

**Exploring novel, minimally invasive, multi-modal bronchoscopic
biopsy tools and techniques using radial endobronchial ultrasound
and the guide sheath to improve the diagnostic yield of peripheral
pulmonary lesions suspected of lung cancer**

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**A thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy**

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ETHICS STATEMENT

All subjects who participated in these studies have provided written informed consent. All protocols have been approved by participating institutional ethics committees in Australia and New Zealand. Maori Ethics have been granted for all required New Zealand studies.

All studies have been registered in clinical trials registries

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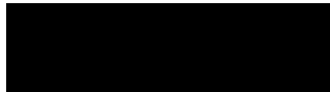
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ABSTRACT

Peripheral pulmonary lesions (PPL) suspected of lung cancer require a safe, high-yield diagnostic biopsy with concurrent mediastinal staging to reduce treatment delays. Emerging lung cancer screening programmes amplify this need. Radial EBUS is a safe bronchoscopy biopsy method for PPL, but the diagnostic yield is low. This thesis hypothesised that it was possible to improve the yield with novel biopsy tools and techniques. Four multi-centre interventional studies were performed. The novel biopsy tools (cryobiopsy and GenCut tool) were examined, and existing biopsy tools (guide sheath and aspiration needle) were optimised and combined as a multi-modal biopsy.

This thesis consists of the first published head-to-head comparison of multi-modal Cryo-Radial biopsy against percutaneous CT-guided biopsy (CT-TTB) for PPL: a study compromised by slow recruitment that resulted in its early termination. Available data showed a modestly lower diagnostic yield than CT-TTB, but significantly fewer critical adverse events, pneumothorax, or haemorrhage.

Bronchoscopic cryobiopsy was introduced to tertiary hospitals in New Zealand, for the first time, through this study in 2015 with relevant safety precautions. To cater for centres where cryobiopsy was not widely available but a core tissue sample was required, a novel GenCut tool was explored as an alternative suitable for sedation bronchoscopy. A multi-modal biopsy approach, rather than reliance on forceps alone, improved the diagnostic yield with adequate tissue obtained for critical molecular testing. This concept of multi-modal biopsy was not reported in Radial EBUS literature at the time of these studies in 2015.

The addition of these safe diagnostic techniques will allow rapid, personalised diagnostic pathways for patients with suspected lung cancer.

Keywords: Radial EBUS, cryobiopsy, cryo-radial, needle aspiration, diagnostic yield, guide sheath, multi-modal biopsy

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LIST OF ABBREVIATIONS AND ACRONYMS

ACCP	American College of Chest Physicians
ANZIPG	Australia New Zealand Interventional Pulmonary Group
AQuIRE	ACCP quality improvement registry, evaluation and education
bMWA	bronchoscopic microwave ablation
bRFA	bronchoscopic radiofrequency ablation
BTPNA	bronchoscopic trans parenchymal nodule access
BTVA	bronchoscopic thermal vapor ablation
CBCT	cone beam computed tomography
CI	confidence interval
CLE	confocal laser endomicroscopy
COPD	chronic obstructive pulmonary disease
CT	computed tomography
CT-TTB	computed tomography-guided transthoracic biopsy
CXR	chest x-ray
DSMB	Data Safety Monitory Board
EBUS	endobronchial ultrasound
EGFR	epidermal growth factor receptor
EMN	electromagnetic navigation
ENB	electromagnetic navigation bronchoscopy
ETT	endotracheal tube
F-EMN	fluoroscopic electromagnetic navigation
FEV	forced expiratory volume
FRC	functional residual capacity
GPS	global positioning system
GS	guide sheath
HREC	Human Rights Ethics Committee
ICI	immune checkpoint inhibitors
ICT	intercostal tube placement
IHC	immunohistochemistry
ILD	interstitial lung disease
L-EBUS	linear endobronchial ultrasound
LMA	laryngeal mask airway
LUL	left upper lobe
MDT	multidisciplinary team
mm	millimetre

NELSON	NEtherlands Leuvens Screening ONderzoek study (Dutch)
NSCLC	non-small cell lung cancer
OR	odds ratio
PD-1	programmed death-1
PD-L1	programmed death ligand 1
PET-CT	positron emission tomography-computed tomography
PPL/s	peripheral pulmonary lesion/s
PR	pulse rate
RACP	Royal Australasian College of Physicians
Radial EBUS	radial endobronchial ultrasound
RCT	randomised control trials
ROSE	rapid onsite examination
TBNA	transbronchial needle aspiration
TKI	tyrosine kinase inhibitor
TSANZ	Thoracic Society of Australia & New Zealand
UTB	ultrathin bronchoscope/bronchoscopy
VBN	virtual bronchoscopic navigation

Explanation of Studies

1. **REBUST 2:** Comparing the diagnostic yield of the large GS and small GS using conventional Radial EBUS sampling methods (forceps/brush), followed by assessing the additional diagnostic yield of adding the needle aspirate to the large GS. This was a randomised control trial
2. **Cryo-feasibility study:** Adding cryobiopsy to Radial EBUS conventional biopsy by using the large GS as a feasibility study to assess safety and diagnostic yield. This was a prospective interventional pilot study to introduce cryobiopsy procedure to New Zealand.
3. **CT-CROP study:** Comparing the diagnostic yield and safety of CT-guided biopsy against multi-modal Radial EBUS and cryobiopsies via the large radial GS. This was a randomised control trial.
4. **R-CORE study:** Using a blunt-ended novel GenCut biopsy via the large GS to determine the safety and ability to perform PD-L1 testing. This was a prospective interventional study with 18 consecutively recruited pilot study.

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LIST OF PUBLICATIONS DURING THE CANDIDATURE

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2. Herath, S., Wong, C., Dawkins, P., Veale, A. Yap, E., Williamson, J., Low, I., Mahajan, H., Pryan, T., Barnard, S., Hawkins, S., Cookson, D., Singh, T., & Ing, A. (2022). Cryobiopsy with radial-endobronchial ultrasound (Cryo-Radial) has comparable diagnosis yield with higher safety in comparison to computed tomography-guarded transthoracic biopsy for peripheral pulmonary lesions: An exploratory randomised study. *Internal Medicine Journal*, Jun 8. <https://doi.org/10.1111/lmj.15833>. Epub ahead of print. PMID: 35675149.
3. Herath, S., Bashirzadeh, F., Mahajan, H., Ing, A., & Fielding, D. (2022). Using the GenCut core biopsy tool with the radial endobronchial ultrasound guide sheath enables a high-quality histology sample capable of programmed cell death ligand 1 (PD-L1) testing. *Respirology Case Reports*, 10(5), e093. <https://doi.org/10.1002/rc2.935>
4. Herath, S., Mahajan, H., Yap, E., & Ing, A. (2022). Radial endobronchial ultrasound guided biopsy for lung lesions: Evaluating the role of the guide sheath size, addition of needle aspirate and multi-modal sampling, in improving the diagnostic yield – An exploratory randomized study. *Journal of Community Medicine and Public Health Reports*, 3(02). <https://doi.org/10.38207/JCMPHR/2022/FEB03020119>
5. Herath, S. (2019). Safe and effective use of cryobiopsy with Radial EBUS for lung masses with a high bleeding risk: Case series and description of technique. *Respirology*, 24(1), 151-152 (Appendix 3).
6. Herath, S., Yap, E., Low, I., Mahjan, H., & Ing, A. (2019). Multicentre randomised controlled trial with Radial EBUS comparing thin versus thick guide sheath (GS) with the addition of an aspiration needle biopsy, in the diagnosis of peripheral lung lesions (REBUST 2). *Respirology*, 24(1), 91 (Appendix 5).
7. Herath, S., Yap, E., Dawkins, P., Veale, A., Williamson, J., & Ing, A. (2019). Multicentre randomised control trial of the diagnostic yield and safety of Radial EBUS guided

cryobiopsy (cryo-radial) versus CT-guided transthoracic biopsy for lung masses demonstrates similar diagnostic success (CT-CROP). *Respirology*, 24(1), 93 (Appendix 6).

8. Herath, S., & Yap, E. (2017). Novel hybrid cryo-radial method: An emerging alternative to CT-guided biopsy in suspected lung cancer. A prospective case series and description of technique. *Respirology Case Reports*, 6(2), e00287.
9. Herath, S., & Low, I. (2016). Needle aspiration using a 21G needle (eXcelon or Wang), via Radial EBUS guide sheath, gives better samples in comparison to cytology brush or forceps biopsies: A descriptive case series. *Respirology*, 21(1), 156.

AUTHORSHIP ATTRIBUTION STATEMENTS

AUTHORSHIP ATTRIBUTION STATEMENT

Chapter 4 of this thesis was published as an original research paper.

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Dr Samantha Herath designed the study, applied, and secured ethics approval, collected the data, performed the procedure, analysed the data, managed the project, wrote, reviewed, and edited the drafts, collaborated with co-authors, and sent the work for publication. Dr Herath was the corresponding author and first named author for this publication.

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A case series extracted from the main study above is also published in Chapter 6:

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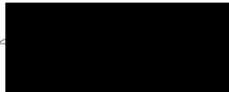
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CHAPTER 1:

INTRODUCTION

1.1 Chapter overview

This chapter provides an overview of the broad problem of peripheral pulmonary lesions (PPLs) suspected of lung cancer and the biopsy modalities available for diagnosis. The unavailability of an optimally safe and effective biopsy technique to diagnose PPLs is highlighted, followed by the pivotal role radial endobronchial ultrasound (Radial EBUS) bronchoscopy plays in this space. This is followed by a summary of the thesis structure including an introduction to the research aims and objectives aiming to improve the diagnostic yield of Radial EBUS.

1.2 Background to the study

1.2.1 Introduction to PPL suspected of lung cancer

PPLs are abnormal areas in the lung parenchyma, noted on radiological imaging. PPLs occur due to a multitude of reasons, including infection, inflammation and malignancies. The radiological appearance of PPLs may vary from ground glass opacity to semi-solid to complete opacified lesion, however, these appearances alone do not delineate if the PPL is of a malignant origin. The risk prediction of a PPL being malignant depends on a variety of factors, including patient risk factors (smoking, occupational exposure, and family history of lung cancer) (Cassidy et al., 2006) and lesion characteristics (spiculated margins in the PPL) (MacMahon et al., 2017). The prevalence of malignancy in non-calcified nodules varies markedly; with total prevalence ranges from 1.1% to 12%. As the size of the PPL increases, the risk of malignancy increases as well, with 0 to 1% risk in a PPL of 5mm, which increases to 64-82% for a 20mm PPL (Wahidi et al., 2007).

Lung cancer is the leading cause of cancer deaths worldwide; 1.79 million deaths per year are recorded due to lung cancer (Bray et al., 2018). The lung cancer mortality outweighs the combined mortalities of the most common cancers including breast, prostate and colon. Worldwide, a diagnosis of lung cancer carries a dismal prognosis with an overall 5-year survival of 15% (World Health Organization, 2021). Australian data is not dissimilar with a 5-year survival rate of only 20% (17% for males and 25% for females).

Every millimetre increase in the size of a PPL suspected of lung cancer worsens the prognosis for the patient. A malignant PPL up to 10mm in diameter has a 92% 5-year survival, which reduces

to 83% (a drop of 9%) for a PPL that is 20mm in diameter, with a further drop in survival to 76% for lesions up to 30mm. The spread of lung cancer to the mediastinal lymph nodes is another poor prognostic indicator. Patients without pulmonary lymph node involvement demonstrate a 60% 5-year survival, whilst patients having ipsilateral mediastinal involvement or N2 disease drop their survival rate to 37% (Rami-Porta et al., 2014).

Due to relatively low symptoms, lung cancer is a silent killer. 57% of lung cancer patients present with metastatic disease at presentation and an accompanying 5-year survival of 5%, compared to patients with localised disease with a five-year survival rate of 57% (Siegel et al., 2020).

1.2.2 Importance of an early diagnosis

Due to this poor prognosis, diagnosing PPLs early and commencing treatment will improve survival. Low dose computed tomography (CT) screening programmes were developed on this premise, which demonstrated success in the relative reduction of mortality by 20% in high-risk individuals (Aberle et al., 2011). However, emanating from the positive group in low dose CT, 96% had false positive results. Similar results have been demonstrated by the more recent NELSON study published in 2020, which demonstrated a reduction in mortality by 24% in men and 33% in women, further strengthening the argument for the implementation of a low dose CT chest screening programme for early detection of lung cancer (de Koning et al., 2020).

These positive findings have led to the implementation of lung cancer screening programmes in the United States in routine clinical practice, on a voluntary basis. A study by Hammer et al. (2020) in real life lung cancer screening programmes had demonstrated a total of 2% true cancer detection and 13% false positive results in a group of 3735 patients. With the execution of the lung cancer screening programme, 1.5 million new PPLs have been detected by chest-CT scans annually in the United States (Gould et al., 2015). With an Australian lung cancer screening programme in the horizon, a similar increase in incidentally detecting PPLs would be expected, therefore, requiring the need to implement a protocolised pathway to manage the burden of PPL (Behar et al., 2022).

The challenge then is to decide on the least invasive biopsy method with lowest adverse events to the patient when obtaining the most adequate biopsy sample.

This biopsy method needs to be minimally invasive to reduce any occurrence of adverse events that may delay definitive treatment or reduce the patient's functional status. In the case of PPLs detected during lung cancer screening programmes, it must be noted that these subjects are not patients, but people without symptoms; only with a high risk of lung cancer. Given the high

probability of a false positive result, a biopsy needs to be of a low risk profile (de Koning et al., 2020; Aberle et al., 2011). A biopsy method that can combine both staging the mediastinum and biopsy the primary PPL during the same procedure will reduce treatment delays and repetitive procedures.

The clinical implications for delays in lung cancer treatment are many. Missed opportunities at following up PPLs suspected of lung cancer and not organising definitive treatment result in an increase in tumour size and the resultant worsened prognosis. This is noted to be most important for patients with Stage 2 primary lung cancer (PPL up to 50mm in diameter and possibility of lymph node involvement of the mediastinum) (Chansky et al., 2017), where staging of the mediastinum becomes very important and noted to have the most impact on survival, with patients only eligible for palliative care having much less impact (Zuniga & Ost, 2021). Thereby the curative stages of lung cancer are affected most by these delays. Lung cancer patients have a high level of anxiety (17.9%) and depression (21.8%), which worsens when delays in treatment occur and can affect the treatment outcome. Significant correlations have been noted between severe depression and increased hospitalisation in lung cancer patients (Margari et al., 2016).

Therefore, a timely and effective biopsy of a suspected PPL is paramount to prevent treatment delays. The safety of the biopsy with an exceedingly low side effect profile is a mandatory requirement.

Currently, two common biopsy modalities are used in the diagnosis of a PPL suspected of lung cancer: (i) CT-guided transthoracic biopsy (CT-TTB); and (ii) bronchoscopic biopsy, including Radial EBUS biopsy. Both methods have been recommended by the American College of Chest Physicians Guidelines (3rd edition) in the management of lung cancer (Rivera et al., 2013).

1.3 Discussion of current biopsy techniques for PPLs

1.3.1 CT-guided Transthoracic biopsies (CT-TTB)

Worldwide, the most widely used biopsy method for diagnosing PPL for decades has been CT-TTB. A meta-analysis in 2015 reviewing 48 papers with 10,383 biopsies demonstrated a diagnostic yield of 92.1% (67-97%) (Lee et al., 2019). This high diagnostic yield persisted even with small lesions less than 20mm (92.8%) in 2718 biopsies. Despite the high diagnostic accuracy, there were two major drawbacks in CT-TTB.

The first major drawback was the high side-effect profile. The pneumothorax rate was 20.5% (8.2-47.3%) with chest tube placement of 7.3% (0-31.1%) and clinically significant haemorrhage

was 2.8% (0-14.7%) (DiBardino et al., 2015). This pneumothorax rate was higher (31.3%) in patients with chronic obstructive airways disease (COPD) (Cruz et al., 2014; Zhou et al., 2021).

The second major drawback in CT-TTB was the inability to stage the mediastinum during the same procedure. At the time of diagnosis, up to 80% of patients had local spread or distant metastatic of disease, requiring mediastinal staging prior to treatment commencement (Chansky et al., 2017). In fact, despite having a negative PET-CT scan in mediastinal lymph nodes (N0 disease) in surgically resected lung cancer patients, 35% had pathological upstaging to mediastinal involvement (pN2 disease), demonstrating the importance of systematic mediastinal staging prior to surgery (Gomez-Caro et al., 2010).

Therefore, even if a diagnosis was made with the CT-TTB procedure, inevitably a staging procedure would be required by most patients, with the additional side effects to consider and additional cost to both the patient and the health system, due to the requirement of another procedure, with the consideration that treatment could not commence without the completion of staging.

Due to this reason, clinicians have investigated alternative diagnostic techniques for PPL with better safety profiles that can combine diagnostic and staging during a single procedure.

1.3.2 Overview of bronchoscopic biopsy methods

The safety profile of bronchoscopy has always been favourable. Despite this safety profile, if the PPL was not visible via the flexible bronchoscope, the diagnosis via the traditional transbronchial biopsy had a low diagnostic yield of 34% for nodules <20mm and 63% for nodules >20mm (Rivera & Mehta, 2007).

Therefore, adjuncts were used to guide the flexible bronchoscope to better visualise the PPL, and methods were explored to “guide” the bronchoscope to the desired location. Multiple “guided” bronchoscopy methods have evolved to increase visualisation of the PPL.

Radial EBUS is one such widely used guided bronchoscopy method that draws on the strength of pulmonologist training, where it is only slightly different to using a blind transbronchial forceps biopsy. The ability to read CT scans is fundamental for a pulmonologist, which can be improved easily to map a bronchoscopic pathway to the PPL of concern (Kho et al., 2020). The now-published Kurimoto method of lung nodule mapping is inexpensive; it broadens the knowledge of the bronchial anatomy and is readily available for respiratory physicians to learn and use (Kurimoto & Morita, 2020). The ability to combine linear endobronchial ultrasound (L-EBUS)

for staging at the same time was an excellent combination to reduce treatment delays and complete the diagnosis and staging in a single procedure.

The greatest strength of the guided bronchoscopy is its highly favourable side effect profile.

The commonly used guided bronchoscopy techniques are briefly explained below, with emphasis on Radial EBUS. The advantage of Radial EBUS is that it is cost-effective and can be used in combination with other guided bronchoscopy methods, including thin bronchoscopy and virtual bronchoscopic navigation (VBN), as well as fluoroscopy. Radial EBUS guided biopsies has been recommended as a diagnostic method for lung cancer by the American College of Chest Physicians Evidence Based Clinical Practice Guidelines, 3rd Edition (Rivera et al., 2013).

In most Australian and New Zealand centres, Radial EBUS is the commonly used as well as the only available guided bronchoscopy system available. It is a rare commodity for a respiratory department to possess costly equipment akin to electromagnetic navigation (EMN) or VBN facility in Australia or New Zealand. The low population density in Australia and New Zealand had always hindered companies and hospitals investing in such expensive equipment.

1.3.3 Currently used guided bronchoscopy methods for PPL: Brief introduction

1. Fluoroscopy
2. Thin and ultrathin bronchoscopy (UTB)
3. Virtual bronchoscopic navigation (VBN)
4. Electromagnetic navigation bronchoscopy (EMN)
5. Radial EBUS ultrasound probe with or without GS (Radial EBUS)

Fluoroscopy

Fluoroscopy is one of the first adjuncts added to flexible bronchoscopy to improve guidance to PPLs not visible endo-bronchially via the flexible bronchoscope. Fluoroscopy is an external X-ray beam that can provide a 2D image of the PPL, whereby, the proceduralist can direct the biopsy instruments. Transbronchial forceps biopsy with fluoroscopy was used as the initial method for improving the bronchoscopic diagnostic yield of PPLs, but the success rate was limited. In a recent study of conventional bronchoscopy with forceps biopsy and fluoroscopy use, the diagnostic yield was only 37% (Tanner et al., 2018).

Fluoroscopy is commonly combined with navigation bronchoscopy and Radial EBUS. A meta-analysis of 31 studies that included 6491 patients demonstrated a diagnostic yield of 70% (Lee & Song, 2023), which begs the question as to the additional value of fluoroscopy during Radial EBUS. There are, however, no head-to-head comparison of Radial EBUS with and without fluoroscopy. Despite this, fluoroscopy continues to be useful in combination with other guided bronchoscopy techniques, as discussed below.

Thin and ultra-thin bronchoscopy

The difference between the thin and ultrathin bronchoscope (UTB) is the size difference and the reach it has to the distal bronchi. The standard therapeutic bronchoscope (BF-1TH190; Olympus; Tokyo, Japan) is traditionally 5mm to 6.2mm in outer diameter and has a 2.8mm or larger working channel that can reach third or fourth generation bronchi. The thin bronchoscope (BF-BF-P190; Olympus; Tokyo, Japan) is less than 4mm in outer diameter with a working channel of 2mm. It can reach to a median of fifth order bronchus. An UTB (BF-XP190 Olympus; Tokyo, Japan) has a 2.8mm outer diameter with a 1.2mm working channel. The UTB can reach up to the ninth order bronchi (Asano et al., 2013).

Thin and ultrathin bronchoscopes have a greater range of motion to improve access to subsegmental bronchi, including those in the upper lobes.

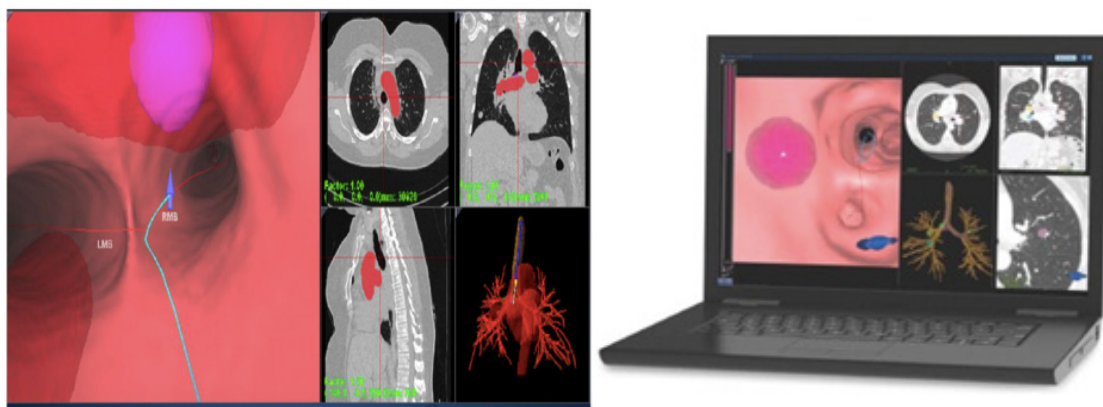


Figure 1.1 Lung point virtual bronchoscopy navigation laptop model

Virtual bronchoscopy navigation (VBN)

VBN is another guided bronchoscopy method that can create a virtual reconstruction of the bronchial tree by using a pre-procedural two-dimensional CT chest. Once a CT scan is uploaded to the software, it projects axial, coronal and sagittal views, allowing the operator to mark the target PPL on the software. The software then generates a pathway to the target, much like the global positioning system (GPS).

There are two commonly used VBN systems: (i) Japanese brand Bf-NAVI® (Cybernet System Inc., Tokyo, Japan) launched in 2008; and (ii) USA brand Lung Point® (Broncus Medical Inc., Mountain View, Calif., USA) launched in 2009.

In both available VBN systems, once CT chest images are uploaded to the software, axial, sagittal and coronal views are displayed. The target lesion is then selected using the provided markers. The main advantage of the VBN system is its ability to provide a GPS to the lesion, simulating an endobronchial navigation pathway. This is most helpful in more peripheral and smaller lesions. Important aspects of VBN include the need for a recent high-quality CT chest with slice thickness of <1mm, but usually requiring a slice thickness of 0.6mm to 0.8mm to provide more peripheral bronchial pathways.

The lung point of VBN exists as a stand-alone desktop navigation, which can be used as an onside companion where an assistant will drive the VBN system whilst the proceduralist operates the bronchoscope. A more expensive version of this system can also synchronize the virtual view with a real time bronchoscopic view to provide a route to the target in real time. The Archimedes navigation system is one such method.

The issue with real-time VBN GPS pathways, similar to the Archimedes system, is that the pathways are pre-formed according to the uploaded CT chest scan. This can create an appreciable difference between the PPL location in the pre-procedural CT chest scan and the PPL location of the patient who is now anesthetised. This lack of adjustment, called a CT to the body divergence can be a cause of reduced diagnostic yields. In the lower lobes, this could be as much as 25mm difference. VBN does not provide real-time biopsy visualisation either. The cost of a stand-alone desktop navigation model is AUD\$75,000 and a real-time Archimedes costs AUD\$330,000, making them expensive to purchase.

Electromagnetic navigation (EMN)

Electromagnetic navigation is another guided bronchoscopy method that can provide real time GPS like navigation guidance to a selected PPL. EMN generates a 3-D reconstruction of the bronchial tree from a recently performed CT chest scan data and then superimposes the real-time position of the bronchoscope using electromagnetic sensors. An electromagnetic field outside the patient's body tracks the position of the sensors. EMN instruments are specialised as they have sensors at their tip incurring an additional cost.

Two commercially ENB systems are available: (i) Super Dimension (Medtronic, Minneapolis, Minnesota, USA); and (ii) SpiNDrive (Veran Medical Technologies, Inc, St Louis, Missouri, USA).

