed to a lesion diameter smaller than 3cm or with the probe situated inside the lesion. The results reiterated the value of EBUS as a diagnostic tool.

All the above studies demonstrating how information from analysis of EBUS images could be utilised for clinical practice relied heavily on the operator having the ability to identify characteristics patterns. This means that they not only relied on an operator's subjective interpretation of the image, but also their previous experience and knowledge with EBUS. Whilst there was often congruence between operators in the above studies; they were all expert and well trained bronchoscopists or radiologists who were familiar with EBUS.

An alternative and objective method of assessing different features of EBUS images is to apply computer-assisted image analysis. This is not a new technique. Haralick et al [99] were the first demonstrate application of easily computable textural features, as based on their grey-scale, to help categorise

or classify pictorial data. They realized that texture was present in all images, and developed a class of textural features which examined the relative frequency distribution. That is, the features were based on the frequency of which one particular grey tone would appear in a specified spatial relationship to another grey tone in the image being examined.

Texture of an image was later further defined as the “appearance, structure and arrangement of the parts of an object within the image” by Castellano [100]. The authors recognized that most medical images were two-dimensional, and composed of little blocks or pixels, which could be further analysed to provide information about the picture. Consequently, grey scale texture analysis was performed on particular regions of interest (ROI) within an image, in order to evaluate the pixels and their grey-level of intensity. As established earlier in Haralick’s paper [99], the textural features assessed were simply mathematical parameters, derived from the distribution of pixels. Their study confirmed that interpretation of the texture features could assist in understanding the underlying structure of the object being imaged [100].

Grey scale textural analysis was subsequently applied to ultrasound images of pathology within different organs such as breast [101], liver [102], endometrium [103] and prostate [104] with success.

The application of grey scale texture analysis to an EBUS image was first described by Nguyen et al [105]. Their study assessed whether computer-assisted grey scale analysis could be applied to EBUS images to help differentiate between malignant and benign lymph nodes. Given the finding by

previous researchers that regions of interest <32 pixels^2 were associated with significant decrease in grey scale texture analysis sensitivity; all of the included ROI were greater than 32 pixels^2. 151 lymph nodes from 136 patients were included, the first 100 of which were used in the prediction set and the remaining in the validation set. 18 patients in this validation set also had FDG-PET-CT results which were available for comparison. 44/51 (86.3%) of the nodes within the validation set were able to be classified correctly. When comparing grey scale texture analysis to FDG-PET-CT, 16/18 (88.9%) were correctly classified according to texture analysis compared to 14/18 lymph nodes (778%) with PET scan criteria.

Given that earlier studies into analysis of peripheral pulmonary lesion images obtained with RP-EBUS were generally descriptive and required expert analysis [97, 98, 106], Nguyen et al subsequently investigated whether greyscale texture analysis could be applied to RP-EBUS images to objectively distinguish benign from malignant aetiology. They only included images where ultrasound contrast and gain settings were set at 4/8 an 10/19 respectively, as any inconsistency with image parameters caught would alter subsequent grey scale features. The proceduralist captured a single still image, as assessed to be most appropriate for performing image analysis of the lesion. Region of interests consisted of 24 x 24 or 32 x 32 pixel squares which could be placed over the EBUS image, depending on the lesion's size. 167 EBUS-MP images were included for analysis – the first 85 in the prediction set, and the following 82 for validation set. The prediction set showed that there were three significant greyscale features differentiating benign lesions from malignant: increased difference

between maximum and minimum pixel values, increased standard deviation of the mean pixel values, and increased entropy. Using these features to help classify lesions in the subsequent validation set, physician 1 was able to correctly classify 63/83 (76.8%) of the lesions, whilst physician 2 correctly identified 62/82 (75.6). NPV for malignancy was 100%, and PPV was 71%. Again by comparing image analysis results to FDG-PET-CT scan results in 34 lesions where PET results were available, both physicians identified 27/34 lesions (79.4%) which was the same as FDG-PET-CT expert opinion.

A similar method of quantitively evaluating EBUS B- mode images was reported by Morikawa et al [107] who used histogram-based analysis of brightness of EBUS images. They retrospectively reviewed clear EBUS images from 38 lung cancer and 22 inflammatory disease patients. For each image, a 400-pixel region of interest was chosen and 6 histogram features were analysed: height, width, height/width ratio, standard deviation, kurtosis and skewness. Of these, height, width, height/width ratio and standard deviation were found to be significantly different between malignant and benign lesions. The histogram feature with highest diagnostic accuracy was standard deviation. Median histogram standard in lung cancer was 11.35 (8.0=14.6) compared to the inflammation group with 9.55 (7.3-14.0). Using a cut-off value of 10.5, this was found to have sensitivity of 78.9%, specificity 86% and diagnostic accuracy of 81.7% for the malignant lesions.

The application of image analysis to EBUS images potentially helps provide further clinical information to clinicians as to whether the image being studied is

benign or malignant. It will never be a substitute for obtaining tissue diagnosis, which would still be the gold standard for confirming evidence of malignancy but could be useful to support diagnosis of benign conditions where biopsies are negative or equivocal. Conversely, it could provide stronger argument for more invasive management such as surgery, in the instances of a negative biopsy but high suspicion for malignancy from greyscale analysis and consideration of the patient's pre-test risk.

CHAPTER 2 (paper)

Radial Endobronchial Ultrasound Greyscale Texture Analysis Using Whole-Lesion Analysis can Characterise Benign and Malignant Lesions without Region-of-Interest Selection Bias

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Radial Endobronchial Ultrasound Greyscale Texture Analysis Using Whole-Lesion Analysis Can Characterise Benign and Malignant Lesions without Region-of-Interest Selection Bias

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Keywords
Bronchoscopy · Endobronchial ultrasonography · Lung cancer · Image analysis

Abstract
Background: Radial-probe endobronchial ultrasound (RP-EBUS) is predominantly used clinically for the localisation of peripheral pulmonary lesions prior to biopsy. However, the RP-EBUS image itself contains information that can characterise the aetiology of lesions. ***Objectives:*** The aim of this study was to show the utility of RP-EBUS image analysis using unconstrained regions of interest (ROIs) that utilise more image information and eliminate ROI selection bias. ***Methods:*** We developed custom software to analyse RP-EBUS images digitally captured during clinical procedures. Unconstrained ROIs were mapped onto lesions. We computed first-order greyscale image statistics of minimum, maximum, mean, standard deviation and range of pixel intensities, and entropy. We also computed second-order greyscale texture features of contrast, correlation, energy and homogeneity. The results of image analysis were compared to gold-standard tissue diagnosis. Features from expert- and non-expert-defined ROIs were also compared. ***Results:*** Eighty-five images were analysed (38 benign and 47 malignant). Five greyscale features were significantly different between benign and malignant lesions. Benign lesions had higher mean ($p < 0.01$) and maximal ($p < 0.001$) intensity, greater range ($p < 0.001$) of pixel intensities and greater entropy ($p < 0.01$). The highest positive predictive values were associated with maximal (87.8%) and range of pixel (83.8%) intensities. There were no significant differences between expert- and non-expert-defined ROIs. ***Conclusion:*** RP-EBUS image analysis using unconstrained ROIs eliminates ROI selection bias and can characterise benign and malignant lesions with an accuracy of up to 85%.

Introduction

Radial-probe endobronchial ultrasonography (RP-EBUS) is a technique that uses high-frequency ultrasonography (20–30 MHz) to visualise peri-tracheal and peri-bronchial tissue and structures. Brightness mode images from this modality reflect tonal and structural

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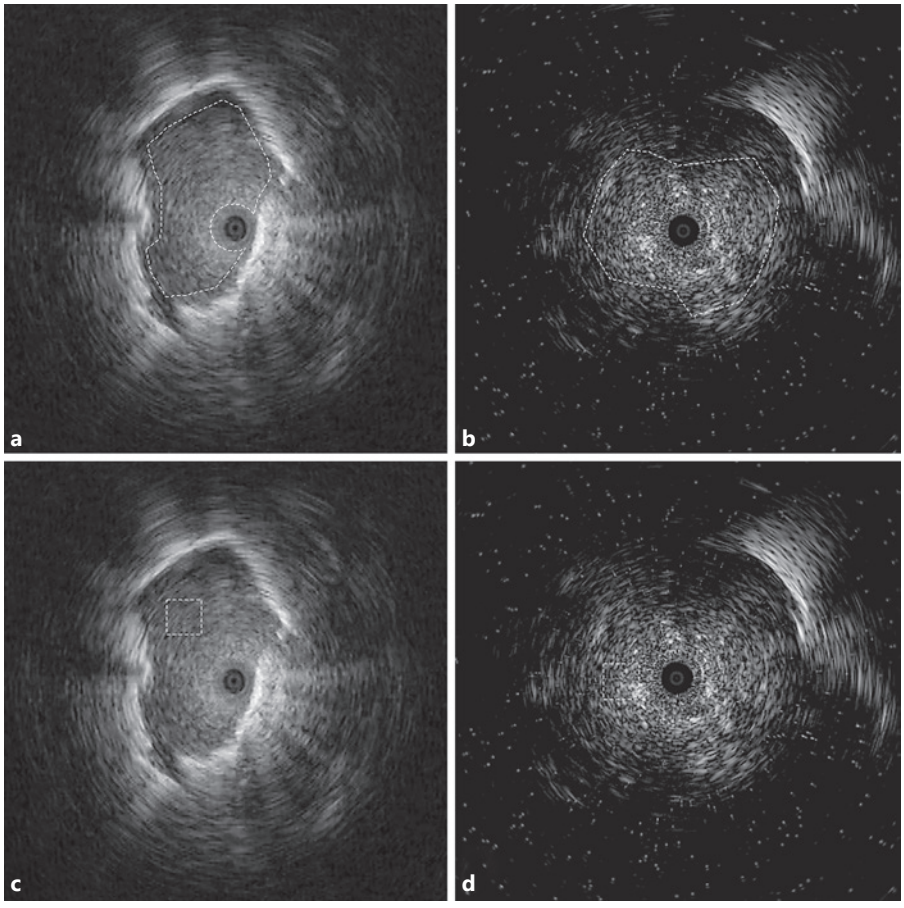

Fig. 1. a Well-defined lesion with polygonal ROI. **b** Ill-defined lesion with polygonal ROI. **c** Well-defined lesion with an example of a 32 × 32 pixel square ROI. **d** Ill-defined lesion with an example of a 32 × 32 pixel square ROI. ROI shown in dashed lines. ROI, region of interest.

properties of the tissue being imaged [1]. It is most commonly used for the localisation of peripheral pulmonary nodules prior to cytological and/or histological sampling, typically via a guide sheath [1–4]. The use of RP-EBUS has been shown to significantly improve diagnostic yield and reduce complications, such as pneumothorax, when compared to computed tomography (CT)-guided biopsy of peripheral pulmonary nodules [2, 5–8]. In the hands of experts and the controlled environment of research studies this technique has been shown to have a diagnostic yield of up to 87% [2]. However, the real-world data indicates a diagnostic yield of approximately 57% [9]. Therefore, a significant percentage of patients with peripheral pulmonary lesions, even when identified by RP-EBUS, do not get a definitive tissue diagnosis. These patients will often undergo a repeat attempt at RP-EBUS-guided biopsy or an alternative modality for tissue diagnosis with associated morbidity and mortality. Hence, tools that can assist in distinguishing lesions as benign or malignant will be useful. This is where the use of the RP-EBUS image itself is proposed as a novel tool.

In 2002, Kurimoto et al. [10] first described the clinical utility of RP-EBUS image characteristics. Images were qualitatively analysed by trained experts to identify image types (homogeneous, hyper-echoic dots and linear arcs or heterogeneous patterns). These were then sub-typed according to the presence or absence of vessels/bronchioles or the presence or absence of hyper-echoic dots or short lines. Comparing these identified image characteristics to resected tissue histology they demonstrated a relationship between the aetiology of lesions and RP-EBUS image patterns [10]. Chao et al. [11] presented similar work from qualitative analysis of RP-EBUS images to identify the presence or absence of 4 image features (continuous hyper-echoic margins, internal echoes, concentric circles and hyper-echoic dots). They also demonstrated a relationship between RP-EBUS image features and the aetiology of the lesion [11]. Similar work has been presented by others [12, 13]; however, these methods all rely on experts to subjectively identify image features or patterns.

To address the subjective nature of prior work, Nguyen et al. [14] developed an objective method to analyse

Table 1. Aetiology of malignant and benign lesions

Malignant		Benign	
Pulmonary adenocarcinomas	19/46 (41%)	Inflammation, infection or normal lung	20/38 (53%)
Pulmonary squamous cell carcinoma	7/46 (15%)	Sarcoidosis or granulomatous inflammation	10/38 (26%)
Non-small-cell lung carcinoma	5/46 (11%)	Cryptogenic organizing pneumonia	4/38 (11%)
Small-cell lung cancer	4/46 (9%)	Langerhans histiocytosis X	1/38 (3%)
Large-cell lung cancer	1/46 (2%)	Eosinophilic pneumonia	1/38 (3%)
Non-pulmonary malignancies	10/46 (21%)	Cryptococcal pneumonia	1/38 (3%)
Carcinomas	7	Pulmonary alveolar proteinosis	1/38 (3%)
Melanoma	1		
Colorectal adenocarcinoma	1		
Renal cell carcinoma	1		

RP-EBUS images. Digitally captured RP-EBUS images were used. A small 25 × 25 or 32 × 32 pixel region of interest (ROI) was selected by an expert and analysed using a computer to calculate greyscale image statistics and texture features. They demonstrated that RP-EBUS greyscale texture analysis can characterise lesions as benign or malignant with relatively good sensitivity and specificity [14]. Criticisms of their study were, firstly, that the ROI was relatively small compared to the total image data available and, secondly, that an expert decided where on the RP-EBUS image the small ROI should be placed, introducing bias. The aim of our study was to demonstrate that RP-EBUS image analysis using unconstrained, whole-lesion ROI, can characterise benign or malignant aetiology and eliminate issues with ROI selection bias.