The electromagnetic field generator is either a board under the patient (Super Dimension) or a square pad (SpiNDrive). The electromagnetic sensor in the Super Dimension is placed within the tip of a steerable guide (locatable guide) which is housed in a larger guide sheath (extended working channel). Once the tip of the locatable guide reaches the target lesion, the locatable guide is removed while the extended working channel is locked onto the bronchoscope. Sampling instruments are inserted into the extended working channel. In contrast, SpiNDrive utilizes a continuous tracking system where electromagnetic sensors are embedded in the tip of the sampling instrument; this allows for continuous electromagnetic tracking, even during sampling.

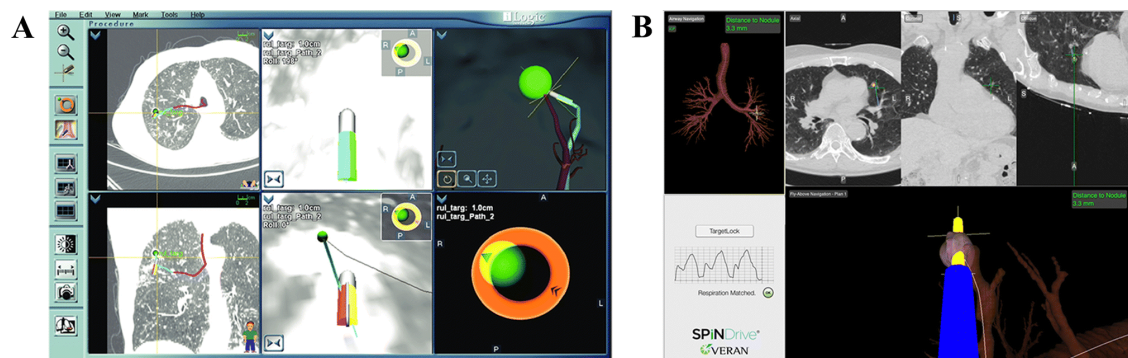


Figure 1.2 EMN platforms: [A] Super dimension; [B] 4D EMN SpiNDrive system

The main disadvantage is the cost of EMN. The cost includes the purchase of the initial device, as well as the magnetic plates that need replacing; the specialised biopsy equipment with the magnetic tip is also expensive. The CT to the body divergence again plays a major role in EMN accuracy.

Kurimoto's method of manual identification of bronchial branches

All the above navigation methods (including VBN and EMN) share one characteristic, that is, the exuberant cost, and many respiratory units cannot afford this cost. In Australia, few respiratory units have navigation capability; and navigation is not available in New Zealand to date.

In Kurimoto's book publication entitled *Actual Identification of Bronchial Branch* (Kurimoto, 3030 pp. 23-125), Kurimoto's method uses a manual drawing of the bronchial branching to the suspected PPL, using the knowledge of the bronchial anatomy and the CT chest image. This cost-effective method allows the user to develop a greater understanding of the bronchial anatomy,

which is available at any time at no cost. Mastering this technique is a fundamental exercise for training interventional bronchoscopists interested in diagnosing PPL and improving their knowledge because this manual method can also be used to double-check the EMN or VBN methods that are not always accurate.

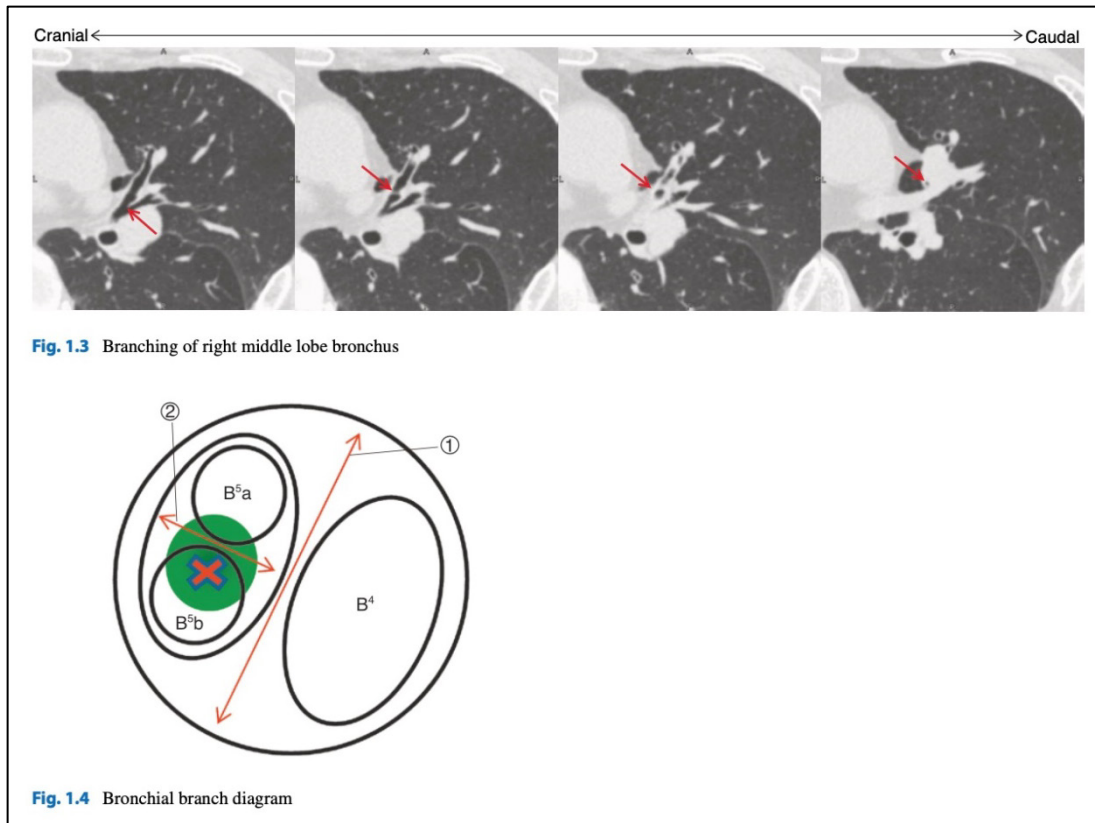


Figure 1.3 Kurimoto's manual method

When using Kurimoto's method, Zong et al. (2022) adjusted it by creating a “clock scale hand-drawn mapping system”. They then compared 48 patients from the hand-drawn group with 42 patients using the VBN method. The comparison resulted in the PPL location and size to be equal in both groups. The total diagnostic yield was 75% for the hand-drawn group and 61.9% for VBN. This was not statistically different. However, when comparing the hand-drawn manual method to VBN, the time of planning pathway (1.32 versus 9.79 min, $P=0.01$), the total time of operation (23.63 vs 28.02 min, $P = 0.002$) and the operating cost (758.31 ± 125.21 vs 1327.70 ± 116.25 USD, $P < 0.001$) were much lower in the manual method. This again demonstrates that manual mapping is a simple and cost-effective method that is well worth spending time to learn it.

Radial endobronchial ultrasound (Radial EBUS) guided biopsy

The main emphasis in this thesis is on Radial EBUS due to its wide availability and cost effectiveness, as well as the ability to combine easily with other navigation methods whilst enhancing their efficacy. More importantly, Radial EBUS is the only guided bronchoscopy method that is widely available in Australia and New Zealand. The use of radial ultrasound was first reported by Hurter and Hanrath (1992). Herth et al. (2002) first published their study on the clinical use of Radial EBUS in 2002. Radial EBUS is a flexible wire-like ultrasound probe that gives a 360-degree radial view of the airway. The length of the probe is 1150mm and reaches beyond the reach of the flexible bronchoscope which has a working length of 500-600mm. It can be used via the working channel of the bronchoscope and advances to the bronchial subsegments beyond the bronchoscopic vision. The radial ultrasound has a GS that fits snugly to the ultrasound, and has a working length of 1050mm.



Figure 1.4 Schematic presentation of the Radial EBUS probe in a PPL

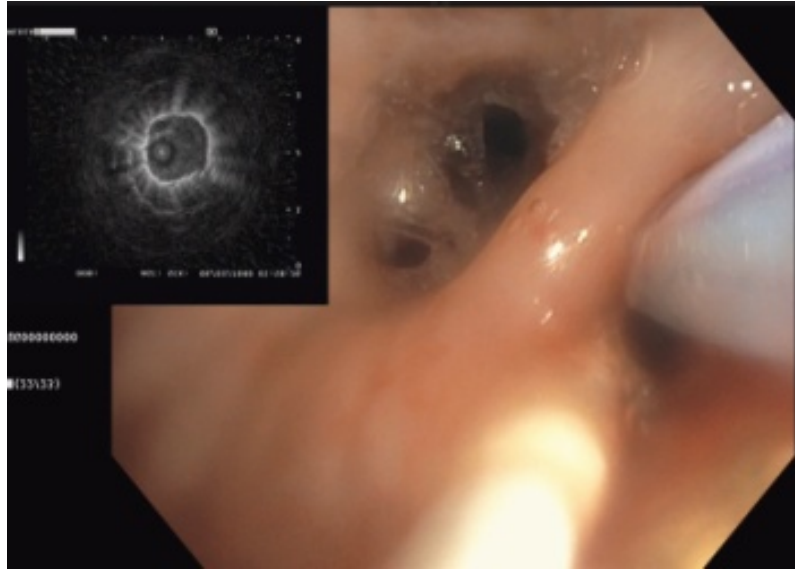


Figure 1.5 Radial EBUS probe with the GS in a PPL with a concentric ultrasound image of the PPL displayed on the left

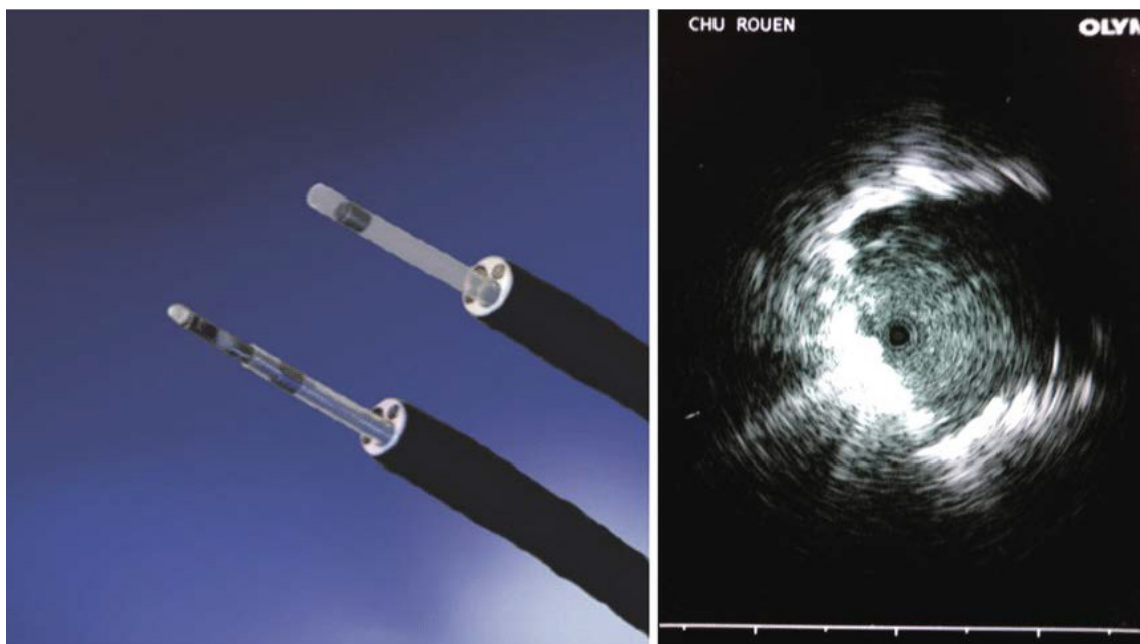


Figure 1.6 Radial EBUS with GS protruding through the working channel of the bronchoscope

The Radial EBUS ultrasound is available in two sizes: (i) 1.4mm outer diameter (UM S20-17S, Olympus, Tokyo, Japan); and (ii) 2.6mm outer diameter (UM S20-20S, Olympus, Tokyo, Japan) (Figure 1.7).

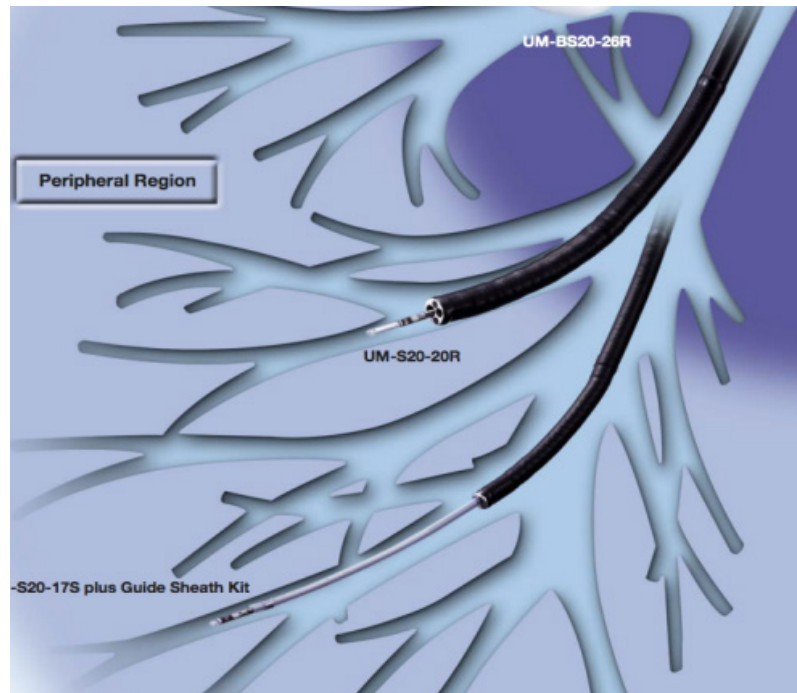


Figure 1.7 Large and small radial endobronchial ultrasound probe in the bronchi

Understanding the airway anatomy and study of the pre-procedural CT to map the correct pathway to the PPL is extremely important. This can be done by manually reading the CT chest and using a combination of fluoroscopy, VBN or EMN to guide the Radial EBUS ultrasound probe into the PPL. The aim of navigation is to place the ultrasound probe ideally inside the PPL, or at least adjacent to the PPL.

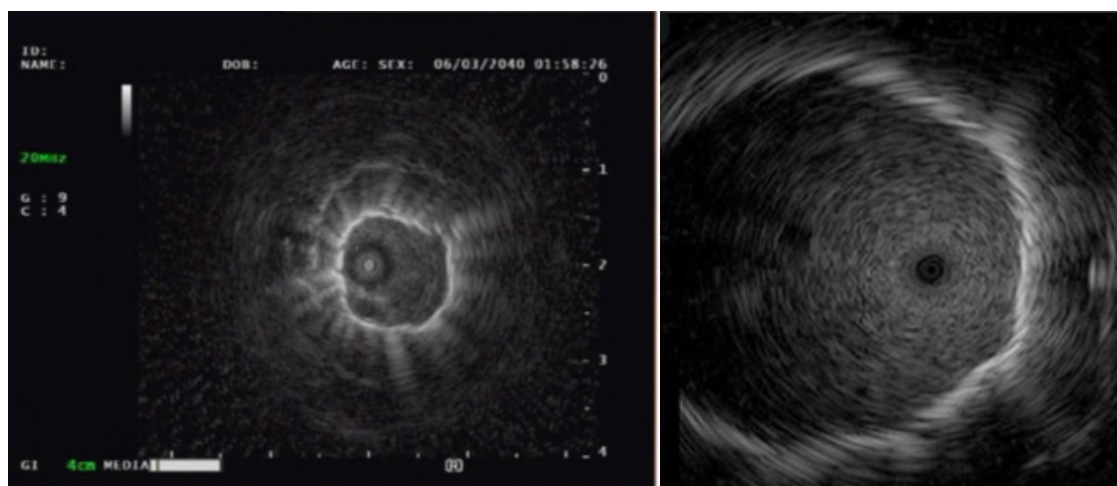


Figure 1.8 Concentric radial ultrasound view: small PPL (5mm) (a) and large PPL (20mm) on Radial EBUS (b)

The ultrasound image then demonstrates the location of the probe in comparison to the PPL. If the ultrasound probe is placed within the lesion this gives a concentric image (Figure 1.8); probe placed adjacent to the PPL (Figure 1.8), gives an eccentric image. The PPL is not visible when the probe is not close enough to the lesion (Figure 1.9) and only shows normal lung parenchyma as a snowstorm appearance due to increased echogenicity (Izumo et al., 2015).

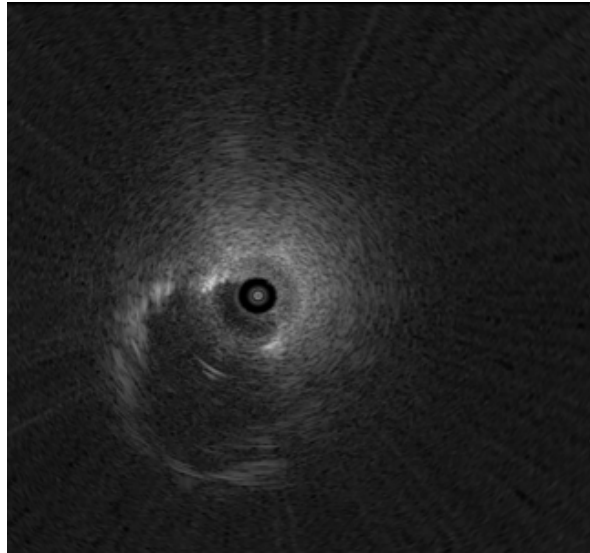


Figure 1.9 Eccentric radial ultrasound view of a PPL on Radial EBUS

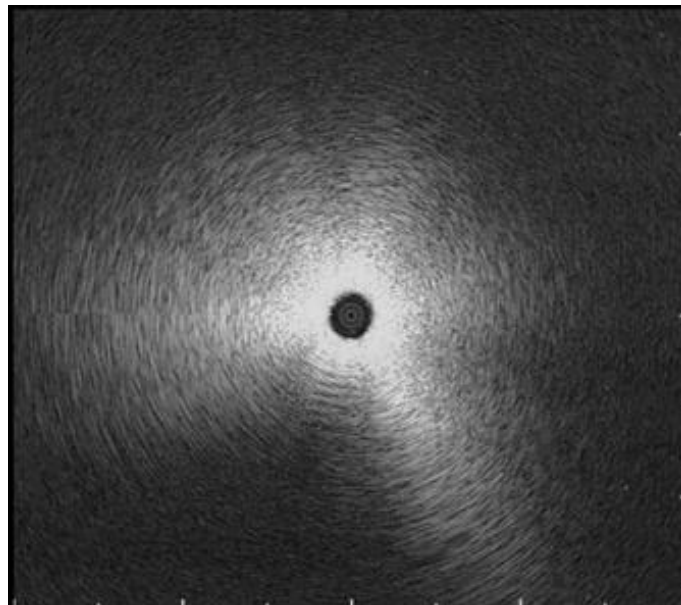


Figure 1.10 Normal lung parenchyma on Radial EBUS

Visualisation of vascularity in close proximity to ultrasound probe

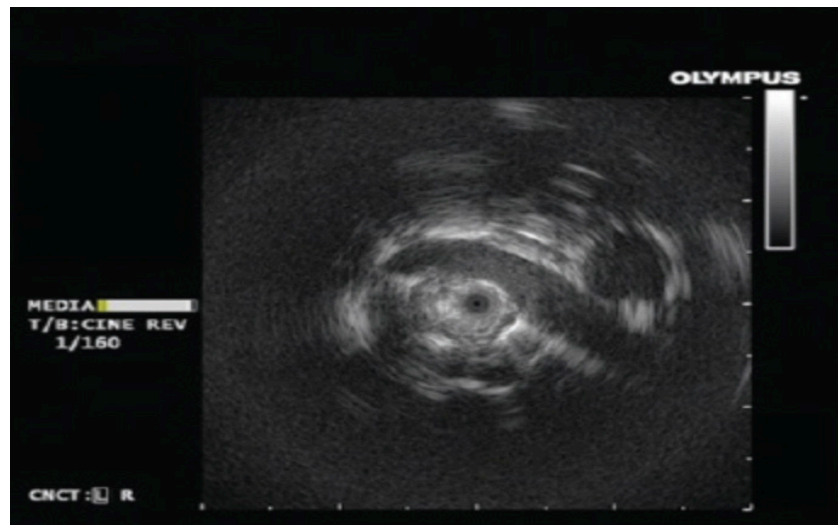


Figure 1.11 Vascular structure at 1 o'clock position in relation to PPL

Another advantage of using Radial EBUS ultrasound is the ability to visualise blood vessels in close proximity to the Radial EBUS probe. If these vessels were located, the probe can be manipulated to a different location with lesser vascularity.

Kurimoto and others introduced the concept of GS in 2004 to act as an extended working channel (Kurimoto et al., 2004). Once placed in the PPL of concern, the ultrasound can be removed. The GS acts as an extended working channel to direct the biopsy instruments. Pre-measured instruments are then used via the GS to take samples. Although fluoroscopy can be used to ensure the biopsy equipment is within the PPL, it is not always accurate due to the limitation of 2-D visualisation and inability to demonstrate “tool-in lesion” in real-time. Therefore, holding the GS in place in relation to the bronchoscope and patient, and frequently checking the location of the GS with the ultrasound probe, and confirming the GS is still located in the PPL is important to increase the diagnostic yield.

There are two sized Radial EBUS ultrasound probes: (i) large ultrasound probe: 20 MHz, UM-BS20-20R-3; Olympus, Tokyo, Japan, outer diameter 1.7mm; and (ii) small ultrasound probe: 20 MHz, UM-BS20-17S outer diameter 1.4mm. To house these ultrasound probes there are matching two sizes of Radial EBUS GS: (i) large GS (K-203), 2.6mm outer diameter, requiring 2.8mm working channel in the bronchoscope); and (ii) small GS (K-201), 1.95mm outer diameter, requiring a 2mm working channel bronchoscope.

Conventional biopsy instruments used in Radial EBUS are forceps and the brush, which are packaged together with their respective large or small GS in a kit. The large GS kit (K-203)

includes a 1.8mm forceps and a 1.8mm cytology brush with a GS of 2.6mm. Similarly, the small GS (K201) includes a small biopsy forceps of 1.4mm, small cytology brush of 1.4mm and a small GS of 1.95mm.

The large GS can accommodate all the standard bronchoscopic equipment (up to 1.9mm) and allows the freedom of using multiple standard bronchoscopic equipment through the GS instead of being limited to what is provided in the GS kit; this is an added advantage of using the large GS.

The smaller GS is expected to provide more visualisation of the PPL due to its ability to travel further peripherally than the large GS, but smaller samples taken via the smaller GS due to the small equipment may get destroyed during the processing of specimens. However, a larger GS can accommodate large instruments and can provide larger sampling that may give a better diagnostic yield.

1.4 Introduction to the research problem

1.4.1 Scope of the research problem

The first meta-analysis of Radial EBUS was published by Steinfert et al. in 2011. This paper consisted of 16 studies with 1420 patient demonstrating a combined diagnostic yield of 73% (95% CI 0.70-0.76). Following this, four other meta-analyses of guided bronchoscopy in which Radial EBUS was used as the predominant guidance technique alone or in combination with thin bronchoscope, EMN and VBN were published with similar results (Wang-Memoli et al., 2012; Ali et al., 2017; McGuire et al., 2020; Sainz Zuniga et al., 2020). This diagnostic yield of 70-73%, is suboptimal compared to CT-TTB.

Despite the extremely low adverse events noted with Radial EBUS in the five meta-analyses (pneumothorax rate of 1.5% and less than 0.7% of chest drain insertion), and despite the ability to combine Radial EBUS with L-EBUS for staging during the same procedure, having a suboptimal diagnostic yield to CT-TTB hinders Radial EBUS being used as a first line biopsy in PPL diagnosis, despite the recommendation by the ACCP (3rd edition) advocating Radial EBUS use (Rivera et al., 2013). Therefore, this low diagnostic yield of Radial EBUS needs to be improved.

At the time of this PhD study in 2015 in New Zealand, Radial EBUS service did not exist in New Zealand. However, in Australia, only three centres had Radial EBUS facilities. Therefore, to commence these studies in all participating centres in Australia and New Zealand, the Radial

EBUS service had to be started from the ground up in most participating centres. The equipment had to be purchased and staff needed to be educated to this innovative technique, including referring respiratory physicians so that confidence in the safety and utilisation of them were assured. All this required pilot studies to be performed and research to be presented in Thoracic Society meetings from 2015 to 2017.

1.4.2 Focus of the research problem

The low diagnostic yield has been commented on by the study authors of meta-analysis on Radial EBUS guided bronchoscopy techniques as a need for future improvement. In attempting to improve the diagnostic yield, the focus has been heavily invested on increasing the visualisation of the PPL by using multi-modal navigation methods to improve the “guidance” to reach the PPL.

The terms of visualisation and diagnostic yield need to be clearly defined. Visualisation is the ability to see the PPL by using one or combined modes of guided bronchoscopy. Diagnostic yield is the ability to obtain a diagnostic biopsy from the visualised PPL. The expectation is for visualisation and diagnostic yield to be equal. That is, every single PPL that is seen by the guided bronchoscopy method or Radial EBUS should have the ability to take a diagnostic sample of the visualised PPL. However, this has not been the case so far.