Methods

The study was performed in the Department of Thoracic Medicine at the Royal Adelaide Hospital, Adelaide, South Australia. The prediction set images from the study by Nguyen et al. [14] (co-author of the current study) were analysed. Appropriate ethics board approvals were obtained as outlined in that study. All RP-EBUS images were obtained by a group of consultants with at least 5 years' experience using EBUS. RP-EBUS images using a contrast level setting of 4 (out of a manufacturer maximum level of 8) and gain level setting of 10 (out of a manufacturer maximum level of 19) were captured and saved in an uncompressed file format (TIFF). We developed a custom software application using Matlab (R2016a, The MathWorks Inc., Natick, MA, USA, 2000) for ROI selection, image processing, greyscale statistic and texture analysis, and data management.

ROI Selection and Probe Artefact Exclusion

Following selection of an image for analysis, the user selects the centre of the radial probe manually with a mouse click. The software then excludes a pre-determined area from the centre of the RP-EBUS field, corresponding to RP-EBUS probe artefact, from analysis. An unconstrained, polygonal ROI was then drawn over the whole lesion (Fig. 1). This ROI was then analysed for the features described below.

Image Statistics and Texture Features

The greyscale image statistics and texture features that were calculated have been described previously [14–16]. Briefly, these consist of the mean, maximum, and minimum pixel intensity, the range (maximum–minimum) and standard deviation of pixel intensities, and entropy (a measure of the degree of disorder in pixel intensities). These are collectively termed first-order features as they do not contain information regarding the spatial relationship of pixels. Second-order features, i.e., contrast, correlation, energy and homogeneity, were also calculated. Contrast is a measure of the weighted mean difference in neighbouring pixel intensities; correlation is a measure of the correlation between pixel intensities; energy is a measure the variety of pixel intensities, and homogeneity is a measure of the frequency of identical pixel intensities in neighbouring pixels. These features are collectively termed second-order features and require calculation of grey-level spatial dependence matrices as described by Haralick et al. [16].

Tissue Diagnosis

The gold-standard diagnosis was that of tissue diagnosis, obtained by either cytology from brushing, or histology of biopsy samples or excised specimens. Lesions were defined as benign based on pathology or stability/resolution on surveillance CT chest imaging for up to 24 months [14]. The aetiology of the malignant and benign lesions is listed in Table 1.

Expert versus Non-Expert

To test inter-user variability in ROI selection, an expert physician was asked to draw unconstrained ROIs on areas they felt were appropriate for all images in the image set. A second non-expert then independently drew unconstrained ROIs for the same images. The "expert" was a consultant with at least 5 years' experience in using RP-EBUS. The "non-expert" was a trainee with 6 months' experience in using RP-EBUS. In all cases, it was an expert who performed the actual EBUS procedure to obtain the image. The trainee was only involved in the image analysis part of the project. The pixel area, greyscale image statistics and texture features where then compared between expert and non-expert selected ROIs.

Table 2. Greyscale statistics and texture features – comparing benign and malignant lesions

Feature	Benign (n = 38)	Malignant (n = 46)	p*
Maximum	244 (230–255)	199 (163–218)	<0.001
Max. – min.	231 (218–255)	186 (153–218)	<0.001
Mean	77.5 (52.0–102.8)	61.6 (48.2–74.3)	<0.01
SD	34.2 (31.3–41.0)	26.7 (24.7–32.5)	<0.001
Entropy	6.9 (6.7–7.1)	6.6 (6.4–6.8)	<0.01

Values are medians (interquartile ranges). SD, standard deviation. * Mann-Whitney *U* test.

Statistical Analyses

All statistical analyses were performed in Matlab (R2016a, The MathWorks Inc., Natick, MA, USA, 2000). The median and interquartile ranges were calculated for all features. The Mann-Whitney *U* test was used to compare greyscale texture features between benign and malignant lesions. Receiver-operating characteristic (ROC) curves were generated for features that were found to be statistically different on Mann-Whitney *U* test ($p < 0.05$). The area under the ROC curve (aROC), a measure of test accuracy, and the optimal cut-off were calculated. An aROC of 0.5–0.7 is considered low accuracy, 0.7–0.9 moderate, and >0.9 high accuracy [14]. RP-EBUS images were then classified as benign or malignant using the optimal cut-off and the result compared to the gold-standard tissue diagnosis. From this, the statistics of sensitivity and specificity, and positive and negative predictive values (PPV and NPV, respectively) were calculated. For comparing the expert and non-expert ROIs, Pearson's correlation coefficient and Mann-Whitney *U* test were used to compare ROI pixel area and greyscale texture features.

Results

In the study by Nguyen et al. [14], a total of 85 cases were used in their prediction set. In total, 47 had malignant and 38 had benign diagnoses. Eight of the benign diagnoses were made based on resolution or stability on imaging at an interval greater than 12 months and up to 24 months. In the current study, one of the malignant cases had to be excluded from analysis due to a corrupt image file. Hence, a total of 46 malignant and 38 benign cases were analysed. The majority of malignant cases were pulmonary adenocarcinoma, and the majority of benign cases were infection/inflammation (Table 1).

Five greyscale texture features were found to be significantly different between benign and malignant lesions (Table 2). Benign lesions had higher mean ($p < 0.01$) and maximal ($p < 0.001$) pixel intensity, greater range ($p < 0.001$) of pixel intensities, and greater degree of disorder (entropy) in pixel intensities ($p < 0.01$). The minimum pixel intensity, standard deviation, contrast, correlation, energy and homogeneity were not statistically significant. At the optimal cut-offs outlined in Table 3, maximal pixel intensity identified malignancy with a sensitivity of 78.3%, specificity of 86.8% and accuracy of 85%; the range of pixel intensities identified malignancy with a sensitivity of 67.4%, specificity of 84.2% and accuracy of 80%; the mean pixel intensity identified malignancy with a sensitivity of 78.3%, specificity of 52.6% and accuracy of 64%; the standard deviation of pixel intensities identified malignancy with a sensitivity of 73.9%, specificity of 74.4% and accuracy of 75%; and entropy identified malignancy with a sensitivity of 63%, specificity of 73.7% and accuracy of 69%. The highest positive predictive values were associated with maximal and range of pixel intensities. The highest negative predicative values were associated with maximal and standard deviation of pixel intensities. These results are summarised in Table 3.

Expert versus Non-Expert

The results comparing the ROI pixel area and greyscale texture features when calculated from ROIs selected by an expert and a non-expert are summarised in Table 4. There were strong correlations ($r > 0.8$) and no significant differences ($p > 0.05$) between the ROI area and greyscale texture features from ROIs selected by the expert and the non-expert.

Discussion

The current clinical use of RP-EBUS is in the localisation of peripheral pulmonary lesions prior to biopsy. Despite the reported high diagnostic yield of RP-EBUS-guided biopsy in the literature, the real-world data suggests that diagnostic yield using this technique is likely of the order of 60% [9]. Thus, a significant number of pulmonary lesions identified by RP-EBUS do not have definitive biopsy results to guide management decisions. Following appropriate validation of the methods described in this paper, the utilisation of RP-EBUS image features could support clinical decision-making, particularly in the setting of non-diagnostic/inconclusive biopsy results. For example, a patient with a low pre-test probability of malignancy, a non-diagnostic biopsy, and EBUS image features suggestive of benign aetiology could undergo clinic surveillance versus a repeat invasive procedure. Equally, a patient with a high pre-test probability of malignancy and non-diagnostic biopsy, with EBUS image

Table 3. Greyscale statistics and texture features to differentiate benign and malignant aetiology using the optimal cut-off calculated using receiver-operating characteristic curves

Feature	aROC	Optimal cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Maximum	0.85	224	78.3	86.8	87.8	76.7
Maximum – minimum	0.80	206	67.4	84.2	83.8	68.1
Mean	0.64	75.6	78.3	52.6	66.7	66.7
Standard deviation	0.75	32.4	73.9	71.1	75.6	69.2
Entropy	0.69	6.7	63.0	73.7	74.4	62.2

aROC, area under the receiver-operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value.

Table 4. ROI pixel area and greyscale statistics and texture features calculated from expert- and non-expert-selected ROIs

	ROI pixel area	Minimum	Maximum	Range	Mean	SD	Entropy
Non-expert	5,139 (3,258–8,389)	11 (0–18)	222 (182–252)	214 (162–239)	70.33 (49.83–84.31)	29.35 (24.37–34.20)	6.70 (6.46–6.95)
Expert	5,007 (3,632–8,895)	9 (0–16)	227 (187–252)	217 (177–239)	65.65 (48.96–85.03)	31.78 (24.92–35.24)	6.69 (6.50–7.00)
p^*	0.72	0.67	0.76	0.73	0.79	0.44	0.54
$r^{\#}$	0.86	0.90	0.83	0.86	0.96	0.90	0.92

Values are medians (interquartile ranges). ROI, region of interest. * Mann-Whitney *U* test. # Pearson's linear correlation coefficient.

features suggestive of malignant aetiology, may proceed to definitive treatment (e.g., surgical resection) versus a repeat invasive diagnostic procedure. Certainly, adding RP-EBUS image features to validated nodule assessment tools [17, 18] is another possible utility of this methodology.

Multiple studies have demonstrated the utility of RP-EBUS image features and patterns in classifying peripheral pulmonary lesions as benign or malignant [10–13]. Most of these studies, however, have been based on subjective assessment of the RP-EBUS images by trained experts. To our knowledge, only 2 studies to date have looked at objective image-based measures. Nguyen et al. [14] used small ROIs selected by an expert and analysed images for greyscale texture-based measures. Morikawa et al. [19] applied a similar methodology but computed greyscale histogram-based measures.

In our study, using unconstrained, whole-lesion ROIs, we have demonstrated that greyscale image statistics and texture measures can classify lesions as benign or malignant with relatively good sensitivity and specificity. As expected, our results mirror those of Nguyen et al. [14] as they are based on the same image set. However, additional measures of mean and maximal pixel intensity were also included in our analysis and shown to be significantly different between benign and malignant lesions. Importantly, using this approach, we have also shown that there is no significant difference in resultant greyscale image statistics and texture features based on ROIs selected by an expert or non-expert.

There are limitations to the work we have presented. The sample size is small, without a dedicated validation set, and images are limited to those RP-EBUS images with constant gain and contrast settings. The latter requirement was to ensure consistency with greyscale image analysis. Despite showing significant differences, we have only presented univariate analyses. The use of logistic regression models that incorporate 2 or more variables to improve the diagnostic accuracy is possible. However, with our small sample size this type of analysis could yield false, misleading results. We were also unable to obtain the actual size of the lesions on CT of the chest. An analysis to investigate if the size of the lesion influences texture analysis/features is planned for future studies. Also, a larger group of experts and non-experts should be tested to verify the inter-user variability in ROI selection.

Probe characteristics and limitations must also be considered. As address by Morikawa et al. [19], image pixels

within a 3-mm radius of the centre of the RP-EBUS probe are artefact. Equally, image pixels further than a 5-mm radius from the centre of the RP-EBUS probe are prone to noise due to attenuation of the ultrasound signal. We excluded an inner area from analysis to eliminate probe artefact; however, we did not exclude image data beyond a 5-mm radius. The effect of any significant ultrasound attenuation on our data is unclear.

To address these limitations, a larger dataset is required. This will also allow validation of the current study technique using a separate validation set of images and to explore appropriate multi-variate models. Further investigation of the probe characteristics to test whether restriction of the ROIs to 3–5 mm from the probe centre is also warranted. To follow up our proof-of-concept study, we plan to expand our image analysis set via recruitment of further patients from multiple centres within our scientific society.

In conclusion, we have demonstrated that RP-EBUS greyscale image statistics and texture analysis using the whole lesion as a ROI eliminates ROI selection bias and can characterise benign and malignant lesions with an accuracy of up to 85%. With ongoing work and appropriate validation, this type of objective, quantitative analysis could be a valuable additional clinical tool.

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Disclosure Statement

The authors report no conflicts of interest.

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CHAPTER 3 (paper)

Radial Endobronchial Ultrasound with Transbronchial Cryobiopsy versus Radial Endobronchial Ultrasound Alone for the Diagnosis of Peripheral Pulmonary Lesions

Title:

Radial Endobronchial Ultrasound with Transbronchial Cryobiopsy versus Radial Endobronchial Ultrasound Alone for the Diagnosis of Peripheral Pulmonary Lesions

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ABSTRACT

Background

The incidence of peripheral pulmonary lesions (PPLs) has been steadily increasing due to lung cancer screening and rising referrals for imaging. Although transbronchial forceps biopsies (TB-FB) has been the conventional bronchoscopic approach to tissue diagnosis, yield can be highly variable. Cryotherapy has recently provided an alternative biopsy tool. We sought to compare transbronchial cryobiopsies (TB-CB) to TB-FB under endobronchial ultrasonography (EBUS) guidance in the diagnosis of PPLs.