Therefore, the hypothesis for this thesis is that if a PPL is “seen” or visualised by the radial ultrasound, yet the biopsy taken is not diagnostic, then one explanation could be due to a deficiency in the biopsy tools causing the low diagnostic yield.

Deficiency in biopsy tool was multifaceted, which included confining the biopsies to a single (mostly forceps) or dual modalities (forceps and brush) and not employing multi-nodal biopsy methods and not using tools, such as the inconsistent use of the GS. Not employing novel biopsy tools, such as cryobiopsy, more frequently may also be a reason for the low diagnostic yield.

There may also be other explanations for this low diagnostic yield. Whilst the PPL is well visualised and if a biopsy is non-diagnostic, then, this could be due to the inability of “real-time” visualisation of the biopsy tool in the PPL. One main advantage noted with CT-guided lung biopsy is to demonstrate whether the biopsy needle was in the desired location of the PPL. With navigation or Radial EBUS bronchoscopic biopsies, this is a major drawback. Surrogate markers are used to confirm the location, including using a GS, fluoroscopy, tomosynthesis and cone beam CT. Using robotic bronchoscopy is a fascinating area of exploring this possibility. However, this remains an area of development and is enthusiastically waited. This topic is also not addressed in this thesis.

Maintaining the position of the GS via shape-sensing robotic equipment and improving the skills of the bronchoscopist to improve the diagnostic yield have not been incorporated in this thesis but have been discussed as future opportunities.

Another explanation for the low diagnostic yield with biopsies was not using Rapid Onsite Cytology Examination (ROSE) consistently to obtain feedback on the correct location of the biopsy tool to relocate them. Whilst noting these findings during the research and describing them in published papers, ROSE was not available at research centres in 2015 when these studies were designed. Therefore, the need for further studies has been highlighted.

Targeting the tools in the toolbox to suit the PPL and patient characteristics also improves the diagnostic yield, which is an area where additional knowledge is acquired as further studies are published. In fact, early studies have provided us with the knowledge to choose suitable biopsy tools for the PPL and patient characteristics, as well as assisted in designing future studies for exploration. The studies in this thesis have focused on introducing new biopsy tools and exploring existing and novel tools to optimise the biopsy procedure with the aim of improving the diagnostic yield and narrowing the gap between visualisation and diagnostic yield.

In the Australian and New Zealand setting, the commencement of these biopsy techniques meant multiple hurdles had to be overcome, both from a technical viewpoint and available facilities. This was the case, especially with cryobiopsy due to its reputation for “high bleeding risks” based on the existing literature on cryobiopsy for ILD.

1.4.3 Introduction to biopsy tools used in Radial EBUS

In this section, the use of the GS, conventional biopsy tools, aspiration needle biopsy and the novel cryobiopsy and GenCut biopsies are discussed. The implementation of these biopsy tools, especially in the New Zealand setting, and adopting safer techniques that were acceptable and doable in local settings, were discussed.

In 2014, cryobiopsy use in PPL was published by a single pilot study by Schuhmann et al. (2014). This group from Heidelberg Germany had markedly different and better logistic settings than those in Australia and New Zealand. Adopting cryobiopsy in the local setting required craftsmanship to deal with the lack of facilities and theatre times necessary to develop various bleeding control methods and safety mechanisms to suit the New Zealand setting and budget.

Use of a GS in Radial EBUS

The use of a GS may improve the diagnostic yield of the Radial EBUS procedure, as demonstrated in multiple studies over the last two decades (Ishida et al., 2012; Kurimoto et al., 2004; Oki et al., 2021). This is especially true for smaller PPLs and PPLs located peripherally.

Size of the Radial EBUS guide sheath

The size of the GS may also influence the diagnostic yield. The smaller GS of 1.95mm can only accommodate small biopsy tools for producing small samples that could be destroyed during processing, compared to the larger GS which may offer larger samples. The only data available is limited, demonstrating no significant difference in using the large or small GS (Kurimoto et al., 2007). Therefore, this is an area that requires further study and exploration.

Conventional Radial EBUS biopsy tools (forceps biopsy and cytology brush)

The conventional forceps biopsy is the main biopsy technique used with Radial EBUS, usually as the sole biopsy method and to a lesser extent, combined with the cytology brush. Both these biopsy tools work well for PPLs located directly on the path of the biopsy tool. Forceps and cytology brush cannot penetrate the bronchial wall and is not an effective biopsy tool for PPLs located adjacent to a bronchus. As demonstrated in Figure 1.12, a biopsy tool similar to a needle aspiration would be better suited for a PPL located adjacent to a bronchus.

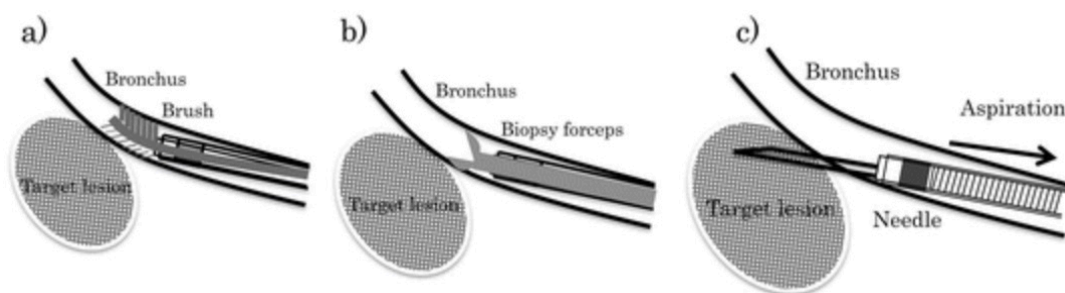


Figure 1.12 Aspiration needle piercing into a PPL adjacent to the bronchi; the forceps biopsy failed to do so

In reflecting on previous published data, most studies have reported a lower yield on PPL adjacent to the bronchi (Radial EBUS giving an eccentric ultrasound image), compared to a PPL located directly in the path of the diagnostic tool (giving a concentric ultrasound image). However, these studies used a forceps or brush as sampling methods. Whilst some early studies, such as Herth et al. (2002) suggested the use of an aspiration needle to improve the diagnostic yield, most

studies have not addressed the pivotal role, and the contribution of a correct biopsy tool to the diagnostic yield.

Aspiration needle biopsy

The needle aspiration has been used as a biopsy tool in few Radial EBUS studies. In a meta-analysis in 2017, Ali et al. (2017) presented 57 studies, all of which used forceps biopsies (57/57 studies) followed by forceps combined with brushings (24/57 studies). There have been a markedly low number of studies (five out of 57) that had used an aspiration needle as a biopsy method in Radial EBUS procedures (Ali et al., 2017) This finding is echoed in the AQuIRE registry data, most interestingly demonstrating that the aspiration needle was used only for 16.4% of cases with Radial EBUS (Ost et al., 2016).

Concept of multi-modal biopsy (combination of biopsy tools in Radial EBUS)

Another interesting finding regarding biopsy tools was the preferential use of a single biopsy tool in most published studies. Only handful of studies have used a combination of biopsy tools (Ali et al., 2017). Whilst forceps can diagnose a concentrically visible PPL, adding an aspiration needle can assist in extracting material from an adjacent PPL. Therefore, combining different biopsy tools may improve the diagnostic yield and not using multi-modal biopsies may be a cause for a lower yield.

Novel biopsy tools with Radial EBUS (cryobiopsy and GenCut tool)

Incorporating novel biopsy tools needs to be explored as a means of improving the diagnostic yield with Radial EBUS.

Cryobiopsy: Cryobiopsy may be one such method that can be beneficial in increasing the diagnostic yield of PPLs. Cryobiopsy is a cold biopsy that uses liquid CO₂. When expanded, it creates a freezing temperature of -59° Celsius; tissues adhere to the biopsy tip that can be pulled out intact. This effect is called the Joule Thompson effect described as far back as the 1940s (Roebuck et al., 1942). The cryoprobe is 1.9mm in diameter, and is reusable. The initial commercially available cryoprobe was 950mm in length, which was shorter than the Radial EBUS GS, being 1050mm. Later on, this reusable cryobiopsy probe was modified and lengthened to 1150mm.

Although reasonable evidence exists on the use of cryobiopsy in interstitial lung disease (ILD), at the time of commencing this study in 2015, there was only a single feasibility study published on the use of cryobiopsy with Radial EBUS for diagnosis of PPLs (Schuhmann et al., 2014). The

Schuhmann study demonstrated acquisition of tissue samples three times larger compared to forceps biopsy. As smaller sized samples from small biopsy tools may get destroyed during processing, the cryoprobe can provide intact tissues that survives processing and are suitable for molecular testing.

Cryobiopsy data is limited, even in early 2023 at the time of this thesis submission; there were only two meta-analyses published in 2021 and 2022 (Giri et al., 2022; Sryma et al., 2021) with a total patient number of 400 in 12 studies. A recent publication of 1024 patient prospective series by Herth et al. (2021) was a welcome addition to this area where data is sorely lacking. Yet, overall, there are only five studies published prospectively. Therefore, further prospective studies in cryobiopsy for PPL would be important to add to the growing body of data, as well as to demonstrate the divergence that was noted between the safety profiles of cryobiopsy for PPL and cryo for ILD.

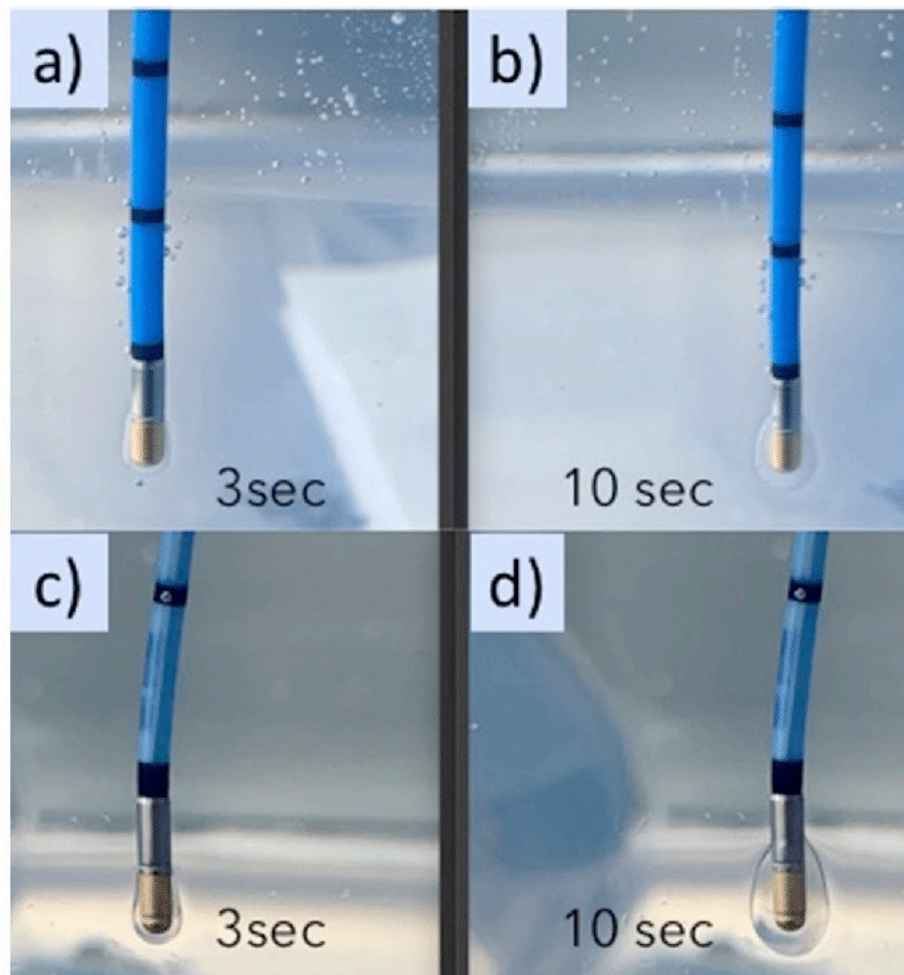


Figure 1.13 Reusable cryobiopsy probe 1.9mm outer diameter (a and b) and 2.4mm outer diameter (c and d) (CRYO2; ERBE, Tuebingen, Germany)

GenCut tool: Another novel biopsy tool that can be used with the Radial EBUS large GS is GenCut (Covidien GenCut core biopsy system). GenCut is a novel blunt biopsy instrument that has demonstrated satisfactory histology samples in porcine models (Khandhar et al., 2015). It is a flexible, 1140mm length, 1.9mm diameter hollow tube with a 10mm blunt metal tip comprising of inwardly placed sharp blades (Figure 1.12). Once in the PPL, suction is applied using a lockable 20ml syringe. The suction ensures the tumour is anchored into the biopsy tool. The tool is then rotated clockwise 45-degrees at a time, whilst maintaining the suction, to complete a 360-degree rotation, ensuring ongoing acquisition of material via suction into the tool.

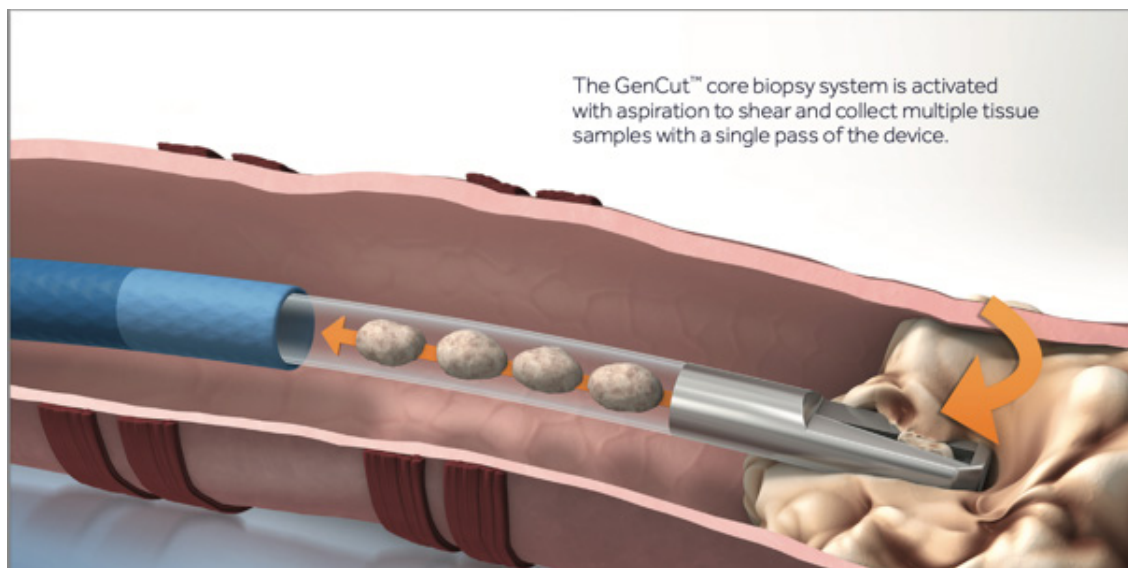


Figure 1.14 GenCut core biopsy tool demonstrating “anchoring” to the tissue via suction and samples drawn into the tool (permission obtained from Medtronic)

1.4.4 Use of Radial EBUS samples for epidermal growth factor receptor (EGFR) and programmed death ligand-1 (PD-L1) expressions in non-small cell lung cancer

In the era of personalised medicine, identifying specific molecular and genetic markers in NSCLC carries important prognostic indicators for the patient.

EGFR mutation is a driver mutation gene found predominantly in NSCLC within the Asian region. A meta-analysis of 456 studies that included 30,466 patients demonstrated an overall pooled prevalence of 32.3% with 38.4% in China, whilst Europe recorded 14.1%. It was also widespread in females (43.7% vs 24%) and non-smokers (49.3% vs 21.5%), as well as in adenocarcinomas (38% vs 11.7%) (Zhang et al., 2016).

The development of drugs that inhibit the EGFR-Tyrosine Kinase Inhibitor (EGFR-TKI) has dramatically changed the prognosis and treatment options in patients with unresectable advanced NSCLC.

Multiple clinical trials and meta-analysis have demonstrated that using EGFR-TKI as a first-line treatment in EGFR mutation positive NSCLC patients improves progression-free survival and adverse events compared to conventional platinum-based chemotherapy (Rosell et al., 2012).

More recently, immune checkpoint inhibitor (ICI) biomarkers, including monoclonal antibodies against PD-1 and PD-L1, have been discovered. The prevalence of PD-L1 expression ranged from 24% to 60% with cut-off positivity at 5%. The PD-L1 expression percentage was of significance as treatment responses above 50% PD-L1 expression correlated with higher treatment responses (Yu, 2016). Patients with these markers experienced dramatic improvement in their survival and quality of life, particularly for NSCLC patients compared with standard chemotherapy (Herbst et al., 2016).

There was also an interplay between the status of EGFR mutant and PD-L1. Patients with EGFR mutant NSCLC showed poor responses to anti-PD-1 or PD-L1 treatment.

The use of an ICI blockade prior to, or concurrent with, Osimertinib has been reported to increase the risk of pulmonary toxicity. Advanced EGFR-mutant patients with strong PD-L1 expressions had a greater chance of a de novo resistance to EGFR-TKIs than patients with PD-L1 negative (Guan et al., 2018).

As mentioned above, a significant proportion of patients will have a biomarker that may change their treatment trajectory. However, targeted therapies are ineffective in most NSCLC patients whose tumours lack oncogenic driver alterations. Therefore, identifying EGFR and PD-L1 biomarker status of a patient's tumour is significant.

Currently, an effective biopsy is the most proven method for obtaining the biomarker status from the primary tumour or from metastatic disease. An analysis of histological (surgical resection specimens) versus cytological samples has not demonstrated any difference in the ability to detect these biomarkers (Ekin et al., 2021).

Radial EBUS samples are small, yet noted to be satisfactory in molecular testing, including EGFR. Guisier et al. reported that 80% of Radial EBUS samples were suited for molecular testing for EGFR (Guisier et al., 2016). The most published results for EGFR testing in Radial EBUS

have been with forceps biopsies followed by radial brush. The ability to perform EGFR testing with a needle aspirate has never been explored. Therefore, this is an area that requires further exploration.

1.4.5 Inability to visualise real time biopsy in tool

Another possibility for a lower diagnostic yield is the lack of real-time visualisation of the biopsy tool in the PPL. The GS acts as a semi-real time method to secure the positioning of the biopsy tools. Once the radial ultrasound is removed after locating the PPL, the GS that is left in situ acts as an extended working channel, with the assumption that this GS would not move and the proceduralist would be able to re-introduce the biopsy tool to the exact location detected by the radial ultrasound. Patient movement of coughing and breathing, as well as external movement at the end of the bronchoscope could cause displacement of the GS.

Not having a real-time ability to visualise the biopsy and relying on surrogate markers, such as fluoroscopy, hinders the accuracy of the location, causing displacement to occur unnoticed. This becomes even more crucial for smaller peripheral PPLs.

When using a GS, checking the location of the GS frequently with the Radial EBUS ultrasound between biopsies need to be adapted to overcome this issue. Steinfert et al. (2011) reported that 23% of studies in the meta-analysis did not use the GS and fluoroscopy was not used in 66% studies. This may have contributed to the lower diagnostic yield.

Better ability to visualise the sample whilst performing the biopsy is now being explored with the use of cone beam computed tomography (CBCT) and robotic bronchoscopy, which is still in its early stage of development. In the future, it is hoped that these novel methods demonstrate a “tool in PPL” to improve the diagnostic yield, especially for smaller PPLs.

At present using the GS seem to be a cost-effective solution to provide a “partial” tool in PPL situation. In addition the size of the GS matters as well, as the GS size determines the size of the biopsy tools it houses.

1.5 Overview of the research topic

This thesis explores novel, minimally invasive, multi-modal bronchoscopic biopsy methods using Radial EBUS and GS to improve the diagnostic yield of peripheral pulmonary lesions suspected of lung cancer.

This thesis also describes how these novel techniques were introduced to the local setting in New Zealand, and how techniques were adopted to improve safety with the local facilities that were available, which were much different to Northern American and European facilities.

1.5.1 Research aims, objective and questions

The well-proven marked safety profile and the ability to combine with L-EBUS during the same procedure makes Radial EBUS to be an excellent choice for diagnosing PPL and performing molecular testing, such as EGFR/PD L1. Despite these advantages, the diagnostic yield of Radial EBUS is inferior to CT-TTB. One explanation for the lower diagnostic yield with Radial EBUS may be due to a deficiency in the biopsy tools used. The aim of this thesis is to investigate whether improving biopsy methods and introducing novel biopsy tools via the Radial EBUS large GS and using a multi-modal biopsy approach would improve the diagnostic yield of PPL whilst maintaining the safety profile.

Another aim is to develop new techniques to improve the safety of these biopsy tools, especially, cryobiopsy when trying to adapt to the Australian New Zealand setting without operating theatre or anaesthetic facilities.

Therefore, the research questions were:

1. Does the addition of an aspiration needle to the Radial EBUS sampling via the large GS improve the diagnostic yield? Will the aspiration needle be useful for EGFR testing as well? Does the size of the GS matter to the diagnostic yield in Radial EBUS?
2. How could the novel cryobiopsy method be introduced in the New Zealand setting without General anaesthetic or operating room facilities? What techniques can be developed to improve the diagnostic yield without compromising safety?
3. How does the novel cryo-radial procedure with the multi-modal biopsy technique compare with the widely used CT-TTB in the diagnostic yield, and its ability to perform EGFR testing and safety?
4. Is the GenCut tool safe and effective in obtaining a core biopsy with sedation Radial EBUS in centres where general anaesthesia is not readily available?
5. Does the combination of biopsy techniques with the large GS (Radial EBUS forceps, brush, and needle aspirate +/- cryobiopsy or GenCut tool), that is, multi-modal biopsy

technique improve the diagnostic yield of PPLs? Does multi-modal biopsy improve the ability to perform EGFR testing?

To explore the above aims, four separate studies were explored, as follows:

1. Comparing the diagnostic yield of the large GS and small GS using conventional Radial EBUS sampling methods (forceps/brush), followed by assessing the additional diagnostic yield of adding the needle aspirate to the large GS. This was a randomised control trial (**REBUST 2 study**).
2. Adding cryobiopsy to Radial EBUS conventional biopsy by using the large GS as a feasibility study to assess safety and diagnostic yield. This was a prospective interventional pilot study to introduce cryobiopsy procedure to New Zealand (**Cryo-feasibility study**).
3. Comparing the diagnostic yield and safety of CT-guided biopsy against multi-modal Radial EBUS and cryobiopsies via the large radial GS. This was a randomised control trial (**CT-CROP study**).
4. Using a blunt-ended novel GenCut biopsy via the large GS to determine the safety and ability to perform PD-L1 testing. This was a prospective interventional study with 18 consecutively recruited pilot study (**R-CORE study**).

1.5.2 Value-added to clinical practice

Radial EBUS is a cost-effective widely available guided bronchoscopy technique in Australia and New Zealand being offered to patients as a safer combined diagnostic and staging modality during a single procedure for the diagnosis of PPLs suspected of lung cancer. By improving the biopsy tools with the addition of new tools, the diagnostic yield may be improved to a comparable level to CT-TTB whilst maintaining a favourable safety profile.

A safer procedure will reduce delays in treatment and improve pre-morbid functional status due to the improved side effect profile. With the higher number of PPLs being discovered and in an era of lung cancer screening programmes in the near future in Australian clinical practices, the ability to offer the safest and effective biopsy of PPLs to make a diagnosis and avoid adverse events is required. The growing number of lung cancer diagnoses in the aging population of Australians who requires a safer procedure needs to be considered.

The study addresses the safety precautions that are required when performing novel biopsy techniques, especially cryobiopsy in clinical practice in Australia and New Zealand, which is much different to the setting of our European or North American colleagues.

I established the first lung cryobiopsy and Radial EBUS service in New Zealand through these studies and incorporated cryo-radial services into the routine clinical practice of two tertiary hospitals in Australia and New Zealand. This is keeping in mind that bronchoscopy in all New Zealand centres and most Australian centres are done under sedation in comparison to most European and North American centres, requiring adaptation of the biopsy techniques to suit the local facilities. All New Zealand and Australian respiratory physicians are not trained in intensive care, unlike their counterparts in the United States, therefore, they require upskilling and development of policies and procedures to improve the efficacy and safety of the novel procedures.

1.5.3 Value-added to the discipline

A head-to-head comparison with the “gold standard” CT-TTB

Cryobiopsy is a novel biopsy procedure that is added to Radial EBUS biopsies. Data and published literature on this novel cryo-radial procedure is sparse and limited to single arm diagnostic studies. The CT-CROP study was the first study that enabled a broad outlook to determine where the cryo-radial procedure stood in terms of the current widely used CT-TTB. This study is an original contribution to the field.

Concept of multi-modal biopsy techniques with Radial EBUS

All four studies (REBUST 2, CT-CROP, R-CORE and Cryo-feasibility) focussed on multi-modal biopsy techniques. All patients were sampled with at least two different types of biopsies; some patients having up to four different types of biopsies. This multi-modal biopsy technique and its ability to improve the diagnostic yield have not previously been extensively and prospectively studied at the time of these studies in 2015.

Utility of aspiration needle in diagnostic yield and EGFR analysis

This study aims to assess whether an aspiration needle has the ability to be used via a large GS to improve the diagnostic yield and the ability to perform EGFR testing. Although previous randomised control trials by Chao et al. (2009) compared the Radial EBUS aspiration needle and forceps proactively, and demonstrated increased diagnostic yield with the needle aspirate; the uptake had been poor. A handful of studies had published the use of aspiration needle since,

however, no study has reported on the ability to perform EGFR testing with a needle aspirate with Radial EBUS.