Methods

A prospective, single-centre, randomised controlled trial of patients referred to the Royal Adelaide Hospital (RAH) with PPLs suspicious for lung cancer was undertaken. Patients were randomised to receive either one TB-CB samples, or 5 TB-FB samples, with the latter being current standard of care.

Results

A total of 28 lesions were evaluated. Overall diagnostic yield was 76.8%. Diagnostic yields of TB-CB and TB-FB were 91.7% and 68.8% respectively ($p=0.14$). Median size of TB-CB was 7.0mm compared to 2.5mm with TB-FB ($p<0.0001$). The EBUS probe was seen adjacent to the lesion in 4/28 cases, and cryobiopsy provided a positive diagnostic yield in 3 of these 4 lesions. There were no major complications with either technique.

Conclusion

TB-CB with EBUS guidance provides larger biopsy specimens with no increased risk of major complications. This technique could potentially provide increased diagnostic yield in smaller PPLs, and in those lesions which are difficult to access.

INTRODUCTION

Peripheral pulmonary lesions (PPLs) are focal parenchymal opacities typically identified on chest imaging. By definition, a PPL is completely surrounded by pulmonary parenchyma and therefore cannot be visualised endobronchially during bronchoscopic examination [3]. The main concern regarding these lesions is their potential to have an underlying malignant aetiology. Obtaining tissue is the only definitive method to confirm diagnosis and transbronchial biopsy is the most conventional bronchoscopic method for doing so. However, even when performed under fluoroscopic guidance and combined with other sampling procedures such as bronchial washings and brushings, diagnostic yield can still be highly variable [20, 21].

The introduction of endobronchial-ultrasonography with a guide-sheath (RP-EBUS) has helped to further improve diagnostic yields, allowing real time visualisation of the bronchoscope location [18]. However, an inherent feature related to forceps sampling is the small biopsies sizes obtained. Tissue samples are also often subjected to forceps crush artefact which can result in poor preservation, or alteration of cellular architecture which can adversely affect histopathologic analysis [64, 67, 69].

Given these limitations of forceps biopsies, more recent attention has been given to cryotherapy. Cryoprobes have been utilised as early as the 1970's where they were primarily used a debulking tool [64]. Subsequent modifications in cryotherapy technology have produced greater freezing power and increased tensile strength allowing it to be used for its adhesive effects. Tumours extracted with this technique have been observed to be of remarkably good

size and quality, despite being frozen and unfrozen [64]. This has led to cryoprobes being utilised for transbronchial cryobiopsies (TB-CB). The reassuring safety profile of TB-CB in diffuse parenchymal lung disease has led to the increasing use of cryoprobes in the investigation of PPLs, and it has been hypothesised that the larger specimens obtained may provide higher diagnostic yields.

In this randomised controlled trial, we therefore sought to evaluate the use of cryoprobes compared to conventional forceps biopsy in the investigation of PPLs. We aimed to demonstrate that TB-CB would produce larger specimens and consequently, higher diagnostic yields when compared to conventional standard forceps biopsies. We also aimed to demonstrate higher diagnostic yields in lesions which were not easily accessible with traditional forceps.

METHODS

Study Population

This was a prospective, randomised single-centre study performed at the Royal Adelaide Hospital (RAH) in Adelaide, Australia. Patients referred for investigation of a peripheral lesion which was suspicious for malignancy were eligible for participation, whereby current standard of care would typically involve performing tissue sampling via RP-EBUS. We excluded patients who had endobronchial disease or mediastinal lymphadenopathy. Eligible patients were randomised to either TB-CB or conventional TB-FB with a computer-generated randomisation schedule. Patients provided signed written consent

and ethics approval was granted by the Royal Adelaide Hospital Ethics Committee.

Procedure

Procedures were performed in the Thoracic Procedure Suite at the RAH by respiratory registrars under the supervision of an experienced interventional pulmonologist. Those patients undergoing conventional RP-EBUS alone were typically performed using local sedation (intravenous midazolam and fentanyl) or under general anaesthesia. TB-CB were all performed under general anaesthesia.

Navigation was performed pre-procedure with CT analysis, using Kurimoto's bronchial branch mapping method to locate the lesion.

Procedures were performed using the radial EBUS probe (K-201/K-203; Olympus, Tokyo, Japan), under fluoroscopic guidance. Following EBUS visualisation of the lesion, the miniprobe was removed with the guide-sheath left in place. Patients randomised to the control group received 5 conventional TB-FB samples, whereas those in the cryotherapy arm received one TB-CB samples (ERBE 1.9 cryoprobe; Germany). The cryoprobe was passed via the guide-sheath (2/2.6mm channels). There was a freeze time of 5 seconds, and the bronchoscope was subsequently removed en-bloc. All patients also received bronchial washings and brushings prior to biopsy as per standard of care.

All TB-CB arm patients were intubated via endotracheal tube (Rusch Bronchoflex), which had two ports, allowing both bronchoscope and a balloon blocker catheter to be passed down simultaneously. The latter could then be

inflated under direct vision to tamponade any potential bleeding whilst the bronchoscope was being removed to retrieve the biopsy.

Any difficulty in accessing a lesion was considered a failed procedure. In cases where the cryoprobe was initially used, the operator would revert to standards methods with forceps to re-attempt biopsy.

Statistical Analysis

Continuous variables are described as mean and standard deviation or median and interquartile range as appropriate to distribution, and dichotomous variables as number and percentage. Baseline characteristics in both groups were compared with Student t tests or Wilcoxon rank-sum tests for continuous variables as per distribution and chi square tests for dichotomous variables. Outcomes in both groups were compared with chi square tests. Analyses were undertaken with Stata version 13.0 (Stata Corporation) and statistical significance set at 0.05.

RESULTS

Patient Characteristics

A total of 32 patients were recruited (19 males; Figure 1 and Table 1). Fifteen patients were randomised into the cryotherapy arm and 17 were randomised to conventional TB-FB. Three patients in the cryotherapy group were subsequently excluded. One of these patients was found to have FDG-avid lymphadenopathy on PET scan with linear EBUS being performed instead. A further two patients were excluded as their lesions were found to have resolved on CT by time of the procedure. Two patients who were randomised to the

cryotherapy arm did not undergo TB-CB as one lesion was located too proximal for safe biopsy, and in the second patient it was not possible for the cryoprobe to be maneuvered into the correct airway. In the conventional forceps group, one case was excluded due to resolution of lesion on CT. As a result, a total of 28 patients were retained for analysis. The mean ages of participants in the cryotherapy and forceps arms were similar at 63 +/- 13 years and 64 +/- 17 years respectively.