At the time of this thesis submission in early 2023, a meta-analysis of needle aspirate use in from 2000 to 2021 have resulted in only seven published studies of 510 patients, thus demonstrating the sparing data that is available on this biopsy tool (Lou et al., 2023). Therefore, this small study adds data to a field that has limited material in terms of diagnostic yield and the ability to perform EGFR testing via a needle aspirate in PPL. Conducting research and collecting data on the ability of the needle aspirate to perform EGFR testing in a PPL is an original contribution to the field.

Use of GenCut with Radial EBUS

As most Australian and New Zealand bronchoscopy units perform procedures under sedation alone, the use of GenCut with Radial EBUS will allow Australian and New Zealand groups to perform core biopsies under sedation without the need for high-end facilities that are required for a cryobiopsy method, which are only available in a few centres in Australia and New Zealand. This is an original contribution to the field.

1.6 Limitations of the studies in this thesis

This thesis explores the widely used and relatively inexpensive Radial EBUS method. It does not cover the use of expensive guided bronchoscopies, such as VBN and EMN, which are not available in Australia and New Zealand.

The cryo-radial procedure was performed in tertiary hospitals with high level equipment and facilities that included rigid bronchoscopy, cryobiopsy, Radial EBUS, L-EBUS, anaesthetic and theatre facilities, which are not available outside tertiary hospitals. Therefore, this procedure is not generalisable to all respiratory units using Radial EBUS. However, to overcome this limitation, the novel GenCut tool used under sedation Radial EBUS bronchoscopy was explored.

During the study, I used either the eXcelon 21G or Medtronic 21G aspiration needle subject to their availability. Therefore, the value of one type of aspiration needle over the other cannot be discussed because the allocation was not randomised and was based on availability. Ground glass lesions were also not discussed during this study and limited to PPLs with at least a part-solid component.

The 1.9mm reusable cryoprobe was a procedural challenge due to the stiff nature of the probe, which caused difficulty in directing it to upper lobar locations. It was further challenging to insert

the 1.9mm cryoprobe into the GS that has a maximum capability of 1.95mm due to the tight fit. This difficulty has been reported by other studies as a major hindrance (Imabayashi et al., 2019). Forcing the cryoprobe into the GS resulted in the cryoprobe being kinked at the proximal end. This resulted in discarding several cryoprobes during the study period. This technical difficulty led to a lower diagnostic yield in the cryobiopsy arm and is discussed further in Chapters 5, 6 and 8.

In this study, rapid onsite cytology (ROSE) was not used at Middlemore Hospital, New Zealand due to a lack of access to ROSE at the time. Therefore, the use of ROSE was up to the proceduralist's preference in Australian centres; ROSE was not mandated by protocols. This hindered the assessment of the value of ROSE with Radial EBUS and cryobiopsy procedures.

This thesis explores the ability to improve the diagnostic yield in Radial EBUS by improving the biopsy tools, introducing new biopsy tools, combining biopsy tools and developing techniques to make them safer for local settings. However, this thesis does not explore other methods that can be used to improve the diagnostic yield, including, exploring methods to real-time, visualising the “tool in PPL” at the time of the biopsy, which are beyond the scope of this thesis. Therefore, further study is required. Table 1.1 outlines Chapters 4-8 in this thesis.

Table 1.1 Chapter overview

Chapter	Content	Published paper/journal
Chapter 4	REBUST 2 Study Published as an original research paper with an additional discussion section included at the end	Radial endobronchial ultrasound guided biopsy for lung lesions: Evaluating the role of the guide sheath size, addition of needle aspirate and multi-modal sampling, in improving the diagnostic yield: An exploratory randomized study. <i>Journal of Community Medicine and Public health reports.</i>
Chapter 5	Cryo-Feasibility Study Published as a case series with an additional discussion section included at the end	Novel hybrid cryo-radial method: an emerging alternative to CT-guided biopsy in suspected lung cancer. A prospective case series and description of technique, and adopting this technique to the New Zealand setting. Respirology case reports.
Chapter 6	CT-CROP Study Published as an original research paper with an additional discussion section included at the end	Cryobiopsy with Radial-EBUS has comparable diagnostic yield with higher safety in comparison to CT-guided transthoracic biopsy for peripheral pulmonary lesions: An exploratory randomised study. <i>Internal Medicine Journal</i>

Chapter	Content	Published paper/journal
Chapter 7	R-CORE study Published as a case series with an additional discussion section included at the end	Using the GenCut core biopsy tool with the radial endobronchial ultrasound guide sheath enables a high-quality histology sample capable of Programmed Cell Death Ligand 1 (PD- L1) testing: Case series. Respirology case reports.
Chapter 8	Conclusion	

CHAPTER 2:

LITERATURE REVIEW

2.1 Chapter overview

This chapter focuses on the importance of a safe and effective biopsy technique in diagnosing PPLs suspected of lung cancer and reviewing the bronchoscopic biopsy techniques. Radial EBUS is identified as the main guided bronchoscopy method discussed in this thesis as it is widely available within Australia and New Zealand and often the only guided bronchoscopy method available locally. Despite the exceedingly high safety profile of Radial EBUS the diagnostic yield is suboptimal compared to CT-TTB. There seem to be gap between visualisation of PPL with Radial EBUS and the diagnostic yield. Therefore, this chapter aims to identify reasons for this low diagnostic yield including the deficiency in biopsy tools.

2.2 Importance of a safe and effective biopsy in PPL

The finding of a PPL in chest imaging is a common presentation in respiratory medicine. In the current environment of multiple radiological investigations for non-malignant conditions, including high usage of CT coronary angiograms and abdominal imaging, as well as high resolution CT chest for various respiratory diseases, PPLs are increasingly being discovered. Lung cancer screening, with its proven mortality benefit and screening programme being incorporated into clinical practice, the diagnosis of PPL had increased (de Koning et al., 2020; Aberle et al., 2011). In the United States, screening programmes alone amount to 1.5 million PPLs being discovered annually (Gould et al., 2015). As the majority (>95%) of PPLs detected in lung cancer screening have been found to be benign (Pinsky et al., 2017), an exceedingly safe biopsy is required to avoid unnecessary harm. A PPL that is too small for a reliable biopsy is followed up in accordance with Fleischner Society Guidelines (MacMahon et al., 2017), however, an enlarging PPL still requires further investigation, including a biopsy. Increasing age is a risk factor for lung cancer with 2/3 patients presenting with lung cancer are over 65 years of age and more than half of this group are over 75 years old (Bravo, 2014; Eguchi et al., 2016). In Australia with its aging population, a safe and effective biopsy would reduce repetitive procedures, adverse events, and treatment delays.

A recent Australian study reviewed health system delays in lung cancer treatment. In this study, the timeframe from a general practitioner referral to being seen by a specialist was a median of seven days, which is within the suggested guidelines of 14 days (Stirling et al., 2014). However,

the time from a specialist visit to treatment was a median of 27 days in 128 studies, which far exceeds the desired 14-day timeframe. The predominant cause of delay was the waiting time for diagnostics to be completed. In 18% of patients in this study, delays were associated with repeated diagnostic investigations, a lack of access to high quality investigations and the need for a further staging procedure after diagnosis (Malalasekera et al., 2018). These findings were echoed by a later meta-analysis by Zhang et al. (2022) documenting that the healthcare system delays, comprised of multiple appointments to establish a diagnosis, multiple specialist consultations and a delay in staging procedures (Zhang et al., 2022).

The challenge for clinicians is to select the optimum biopsy technique based on high-yield and low complication rates to obtain sufficient samples for immunohistochemistry (IHC) and molecular testing from PPLs (Rivera et al., 2013).

2.3 CT-guided transthoracic biopsy for PPLs

CT-TTB is the most widely employed biopsy tool for PPLs. In 48 studies which included 2718 biopsies, the diagnostic yield was noted to be high (92%), including for smaller PPLs less than 20mm (92.8%). However, the associated pneumothorax rate was 20.5% (8.2-47.3%) with chest tube placement of 7.3% (0-31.1%), and clinically significant haemorrhage was 2.8% (0-14.7%) (DiBardino. et al., 2015).

The risk of pneumothorax with CT-TTB was further increased to 31% if emphysema was present in the needle pathway (Zhou et al., 2021). Given that 80% of lung cancer diagnoses occur in current or ex-smokers, emphysema is a common underlying co-morbidity in this group (Kolderman et al., 2019; Zhou et al., 2021). The absence of pleural contact and smaller size of the PPL were independent risk factors for pneumothorax, with smaller PPLs less than 20mm reporting a 31% pneumothorax rate compared to PPLs more than 40mm reporting 15.4% (Cruz et al., 2014).

Other serious side effects, including intraventricular air embolism (Dietrich, et al., 2017) and cerebral air embolism have been reported. CT-TTB has been associated with rare causes of death due to massive lung haemorrhaging (Yamamoto et al., 2016; Hung et al., 2015, 2018; Piccoli et al., 2019).

The inability to perform mediastinal staging during the same procedure is another drawback with CT-TTB. Therefore, clinicians have set themselves on the path of investigating alternative

diagnostic modalities that can overcome these limitations and provide an efficacious biopsy with a much safer adverse event occurrence, as well as perform staging during the same procedure.

2.4 Bronchoscopic biopsy methods for PPLs

Bronchoscopic diagnostic techniques have repeatedly been evaluated as a far safer biopsy method for PPLs for decades. Gustav Killian was considered the father of modern bronchoscopy. In 1876, he removed a pork bone from a farmer's airway using an oesophagoscope. Following this, in the early 20th century, Chevalier Jackson modified the oesophagoscope and developed a rigid tube with illumination and suction tubing that could be inserted into the patient's airway, which has now evolved as a rigid bronchoscopy. This rigid bronchoscopy procedure was difficult to tolerate and was revolutionised by a Japanese thoracic surgeon, Shigeto Ikeda, by developing the first flexible bronchoscopy in 1966, which became commercially available in 1968 (Panchabhai & Mehta, 2015).

In a Japanese study of 421 facilities performing 98,497 bronchoscopies, the death rate related to bronchoscopy was 0.011%, total incidence of complications was 1.84%, and the most frequent complications related pneumothorax 0.7%, pneumonia/pleurisy 0.46%, and haemorrhaging 0.45%, all of which were significantly less than the CT-guided biopsy (Horinouchi et al., 2020).

Early flexible bronchoscopic diagnoses had an acceptable diagnostic yield of 88% for central lesions that were visualised via the bronchoscope but much less for PPLs not visible to flexible bronchoscope and varied from 18-75% (Torrington & Kern, 1993). Incorporating various "guiding" systems with the flexible bronchoscopy was explored to overcome this issue with visibility.

2.5 Emergence and development of guided bronchoscopy

If a visualisation method could be added to this safe method of flexible bronchoscopy, diagnostic yield would increase. Therefore, methods to "guide" the bronchoscope to the required location were explored, and multiple "guided bronchoscopy" methods have evolved to improve the visualisation of PPLs. The first attempt at guided bronchoscopy using endobronchial ultrasound, was reported in 1992 by Hürter and Hanrath (1992).

Since then, the last 20 years have seen a marked advancement in guided bronchoscopy techniques. The commonly used guided bronchoscopy techniques are discussed below. The predominant guided bronchoscopy method in this thesis is Radial EBUS and is discussed in-depth.

Another adjunct that was added early in the history of bronchoscopy to improve the visualisation of lesions that are not seen endobronchially via the flexible bronchoscope is fluoroscopy. The inability to locate the tip of the biopsy tool in a three-dimensional setting was a major disadvantage for fluoroscopy, thus limiting its success rate in the biopsy of PPLs, especially for PPLs <30mm. In one recent study, the success of transbronchial forceps biopsies using fluoroscopy was stated as 34% with PPLs <20mm and 63% for PPLs >20mm (Rivera et al., 2013). Despite this, fluoroscopy is being used in combination with nearly all other guided bronchoscopy methods and has proven to be a valuable adjunct to improving the diagnostic yield of PPLs.

The commonly used guided bronchoscopy methods are listed below:

1. Radial endobronchial ultrasound (Radial EBUS) guided biopsy
2. Thin and ultra-thin bronchoscopy
3. Virtual bronchoscopic navigation (VBN)
4. Electromagnetic navigation bronchoscopy (EMN)

2.5.1 Radial EBUS guided bronchoscopy/biopsy

Endobronchial sonography was first reported by Hurter and Hanrath (1992) when they demonstrated the use of ultrasonography in lung tissue. In their study of 100 consecutive patients, they noted the difference in echogenicity with the normal lung parenchyma being echo rich and lung cancers being echo poor. They also noted that arteries can be identified in lung parenchyma as pulsatile echoes. This method was revolutionary as an innovative method of examining lung parenchyma with minimal complication and radiation exposure and was well tolerated by patients. Hurter and Hanrath (1992) suggested this sonography method as an alternative to fluoroscopy and this technique rapidly developed into a method used in routine respiratory practice.

This finding was further studied by Herth et al. (2002) who published the first feasibility study of Radial EBUS as we know it today. Herth et al. (2002) performed transbronchial biopsies on 50 consecutive patients who were given either fluoroscopy guidance or Radial EBUS guidance. The diagnostic success was 80% for Radial EBUS and 76% for fluoroscopy, however, smaller lesions of <30mm were diagnosed better with Radial EBUS (Herth et al., 2002). Herth et al. (2002) commented that Radial EBUS is as effective as fluoroscopy. In keeping with the previous study

by Hurter and Hanrath (1992), Herth et al. (2002) also commented on the high safety profile of the Radial EBUS procedure.

One comment made by Herth et al. (2002) was the learning skill of the proceduralist for Radial EBUS, suggesting that 40-50 procedures is required to be skilled in the process. Having another guiding method, such as fluoroscopy, while Radial EBUS forceps biopsies are being performed (as the Radial EBUS ultrasound has to be removed during the biopsy) was thought to increase the yield.

However, the ongoing issue was that once the PPL is located by Radial EBUS and the radial ultrasound is removed, finding the same position again with the biopsy tools was difficult because there was no true guiding pathway and reliance made on fluoroscopy, which was not always accurate.

Kurimoto et al. (2004) introduced the Radial EBUS GS to overcome this issue and demonstrated using a GS to cover the Radial EBUS ultrasound and leaving it in situ as an extended working channel would improve the diagnostic yield. By using a GS repeated biopsies could be accurately performed. Bleeding was minimal due to the tamponade effect of the GS (Kurimoto et al., 2004).

Use of the GS and its effect on the diagnostic yield of Radial EBUS

The use and size of the GS may have an impact on the diagnostic yield of a PPL due to the biopsy tools used via the GS is limited by the size of the GS. With only two sizes of GS commercially available, the small GS 1.95mm outer diameter and the large GS 2.6mm outer diameter, they have been compared head to head in a single study, demonstrating that the large GS had 93% visualisation and 77% diagnostic yield whilst the small GS had 97% visualisation and 83% diagnosis (Kurimoto et al., 2007). Kurimoto et al. (2007) concluded that the small GS had an increased diagnostic yield and visualisation despite there being no statistical significance. Since then, studies seemed to have favoured the use of the small GS.

Direct comparisons of these two GS methods have not been prospectively randomised and undertaken and limited to this single abstract presentation by Kurimoto et al. (2007).

Ishida et al. (2012) investigated the use of forceps biopsy with a Radial EBUS small GS and forceps biopsy without Radial EBUS GS. They demonstrated that a Radial EBUS GS forceps biopsy had a better success rate of 64.6% compared to 46.7% with forceps biopsy without the GS. The small GS and the forceps biopsy method used in this study (Ishida et al., 2012) could have

resulted in smaller samples as the diagnostic yield was much lower than the published meta-analysis data at the time of 70-73% diagnostic yield (Steinfort et al., 2011).

Using a GS was noted to be extremely useful in most studies over the last two decades (Bo et al., 2019; Kurimoto et al., 2004; Oki et al., 2012, 2021). Yet some authors have explored the utility of a method called the “measured distance” instead of the GS. In this method, the Radial EBUS ultrasound is used to locate the PPL, followed by measuring the distance from the PPL to the working channel of the bronchoscope. The distance measured is then transferred to the measurement of the biopsy tools.

Zhang et al. (2016) found no difference after recording the distance measured from a PPL to the working channel when comparing it with Radial EBUS GS biopsies (S. Zhang et al., 2016). However, in this study, the sizes of the biopsy tools were different. The distance measured arm used a 1.8mm diameter forceps, resulting in a diagnostic yield of 76% versus the small GS arm using a 1.4mm forceps with a diagnostic yield of 72%. Although the difference was not statistically significance, the comparison between biopsy tools of different sizes may have made a difference in the diagnostic yield. The study authors did not comment on this possibility. However, the study has highlighted one advantage of the distance measured method, that is, the ability to use large biopsy equipment without the constriction of the size of the small GS.

But there are disadvantages of not using a GS in the distance measured method, as highlighted by Zhang et al. in 2018 (S. Zhang et al., 2018). The bleeding risk of 3.96% was higher than most published studies on Radial EBUS and was likely due to the lack of the GS to tamponade a bleed. This distance measured method also demonstrated 85% visualisation and 55% diagnostic yield. Although authors have concluded this to be of high sensitivity, the yield is suboptimal when compared to published meta-analysis on Radial EBUS yield of 70%, around the same time period of (Ali et al., 2017). The gap between visualisation and diagnosis was almost 30%, which was significantly higher than the studies using the GS resulting in a gap of 10-20%, therefore, the consistent use of a GS may improve the diagnostic yield.

A recent paper published by Goel et al. (2021) had reported retrospectively on 126 patients using Radial EBUS without GS and cryobiopsy with 1.9mm cryoprobe. The overall diagnostic yield was only 78.6%. Out of this 69% were larger than 30mm and 90% of the PPL were concentric PPL. Therefore, one could argue that if the GS was used, the diagnostic yield may have been much higher, displaying a selection bias in a retrospective study.

Studies had ventured to understand Radial EBUS and the factors that would indicate a higher diagnostic yield in PPL.

Discovery of factors that affect the Radial EBUS diagnostic yield through time

Kurimoto et al. (2004) published the first paper to demonstrate that if the ultrasound was within the PPL (concentric image) yielding an 87% success rate whilst it was placed adjacent to the PPL (eccentric ultrasound image), there would be a reduction of diagnostic yield to 42%. Yamada et al. (2007) concurred that the ultrasound probe being located in the PPL gave a better result of 83% compared to 61%.

Yamada et al. also explored the use of Radial EBUS with a GS to determine other factors that would decide the diagnostic yield. They found similar findings to Kurimoto et al. (2004), Herth et al. (2002) and Yoshikawa et al. (2007), demonstrating that larger PPLs have a better diagnostic yield, with PPL <15mm at a 40% success rate and >15mm at 76%.

Yoshikawa et al. (2007) were the first to report on the utility of the air bronchogram traversing the PPL (CT bronchus sign). PPLs presenting with a CT bronchus sign had a yield of 79.2% versus 56.7%, without. The CT bronchus sign was associated with a markedly higher prediction success rate from Radial EBUS procedures in multiple later studies (Guvenc et al., 2015; Jiang et al., 2016).

Since then, studies have identified the factors related to the higher diagnostic yield in PPL via Radial EBUS, as: (i) larger size (Ikezawa et al., 2014; Wang-Memoli et al., 2012); (ii) more solid lesions giving a better yield (Yoshikawa et al., 2007); (iii) the presence of a CT bronchus sign (Evison et al., 2014; Guvenc et al., 2015)); (iv) the location of PPLs (concentric PPLs produce better results than eccentric PPLs) (Ali et al., 2017; Guvenc et al., 2015; Okachi et al., 2016).

Some studies have addressed the factors that affect the visualisation of PPLs. Tay et al. (2013) demonstrated that the size of the lesion was important with 85% visualisation for >20mm, 66% for <20mm, with distance from the hilum, 91% for <50mm and 55% for >50mm (Tay et al., 2013).

Only a few studies have looked at the biopsy tools and their impact on the diagnostic yield. Since the earlier studies published by Kurimoto et al. (2004), the biopsy tools used with Radial EBUS were limited to forceps biopsy and cytology brush alone. Kurimoto et al. suggested evaluating the role of needle aspiration with Radial EBUS guidance as a future study proposal to improve the diagnostic yield (Kurimoto et al., 2004). Chao et al. (2009) performed a randomised control trial

(RCT) comparing a Radial EBUS biopsy with forceps and bronchial wash against forceps, bronchial wash and aspiration needle biopsy. The diagnostic yield was 60.6% versus 78.4% favouring the aspiration needle arm. In the arm with three biopsy modalities, the aspiration needle gave a 62.5% diagnostic yield followed by 48.9% for forceps and 19.8% for bronchial wash. Whilst the paper concluded that the aspiration needle improved the diagnostic yield, the authors had not appreciated the increased diagnostic yield when three types of biopsy tools were combined (Chao et al., 2009).

Yet, there was not much uptake in using the aspiration needle and not many published studies on the subject. This may be due to the lack of a lung parenchyma specific, flexible aspiration needle. Chen et al. (2014) reported on the use of needle aspirate in a retrospective analysis of 496 patients who underwent Radial EBUS procedures over a 5-year period (A. Chen et al., 2014). The sampling instruments used included a 1.8mm forceps and a 21G aspiration needle, resulting in a diagnostic yield of 77% with forceps and 80% with an aspiration needle, demonstrating the aspiration needle yield to be similar to a forceps biopsy.

Many years later, the biopsy tools that were utilised have not changed and are predominantly limited to a forceps biopsy followed by cytology brush. In a meta-analysis of 57 studies by Ali et al. in 2017, only five studies had used an aspiration needle as a biopsy tool (Ali et al., 2017). As described later in this chapter, the importance of biopsy tools in improving the diagnostic yield was not appreciated until more recently when Gildea et al. (2021) reported on the value of the use of multiple biopsy tools in late 2021. This retrospective study, analysing the Navigate study data, was the first published study to coin the words “multi-modal biopsy” and to recognise its ability in increasing diagnostic yield.

Instead, the ongoing focus has always been on combining Radial EBUS with other guided bronchoscopy techniques as a means of improving the navigation to the PPL and improve “visualisation” or “seeing” the PPL.

In the following section, the utility of combined navigation methods of VBN, EMN and thin bronchoscopy with Radial EBUS are explored to demonstrate the increased visualisation with the combined navigation methods and to highlight that despite the increased visualisation, the diagnostic yield continues to lag.

2.5.2 Virtual bronchoscopy navigation (VBN)

VBN was initially reported by Asahina et al. (2005). VBN is a navigation and sampling method used with brushing and forceps, most often performed in combination with the Radial EBUS GS.

Ishida et al. (2011) published a 200-patient RCT comparing the diagnostic yield in PPLs <30mm with the use of VBN with Radial EBUS and using the small GS in comparison to the non-VBN group, using axial CT images as a reference for Radial EBUS (Ishida et al., 2011). The success rate was 80.4% for combined Radial EBUS and VBN against 67% for the group without VBN. The results were in keeping with other studies (Eberhardt et al., 2007), demonstrating that combined guided methods of VBN and Radial EBUS improve the diagnostic yield.

Asano et al. (2013) published a second RCT, allocating 350 patients with small lung nodules, PPL <30mm to two groups. In this study, VBN was used with an UTB, which had a 1.2mm working channel. In the VBN group, the UTB was guided to the PPL using VBN and in the non-VBN group, axial CT images were used. Biopsies were performed using fluoroscopy for both groups. The diagnostic success of using the UTB was 67.1% for the VBN group and 59.9% for the non-VBN group. Radial EBUS was not used in this study, due to the small working channel of 1.2mm in the UTB and may have been the cause of this lower diagnostic yield. Given the small size of the samples produced, it is possible the samples may have got destroyed during processing. Asano et al. (2013) had performed only five forceps biopsies and suggested that six biopsies per PPL when using these small instruments may be important in keeping with the work done by Saka et al. (2004) and Yamada et al. (2007) to ascertain an adequate number of passes to obtain adequate tissue to compensate for the small-sized biopsies from the small instruments used.

Compared to Ishida et al. (2012), the diagnostic yield was lower when using the UTB with VBN as opposed to the small Radial EBUS with VBN. This may be due to the ability of the Radial EBUS GS biopsy tools to be better placed in the PPL compared to fluoroscopy that only provides a two-dimensional imaging. Another reason may be the small cytology brush and forceps biopsy used via the 1.2mm working channel with the UTB may have not survived processing due to the smaller size.

The use of VBN with Radial EBUS demonstrating a higher diagnostic yield was also reported by Wong et al. (2014). Takashina et al. (2011) also demonstrated that the addition of VBN to Radial EBUS improves the diagnostic yield. In this study of 199 patients with lesions <30mm, Radial EBUS was performed with and without VBN, which resulted in a significantly higher diagnostic yield with VBN +Radial EBUS (80% versus 67%) (Takashina et al., 2011).

This higher diagnostic value, when using VBN combined with other guidance methods, was more pronounced when the lesion size was smaller. Asano et al. (2014) review study of 13 studies with 868 patients demonstrated the combination of different guided bronchoscopy methods with VBN

resulting in an overall diagnostic yield of 73.8% (95% CI 70.9-76.7%) for lesions ≥ 20 mm was 67.4% (95% CI 63.3-71.5%).

VBN has been shown to reduce the time to locate a lesion, as well as reduce the overall procedure time in a 33-patient study, which demonstrated the time to locate lesions was 5.3 minutes versus 10.5 minutes ($P = 0.04$) and the overall procedure time was 22.4 minutes versus 29.9 minutes ($P = 0.044$) respectively (Wong et al., 2014).

These findings on the diagnostic yield with VBN have not changed over time. In a more recent RCT of 105 patients using Radial EBUS with and without VBN (Xu et al., 2021), a diagnostic yield of 76% in VBN with the Radial EBUS group and 65.5% in the non-VBN group ($P = 0.287$) was revealed. The procedure time for Radial EBUS + VBN (20.6 mins) was significantly shorter than the non VBN group (28.6 mins) which was in keeping with the study by Wong et al. (2014).

Despite the markedly favourable safety profile of 1.5% for major adverse events, the diagnostic yield of VBN guided bronchoscopy as noted above (80%-65%) is suboptimal compared to CT-TTB diagnostic yield of 92% (Asano et al., 2014).

The lower diagnostic yield with VBN could be due to the body to CT divergence, collapse of the lung with anaesthesia and changes in the lungs since the CT scan was performed leading to the mis-registration of the PPL location. However, this does not seem to be the only reason for the lower diagnostic yield. There appeared to be a discrepancy between successfully reaching the target PPL (visualisation of the PPL) and obtaining a diagnostic specimen, which may be due to a deficiency in the biopsy techniques.

Improving diagnostic yield of PPLs without bronchus sign via VBN-BTPNA/TBAT

The presence of a bronchus sign leading to the target PPL is a strong predictive factor for successful diagnosis of PPLs with techniques such as Radial EBUS and VBN. Bronchoscopic trans parenchymal nodule access (BTPNA) and the transbronchial access tool (TBAT) are emerging techniques for overcoming limitations of low diagnostic yields in PPLs without a bronchus sign.

BTPNA generates a route to the PPL through the lung parenchyma, integrated as part of the larger Archimedes platform (Broncus Medical, Inc, San Jose, California, USA). Archimedes is a VBN system that integrates bronchoscopic images, CT data and fluoroscopic images to provide a 3-D reconstructed real-time airway image. This includes highlighting a point-of-entry in the central airway and a lung parenchymal route that avoids blood vessels. Based on this virtual guidance, a

coring needle punctures the planned point-of-entry, which is dilated by a balloon catheter. A sheath with a blunt dissection stylet is then advanced to the target PPL.

The first report of BTPNA in humans by Herth et al. (2015). This study showed that adequate sampling was achieved in 10 out of 12 patients (83%) using Archimedes and fluoroscopy guidance, however, nodules were not visible by fluoroscopy in seven patients. Although no severe post-procedural complications in BTPNA have been reported to date, further investigation on safety is necessary. A large multicentre international trial to assess the utility of BTPNA is currently recruiting patients, which will likely provide clearer data on the technique's safety (EAST-2 trial: ClinicalTrial.gov registration no. NCT02867371).

2.5.3 Electromagnetic navigation (EMN) bronchoscopy/biopsy

EMN is another guided bronchoscopy method that has been used alone and in combination with Radial EBUS to improve the visualisation of PPL. This method of EMN was first described by Schwarz et al. (2003) in a swine lung model with artificially created PPLs to demonstrate the accuracy of the technique when EMN was used in real-time with the previously acquired CT chest. Herth and Eberhardt (2008) demonstrated the effective and safe use of EMN and Radial EBUS with a GS by reiterating the previous feasibility study performed by Eberhardt et al. (2007).

Studies comparing the diagnostic yield with EMN and Radial EBUS, and Radial EBUS alone revealed that the diagnostic yield was no different, however, there was a tendency for patients with a CT bronchus sign to do better with Radial EBUS, and patients with Radial EBUS and EMN to do better if there was no CT bronchus sign, although the results were not statistically significant (Ma et al., 2020).

Similarly, studies have not demonstrated a difference in diagnostic yield between EMN versus non-EMN bronchoscopy. In a meta-analysis of lesions >20mm in size, 1037 patients undergoing EMN navigation and 1094 in non-EMN bronchoscopy indicated no difference in diagnostic value between EMN and non-EMN bronchoscopy (76.4% for non-EMN versus 79.27%). A separate meta-analysis in EMN demonstrated the pooled diagnostic yield to be 65% of 15 studies in 2015 involving 1033 lung nodules (95% CI 59.2-70.3) (Gex et al., 2014).

However, EMN had a better yield for PPLs <20mm (64.09% versus 48.67%; OR 2.09; 95% CI 1.44-3.03; $P < 0.001$); It was also better for lesions in the outer one-third of the lungs (70.59% versus 50.36%; OR 2.26; 95% CI 1.48-5.92; $P < 0.001$) (S. Jiang et al., 2020).

This lower diagnostic yield again, may be due to the CT to body divergence experienced with EMN. Whilst the usual pre-procedural CT scan was performed at total lung capacity (TLC), the anaesthetised patient's lung volume may be at functional residual capacity (FRC). Also, respiration, cardiac motion and atelectasis body position can all alter the location of the PPL in real-time compared to the pre-procedural CT. In fact, a PPL on the lower lobes can vary in distance as much as 25mm between inspiration and expiration (Wang-Memoli et al., 2012).

Fluoroscopic tomosynthesis-guided EMN (f-EMN)

To overcome this issue, of CT to body divergence, one of the developers of EMN, Medtronic provided real-time fluoroscopic imaging to correct the discrepancy (fluoroscopic tomosynthesis-guided EMN or F-EMN), which demonstrated a 25% increase in the yield with F-EMN fluoroscopy (54% with EMN and 79% with F-EMN) (Aboudara et al., 2020).

However, despite these expensive navigation techniques being used, the diagnostic yield continues to be suboptimal (54%-79%), compared to CT-TTB. Again, the biopsy tools are an area where little focus has been applied; it is an area that may play a pivotal role in improving the diagnostic yield in guided bronchoscopy. Biopsy tools again seem to be limited to forceps biopsy or cytology brush.

2.5.4 Thin and ultra-thin bronchoscopy/biopsy

Ultra-thin (Olympus BF-XP190) 3.1mm outer diameter, 1.2mm working channel) bronchoscope and thin (Olympus BF-P190) (4.2mm outer diameter) bronchoscope. The thin and ultra-thin bronchoscopy improved the ability of the bronchoscopist to visualise much further into the bronchial system.

Thin bronchoscope

Iyoda et al. (2003) introduced the thin bronchoscope, measuring 4.2mm outer diameter and 2mm working channel. They demonstrated that the thin bronchoscope could travel further into the bronchial tree than the much larger video-bronchoscope (6mm outer diameter) used at the time; it demonstrated that the diagnosis from the upper lobes were markedly favourable with the thin bronchoscope using forceps biopsy or cytology brushing (Iyoda et al., 2003).

A randomised controlled trial (RCT) compared the standard sized bronchoscope (outer channel diameter >6mm) with fluoroscopy versus thin bronchoscopy (BF-P190; Olympus) with a 4.2mm outer diameter coupled with the small Radial EBUS ultrasound (UM-S20-17S; Olympus) with or without the small 2mm guide sheath (K201). This study demonstrated 37% diagnostic success for

the standard bronchoscope with fluoroscopy and 49% success for the thin bronchoscopy and Radial EBUS (Tanner et al., 2018). The diagnostic yield in this study was much lower than the meta-analysis published on guided bronchoscopy of 72%-73% (Sainz Zuniga, 2020; Wang-Memoli et al., 2012). Tanner et al. (2018) had commented on the inability to use a needle aspiration as a biopsy tool as one reason for the resultant low yield and variable proceduralist skill as another.

The thin bronchoscope was investigated against the Radial EBUS GS (with the small GS), which demonstrated a similar diagnostic yield. However, in both procedures, the diagnostic yield was suboptimal compared to CT-TTB with a thin bronchoscope yield of 65% versus Radial EBUS (small GS) diagnostic yield of 62%. In both procedures, small forceps biopsies were the only sampling method used (Oki et al., 2012).

Ultra-thin bronchoscope

With the development of the ultra-thin bronchoscope, the visualisation of peripherally located PPLs has become even more achievable. However, the working channel of the UTB is 1.2mm, hence, only small biopsy equipment can be used. A comparison between Radial EBUS with a 4.2mm outer diameter versus an ultra-thin prototype bronchoscope measuring 3.4mm outer diameter and 1.7mm inner working channel showed non-inferiority (65% versus 62%) by Oki et al. (2012).

Both the thin bronchoscope and UTB had been combined with other navigation methods and demonstrated a higher diagnostic yield with combination methods. When the UTB was combined with other navigation methods, there was improved diagnostic yield. Ishida et al. (2011) used UTB with Radial EBUS and VBN, the diagnostic yield for lesions measuring 30mm was 74%. The combination of Radial EBUS, VBN and an UTB seemed to be a promising combination in the diagnosis of PPLs. The procedure also appeared to be less time-consuming (Ishida et al., 2011).

This diagnostic yield with combined methods continued to improve when a thin bronchoscope was used. Asano et al. (2013) compared the thin bronchoscope with a 1.4mm ultrasound GS and VBN. This combined modality improved the diagnostic success of lesions <30mm with 93.8% visualisation and 84.4% diagnosed using a TTB. The median examination time was 22.3 minutes (9.8-41.5 mins) (Asano et al., 2013). For smaller PPL this diagnostic yield of 84.4% was indeed high with combination of navigation modalities, this was higher than UTB, possibly due to the larger sample size taken via the 2mm working channel.

Despite the high visualisation, diagnostic yield is still approximately 10-15% lower compared to visualisation. This gap in diagnostic yield may be due to inefficiency in biopsy tools, as well as due to smaller biopsy tools with the resultant small sample size. The need to consider the biopsy tools to improve the diagnostic success with VBN was raised by Asano et al. (2013) in their review paper on VBN. The inability to reach a PPL that does not have a bronchus sign, with the conventional biopsy tools (cytology brush and forceps biopsy) was another reason given for the low diagnostic yield by Asano et al. (2013).

2.6 Utility of combined guided bronchoscopy methods

A combined approach using multi-modal navigation methods with PPLs was first demonstrated by Eberhardt et al. (2007) who compared electromagnetic navigation bronchoscopy (ENB) only, Radial EBUS only or a combination of ENB and Radial EBUS. The authors coined the term “multi-modal” for the first time in literature for these combined navigation methods. The authors demonstrated that the combined procedure of Radial EBUS and EMN resulted in a success rate of 88% whilst Radial EBUS alone was 69% and EMN was 59%. Fluoroscopy was not used during this study for the purpose of reducing radiation exposure to the patient and staff. This practice was later shown by Ishida et al., (2011), demonstrating an 80% success with VBN assistance and 67% without.

Asano et al. (2014) commented that the diagnostic yield by UTB in combination with CT and VBN was within the range of 65.4% to 81.6%. Using a Radial EBUS GS and VBN, the result was between 63.3% and 84.4%, and using fluoroscopy and VBN, the range was from 62.5% to 78.7%.

Even with using a navigation desktop workstation (not in real-time) with Radial EBUS, the diagnostic success increased to 77.7% (with VBN) versus 64.6% ($P = 0.03$) which significantly reduced the procedure time (Matsumoto et al., 2015). This reduction in procedural time and value in combined diagnostic guided methods were demonstrated by Bo et al. (2019) and Xu et al. (2021). Therefore, it was evident that multi-modal navigation methods would improve the diagnostic yield of bronchoscopy procedures.

This similar principle of combining different biopsy tools (multi-modal biopsy tool) to improve the diagnostic yield is not well explored. In these studies, the biopsy tools used were frequently forceps biopsy alone and occasionally combining with the cytology brush.

A recent retrospective analysis of the Navigate study was one of the few studies with 1215 subjects demonstrated, by using extensive multi-modal biopsy (forceps, brush, aspiration needle,

needle tipped cytology brush) improved the diagnostic yield when compared to the restricted number of tools or conventional tools alone, which included forceps, and a brush with lavage (Gildea et al., 2021). This paper adds to the extremely sparse data on multi-modal biopsy techniques in the diagnostic yield of PPL.

2.7 Meta-analysis on guided bronchoscopies

There have been five meta-analyses published in the last decade on using Radial EBUS alone or in combination with other navigation methods described above. The first publication was in 2011 by Steinfort et al. (Steinfort et al., 2011) who incorporated 16 studies with 1420 patient, using Radial EBUS, reporting a pooled diagnostic yield of 73% (95% CI 0.70-0.76). The study included GS and non-GS patients. The complication risk varied from zero to 7.4%. The pooled rate of pneumothorax risk was 1.0% with intercostal catheter drainage of pneumothorax at 0.04%. In this meta-analysis, PPLs where GS was used had a success rate of 73% versus no GS use at 69%. The majority of the studies (10/13) had used the GS, however, the type of GS (large or small) used was not specified. Five studies used fluoroscopy and eight did not, but there was no difference in the pooled sensitivity for diagnosis, which remained at 73%. The diagnostic yield across all studies included in this meta-analysis varied vastly from 49% to 88%.

The next meta-analysis was published by Wang-Memoli et al. (2012) who demonstrated and reviewed all guided bronchoscopy methods, namely, fluoroscopy, ENB and VBN with Radial EBUS in 39 studies with 3052 lesions pooling the diagnostic yield at 70% (46-86.2%). The pneumothorax rate was 1.5% with 0.6% requiring a chest drain placement. In this study, 11/39 used the GS and 28/39 did not use the GS. Using a combination of guided bronchoscopy methods demonstrated improved yield. The three significant findings that improved the diagnostic yield were: (i) using the GS; (ii) adding VBN; and (iii) using Radial EBUS.

Another systematic review and meta-analysis conducted in 2017 by Ali et al. (2017) consisted of 57 studies with 7872 lesions with a diagnostic yield of 70.6% (95% CI 68-73.1). The researchers commented on an overall diagnostic yield of 70.6% (95% CI 68-73.1%). PPLs with the CT bronchus sign resulted in a higher diagnostic yield of 74.1% (68.3-79.5%) compared to without 49.6% (39.6-59.5%); this is in keeping with previous literature that have demonstrated better success rates with the CT bronchus sign. The overall complication rate was 2.8%, pneumothorax rate was 1.4% and chest drain insertion rate was 0.2%. The diagnostic yield, varied considerably across the studies in this meta-analysis from 49.3% to 92.26%. Ali et al. (2017) meta-analysis contained valuable data on the use of biopsy instruments.

The biopsy instruments used by Ali et al. (2017) in 57 studies were mainly forceps (57/57), followed by a cytology brush (23/57). The sole biopsy technique was forceps in 25/57 studies. Only five out of the 57 studies used an aspiration needle. Similar to previously published studies, this study also demonstrated four factors that impacted the diagnosis success from Radial EBUS: (i) larger size of the PPL <20mm versus >20mm; (ii) malignant lesions; (iii) presence of a bronchus sign on the CT; and (iv) concentric position on ultrasound. However, the impact on biopsy tool was not appreciated (Ali et al., 2017).

A more recent meta-analysis of radial probe ultrasound and ENB was published by McGuire et al. (2020) which consisted of 41 studies and 3204 patients (Radial EBUS 2097; ENB 1107). The overall diagnostic accuracy was no different, with Radial EBUS at 70.5% (95% CI 66.1-74.8) and ENB at 70.7% (95% CI 64.7-76.8). The complication rate was <2% for both modalities. The chest drain was 0.9%.

Another recent updated meta-analysis by Sainz Zuniga et al. (2020) who demonstrated a similar diagnostic yield of 72% (70-75%) in 51 studies with 7601 PPLs. Again, the extremely favourable side effect profile was highlighted with a pneumothorax rate of 0.7%.

The greatest strength of Radial EBUS resulted in a very favourable side effect profile. In five meta-analyses (Ali et al., 2017; McGuire et al., 2020; Steinfort et al., 2011; Sainz Zuniga et al., 2020; Wang-Memoli et al., 2012)), the pneumothorax rate was highest at 2% and the chest drain rate was 0.9%. Even with advanced COPD in 50 patients with FEV1 1.01 ± 0.28 L (FEV1 percentage predicted) 39.7 ± 8.2 , the pneumothorax risk was only 2.1% (Georgiou et al., 2016). Using a GS seemed to reduce haemorrhagic complications even further. The incidence of haemorrhagic complications was less in the GS group (0.29%) compared to the non-GS group (0.58%) (Horinouchi et al., 2020).

2.8 Issues noted with Radial EBUS bronchoscopy

2.8.1 Unresolved gap between visualisation and diagnostic yield in Radial EBUS

Despite improved visualisation with the Radial EBUS alone or in combination with other guided techniques, including fluoroscopy, VBN or EMN, and thin and ultra-thin bronchoscopy, the diagnostic yield seemed to be 10-20% lower than visualisation. This gap had been present throughout the history and continues to be an issue today, despite the use of advanced navigation methods.

Herth et al. (2006) demonstrated that Radial EBUS can be used successfully in fluoroscopy negative PPL <30mm with the avoidance of unnecessary radiation exposure. However, although visualisation was 89%, the diagnostic success was only 70%. This is the first literature available that has demonstrated the gap between visualisation and diagnostic yield with Radial EBUS, possibly due to only forceps biopsy being used as the sole sampling method (Herth et al., 2006).

Yamada et al. (2007) explored the use of Radial EBUS with a GS to determine factors that would decide the diagnostic yield. The diagnostic yield in 106 patients was 65%. Yet, the visualisation of the PPL was much higher at 85%. The study authors did not comment on this gap between the diagnosis and visualisation. There was a preference for using the 1.4mm outer diameter small radial ultrasound with its respective GS. This is the first study that has documented the preference for the use of a small GS (Yamada et al., 2007).

Another study by Yoshikawa et al. (2007) demonstrated that Radial EBUS had an acceptable diagnostic yield without the use of fluoroscopy. Again, overall in this study, the diagnostic success was 61.8%, despite higher visualisation at 76.6%. This could be due to using the conventional biopsy equipment of radial forceps and a brush that does not penetrate or biopsy PPLs adjacent to the bronchial wall. This study also used the small GS with the small Radial EBUS ultrasound probe, which may account for the smaller sample size and lower yield (Yoshikawa et al., 2007).

A. Chen et al. (2014) performed a retrospective review of 496 patients who underwent Radial EBUS in a 5-year period. In this study visualisation of the PPL via Radial EBUS was 96% but the diagnostic yield was 69%.

Since then, multiple studies have used Radial EBUS as a guided method and have continued to demonstrate the gap between visualisation and diagnostic yield, with the diagnostic yield being 10-20% lower than visualisation, but there has been no attempt to explain the gap in any published studies.

The Ostendorf et al. (2011) study on Radial EBUS demonstrated a visibility of 79.4% and diagnostic yield of 54% when a large GS was mostly used with forceps biopsies. Hence, the biopsy size would be expected to be larger, resulting in a better yield and the survival processing. At least four forceps biopsies were performed, showing adequacy of sampling in keeping with the previous recommendation from Yamada et al. (2007). Despite this, the overall diagnostic yield was lower than the published meta-analysis on Radial EBUS of 70-73% (Ali et al., 2017; McGuire et al., 2020; Wang-Memoli et al., 2012; Sainz Zuniga et al., 2020; Steinfort et al., 2011).

One explanation for the lower diagnostic yield could be due to the predominance in small PPLs in this group, where 62% were below 30mm in size and 30% of PPLs were less than 20mm in size, thus explaining the low diagnostic yield despite using a large GS and resultant larger forceps, as well as performing at least four forceps biopsies.

Another possibility is the inability to “real time” see the biopsy tools in the PPL specially in the context of the study consisting of smaller PPL. With the increased use of Radial EBUS for PPL, using a targeted biopsy tool to suit the patient and PPL would be another step to improve the diagnostic yield,

In all these studies, visualisation and the diagnostic yield were separately reported, yet were not considered to be a deficit and an issue that required addressing to improve the diagnostic yield in Radial EBUS. Realistically, despite visualisation, if a diagnosis is not made the high visualisations does not matter. In fact, recording a visualisation seem quite pointless. Yet, this position has not changed over time and a recent letter to the editor by Kramer et al. (2021) has raised this very question.

2.8.2 Ongoing low diagnostic yield of Radial EBUS in “real life”

Despite advancements in guided bronchoscopy methods and use of these methods in combination, the pooled diagnostic yield has not exceeded 73%. There is a large heterogeneity between studies included in the meta-analysis of guided bronchoscopies and significant variations between the diagnostic yields in the studies included in the meta-analysis being noted. In fact, the diagnostic yield appears to be even lower outside trial situations in real life clinical practice.

Real life registries such as the AQuIRE Registry have reported worse results when data was analysed from 581 patients with 22 physicians, with only 53.7% having a diagnosis for PPL from the bronchoscopy procedure. This was 63.7% without Radial EBUS or EMN, 57% for Radial EBUS alone, 38.5% for EMN alone and 47.1% when EMN and Radial EBUS were combined (Ost et al., 2016). A similar result of the low diagnostic yield in clinical practice was recorded by Tanner et al. (2018) with their publication of thin bronchoscope use with Radial EBUS demonstrating a 49% yield, with five participating sites.

Therefore, the diagnostic yield may be affected by the training and experience as well as the ongoing high volume skill maintenance, of the proceduralist. Tanner et al. (2018) commented that this trial was a multi-centre trial (5 centres) with various proceduralists having different skill sets that may have caused lower results than studies in larger tertiary centres with expert interventional bronchoscopists, therefore, suggesting the importance of upskilling proceduralists to improve the

diagnostic yield (Tanner et al., 2018); this is in keeping with comments made by Herth et al. (2002) during their first feasibility study of Radial EBUS, stating that 40-50 procedures are required for competence with Radial EBUS.

2.8.3 Ongoing lower diagnostic yield of Radial EBUS, in comparison to CT-TTB

Radial EBUS has been compared with CT-guided biopsy through multiple studies. Whilst an earlier RCT by Steinfort et al. (2011) demonstrated similar results between CT-TTB and Radial EBUS diagnostic yield (CT-TTB 81.3% versus Radial EBUS 78.1%) (Steinfort et al., 2011), more recent RCTs have demonstrated CT-TTB to be of a higher yield, for example, Fielding et al. (2012) (CT-TTB 78.8% versus Radial EBUS 67.6%), C. Wang et al. (2015) (CT-TTB 84.6% versus Radial EBUS 59.6%), L. Zhang et al. (2017) (CT-TTB 97.7% versus Radial EBUS 64%), Zhu et al. (2018) (CT-TTB 94.6% versus Radial EBUS 78.2%), and Zhu and Gu (2019) (CT-TTB 94.6% versus Radial EBUS 78.2%).

Jiang et al. (2016) analysed 187 patients undergoing CT-TTB versus Radial EBUS with a GS. This was performed using data from a prospective registry but analysed retrospectively. The diagnostic yield was 81% for the EBUS GS and 87% for CT-TTB, resulting in an overall complication rate of 13% in the CT group and 2% in the Radial EBUS group (G. Jiang et al., 2016).

A 2018 meta-analysis by Chang et al. (2018) consisting of nine studies with 813 procedures in bronchoscopic biopsy versus 15 studies on CT-TTB with 3463 procedures demonstrated a CT-guided biopsy yield of 93% (95% CI 90-96%) versus a Radial EBUS yield of 75% (95% CI 69-80%). PPLs <20mm noted a more pronounced difference with CT-TTB of 92% and for Radial EBUS, a much lower yield of 66%. The incidence of complications in CT-TTB was 26%, the CT percutaneous needle biopsy group (26%) (95% CI 21-32%) with chest tube insertion rate of 3%, which was markedly higher than those in the Radial EBUS group 2% pneumothorax rate and chest tube insertion <1%.

A meta-analysis by Fu et al. (2021) of nine studies with 2025 patients comparing Radial EBUS (n=994) and CT-TTB (n=1041) demonstrated CT-TTB to be associated with a higher diagnostic yield (OR 0.23; $P < 0.00001$), and higher rates of complications (OR 7.27; $P < 0.0001$). CT-TTB produced a markedly higher diagnostic yield for PPL <30mm (OR 0.24; 95% CI 0.14-0.41; $P < 0.00001$) and lesions close to the pleural.

The most recent meta-analysis on randomised controlled trials on Radial EBUS vs CT-TTB was published in 2023 by Ho et al. (2023), which included four RCTs with a total of 325 patients.

CT-TTB had a higher diagnostic yield than Radial EBUS/TTB (83.45% vs 68.82%). Radial EBUS, however, was significantly safer with 5-8 times lower risk of pneumothorax hospital admissions and bleeding.

Therefore, these comparison studies between CT-TTB and Radial EBUS have highlighted the marked safety profile of the Radial EBUS procedure but also the ongoing drawback of the low diagnostic yield with Radial EBUS. The visualisation of PPL with Radial EBUS in combination with guided bronchoscopies (up to 93%) is similar to the diagnostic yield of CT-TTB. Yet, the diagnostic yield of Radial EBUS sits at approximately 73%, and has not changed over the last decade, despite the combination of advanced navigation methods to Radial EBUS.

2.8.4 What do we know about the biopsy tools in Radial EBUS?

The thin bronchoscope, UTB, EMN and VBN, have been used in combination with Radial EBUS to improve visualisation and to achieve a higher diagnostic yield. With improved visualisation with guided bronchoscopy, the diagnosis improved, but the gap between visualisation and diagnostic yield, although slightly better, continue to exist.

Biopsy tools could play a major role and maybe one solution that improves the diagnostic yield by narrowing the gap between visualisation and diagnostic yield in Radial EBUS; however, they have not been well explored and consists of a gap in knowledge.