Lesion Characteristics

Average dimensions of the TB-CB lesions were 19x21 millimetres compared to 25x23 millimetres in the TB-FB arm (p=0.71 and 0.22 for shortest and longest dimensions respectively; Table 1). Median lesion area in the cryotherapy arm was slightly smaller compared to the conventional forceps group (360 [IQR 192-800] versus 459 [IQR 276-1012] mm^2, p=0.38). Distance from the pleura was also very similar between lesions being biopsied in both arms, with median distances of 10 (IQR 3-30) and 8 (IQR 3-30) mm in the cryotherapy and forceps groups respectively (p=0.94). 50% of participants had lesions located within the right upper lobe, and 25% had lesions within the left upper lobe. The remainder of lesions were found within the right lower lobe, lingula and left lower lobe.

Biopsy Characteristics

Measurement of biopsy sample sizes confirmed that cryobiopsies were generally larger with median biopsy size 7 (IQR 2-22) mm, and with a greater range in biopsy sizes, as compared to the forceps group which had median size 2.5 (IQR 1-5) mm (p<0.0001).

The EBUS probe was observed to be within the lesion in 22/28 patients. The lesion was seen to be adjacent to the probe in 4/28 patients, with 3 of these patients having been randomised to cryobiopsy. In 2/28 patients, the lesion could not be identified.

Diagnostic Yield

The overall diagnostic yield was 78% (22 out of 28 patients). A numerically higher diagnostic yield of 91.7% (11 out of 12 patients) was demonstrated with cryotherapy compared to the forceps group (68.8%, 11/16 patients), however this did not reach statistical significance (p=0.14).

Where the lesion was visualised with EBUS, cryotherapy again demonstrated a numerically higher yield compared to forceps (Table 2). Of the 2 lesions which could not be identified by EBUS, a diagnosis was still achieved nonetheless in the one patient randomised to cryobiopsy.

When the probe was seen within the lesion, TB-CB had diagnostic yield of 87.5% (7 out of 8 patients), as opposed to 71.4% (10 out of 14 patients) (p=0.61). In the 4 patients where the lesion was seen adjacent to the probe, of which 3 were randomised to cryotherapy, a diagnosis was achieved in all cases.

There appeared to be a trend towards increased diagnostic yield for cryobiopsy of lesions located within the left upper lobe (p=0.053).

A total of 22 of 28 lesions were found to be malignant, with 13 (47%) confirmed as pulmonary adenocarcinoma. The remaining 6 patients (21%) had benign aetiology with evidence of infection or inflammation on biopsy. Diagnostic yields

for malignant and benign lesions were both higher with TB-CB, although this did not reach statistical significance. Positive predictive value for malignancy overall and with TB-CB were 100% respectively.

A small subset of patients randomised to cryotherapy also received TB-FB biopsies in addition to their one TB-CB at the discretion of the proceduralist, thus allowing a direct comparison of the biopsy technique in the same patient. There were 3 patients in whom TB-CB provided a diagnosis whereas conventional TB-FB was inconclusive. Conversely, there were 4 patients where a diagnosis was not obtained by TB-CB, but was confirmed with the forceps. In 2 of these 4 cases, TB-CB could not be performed due to the lesion being located too proximal for safe biopsy, or where the cryoprobe could not be maneuvered into the correct airway.

For lesions where a diagnosis was diagnosed only via cryobiopsy, median lesion size as measured from CT imaging was 117 (IQR 114-225) mm^2. In comparison, there was a median size of 619 (869-7324) mm^2 for those lesions in which a diagnosis was only achieved via forceps, and not via cryoprobe.

Repeat Procedures and Complications

Further diagnostic procedures were required in 7 patients. 3 patients with non-diagnostic TB-CB were referred on for CT-guided biopsy. Of the 4 patients with negative TB-FB results, 2 were referred for CT-guided biopsy, one patient underwent repeat RP-EBUS, and the final patient had aspiration of pleural effusion.

The most common complication observed was bleeding, with incidence of 28.6% in cryotherapy group, and 3.6% with TB-FB. However, these were all relatively mild and resolved with the used of suction, iced normal saline and/or adrenaline alone. There was no major bleeding or pneumothorax reported.

DISCUSSION

Major Findings

In this randomised controlled trial, we compared the use of TB-CB to conventional TB-FB in evaluating PPLs with the use of RP-EBUS. Cryotherapy produced significantly larger biopsy sizes. Whilst there was a numerically higher diagnostic yield within the cryotherapy group, this did not reach statistical significance. The overall diagnostic yield of 78% from our cohort, was very much in keeping with the historical diagnostic yield at the Royal Adelaide Hospital. For the proceduralist involved, historically his overall yield was 70-80% over many yearsfor transbronchial biopsies. There was no difference in the incidence of major complications.

Investigation of PPLs

The incidence of peripheral pulmonary lesions has been steadily increasingly, particularly with increased usage of computed tomography imaging generally, and also due to lung cancer screening [8, 71, 108]. Results from the recent National Lung Screening Trial demonstrated a 20% relative reduction in lung cancer mortality when participants underwent annually screening with low-dose CT [8].

For over 40 years, bronchoscopy has had a pivotal role in the investigation of PPLs [18]. However, its success relies significantly upon the bronchoscopist's ability to navigate the bronchial system and obtain samples successfully from the target lesion. Even with the assistance of fluoroscopy, diagnostic yield for transbronchial biopsies is highly variable. For nodules less than 2 centimetres,

sensitivity can be as low as 14% [23, 33]. The introduction of radial EBUS has not only been useful for smaller lesions which are fluoroscopically invisible [18, 39, 40], but has also allowed for an increased reliability and yield of the sampling through use of the guide sheath.

Schuhmann et al [71] were the first to evaluate safety and feasibility of radial EBUS with cryobiopsy in peripheral lesion. 39 patients were enrolled to receive both forceps transbronchial biopsies and cryobiopsies, with the order determined by randomisation. They reported overall diagnostic yield of 60.5% (23/39 patients), which increased to 74.2% when accounting for lesions visualised by EBUS (23/31 patients).

EBUS visualisation was already known to be strongly associated with diagnostic yield [3, 18, 25, 39, 44, 45, 47, 50] In the first randomised trial of RP-EBUS versus CT-guided biopsy, Steinfort et al not only demonstrated increased yield with EBUS visualisation (100% vs 50%), but that EBUS visualisation was also significantly associated with a subsequent diagnosis of lung cancer [1].

Our study did not demonstrate any overall difference in diagnostic yield between TB-CB and TB-FB, which is not dissimilar to previous studies using RP-EBUS guidance [71, 76, 77]. Although there was improved diagnostic yield for TB-CB with positive EBUS visualisation, this did not meet statistical significance.