2.9 Gaps in knowledge of biopsy tools

The following gaps in knowledge regarding biopsy tools and techniques that could lead to a low diagnostic yield were noted and discussed in detail below.

2.9.1 Sparse data on comparison of large versus small GS

The GS is an important conduit to guide the Radial EBUS ultrasound to the accurate location to obtain a diagnostic biopsy from a PPL. As Radial EBUS does not have the real time capability of visualisation of the biopsy tool in a PPL, the GS acts as a “semi-real time” measure to ascertain the tool is in the PPL. Once the PPL is visualised, by not using a GS, the probability of returning to the PPL is reduced, especially when VBN or EMN is not used, which is the case in most Australian and New Zealand centres currently.

Even when the GS was used, dislodgement is an issue. The methodology of most studies does not mention measures taken to assure the GS location was checked, including if the radial ultrasound was reintroduced between biopsies and whether the GS was checked to determine if it was still

within the PPL by using fluoroscopy. This is vital information for determining the diagnostic yield. A dedicated assistant holding the GS and the bronchoscope at the patient's mouth is another parameter that would assist in confirming the GS had not dislodged.

The size of the GS may matter as well. There are two sizes of Radial EBUS GS in commercial use, with each GS having similar sized biopsy forceps and a brush in a pack. The large 2.6mm GS pack (K-203 or K-204) includes a 1.8mm forceps and a 1.8mm diameter brush. The small 1.95mm GS pack (K-201 and K-202) includes small 1.4mm forceps and a 1.4mm diameter brush.

In the Radial EBUS studies where a GS had been used, the small GS was favoured. There is hesitancy in using the large GS, based on a review of 14 studies that only had three using the large GS (L. Zhang et al., 2017). In these studies, using the large GS seemed to provide a better diagnostic yield (77-84.2%) compared to using the small GS (58.3-83.3%), keeping in mind that only two studies out of 14 had used the large GS exclusively (Kurimoto et al., 2004; Oshige et al., 2011).

The size of the Radial EBUS GS determines the size of the biopsy equipment that is used and may be instrumental in improving the diagnostic yield. Small forceps biopsies can get destroyed during the processing. At the same time, forceps biopsies are prone to crush artefacts, making it difficult to analyse. Therefore, using smaller forceps and a smaller brush may have less diagnostic efficacy compared to a large GS and the larger equipment that it houses.

Oki et al. (2021) performed a RCT with 596 patients with PPL less than 30mm. The patients were randomised to GS arm and non GS arm with Radial EBUS. The GS arm had a diagnostic yield of 55.3% and the non-GS arm had a yield of 46.6%. Whilst this RCT demonstrated the importance of using a GS to improve the diagnostic yield, the size of the GS was not discussed. The GS used here is the small GS and the lower diagnostic yield may be a representation of smaller samples obtained with the small biopsy tools (Oki et al., 2021).

Although there are extensive studies on Radial EBUS, there is sparse data comparing the two types of GS (small 1.95mm GS (K 201) and large 2.6mm GS (K-203) with their respective sized instruments). The evidence is limited to a single abstract presentation, with the authors suggesting the smaller GS to be better in diagnostic yield despite there being no significant difference in the diagnostic yield overall (Kurimoto et al., 2007). However, a better yield was demonstrated with the small GS when used in the left upper lobe (LUL) (apicoposterior segment) with an 88% diagnostic yield in the small GS arm versus 40% in the large GS arm. This may be due to the

large GS housing the larger equipment; however, the small GS has a better reach to more peripherally located PPL.

Due to sparse data currently available, further research is required to determine the correct sized GS (and accompanying equipment) to be used, to improve the diagnostic yield and the choice of biopsy tool, depending on the location of the PPL.

2.9.2 Under-utilisation of the aspiration needle

There is sparse data on prospective studies using the aspiration needle (Chao et al., 2009; A. Chen et al., 2014; Gildea et al., 2021; Hayama et al., 2015; Meena et al., 2022). There is no data on the ability of the needle aspirate to perform molecular testing (EGFR mutation) in PPL.

Adding an aspiration needle to improve the yield has been discussed in previous studies. Herth et al. (2006) mentioned that an aspiration needle may improve the diagnostic yield, although they did not use an aspiration needle as a biopsy technique. Furthermore, Asano et al. (2008) commented on adding an aspiration needle to improve diagnosis, however, he also did not use an aspiration needle in his 2008 study.

Since 2009, few studies have used the aspiration needle and demonstrated its benefit. Chao et al. (2009) performed a randomised control trial of 202 patients that reviewed biopsy methods has demonstrated an improved yield when using a Wang needle with forceps biopsy and bronchial wash in comparison to forceps biopsy and bronchial wash alone (78% versus 60%). The authors also demonstrated, the aspiration needle has been the sole diagnostic sample in 62.5%; this did not change for eccentric and concentric PPL. The diagnostic yield of forceps alone was 48.9% and bronchial wash 19.8% (Chao et al., 2009).

Forceps biopsy was compared to aspiration needle retrospectively by A. Chen et al. (2014) with the variable use of the GS demonstrating a diagnostic yield of 80% for the aspiration needle; this was similar to the forceps biopsy diagnostic yield of 77%.

Using an aspiration needle via the EBUS GS has been reported in cases where the PPL was not detected via Radial EBUS or Radial EBUS “invisible” PPL. Even these PPLs from using the aspiration needle via a GS have yielded a higher diagnosis rate (54.5%) compared to the group without the aspiration needle (17.8%) (Hayama et al., 2015). This was again noted in a retrospective assessment of 160 PPLs that were adjacent or not seen; 64 had an aspiration needle biopsy. There was a tendency for a higher diagnostic yield in the aspiration needle group (60.9%) compared to the non-aspiration needle group.

Despite these favourable data, albeit in few studies, in a meta-analysis by Ali et al. (2017) only five out of 57 studies used needle aspirate as a sampling method. In Radial EBUS, few studies have used a 21G aspiration needle, which was used with and without the GS. The aspiration needles used in the literature were predominantly 21G needles with eXcelon or the Wang needle.

There is an under-utilisation of the aspiration needle, even when it has been shown to produce a better yield. The AQUIRE registry data have demonstrated that the Radial EBUS aspiration needle was able to make a diagnosis in 9.5% of cases where the forceps biopsy was negative. In 2006, it was reported that an aspiration needle for PPL was used by only 11.8% of pulmonologists (Chhajed & Tamm, 2006). This has not changed over time. In 2016, AQUIRE registry data demonstrated the use of aspiration needles by respiratory physicians to be only 16.4% (Ost et al., 2016).

There is an additional advantage of being able to use ROSE at the time of aspiration needle sampling to review the sample for diagnostic success (Chhajed & Tamm, 2006) and re-position the tools to obtain a better yield. This higher yield of aspiration needle was again demonstrated by Gildea et al. (2021), as many intervening years continued to underutilise the aspiration needle in Radial EBUS. It is encouraging to see improved interest more recently in the use of needle aspiration with ROSE, which demonstrates that the constancy of a needle aspirate sample may give a clue to the diagnostic yield (Meena et al., 2022).

Yet, the number of studies demonstrating the benefit of aspiration needle in Radial EBUS biopsy of PPL are sparse. A meta-analysis in 2023 on aspiration needle use in PPL during the period 2000 to 2021 demonstrated only seven studies involving 510 patients (Lou et al., 2023). Further prospective studies are necessary to add to the sparse body of evidence to improve confidence in pulmonologists in adding this tool to the armoury of biopsy tools with Radial EBUS.

Ability of aspiration needle to perform EGFR testing

Whilst studies have reported on the diagnostic ability of Radial EBUS forceps and brush to perform EGFR testing, no study has utilised the aspiration needle for EGFR analysis in the biopsy of PPL.

If a diagnosis of malignancy is made on a biopsy, performing EGFR mutation analysis could be done in 80-96.5% of Radial EBUS samples (Guisier et al., 2016; Yu et al., 2018). Radial EBUS brush samples in 941 consecutive patients demonstrated a diagnostic success rate of 66.3%, of which 96.5% of these samples could be used for EGFR mutation analysis (Yu et al., 2018).

Guisier et al. (2016) demonstrated that 80% of Radial EBUS conventional sampling is able to perform molecular analysis (EGFR, Alk, HER 2, BRAF). Radial EBUS and forceps biopsy brushings both have been proven for use in EGFR testing. In 23.1% of patients where EGFR mutation could not be performed by forceps biopsy, 39.6% of the diagnosis could be made on brushings (Yu et al., 2018). As more than 60% of patients with lung cancer are diagnosed with advanced disease, Sanchez-Font et al. (2018) demonstrated that EGFR mutation could be tested in 100% adenocarcinoma specimens from brushings in Radial EBUS.

There is evidence on using transbronchial needle aspiration from mediastinal lymph node to obtain EGFR status (Hsu et al., 2019), and a meta-analysis of 33 studies with 2698 participants demonstrating a sufficient sample in 94.5% (Labarca et al., 2018).

Yet, the use of a needle aspirate for EGFR status in PPL has not been explored.

2.9.3 Lack of efficient biopsy techniques

A lack of efficient biopsy techniques to obtain larger non-crushed biopsies and the heavy reliance on forceps biopsy most often used as the sole biopsy tool may be a cause of the low diagnostic yield in Radial EBUS. The usual biopsy tool in Radial EBUS for PPLs are predominantly forceps biopsy, and occasionally combined with brushing. The forceps biopsy and brush method have an effective diagnostic utility when the PPL is located within the bronchi giving an “end on” appearance resulting in a concentric radial ultrasound view. For PPLs located adjacent to the bronchus, giving an eccentric ultrasound view, the forceps method is not useful because the forceps cannot penetrate the bronchial wall. Another problem with forceps biopsy is the “teeth” of the forceps giving a crush artefact to the sample, which can lead to difficulty in interpreting the biopsy sample.

Asano et al. (2008) commented that the sampling with conventional biopsy equipment and crush artefact with small forceps may have led to lower results. Despite this, there is a heavy reliance on forceps biopsy as the sole biopsy tool which was predominantly present in older studies. In Ali et al. (2017) meta-analysis of 57 studies, all used forceps biopsy, out of which 25/57 (43.8%) used forceps biopsy as the sole biopsy method.

The next most frequently used biopsy method was cytology brush. The drawback of the brush sample is apart from not being able to pierce the bronchial wall, its inability to be used as a histology sample is also an issue; it can only be used for cytology and cell block. In 23/57 (40%) studies, forceps was combined with the cytology brush (Ali et al., 2017). There is a lack of data on efficient biopsy techniques to obtain larger non-crushed biopsies.

By using the GS and also preferentially limiting to the small GS, a size restraint has been imposed to biopsy tools. Novel biopsy tools that can still be used via the GS without restricting the size of the sample need to be explored. Cryobiopsy and the GenCut tool are two of these methods that have limited data to date.

2.9.4 Limited data on novel biopsy tools for diagnosis of PPL: Cryobiopsy

The novel biopsy tool of cryobiopsy can improve this situation and provide larger non crushed samples. It can be used via the GS, yet obtain much larger samples. It can be used for eccentrically PPLs, as well as concentric PPLs, as evidenced in studies below. Yet the studies in cryobiopsy for PPL are limited to 12 published papers, of which there are five prospective studies that include 196 patients (Sryma et al., 2023; Tanaka et al., 2022).

In Australia and New Zealand, the cryogen used is liquid CO₂, which when expanded generates a freezing temperature of 59°C and adheres the tissue, called the Joule-Thompson effect (Roebuck et al., 1942). The cryoprobe is 1.9mm in diameter, 950mm to 1150mm in length, and is reusable.

Although there is a body of data referencing the use of cryobiopsy for ILD, data for cryobiopsy use in PPL is limited. The first feasibility study concerning using cryobiopsy for PPLs was performed by Schuhmann et al. (2014). They reported on 39 patients with a mean PPL size of 29.7mm, using cryobiopsy with the Radial EBUS large GS. The biopsy tools used were cryobiopsy and forceps biopsy. The freeing time for the cryobiopsy was four seconds. The overall diagnostic yield was 74%. Four from 31 patients revived the diagnosis from cryobiopsy only. There was no pneumothorax, however, one patient had a moderate bleed. In this study a rigid bronchoscope under general anaesthetic was used (Schuhmann et al., 2014).

Imabayashi et al. (2019) reported on retrospective analysis of cryobiopsy use via Radial EBUS guidance using the 1.4mm small ultrasound probe for PPLs in 36 patients, with a mean PPL size of 37.2mm. Radial EBUS was used without the GS, but VBN was used for navigation. The freezing time varied from 2-5 seconds, with the majority of patients (27/36) having a 3-second freezing time. A bronchi blocker (5-7 Arndt) was used in a small number of patients (7/36). The overall diagnostic yield was 86.1%, pneumothorax rate 2/35 (5.7%) and moderate bleeding in one patient (Imabayashi et al., 2019). In comparison to the Schuhmann et al. (2014) finding, a higher bleeding risk was reported. This may be due to not using rigid bronchoscopy and inconsistent use of a bronchial blocker. This study also noted that the bleeding incidence rose with the increase in freeze time with 37.5% in two seconds, 57.1% in three seconds, 70.5% in four seconds and 80% in five seconds.

Matsumoto et al. (2019) reported on 211 patients retrospectively, each with a PPL size of 22.8mm, using Radial EBUS, VBN and fluoroscopy. The resultant overall diagnostic yield was 90.0%, with 19 cases diagnosed only by cryobiopsy. The diagnostic yield was improved compared to Radial EBUS alone. Complications were experienced due to one pneumothorax, two lung abscesses and 65 moderate-to-severe bleeds. The moderate to severe bleeding may have been caused by many factors. One factor is the procedure was performed under sedation which would have made it difficult to manage the bleed when the patient had an increased cough reflex with blood in the bronchi. The other possibility was the lack of a bronchial blocker and rigid bronchoscopy that would have either contained the bleed or enabled to attend to the bleed quicker than when a patient is under sedation (Matsumoto, et al., 2019).

Taton et al. (2018) investigated prospectively the use of cryobiopsy in combination with EMN in small PPL <20mm. Radial EBUS with a GS was used, and a prophylactic bronchial blocker was used for the cryobiopsy. Rigid bronchoscopy was also used. In 32 patients with <20mm PPLs, they demonstrated that cryobiopsy was more efficacious than forceps biopsies, giving five times larger samples. The large Radial EBUS GS was used and the cryobiopsy was performed through the GS. Freezing time was higher at 7-8 seconds for each cryobiopsy, which would explain the larger size of the biopsy. A maximum of two cryobiopsy samples were taken and one pneumothorax in this study. The study used EMN with cryobiopsy and noted 69% diagnostic success with cryobiopsy compared to forceps biopsy of 38%. There were four moderate bleeds and one pneumothorax in the 32 patients (Taton et al., 2018). This study presented a high bleeding risk of 12.5%, although a bronchia blocker and rigid bronchoscopy were used with the procedure performed under general anaesthetic. This was most likely attributed to the higher freezing time associated with the higher bleeding risk as noted by Imabayashi et al. (2019). Given the small size of the PPL (16+/-3mm), it was possible that the high freeze time may have caused freezing of the adjacent lung tissue outside the PPL, due to the small size of the target PPL and caused a higher bleeding risk.

Schuhmann et al. (2014) also established the importance of the number of passes in cryobiopsy demonstrating that by using one pass the diagnostic yield was 61%, two passes increased to 72% and three passes increased to 74%.

The advantage in cryobiopsy is its ability to freeze and extract tissue. There is no requirement to “grasp” at tissues, therefore, it is well suited to biopsy adjacent PPLs to the bronchus, as well as the PPL located “end on” to a bronchus. Most studies did not comment on any freezing artefact caused by the freeze effect of the tissue sample during biopsy. However, in one recent study (Zarogoulidis et al., 2022), the pathologists commented that freezing had partially destroyed the

sample. This study used liquid nitrous oxide which has a freezing temperature of -80° Celsius. Whereas in Australian and New Zealand centres, the available cryogen is liquid carbon dioxide which has a lower freeze nadir to -59° Celsius and may be better suited for preservation of biopsy.

There are multiple studies demonstrating the advantage of cryobiopsy over forceps biopsy in the diagnosis of PPLs. The larger size cryobiopsy and the well-preserved sample quality is evident in studies. Schuhmann et al. (2014) demonstrated that the cryobiopsy produces a sample three times larger (11.17mm^2 versus 4.69mm^2 , $p = 0.001$) than forceps biopsy; and Taton et al. (2018) demonstrated the cryobiopsy sample to be five times larger than the forceps biopsy.

The ability to perform a biopsy in a PPL adjacent to the bronchi and obtain a higher diagnostic yield with cryobiopsy was also noted. Kho et al. (2019) reported on 38 patients with Radial EBUS guidance using forceps and cryobiopsy. They noted that cryobiopsy in adjacent lesions gave the diagnosis in 18/24 case (75%) compared to forceps 20/41 (48.8%). Matsumoto et al. (2019) demonstrated an advantage in using cryobiopsy for adjacent PPLs, resulting in a success rate of 83.3% for cryobiopsy and 67.7% for forceps.

Cryobiopsy samples are also more favourable in providing large uncrushed DNA and RNA material. Udagawa et al. (2020) performed a prospective study comparing cryobiopsy and forceps biopsy without Radial EBUS and using fluoroscopy guidance. The freezing time used was 3-5 seconds. The cryoprobe was applied either with direct vision or fluoroscopy. In fact, if this was a visible endobronchial lesion, it would have sufficed as an endobronchial cryobiopsy, as opposed to a cryobiopsy from a PPL, therefore, it contradicts the lesion being classified as a PPL. A bronchial blocker was not used. The PPL sizes ranged from 11-90mm, including large tumours. Despite these discrepancies in this study, when comparing forceps biopsy to cryobiopsy, cryobiopsy samples were seven times larger than forceps biopsies (15mm^2 versus 2mm^2). The cryoprobe also had larger amounts of RNA and DNA (Udagawa et al., 2020). This extremely large cryobiopsy sample with only 3-5 second freezing time was an outlier when compared with Schuhmann et al. (2014) and Imabayashi et al. (2019), as it is in keeping with an endobronchial cryobiopsy rather than a biopsy of a PPL.

Udagawa et al. (2020) also recorded higher severe adverse events at 4% (90% CI 2-9). This may be due to the cryoprobe not being placed in the PPL due to the lack of an accurate guidance method (not using Radial EBUS) or the biopsy was endobronchial. The moderate bleeding risk of 74% with cryobiopsy and 61% with forceps biopsy was also higher than other previously published studies, which may be due to a multitude of reasons, including the procedure was performed under sedation, and not using a bronchial blocker or rigid bronchoscopy. Although this

study proves the capacity for larger samples with cryobiopsy compared to forceps, it is an outlier to all previous studies that have researched the use of cryobiopsy with Radial EBUS due to its inability to define the PPL as lesions not visible endobronchially.

All above studies in cryobiopsy mounts to 10 small studies with up to 400 patients. Although each of the study was able to give valuable information on smaller components of the procedure, for example, bleeding risk versus freezing time, sample size versus freezing time etc, a meta-analysis was needed to assess the overall diagnostic yield and adverse events.

To date, there are only two meta-analysis publication of cryobiopsy: (i) Sryma et al. (2021); and (ii) Giri et al. (2022). In Sryma et al.'s (2021) nine studies with 300 patients, the pooled diagnostic yield of transbronchial cryobiopsy was 77% (95% CI 71-84). One severe bleeding and three pneumothoraxes requiring intercostal tube placement (ICT) (major complication rate 4/122, 1.8%) were reported with transbronchial cryobiopsy. Giri et al. (2021) had 780 patients in 11 studies comparing the use of cryobiopsy on lung tumours and interstitial lung disease together, which may not have yielded the true contribution by cryobiopsy to the diagnosis of a PPL. A recent retrospective cohort of 1024 patients, which consisted of more patients than the most recent meta-analysis, demonstrated an acceptable safety profile of 3.5% bleeding risk and 6.3% pneumothorax (Herth et al., 2021).

The above studies were carried out using the 1.9mm reusable stiff cryoprobe. Both Arimura et al. (2019) and Imabayashi et al. (2019) commented on the stiff cryoprobe as being a major hindrance due to its stiff nature and difficulty in manoeuvring the upper lobar locations.

With the development of a single use flexible 1.7mm and 1.1mm cryoprobes, there was expectation for a marked improvement of flexibility to reach the upper lobes with a resultant increase in diagnostic yield. This was proven by Tanaka et al. (2021) who demonstrated in a study consisting of 50 consecutive patients the use of the new single use flexible 1.7mm cryobiopsy probe. This was the first study to use the 1.7mm flexible, single use probe on patients with a mean PPL size of 20mm. In the Tanaka et al. (2021) study, the GS was not used; the freezing time was three seconds with a maximum of four passes recorded. VBN and fluoroscopy were used for guidance. A Radial EBUS was inserted, as needed, to check the position. A bronchial blocker was not used, however, the second bronchoscopist immediately assisted with a second bronchoscope and tamponade a bleed once the biopsy was taken (two scope approach). The diagnostic success was 94% (83.5-98.8%). There was moderate bleeding (62%), which was higher than previously reported, possibly due to not using the prophylactic bronchial blocker and not using general anaesthetic. Pneumothorax was seen in one patient (Tanaka et al., 2021). The diagnostic yield of

94% in a mean PPL size of 20.8 was a significant improvement, likely due to the flexibility of the cryoprobe.

A higher bleeding risk in small PPLs was noted by Tanaka et al. (2021) on a 20.8mm PPL with 62% moderate bleed with a 3-second freezing time and Taton et al. (2018) on a 16mm PPL with 12.5% moderate bleed with a 6-7 second freezing time, which were both much higher than the meta-analysis of 1.5% severe adverse reaction rate (Sryma et al., 2021). However, the difference in technique was that Taton et al. (2018) used all the safety precautions against a bleed, including a bronchial blocker, rigid bronchoscopy and general anaesthetic, while Tanaka et al. (2021) did not use any safety measure that may have resulted in five times higher moderate bleed risk (62%) for similar sized PPLs, as in Tanton et al. (2018) (12.5%).

The 1.1mm new flexible cryoprobe is expected to revolutionise the way we approach PPL. Preliminary studies have already demonstrated the utilisation of this cryoprobe, which is flexible, small and can be used via the small GS (Kho, SS, 2020), the ultra-thin bronchoscope (Oki, 2022) and the guide sheath of robotic bronchoscopy (Oberg et al., 2022). Further studies are eagerly awaited with this small 1.1mm cryoprobe, which has the marked potential to improve the diagnostic yield, as well as take cryobiopsy from tertiary centres to a general usage with the correct adoption of techniques. Using cryobiopsy with ultrathin bronchoscopy has sparked new interest due to the ability to use cryobiopsy without advanced equipment such as rigid bronchoscopy (Ing, 2023; Oki, 2023).

Using the 1.1mm cryoprobe through the small and large GS had been published as case reports awaiting larger studies (Herath, 2023). Cryobiopsy was initially used in the lung parenchyma for ILD, which showed moderate to severe bleeding (12%) and pneumothorax (39%) in a meta-analysis of 11 studies totalling 731 patients (Johannson et al., 2016). This higher bleeding risk resulted in consensus guidelines being developed to standardise the procedure (Hetzl et al., 2018; Korevaar et al., 2022).

Cryobiopsy in ILD had also reported life-threatening complications of haemothorax (Michimata et al., 2022), as well as air embolism (Kawataki et al., 2022). This could be due to the wider use of cryobiopsy in ILD compared to PPL or the potentially better safety profile with cryobiopsy in PPL. Despite life-threatening complications not being reported, safety precautions are of utmost importance in cryobiopsy in PPL due to the scarcity of existing data.

In contrast to the cryobiopsy for PPLs, using Radial EBUS showed a much better safety profile with a very low bleeding risk and lower pneumothorax risk. This may be due to the cryoprobe

probe being placed in the PPL and not in the lung parenchyma. The possibility of having pulmonary hypertension was also much lower in the patient's cohort with PPL as opposed to ILD patients. The fact that a PPL requires a smaller amount of tissue and a freezing time of 3-4 seconds seemed to provide adequate tissue samples compared to ILD cryobiopsies that need a much larger sample to assess the architecture (Giri et al., 2022; Herth et al., 2021; Sryma et al., 2021).

Cryobiopsy with Radial EBUS when used for PPL appears to provide a much higher diagnostic yield, as well as often noted to be the sole diagnostic sample in some patients, thus increasing the diagnostic yield (Schuhmann et al., 2014; Giri et al., 2022; Chen et al., 2023).

The balance between freezing time and adequate samples lies around 3-4 seconds according to these studies. Research using a bronchial blocker and general anaesthetic with rigid bronchoscopy have reported less bleeding either by containing the bleed with a blocker (Arndt or Fogarty) or the ability to control the bleed due to anaesthetic support. All these studies demonstrate the advantage in using cryobiopsy with Radial EBUS guidance, therefore, it is a promising tool for improving the diagnostic yield.

Whilst there are multiple studies comparing the use of Radial EBUS against CT-TTB, there are no published studies comparing Radial EBUS with cryobiopsy head-to-head against the presumed "gold standard" CT-TTB and is an area severely lacking a RCT.