Our protocol differed to Schuhnann et al's in that patients were otherwise randomised to either TB-CB or TB-FB. Whilst there were 7 patients who received both biopsy methods at discretion of the proceduralist, there were too

few cases in this subset to definitively compare diagnostic yield. A diagnosis was achieved via only TB-CB in 4 of these patients, and only TB-FB in the other 3. Nasu et al [76] had similar results where 7 patients were positive on forceps, but negative on cryobiopsy. Their study differed in that TB-CB was performed without guidesheath. The authors commented on difficulty advancing the cryoprobe where there were multiple airways, and there was thus a possibility that TB-CB was not obtained from same site as TB-FB. This was the case with one patient in our study where the probe could not be properly introduced in to the correct airway. It was notable in our study that lesions in which only cryobiopsy was successful were of markedly smaller size, when measured from CT imaging.

The position of the radial EBUS probe in relation to the lesion as seen during bronchoscopy was also recorded as this has previously been demonstrated to be an important factor influencing diagnostic yield [47, 50]. Yamada et al [50] showed this to be the only significant factor on multivariate analysis for lesions smaller than 3 centimetres. Kho et al [77] specifically assessed the performance of TB-CB in eccentrically and adjacently-orientated PPLs. They showed cryobiopsy increased diagnostic yield in adjacent lesions from 22% to 66.7%, although this also did not reach significance. The advantage of the cryoprobe is its ability to obtain a sphere of frozen tissue, hence taking lateral biopsies as opposed to just sampling in a forward direction [71, 78]. In Kho et al's study, 57.2% of inconclusive TB-FB were found to be bronchial epithelium, likely due to the inadequate depth of lateral biopsy [77]. In our cohort, there

were 4 patients where the probe was seen adjacent to the lesion. A diagnosis was achieved in all, with 3 of these 4 undergoing cryobiopsy.

It is not unusual to have increased episodes of mild bleeding with cryobiopsy, as reported in a large multicentre trial by Hetzel et al [75]. This was attributed to the need for the whole removal of the cryoprobe and bronchoscope en-bloc, for retrieval of the sample, resulting in a longer duration for blood to pool at the biopsy site. This was opposed to conventional forceps where any minor bleeding could be suctioned/tamponaded immediately whilst the scope remained in place. Our study showed consistent results with increased incidences on bleeding, but with only minimal intervention required in all cases. . Similar to Schuhmann's feasibility study, there was no pneumothoraces in our patients. Factors minimising risk of damage to the pleura likely included use of fluoroscopy, use of the radial sheath, and that only lesions visually identified with EBUS were biopsied.

Study Limitations

There were several limitations to our study. Complete blinding of investigators and participants to randomisation was not possible, due to the need for TB-CB to be performed under general anaesthesia. Our sample size was also small and therefore not powered to demonstrate superiority. Longer waiting times for availability on a general anaesthetic list at our institution impacted our ability to recruit. It was not appropriate for potential participants to wait longer for the purposes of this trial, if they could access TB-FB with local sedation in a timely manner, especially given concern for underlying malignancy.

Our study was commenced prior to the availability of newer disposable cryoprobes with their thinner 1.1 millimetre diameter, which could be passed through the thin guide sheath channels. These could provide improved manoeuvrability, facilitate more accurate placement and further improve diagnostic yield.

CONCLUSION

In conclusion, our study confirmed that cryobiopsy provides larger biopsy specimens and could potentially be more useful in smaller peripheral lesions, and those which are more difficult to access such as those located adjacent to the probe. Furthermore, we observed no increase in major complications, Cryobiopsy may therefore provide an alternative method to investigating PPLs. Further prospective randomised trials, with larger participants numbers, would be required to further confirm our results.

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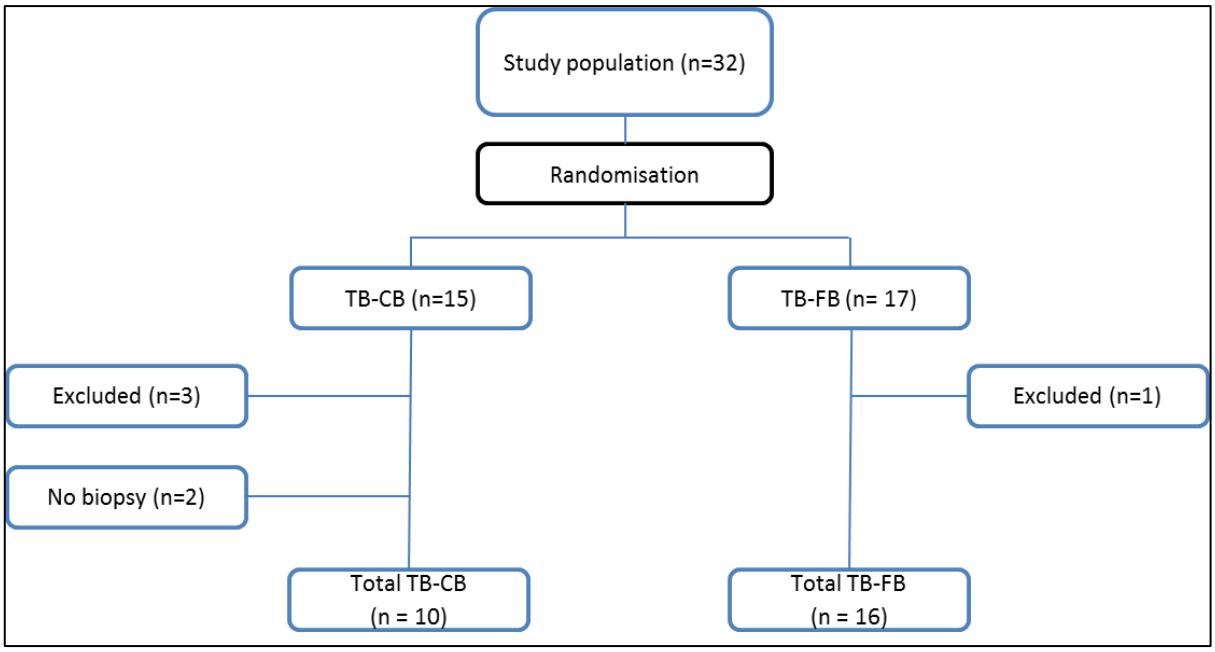
Table 1:

	Forceps Biopsy	Cryobiopsy	p-value
Patients			
Age	64 +/- 17	63 +/- 13	1.00
Gender			
- Male	10 (58.8)	9 (60.0)	0.95
- Female	7 (41.2)	6 (40.0)	
Lesion			
Size	25x23	19x21	
Area (mm^2)	459 (275-1012)	360 (192-800)	0.38
Distance from pleura (mm)	8 (3-30)	10 (3-30)	0.94

Table 2:

	Forceps Biopsy	Cryobiopsy	p- value
Biopsy Method	68.8 (11/16)	91.7 (11/12)	0.14
EBUS visualisation of lesion			
Positive	73.3 (11/15)	90.9 (10/11)	0.26
Negative	0 (0/1)	100 (1/1)	1.00
Probe Position			
Within Lesion	71.4 (10/14)	87.5 (7/8)	0.61
Adjacent to Lesion	100 (1/1)	100 (3/3)	-
Diagnoses			
Malignant	66.7 (8/12)	90.0 (9/10)	0.19
Benign	75.0 (3/4)	100 (2/2)	0.44

Figure 1:


Study population (n=32)
Randomisation
TB-CB (n=15)
TB-FB (n= 17)
Excluded (n=3)
Excluded (n=1)
No biopsy (n=2)
Total TB-CB
(n = 10)
Total TB-FB
(n = 16)

CHAPTER 4: FINAL DISCUSSION, LIMITATIONS AND CONCLUSION

This thesis has examined aspects related to the investigation of peripheral pulmonary lesions, the incidence of which continues to rise with increased CT imaging and with lung cancer screening. This is of particular relevance in Australia where lung cancer is not only the fifth most commonly diagnosed cancer, but also contributes to the most number of cancer-related deaths. Whilst there are several well-established international guidelines regarding management and potential surveillance of these lesions, obtaining tissue is the only way for obtaining an accurate diagnosis. As survival rates fall with advanced disease, the ability to identify lung cancer early is critical to preserve quality of life and survival.

The aim of this thesis was to evaluate alternative investigative techniques which could be used alongside conventional transbronchial forceps biopsies, in the bronchoscopic investigation of peripheral lesions.

In Chapter 2, we demonstrated how objective greyscale analysis of RP-EBUS images could further assist in differentiating benign from malignant disease. This is particularly relevant in patients with the former, who might be saved from unnecessary repeat invasive procedures in the situation where there is no evidence of malignancy demonstrated on initial sampling.

Our study involved assessment of 84 RP-EBUS images for image analysis. 46 lesions had underlying malignant aetiology. We identified five greyscale features which were found to significantly differ between benign and malignant lesions. By utilising the RP-EBUS image of the (unconstrained) whole lesion as

region of interest, this method could differentiate lesions with diagnostic sensitivity up to 78%, specificity up to 86% and positive predictive values up to 88%. The highest positive predictive values were seen with maximal pixel intensity and range of pixel intensity.

Whilst the current use of RP-EBUS is to aid the bronchoscopist as to the optimal biopsy site, the RP-EBUS image itself has also been shown to provide further clinical information regarding the lesion's underlying nature. Earlier studies demonstrated that this information could be utilised in clinical practice, however to do so relied solely on the operator's subjective ability to identify and interpret image characteristics, as well as their previous experience and knowledge with RP-EBUS. However, it is recognised that digital images contain more information that can be perceived by the human eye. Our results reiterates earlier findings that application of computer-assisted image analysis could provide an alternative and more objective method of assessing different features of these EBUS images. Greyscale texture analysis had already been successfully applied to ultrasound images of pathology in non-pulmonary organs. It was then successfully used to objectively distinguish benign from malignant aetiology in the analysis of RP-EBUS images. Our study progressed from previously demonstrated results by using a larger, whole-lesion, unconstrained region of interest. We hypothesised that the larger unconstrained ROI would not only provide more pixel information, but would also reduce ROI selection bias and thus be less dependent on user expertise.

Whilst we recognise histology remains the gold standard in diagnosis, greyscale image analysis could further aid clinical decision making. This would

be especially relevant in situations where there is uncertainty regarding adequacy of biopsy samples, with inconclusive or non-diagnostic results.

Chapter 3 further investigated the utility of cryobiopsy as an alternative biopsy to conventional forceps for sampling peripheral lesions. Whilst bronchoscopy has been a fundamental tool in the investigation of peripheral lesions, its success relies heavily on the proceduralist's ability and diagnostic yield can be highly variable. Sensitivity also correlates to the size of the target lesion, and is lower with smaller nodules. The introduction of radial EBUS improved diagnosis in smaller and even fluoroscopically-invisible lesions. However an inherent feature of these forceps biopsies samples was their small size, and predisposition to crush artefact.

Initially, transbronchial cryobiopsies had primarily been used for the evaluation of diffuse parenchymal lung diseases. In light of their favourable safety profile, we have recently seen increasing data regarding its use for peripheral lesions. We aimed to demonstrate that cryobiopsy with RP-EBUS would provide larger biopsy specimens compared to conventional forceps. This was essential not only for improved diagnostic yield, but also for guiding further immuno-histochemical, and molecular analysis which may be relevant for treatment. We also hypothesised that cryobiopsy might have increased diagnostic yield in lesions which were typically difficult to biopsy with conventional transbronchial forceps.

Our results confirmed that much larger biopsy sizes could be achieved with cryotherapy compared to that with forceps, with median sizes 7mm and 2.5mm respectively ($p<0.0001$). Of the 28 lesions evaluated, there was overall

diagnostic yield of 76.8%. There was a trend towards improved diagnostic yield with TB-CB than TB-FB. In particular, our results suggested that cryotherapy could potentially be of increased utility in patients with smaller lesions, or those which were difficult to access.

There are limitations to both studies in Chapter 2 and 3. The image analysis study in Chapter 2 was only of small sample size with images limited to only those RP-EBUS images with constant gain and contrast settings. Whilst significant differences were seen on univariate analysis, it is possible that multivariate analyses could provide improved differentiation between malignant and benign disease. We acknowledge that the user would still be required to have some understanding of interpreting RP-EBUS images in order to draw the region of interest, and the analysis could only occur post-procedure. This method did not currently assist a proceduralist real-time during a procedure.

Further studies with larger datasets, separate validation sets and further multi-variate analysis would be of benefit to validate the applicability of this method further. Similarly, larger number of experts and non-experts would also provide more information regarding the inter-user variability or region of interest selection.

Our study on transbronchial cryobiopsy in Chapter 3 was also limited by small sample size. As cryobiopsy patients required their procedure to be performed under general anaesthesia with potential longer waiting times for investigation, this had significant impact on our ability to recruit all suitable patients. As the study was performed in a single tertiary referral hospital, it is also possible that the generalisability of these findings is limited. Larger prospective, multi-centre

trials would be required to further clarify the potential superiority of cryobiopsy in the management of these patients. Future directions could also include comparison of different sized cryoprobes, assessing adequate number of cryobiospy samples, and further evaluation of its use in lesions which are typically difficult to sample via conventional transbronchial forceps.

In conclusion, the timely management and accurate diagnosis of the peripheral pulmonary lesion will continue to be critical for respiratory physicians in order to maintain best patient outcomes. Whilst there can be wide variety in how these lesions are approached with multiple different international guidelines available, obtaining tissue remains the gold standard for diagnosis. This thesis has explored the different bronchoscopic methods for sampling target lesions. We have shown the advantage of cryotherapy in providing larger sample sizes with similar safety profile to transbronchial forceps biopsies. Furthermore, we have demonstrated the value and accuracy of greyscale image analysis, which could be especially useful as an adjunct, particularly if there is uncertainty regarding a biopsy being truly non-diagnostic or inconclusive.

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