2.9.5 Limited data on novel biopsy tools for diagnosis of PPL: GenCut

The use of cryobiopsy is resource-intensive due to the requirement of general anaesthetic facilities, equipment and skilled proceduralist and bronchoscopy nurses that is not available in a generalizable fashion in most Australian and New Zealand centres performing Radial EBUS under sedation. There is a lack of biopsy tools that can perform core biopsies (without the crush artefact) with sedation and not require expensive equipment.

GenCut is a novel biopsy tool that was initially designed to be used with EMN. It is a flexible, 1140mm in length; 1.9mm in diameter, and has a hollow tube with a 10mm blunt metal tip comprising of inwardly placed sharp blades (Figure 1.12). The tip of the GenCut is blunt to ensure the chance of pneumothorax is reduced. The sheath is extremely flexible and moves effectively to the upper lobes.

As mentioned above, a cryobiopsy sampling requires a large amount of equipment, including cryobiopsy, bronchial blockers, rigid bronchoscopy or endotracheal tube (ETT) with airway support and anaesthetic support with operating room facilities. Therefore, in most Australian and

New Zealand centres where flexible bronchoscopy is being used under sedation, this type of biopsy is not possible.

In comparison, GenCut is a simpler biopsy tool that can provide large core biopsy samples for testing PD-L1. GenCut (Covidien GenCut core biopsy system) has demonstrated satisfactory histology samples in porcine models (Khandhar et al., 2015). Khandhar et al. (2015) demonstrated that GenCut core biopsy samples are superior to forceps biopsies.

A recent publication by Orr et al. (2021) compared GenCut core biopsy to forceps biopsy for PPLs in a prospective observational study of 324 consecutive patients. The patients had five passes of forceps biopsy and three passes of GenCut core biopsy. The diagnostic yield overall from both biopsy methods was 50.5%. Although this was a lower yield than anticipated, 14.6% patients were diagnosed only from the GenCut core biopsy system. The procedure was performed using EMN (49% patients) and Radial EBUS (96% patients).

In Australia, EMN is not currently available, however, the utility of the GenCut core biopsy tool in combination with Radial EBUS without EMN is plausible and is a good opportunity to improve the diagnostic yield and to obtain a core biopsy sample using Radial EBUS. The safety and yield of this procedure needed to be assessed as a proof-of-concept study.

2.9.6 Multi-modal biopsy techniques

Multi-modal guided bronchoscopy techniques have improved the diagnostic yield in comparison to using a single guiding method for biopsy of PPL. This principle may also be applied to biopsy tools.

Most Radial EBUS biopsies generally have used only one biopsy tool, that is, forceps biopsy. There seems to be a reluctance to use combined multi-modal biopsy methods. Ali et al. (2017) performed a meta-analysis of 57 studies using a single biopsy modality of forceps in 27/57 (47%) of patients, a dual biopsy modality with forceps and brush in 25/57 (43%), and only 5/57 (8%) had an aspiration needle used in combination with brush and forceps as a multi-modal biopsy. Combining biopsy tools may improve the diagnostic yield but has not been explored in Radial EBUS.

A recent study presented for the first time, the improved diagnostic yields of multi-modal biopsy in comparison to a single or dual biopsy techniques in Radial EBUS and demonstrated no increase in side effects (Gildea et al., 2021). Yet there is marked paucity in data demonstrating the value in combining biopsy tools to improve the diagnostic yield and the need to match the biopsy tools to

the PPL. To improve uptake of multi-modal biopsy with pulmonologists the safety of multi-modal biopsy needs to be demonstrated in more prospective studies.

2.9.7 Use of rapid onsite examination (ROSE) in Radial EBUS

ROSE has been demonstrated to improve the diagnostic yield in bronchoscopy by providing immediate feedback on the diagnostic success. When using a brush or a needle aspirate, ROSE assists in providing an onsite cytological evaluation that would assist the proceduralist in directing the probe to the correct location. When using an aspiration needle, ROSE improves the fluoroscopy guided bronchoscopy diagnostic yield of the aspiration needle and increases the yield (Leoncini et al., 2020).

ROSE can be used to confirm the correct placement of the GS, especially prior to a procedure like cryobiopsy. C. Chen et al. (2015) demonstrated the value of ROSE, especially if the PPL is apical, small or the probe is not within the PPL. ROSE can assist the bronchoscopist in modifying the procedure and finding the PPL.

ROSE can also reduce the procedural time. Xu et al. (2020) demonstrated the ROSE group in Radial EBUS having significant differences between the diagnostic yield and the operation time. In total, 158 patients were placed on Radial EBUS with ROSE (84/158), and 74 patients were without ROSE. The ROSE group had a diagnostic yield of 85.7%. In the group without ROSE, the diagnostic yield was 70.3%. The operation time was 24.6 ± 6.3 minutes with ROSE; the time was 31.5 ± 6.8 minutes without ROSE.

Studies on cryobiopsy also demonstrated the value in using ROSE during the cryobiopsy procedure. Arimura et al. (2019) demonstrated a 70% sensitivity with ROSE, which had also been used successfully in other studies that used cryobiopsy, such as Taton et al. (2018) and Imabayashi et al. (2019) who demonstrated the value of imprint cytology with cryobiopsy. Gildea et al. (2021) also commented that by using ROSE the number of biopsy tools that are used can be reduced and also noted that ROSE was capable of recognising a malignant diagnosis in 71% cases.

Therefore, ROSE can play an integral role Radial EBUS procedures to improve the diagnostic yield and to direct the biopsy tool to allow for feedback at the time of procedure. ROSE has become an integral part of assessments in bronchoscopic units today (Manley et al., 2022). In 2015, this thesis had not incorporated ROSE due to the unavailability of this facility in most study centres.

2.9.8 Inability to confirm the biopsy tool in PPL “real time”

Another possibility for this low diagnostic yield was the inability of real time visualisation of the biopsy tool in the PPL. In comparison to CT-TTB which has the advantage of visualising the core biopsy needle whilst the biopsy is being taken, in Radial EBUS which lacks this capacity is relaying on surrogate measures like the GS to accurately direct the biopsy tool to the target.

A major drawback in the Radial EBUS technique by not having real time visualisation of the tool in the PPL. Researchers have explored the use of different methods to improve this situation. Three methods that are currently in development and show promise for the future are: (i) hybrid needle aspiration in combination with Radial EBUS; (ii) CBCT; and (iii) robotic bronchoscopy.

Use of hybrid real time aspiration needle

Yarmus et al. (2019) attempted to visualise the aspiration needle during biopsy in the PPL by performing a hybrid needle aspiration while continuously monitoring with a Radial EBUS ultrasound. This first attempt at exploring a method in monitoring a biopsy in real time resulted in a diagnostic yield of 70%.

Use of cone beam computed tomography (CBCT)

CBCT is a recent CT modality that was adopted widely by interventional radiology and vascular surgery, and only recently introduced to pulmonology. CBCT is a compact system that can be mounted onto a moving C-arm. C-arm CBCT has a flat panel detector placed under the patient. Whilst the patient remains anaesthetised and stationary, CBCT performs a complete data volume set in a single rotation of the source and detector. The time taken for a single rotation is 4-10 seconds. CBCT can also produce stranded fluoroscopy ability and confirm the tool in the lesion (Cheng et al., 2020; Orth et al., 2009).

There is a multitude of advantages of CBCT over the existing methods of VBN and EMN. One main advantage is its ability to gain a better acquisition of the image and to reduce the CT to body divergence. CBCT is performed while the patient is under anaesthesia. In addition, being able to use standard fluoroscopy images assist in making sure the tool is in the lesion. This also leads to another recent methodology, augmented fluoroscopy, which is using a dedicated software to create an overlying target on the fluoroscopy image. (Cheng et al., 2020).

Pritchett et al. (2018) published his findings on using CBCT with augmented fluoroscopy and EMN. This technique was utilized for 93 lesions in 75 consecutive patients. The median size of these lesions was 20mm (7-55mm). The overall diagnostic yield was 83.7% (95% CI 74.8-89.9)

and pneumothorax occurred in three patients (4%). The diagnostic yield was higher than Radial EBUS studies with EMN and VBN at 83.7% for small PPLs with a mean size of 20mm. However, this was also a single centred operation and cannot be generalised.

Data on CBCT-guided bronchoscopy are still in its infancy. Other published notable studies include a retrospective study by Sobieszczyk et al. (2018). With a median nodule size of 21mm (7-52mm) a success rate of 77% in 22 patients, which was much higher than Radial EBUS alone for small PPL<20mm. Another study by Bowling et al. (2017) using CBCT with ENB and TBAT also demonstrated similar success of 71% in lesions with a median size of 18mm (9-30mm) in 14 patients. This was also a retrospective study. These were four lesions without a bronchus sign reporting an overall diagnostic yield of 71%, which was significantly higher than all navigation methods used previously (49.6% for Radial EBUS) for lesions without a bronchus sign.

Whilst this increased diagnostic yield in smaller PPLs is encouraging, the ongoing gap between visualisation and diagnostic yield continues to persist even with the use of CBCT. A recent study by Verhoeven et al. (2021) demonstrating the use of CBCT and EMN reported visualisation at 87.5% but the diagnostic yield was 72.4% in small PPLs (14.2-16.6mm). Again, an increased diagnostic yield for the small PPL size has been reflected, however, with a discrepancy between visualisation and the diagnostic yield persists.

These new techniques are promising for sampling smaller PPLs and PPLs without a bronchus sign. Despite its invasiveness, no severe adverse events have been reported. Further studies are needed to confirm the cost and diagnostic yield, as well as the general applicability to clinical practice. In the era of lung cancer screening programmes in the horizon for Australia, the discovery of small PPL and the need to have reliable and safe biopsy techniques like CBCT becomes important.

Use of robotic bronchoscopy

Since the first robotic guided neurosurgical biopsy was performed in 1985, robotic surgery has progressed and is now widely used for thoracic, urological and general surgery. It is similarly applied to interventional bronchoscopy in the hope of overcoming limitations of conventional bronchoscopy and current navigation technologies. There are currently two robotic bronchoscopy platforms approved by the FDA: (i) Monarch by Auris Health Inc. (Redwood City, California, USA) approved in 2018; and (ii) Ion by Intuitive Surgical Inc. (Sunnyvale, California, USA) approved in 2019. The Monarch system uses a cart with two robotic arms, a tower with a monitor and a controller. The CT is incorporated into the software and navigation is supplied by electromagnetic navigation. The inner bronchoscope is 4.2mm with an 6mm outer sheath and a

2.1mm working channel. The Monarch also has constant peripheral visualisation that allows instruments to be directed during biopsy.

The Ion system has a single robotic arm and a trackball for navigation so that the physician can direct the scope to the pre-planned airway. The precision location is provided by the fibre optic shape sensor. Ion also has a biopsy needle in 19G to 23G. It can also use conventional bronchoscopic forceps and brushes, as well as Radial EBUS. The fully articulating catheter is 3.5mm and the working channel is 2mm. With Ion the outer sheath can be locked to allow greater stability for the doctor holding it. The ion system had successfully used the new flexible 1.1mm cryoprobe via its GS as well (Oberg et al., 2022).

In a cadaveric human study of tumours 10-130mm in size, robotic bronchoscopy obtained a significant 97.5% success rate. Despite the combination of multiple advanced image-guided methods, this consistent high success rate was not demonstrated previously (A. C. Chen et al., 2020). The first human study using Monarch in 2018 resulted in 14/15 with a success rate of 93% in PPL of mean diameter of 25mm without using Radial EBUS. The pneumothorax rate was zero (Rojas-Solano et al., 2018).

The first human study by Ion demonstrated 96.5% visualisation, with 79.3% success rate, which was an excellent result as the mean lesion size was 12.2mm. There were no adverse events in the Ion system (Fielding et al., 2019). However, given the small lesion size and only 60% of lesions had a bronchus sign, the ability to visualise almost 96.5% was a remarkable success.

Robotic bronchoscopies seem to overcome issues of precision, reach and flexibility that are missing with existing methods. Further studies are eagerly awaited as the cost of the equipment is high and not achievable by many institutions worldwide. Furthermore, results from the TARGET study, a multi-centre post-marketing study with the Monarch system aimed at enrolling 1200 patients across 30 sites over the next four years is anticipated (NCT 04182815), as well as the Ion study PRECISE (NCT 03893539) which is planning to enrol 360 patients. The use of robotic bronchoscopy with navigation and cryobiopsy is explored as a way towards the future for obtaining real-time visualisation and adequate sampling (Oberg et al., 2022; Patel, 2022).

Robotic bronchoscopy is rapidly leading the way in the diagnosis of PPL. A recent multi-centre retrospective study of 225 patients demonstrated that robotic bronchoscopy had a similar diagnostic yield to CT-TTB (87.6% vs 88.4%), however, with fewer complications (Yu Lee-Matus et al., 2023). This technology is perhaps 5-10 years away from wider use in Australia and New Zealand.

CHAPTER 3:

METHODOLOGY

3.1 Chapter overview

Chapter 3 provides an overview of the methodology used in this thesis. This thesis comprises of four research studies and four published papers (Chapters 4-7). The specific methodology pertaining to each study is outlined in these papers.

In this chapter, the methodology of data collection, analysis, repeatability and the problems occurred during applying this methodology in the studies are outlined. The procedural details, specifically the modifications that were made in adopting these techniques to the New Zealand and Australian settings were highlighted, which enabled other proceduralists to follow the sequence of the procedure.

3.2 Significance of study questions

Despite increased visualisation of the PPL via Radial EBUS alone and in combination with other guided bronchoscopy techniques, the diagnostic yield is 10-20% lower than visualisation. This may be due to an inefficiency in the biopsy tools used with Radial EBUS, which have not been well explored. Forceps biopsies seem to be the most frequently used and often is the only mode of biopsy appearing in published papers on Radial EBUS. However, it may not be the most successful biopsy tool for all PPLs. A PPL that is placed eccentrically to the airway cannot be reached by conventional biopsy techniques, including forceps or brush.

Therefore, although the PPL is visualised, a diagnostic sample is either not extracted from the PPL (tools are not accurately placed in the PPL) or the extracted material is not adequate or too small (suboptimal biopsy tools), therefore, it gets destroyed during processing.

3.3 Study hypothesis

Despite improved visualisation with Radial EBUS in combination with other guided bronchoscopy methods, the yield remains suboptimal compared to CT-TTB. One reason for the lower yield may be due to the biopsy techniques and biopsy tools used. Conventional biopsy tools used included Radial EBUS forceps alone or in combination with a brush. The addition of novel biopsy tools and improving the biopsy techniques that are already in use whilst combining biopsy

tools as “multi-modal biopsy” may improve the diagnostic yield by bridging the gap between visualisation and diagnosis.

Therefore, several hypotheses of this common practice of utilising biopsy tools and techniques more effectively have been explored. One theory is the addition of an aspiration needle to the Radial EBUS sampling via the large GS to improve the diagnostic yield, which may be useful for EGFR testing due to the ability to use the larger instruments and aspiration needles that cannot be used via the smaller GS. We hypothesise that the size of the GS may matter to the diagnostic yield in Radial EBUS.

Another hypothesis is to introduce cryobiopsy to a conventional EBUS sampling to improve the diagnostic yield. Feasibility studies in Germany have shown that cryobiopsy produces large non-crushed samples (Schuhmann et al., 2014). However, introducing this technique in New Zealand may pose challenges because general anaesthetic and operating room facilities are not available, however, this will be taken into account in the study design.

Another hypothesis is that the novel cryo-radial procedure with multi-modal biopsy techniques may compare favourably with the widely used CT-TTB in the diagnostic yield due to its ability to perform EGFR testing safely.

Due to the lack of general anaesthetic and operating room facilities in the majority of bronchoscopy units in Australia and New Zealand, a biopsy tool, such as the GenCut, that can be used safely via sedation alone would have a wider usage and may be able to obtain a core biopsy sample effectively.

The final hypothesis is that a multi-modal biopsy technique with the large GS (Radial EBUS forceps, brush, and needle aspirate +/- cryobiopsy or GenCut tool) may improve the diagnostic yield of PPLs and the ability to perform EGFR testing because it is able to sample both extrinsically and intrinsically placed PPL due to the different techniques employed.

3.4 The aim

The aim of this thesis is to investigate whether improving existing biopsy techniques and introducing novel biopsy tools via Radial EBUS and using a multi-modal biopsy approach would enhance the diagnostic yield of PPLs while maintaining the safety profile. Another aim is to modify and develop interventional techniques for the local New Zealand setting, especially when introducing cryobiopsy for the first time in New Zealand.

To explore the aim, the following questions have been compiled:

3.4.1 Study questions/objectives

1. Does the addition of an aspiration needle to the Radial EBUS sampling via the large GS improve the diagnostic yield? Will the aspiration needle be useful for EGFR testing as well? Does the size of the GS matter to the diagnostic yield in Radial EBUS?
2. How could the novel cryobiopsy method be introduced in the New Zealand setting without General anaesthetic or operating room facilities? What techniques can be developed to improve the diagnostic yield without compromising safety?
3. How does the novel cryo-radial procedure with the multi-modal biopsy technique compare with the widely used CT-TTB in the diagnostic yield, and its ability to perform EGFR testing and safety?
4. Is the GenCut tool safe and effective in obtaining a core biopsy with sedation Radial EBUS in centres where general anaesthesia is not readily available?
5. Does the combination of biopsy techniques with the large GS (Radial EBUS forceps, brush, and needle aspirate +/- cryobiopsy or GenCut tool), that is, the multi-modal biopsy technique improve the diagnostic yield of PPLs? Does multi-modal biopsy improve the ability to perform EGFR testing?

3.4.2 Studies performed to explore the study questions

1. Comparing the large GS and small GS using conventional Radial EBUS sampling methods (forceps/brush) and assessing the additional diagnostic yield of needle aspirate to the large GS. This was a randomised control trial (**REBUST 2**).
2. Introducing the cryobiopsy procedure to New Zealand. Adding a novel cryobiopsy to Radial EBUS conventional biopsy using the large GS as a feasibility study to assess safety and the combined diagnostic yield. This was a prospective, consecutively recruited interventional pilot study (**Cryo-feasibility study**).
3. Comparing the new cryobiopsy and Radial EBUS with the “Gold standard” CT-TTB. Comparing the diagnostic yield and safety of CT-TTB against multi-modal Radial EBUS and cryobiopsy, using the large radial GS. This was a randomised control trial with methods taken to improve the safety of the cryo-radial procedure (**CT-CROP study**).

4. Exploring the use of a novel biopsy technique, GenCut. A blunt-ended novel GenCut biopsy via the large GS was used to determine the safety and ability to perform a core biopsy capable of PD-L1 testing without the need for expensive equipment or general anaesthetic facilities. This was a prospective consecutively recruited interventional pilot study (**R-CORE study**).

3.5 Research design

This thesis comprised of four studies exploring biopsy techniques and tools to improve the diagnostic yield as above. An interventional and prospective approach was chosen to collect data for these studies and to investigate the direct impact of biopsy tools and techniques on the diagnostic yield. The prospective approach is in keeping with the established methodology, demonstrating causation on the diagnostic yield from the biopsy tools and techniques used. The research studies in this thesis collected primary data from all participants. A multicentred approach was needed to improve the generalisability of the biopsy techniques. All studies were experimental, interventional studies. There were two studies exploring novel biopsy techniques, the cryobiopsy and GenCut tool. To explore the diagnostic yield and safety, a feasibility pilot studies were designed (Cryo-Feasibility study and R-CORE study for the GenCut tool). To explore the two sizes of the GS (REBUST 2) and to compare the CT guided biopsy with cryo-radial procedure (CT-CROP), randomised controlled trials were designed as outlined in Table 3.1.

Table 3.1 Design outline of four studies and biopsy tools explored

Chapter	Biopsy tool	Study	Acronym	Trial Design
3	Large GS biopsy versus small GS biopsy	Comparing diagnostic yield with use of large GS versus small GS and respective conventional instruments with sedation bronchoscopy	REBUST 2	RCT
	21G aspiration needle Medtronic and eXcelon)	Addition of aspiration needle via large GS	REBUST 2	Single arm study as part of the RCT
4	Cryobiopsy-feasibility study	Exploring safety and efficacy in adding cryobiopsy to Radial EBUS (first human study in New Zealand)	Cryo-feasibility study	Prospective pilot study
5	Cryo-radial biopsy against CT-TTB	RCT of cryo-radial versus CT-TTB (RCT against current gold standard CT-TTB)	CT-CROP	RCT
6	GenCut core biopsy tool	GenCut core biopsy tool via Radial EBUS GS with sedation bronchoscopy	R-CORE	Prospective pilot study

For ease and clarity, these studies are referred to by their acronym from here on.

3.5.1 Randomised control studies (REBUST 2 and CT-CROP)

Two of the four studies (REBUST 2 and CT-CROP) were randomised control trials. With randomisation, it was assumed that all parameters in the two arms of the study were equal except for the biopsy tool. This was deemed to be the best choice of study design to compare the different biopsy tools used via the two different GS, as well as comparing two separate types of biopsy methods (cryo-radial versus CT-TTB).

In these two randomised control studies, intervention was randomly assigned based on a permuted randomisation sequence with a block size of four. For both studies, sequence each of the biopsy tools used in the Radial EBUS arm was also randomly allocated. This allocation was performed by the study statistician and was contained in a sealed envelope and was opened by a non-investigative medical staff member immediately prior to the commencement of the procedure.

The REBUST 2 study was single-blinded as only the patient was blinded. Due to the obvious difference in the size of the GS, the proceduralist could not be blinded. The CT-CROP study was a non-blinded study, as the two types of biopsy modalities (cryo-radial versus CT-TTB) were different from each other.

3.5.2 Non-randomised pilot studies (Cryo-feasibility and R-CORE)

The Cryo-feasibility and R-CORE studies were interventional prospective pilot studies. In these research studies, a novel biopsy tool was added to existing conventional biopsy tools of Radial EBUS (forceps and brush) and a comparison was made between the conventional method and the novel method. Given both these novel methods, (cryobiopsy and GenCut), had sparse data on their safety profile, this prospective pilot was aimed at identifying the safety of the procedure.

The sequence of biopsy tool use was not random in these pilot studies due to reasons explained below.

In the Cryo-feasibility study, the novel biopsy tool cryobiopsy was performed as the last biopsy method. This was performed to secure a diagnosis from a proven Radial EBUS biopsy to the patient. Furthermore, due to the higher bleeding tendency of cryobiopsy, if it was done as the initial biopsy, further biopsies would have been hindered due to the bleeding risk and soiling of the airways with blood. Another reason was the cryobiopsy tended to produce three times larger samples compared to forceps biopsy according to the only published paper on cryo-radial procedure by the time this study commenced (Schuhmann et al., 2014). Therefore, using

cryobiopsy first may have the theoretical possibility of not leaving much tissue for the other biopsy methods to sample.

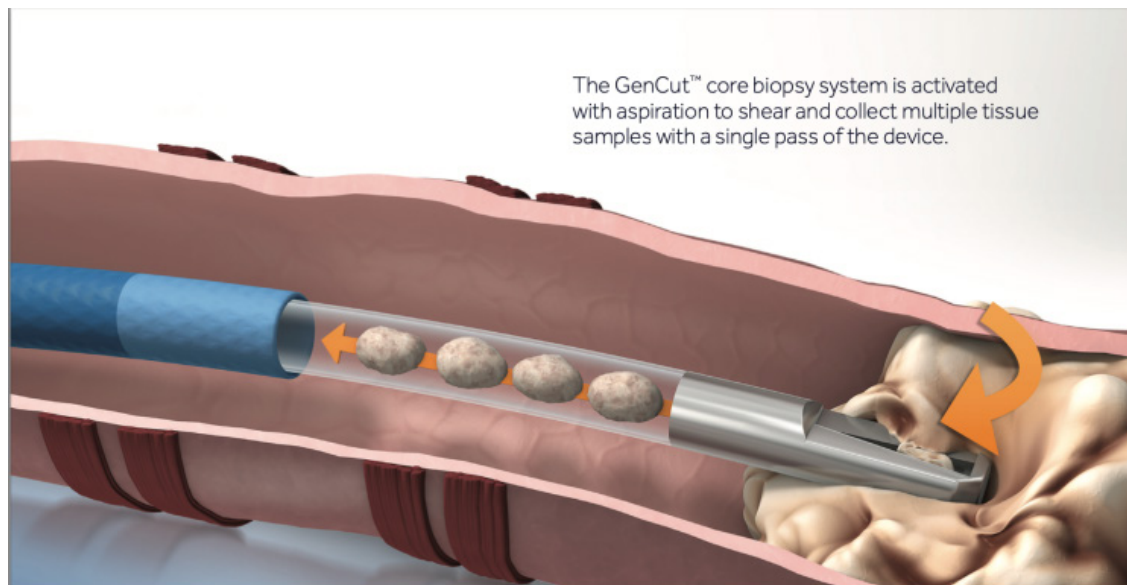


Figure 3.1 GenCut core biopsy tool demonstrating suction of sample via blunt tool sheath during rotation

In the R-CORE study, the opposite was performed using the novel GenCut tool as the first biopsy technique before sampling with the conventional Radial EBUS. This was done to assess the risk of pneumothorax of the GenCut biopsy, as there was suction applied to the biopsy tool to extract tissue. After each GenCut biopsy, a bedside ultrasound was performed by the respiratory physician with ultrasound training and competence in accordance with the Thoracic Society of Australia and New Zealand competence certificate.

Two ultrasonic determinants of pneumothorax were assessed: (i) loss of sliding sign; and (ii) bar code in the M band to confirm pneumothorax. The ultrasound was performed on the second intercostal space at the mid-clavicular line when the patient was supine on the ipsilateral side of the biopsy. If there was detection of a pneumothorax or any doubt about a pneumothorax, the procedure was terminated and a routine CXR performed to determine the existence of the pneumothorax.

GenCut biopsies were performed via the Radial EBUS large GS and deemed to be less of a risk of bleeding.

3.5.3 Sample size calculation

Cryo-feasibility study: A sample size of six patients was planned by the study statistician and research committee for this pilot study. Consenting patients were subsequently enrolled.

R-CORE study: To explore the safety of the GenCut tool, a sample size of 18 patients was planned by the study statistician and research group for this pilot study.

REBUST 2 study: This study compared the large GS with its conventional instruments of forceps and cytology brush against the smaller GS with the smaller forceps and brush. The data on GS use was limited to a single study that demonstrated a much higher diagnostic yield with small GS use (88%) compared to the large GS use (40%) in left upper lobe apicoposterior segment (Kurimoto et al., 2007). To cope with the confounding position of PPLs with the size of the probe, a stratified design was used to allocate the diagnostic method.

A statistical power of 0.85 and a Type 1 error of 0.05 (adjustment for 5% missing data for 88 patients) were required for the study. This calculation was based on a single available publication (Kurimoto et al., 2007) and an interim analysis planned at 25% recruitment to recalculate the sample size.

CT-CROP study: In this study combined cryo-radial procedure is compared to CT-TTB which is the most widely used “gold standard” lung biopsy method. It has been assumed that a CT-TTB has a sensitivity of 90%, and cryobiopsy has a sensitivity of 70%, according to the only published study on the cryo-radial procedure for PPL, by the time this study was designed by Schuhmann et al. (2014). Each arm of 79 patients detected a difference of 20% in sensitivity with a statistical power of 0.85 at a significance level of 0.05. The proposed sample size of 79 for the CT-TTB arm achieved a marginal error rate of 7% for 90% sensitivity. The proposed sample size of 79 for the cryobiopsy arm achieved a margin of error at 10-13% with 70% sensitivity of the cryobiopsy.

Of note, the sample size calibration was planned at the interim analysis (25% recruitment) due to the sparsity of data available on the use of cryobiopsy on PPLs at the time (Schuhmann et al., 2014).

3.5.4 Research setting

All four studies were conducted in Australia and New Zealand across four tertiary academic teaching hospitals. (i) Middlemore Hospital in Auckland, New Zealand; and (ii) Westmead

Hospital in Sydney, Australia; (iii) Macquarie University Hospital in Sydney, Australia; and (iv) Royal Brisbane and Women's Hospital in Brisbane, Australia.

Tertiary hospitals were chosen due to the equipment availability, the necessary Radial EBUS expertise including experienced proceduralists and skilled nursing staff. In the event of an adverse result, intensive care support and resuscitation facilities were readily available. These hospitals are also located in lower socioeconomic areas, with higher smoking rates and higher lung cancer prevalence. The patients were recruited from the referrals made to the outpatient lung cancer clinics.

In New Zealand, Middlemore Hospital is a public hospital located in South Auckland. Auckland is the highest populous city in New Zealand with a population of 1.57 million people in 2015 at the time of commencing these studies. South Auckland is the catchment area of Middlemore Hospital that caters to a large majority of migrant, Maori and Pacifica population. The lung cancer registration and mortality rates in both male and female Maori patients are three to four times higher than non-Maori patients, with late presentations being common (New Zealand Cancer Registry, 2018).

In Australia, Westmead Public Hospital and Macquarie University Hospital are situated Sydney, New South Wales, which is the most populous state out of the six states in Australia; it has a population of 7.4 million according to the 2016 Census from Australian Bureau of Statistics (2017). Westmead Hospital is situated in a low socioeconomic area in Western Sydney with a predominate migrant population.

Macquarie University Hospital is a university academic hospital located 20 kilometres from Westmead Hospital in low to medium income suburb of Ryde in Sydney; it has well-equipped theatre facilities and was used for some of the bronchoscopic procedures in the studies.

The Royal Brisbane and Women's Hospital is a major public teaching hospital situated in Queensland, Australia with 4.7 million people (2016 Australian Census); it is positioned in a low socioeconomic area of South Brisbane.

3.5.5 Research sample of participants

The target population were patients presenting to these tertiary hospital respiratory clinics with a PPL suspected to be lung cancer between 2015 and 2019. This sample of patients were chosen as they required a biopsy to diagnose the abnormal PPL as per usual clinical management.

3.5.6 Patient recruitment and consent

The Royal Brisbane and Women's Hospital recruited patients for one study, using the GenCut biopsy tool. The Macquarie University Hospital recruited for the CT-CROP study alone. The Middlemore Hospital recruited patients for the REBUST 2 and CT-CROP studies, and Westmead Hospital recruited for three studies (REBUST 2, CT-CROP and R-CORE).

At Westmead Hospital and Middlemore Hospital, where competing studies were carried out concurrently, as patients were being referred by the referring clinician to participate in any study without indicating a specific one, they were recruited to a study where they could have the biopsy at the earliest opportunity (e.g. cryobiopsy in the CT-CROP study required operating room facilities and a longer wait time compared to the REBUST 2 study which could be performed under sedation).

Patients were recruited during their outpatient respiratory clinic appointment. Eligible patients were provided with an information sheet regarding the proposed research by the clinician attending to them. The patient was given time to study the sheet and to seek advice from his/her general practitioner, family member or Maori elders before deciding whether to participate in the study. All patients were required to read and understand the consent form and information sheet, as well as able to give informed consent.

Obtaining a biopsy from a PPL suspected of lung cancer was time sensitive. Therefore, the patient had 48 hours to decide if they wish to participate in the study. The study nurse contacted the patient within 48 hours to confirm the patient's decision, and if the patient wished to proceed, an appointment was arranged with a study investigator to discuss any concerns with the patient and to answer all questions. If the patient was satisfied, he/she signed the consent papers, which were then witnessed and countersigned by a medical staff member not affiliated with the study. If the patient decided not to participate in the study, the nurse made an appointment for his/her biopsy in accordance with usual clinical care, as advised by the referring respiratory physician.

Each study consisted of a participant information sheet and a study specific consent form.

1. REBUST 2 patient information sheet and consent (Appendix 7)
2. Cryo-feasibility patient information sheet and consent (Appendix 8)
3. CT-CROP patient information sheet and consent (Appendix 9)
4. R-CORE patient information sheet and consent (Appendix 10)

Apart from the study specific consent form, the patient was also required to sign the institutional consent form for a bronchoscopic procedure.

3.5.7 Inclusion/exclusion criteria

All patients aged 18 years or older with a PPL >10mm, and suitable for a biopsy (bronchoscopic procedure or CT guided biopsy procedure), were included in this study. Patients who were pregnant, on anticoagulation or anti-platelets (except aspirin) that could not be stopped for five days for the procedure due to medical reasons were excluded. Patients with endobronchial disease and significant mediastinal lymphadenopathy were excluded from the studies. Some institutional guidelines required to perform a staging procedure with L-EBUS on none size significant lymph nodes if the PPL size was >30mm or the PPL that were located in the inner 2/3 of the lung parenchyma. When this requirement was present, patients had the L-EBUS performed with on-site ROSE. If the lymph node was negative, the patient continued with the allocated study. However, if the lymph node was positive, the patient was excluded from the study. If there was a less invasive biopsy (e.g. a supraclavicular lymph node) that could be performed for diagnosis and staging at the same time, the patient was excluded from the study. If patients were not suitable for a biopsy (INR >1.5 platelets <100, Hb >100g/l, neutrophil count <1.0, EGFR <30ml/kg/min, liver function tests (AST/ALT) >2 times upper limit of normal), they were excluded from the study. If a patient from a non-English background was participating, the consent was obtained in the presence of a certified interpreter by the respective hospital.

3.5.8 Clarification on selection of PPL for biopsy

PPLs greater than 10mm were selected for the study. In the event the patient had more than one PPL on the CT imaging, the respiratory physician in charge of the patient's care decided on the lesion that required a biopsy based on the patient's current clinical situation (e.g. avidity of the PET-CT). In the event there were multiple PPLs and no preference by the referring respiratory physician as to which PPL should be biopsied, the proceduralist decided on the PPL that would provide the best yield with an acceptable safety outcome.

Once the biopsy arm was allocated, the proceduralist reviewed the CT images and assessed the suitability for biopsy. If the proceduralist decided that the procedure was technically not possible or too risky (depending on the location of the lesion or proximity to a large vessel), the patient was redirected to another biopsy method without delay.

The reason for not undergoing the allocated procedure was clearly documented. The patient was not permitted to re-enter the study.

3.5.9 Materials and intervention

Materials

Below is a list of materials used in all four studies that researched the Radial EBUS procedure. However, each study also used materials specific to that study, therefore, they are listed separately in Chapters 4-7 in the published/submitted papers.

1. The Radial EBUS GS is available in two sizes: (i) small outer diameter 1.95mm (K201; Olympus, Tokyo, Japan); and (ii) large outer diameter 2.6mm (K203; Olympus, Tokyo, Japan).
2. The Radial EBUS small GS kit 201 is packaged with accompanying forceps and a small brush, both with an outer diameter of 1.4mm.
3. The Radial EBUS large GS kit K-203 is packaged with accompanying forceps and a large brush both with an outer diameter of 1.8mm.
4. For all Radial EBUS procedures, the Radial EBUS small ultrasound probe with outer diameter of 1.7mm (20 MHz, UM-BS20-17S; Olympus, Tokyo, Japan) was used. The small ultrasound probe can be used with both the large and small GS.
5. Two types of 21G aspiration needles were used during this study: (i) Medtronic 21G 8mm aspiration needle (1.8mm diameter/8mm in length); and (ii) eXcelon Boston scientific 21G aspiration needle (1.8mm diameter/15mm in length).
6. The GenCut core biopsy blunt-ended tool was sized 1.9mm, 1140mm length (Covidien).
7. Two pathology collection types for samples were used: (i) Hanks solution and CytoLyte solution for cytology samples (needle aspirate and cytology brush); and (ii) Formalin for histology samples (cryobiopsy/GenCut biopsy and forceps biopsy).
8. Therapeutic flexible bronchoscope was used (Olympus: Outer diameter >4.9mm and working channel >2.8mm).
9. Cryobiopsy probes were reusable. 1.9mm outer diameter probe was used. The initial probe used in the Cryo-feasibility study was 950mm. This was later upgraded to a 1150mm probe which was used in the CT-CROP study.

10. CT guided biopsy patients were randomised to the CT-TTB arm in the CT-CROP study; they had core biopsies using coaxial needle 17-19G and core needle 18-20G.

Intervention

The REBUST 2, GenCut and Cryo-feasibility and cryo-radial arms of the CT-CROP study used the Radial EBUS procedure. CT-CROP study patients were randomised to the CT-guided biopsy arm or Radial EBUS procedure, and detail explanation of methodology of CT-TTB was in the methodology section of the respective paper.

The base procedure for all studies was Radial EBUS, which was used with the GS, as explained below.

All patients had a recently performed (within one month) CT chest with thin slices of less than 1mm. For patient's undergoing the Radial EBUS procedure, the PPL in question was identified by the proceduralist, and a planning pathway was performed manually by studying the CT chest scan prior to the procedure. VBN and EMN were not available for these studies. Kurimoto's mapping was used for manual planning the bronchoscopic pathway in most cases.

On the day of the procedure, the patient underwent flexible bronchoscopy in accordance with institutional guidelines. A therapeutic flexible bronchoscope (Olympus Flexible bronchoscope outer diameter >4.9mm and working channel >2.8mm) was used for all procedures.

Once the segment where the PPL was located had been reached, the Radial EBUS GS with the ultrasound, was introduced through the working channel of the bronchoscope. All procedures in this study were performed using a GS. The size of the GS depended on the study (either large or small GS).

Positioning the ultrasound probe

Once the PPL was located via the ultrasound, the ultrasound is moved distally and proximally to assess the best position. At the same time, the vascularity of the PPL was also assessed by the ultrasound. Care was taken to position the ultrasound away from vascular areas and to direct it to a non-vascular or less vascular area. This was especially done with care during the cryobiopsy procedures. Once the location was confirmed by the proceduralist, the ultrasound was removed and the GS was left in situ as an extended working channel. Sampling was performed through this GS. The biopsy tool differed according to each study's methodology, which is explained in detail in the methodology section of the published paper (Chapters 4-7).

A CXR was performed one hour after the procedure for all study patients to assess for pneumothorax. Prior to discharging the patient, the proceduralist reviewed the results, as per routine institutional guidelines for Radial EBUS bronchoscopy.

Anaesthetic support

REBUST 2 study: This study was performed in endoscopy with sedation only. The proceduralist also supervised the sedation of the patient. There was a respiratory registrar or trainee to assist with sedation.

R-CORE study: The GenCut and Radial EBUS was performed in endoscopy with sedation only. The proceduralist also supervised the sedation of the patient. There was a respiratory registrar or trainee to assist with sedation.

Cryo-feasibility study: This study was done under sedation for five patients and general anaesthetic for one patient. When the procedure was done under sedation, a second bronchoscopist was present to assist with any adverse events and sedation. For the patient requiring general anaesthesia (due to underlying comorbidities) the procedure was performed in the operating rooms with a laryngeal mask airway and spontaneous breathing. Rigid bronchoscopy and jet ventilation was not used.

CT-CROP study

Cryo-radial arm: All patients had general anaesthesia with spontaneous breathing. Some patients had a rigid bronchoscopy insertion and ventilation was carried through the rigid bronchoscope. Jet ventilation was not used. Three patients had the procedure performed in endoscopy with anaesthetic support and all others had the procedure performed in operating rooms.

CT-TTB arm: The procedure was performed in radiology suites under local anaesthesia. The proceduralist supervised the anaesthesia as well.

Fluoroscopy: The patients had fluoroscopy for the Radial EBUS procedure according to the proceduralist's preference.

3.5.10 Data collection

Data collection comprised of standardised data collection sheets for use across all Australian and New Zealand study sites. The following information was collected from each study participant:

1. Data collection (patient demographic and medical/radiological – all procedures) (Appendix 11)
2. Data collection (pneumothorax and bleeding – all procedures) (Appendix 12)
3. Data collection forms on the Radial EBUS bronchoscopy procedure (separate questionnaires for each of the four studies due to the use of a different biopsy tools) are listed below:
 - a) Data collection form – bronchoscopy (REBUST 2) (Appendix 13)
 - b) Data collection form – bronchoscopy (Cryo-feasibility study) (Appendix 14)
 - c) Data collection form – bronchoscopy (CT-CROP) (Appendix 15)
 - d) Data collection form – bronchoscopy (R-CORE) (Appendix 16)
 - e) Data collection form – radiology (CT-CROP) (Appendix 17)

Data collection forms on pathology samples were collected for all studies in the thesis, and a standardised two-part generic pathology data collection form was used for the Radial EBUS biopsies.

Pathology forms for the two novel biopsy tools are listed below as an example:

1. Data collection form – pathology (CT-CROP) (Appendix 18)
2. Data collection form – pathology (R-CORE) (Appendix 19)

Data collection on patient demographic

Patient demographic data collected (7 questions) included patient demographic characteristics, study ID, study site, date of procedure, study arm, age, sex and employment. The patient's medical details (13 questions) included presenting complaint, presence of COPD, COPD X categorisation, lung function in FEV1 as a percentage, diffusion capacity, hypertension, chronic renal failure, obstructive sleep apnoea, ischemic heart disease, American Society of Anaesthesiology and Eastern Cooperative Oncology Group score. The CT chest characterises (4 questions) included the size of PPL, largest diameter on axial CT chest, location of PPL (outer 1/3, middle 1/3 or inner 1/3), lobar location, and the presence of air bronchogram on CT chest imaging.

Data collection on adverse events

The Common Terminology Criteria for Adverse Events was used to define adverse events.

Pneumothorax was defined as: Grade 1 – Asymptomatic: Clinical or diagnostic observation; no intervention required; Grade 2 – Symptomatic: intervention required; Grade 3 – Symptomatic: intervention and hospital admission; Grade 4 – Life threatening; urgent treatment; and Grade 5 – Death.

Haemorrhage/bleeding was defined as: Grade 1 – Mild: intervention not required; Grade 2 – Symptomatic: requiring medical intervention; Grade 3 – Transfusion and operative: endoscopic or operative; Grade 4 – Life-threatening; and Grade 5 – Death.

Data collection on CT guided biopsy

This was completed by the radiologist and was only required for CT-CROP patients who were randomised to the CT-TTB arm. The datasheet comprised of sedation requirement, size of coaxial needle, size of core needle, number of passes made and safety precautions.

Data collection on bronchoscopy procedure

In the bronchoscopy procedure forms for all four studies, the same procedure data collection sheet included questions for the conventional Radial EBUS component of the procedure. These included salient questions of type of sedation (general anaesthetic or conscious sedation), amount of sedation (amount of midazolam; method of administration), ability to see the PPL on the ultrasound, time taken to visualise the PPL, number of biopsies performed with each conventional Radial EBUS sampling modality, and safety precautions taken. For the novel biopsy tool in the two studies, additional questions were included specific to the biopsy technique, as mentioned in separate bronchoscopy data collection sheets.

Data collection on pathology sample

The gold/referenced standard for diagnosis is the pathologist's confirmation of observing malignant cells/diagnostic cells in the case of non-malignant disease, in the biopsy specimen. The pathology data collection sheet was completed by two independent pathologists blinded to the procedure who analysed the samples for quality and quantity; they agreed on the final diagnosis. If there was a discrepancy, a third opinion was sought. The analysis of each biopsy was documented on a pathology data collection sheet dedicated to each study depending on the biopsy sample.

All samples were assessed for their suitability to perform IHC and EGFR analysis. If the diagnosis resulted in non-malignancy but confirmative, such as sarcoidosis, then this was recorded as a true benign diagnosis. When a diagnosis was made of a non-malignant condition (inflammation/sarcoidosis/organising pneumonia) based on the information from the biopsy sample, the patient was followed up at six months and 12 months with CT chest scans to confirm the diagnosis.

The pathology data collection sheet recorded the diagnosis describing the quantity and quality of the diagnostic material, measured size of the tissue, percentage of tumour involvement, suitability of IHC, suitability for molecular testing, and finally the best overall sample from the study and best sample from the different Radial EBUS biopsy tools.

3.6 Follow-up

The patient was contacted by study investigators 24 hours after the procedure to assess for any delayed side effects. The referring physician followed up the patient after one week, as per usual clinical practice. Patients were further followed up at six months and 12 months if a diagnosis of malignancy was not made to confirm the benign nature of the PPL by the study investigators with repeat CT chest surveillance.

Dealing with negative samples: If the diagnosis was non-malignant and the sample was inadequate or inconclusive, the patient was directed to another biopsy method or for close monitoring with CT scans, as directed by the treating respiratory physician. The patient's records were followed up until a definitive diagnosis was made or until twelve months had passed to confirm the benign nature of the disease. These negative samples are considered non-diagnostic or a negative biopsy.

3.7 Protection of human ethics and data handling

This study was granted institutional ethics approval from the respective Human Research Ethics Committee (HREC) of the hospitals and were registered in the relevant trial registries, as mentioned in Table 3.2. Data collected from all sites were stored at each site's research office as de-identified data. Electronic copies were kept with the principal investigator in the sponsoring institution's computer under password protection.

Internal data safety monitoring board: The CT-CROP and R-CORE GenCut studies had an internal data safety monitoring board that consisted of two specialist physicians in the participating institutions who were not involved in the study (respiratory physician and

pathologist) and the study statistician. All adverse events were reported within 24 hours to the HREC, followed by a three-monthly report. Each study was registered with the appropriate clinical trials registration.

Table 3.2 Ethics approval numbers and clinical trials registration numbers

Study acronym	Ethics approval	Study centres	Clinical trials registration no.
REBUST 2	Health and disability ethics committee: Northern regional Ethics Committee: 15/STH/3 HREC 2019/ETH02599 Appendix 20	Middlemore Hospital, Auckland Westmead Hospital, Sydney	ClinicalTrials.gov Identifier: NCT02739126
Cryo-feasibility	Health and disability ethics committee: Northern Regional Ethics Committee/CEN/5 Appendix 21	Middlemore Hospital, Auckland	Not registered in clinical trials
CT-CROP	HREC/16/WMEAD/337 New Zealand: 15/NTA/48. Appendices 22 and 23	Westmead Hospital, Sydney Middlemore Hospital, Auckland Macquarie University Hospital	clinicaltrials.gov: NCT02395939
R-CORE	RED HREC/17/WMEAD/461 Appendix 24	Westmead Hospital, Sydney Royal Brisbane and Women's Hospital	ANCTR-U1111-1204-2333

3.8 Data analysis

Interim analysis: Both the CT-CROP and REBUST 2 randomised control trials had only a single published study each to derive data to calculate a sample size for the study. Therefore, a pre-defined interim data analysis was planned for the CT-CROP study at 25% recruitment and the R-CORE study at 25% recruitment.

Final analysis: Data was analysed using IBM SPSS version 25 and Stata 17 software. Descriptive data was analysed with means and standard deviations. The Fisher's exact and Chi-square tests were used to compare proportions and smaller sample sizes. A statistical significance was set at a P value of < 0.05.

3.9 Problems encountered

3.9.1 Early termination of the REBUST 2 Study

The REBUST 2 study had acceptable recruitment at the beginning of the study in 2016. A pre-defined initial analysis was planned for recalculating the sample size at 25% recruitment. Towards the end of this goal, study investigators noted a slowing down in recruitment.

This was due to referring clinicians noting that patients who were randomised to the large GS had better results and patients who were randomised to the smaller GS required a repeat procedure. The referring physicians commented on the lower diagnostic yield with the small GS arm. There seemed to be a reluctance to refer patients for the study to be randomised. Whilst the recruitment was being undertaken at Westmead and Middlemore Hospitals, there were competing studies (CT-CROP/GenCut) starting around 2017, which also resulted in further reduction in recruitment.

While this situation was occurring, a pre-planned analysis was performed to recalculate the sample size. During the interim analysis it was noted that the large GS arm did have a tendency for better results than the small GS arm although not statistically significant. Following a discussion with the steering committee, it was deemed unethical to randomise and continue the study due to the perceived loss of clinical equipoise among the referring clinicians which was supported by the interim analysis.

Therefore, the study was terminated early, which created a major drawback in this study; loss of power to statistically differentiate the diagnostic yield between the arms and confirm the clinical difference. The study was also planned to identify any differences between upper and lower lobes, but this was also not possible due to the low numbers and reduced power and the Type 2 error that occurred due to early termination of the study.

3.9.2 Slow recruitment in the CT-CROP study

The CT-CROP study experienced a slow recruitment process as well. However, this was different to the REBUST 2 study as recruitment was slow from the onset. There were multiple reasons for this.

The CT-CROP study recruited from major tertiary centres, including Middlemore and Westmead Hospitals. These hospitals were chosen for their lower socio-economic status of the population and higher lung cancers. Whilst the incidence was high, the patients presented late for treatment with over 75% patients presenting with nodal disease. The CT-CROP study excluded patients with enlarged mediastinal adenopathy, inadvertently targeting early disease. Unfortunately, early

disease was not common in this population; this resulted in a much lower than expected number of patients available for these studies.

Another reason was the strict randomisation criteria of randomising all PPLs to either CT-TTB or cryo-radial without considering patient or PPL characteristic. There was much debate among the clinicians when this study protocol was discussed at our clinical meetings in choosing randomisation criteria. Clinicians have argued that patients should be selected by a multidisciplinary team (MDT) after discussion about the patient's lung cancer, whilst other clinicians have argued that a MDT discussion would add significant bias to the selection process, based on the experience and skill of the MDT at its respective hospital.

At the time of this study design phase in early 2015, Middlemore Hospital in Auckland had only just begun the lung cancer MDT. Most clinicians were not aware of Radial EBUS at the time because it was a new service commenced in New Zealand for the first time through these studies. Hence an MDT decision at this point was considered suboptimal in choosing the biopsy method.

Therefore, following six months of discussions without a solid answer, this study was discussed at the ANZIPG meeting. Again, randomisation methods were highly debated, however, approval was finally granted to proceed with randomising all consented patients using computer-generated randomisation sequences without taking into account the PPL or patient characteristics.

The ANZIPG acknowledged that this study would experience difficulty in finding recruits. Therefore, a lower than anticipated number of referring centres participated in this study, which reduced the opportunity for recruitment.

One other factor in the CT-CROP study was that if the patient was randomised to the cryo-radial arm, then the procedure had to occur in an operating room. During this period in 2016-2018, throughout New Zealand and most of Australia, bronchoscopies were performed under sedation in endoscopy suites. The operating room needed to be booked and identified as an acute case with patients having to wait for an allocated time; there was no fixed theatre list for the respiratory team. Therefore, the referring physician felt the patient would have a longer wait time, so the patient was referred to another study, such as the R-CORE study or REBUST 2 that could be performed under sedation. Having competing studies at the same time with same selection criteria hindered the recruitment for CT-CROP.

3.9.3 Methods to overcome slow recruitment

The main avenue taken to improve recruitment was to increase the number of participating sites. The research in this thesis was conducted in Australia and New Zealand. All respiratory physicians in Australia and New Zealand are under the governance of the same Royal College of Physicians and both countries practice according to the guidelines set by the Thoracic Society for Australia and New Zealand for interventional procedures. However, there is no agreement between the Australia and New Zealand in honouring the same ethics approval.

The ethics for New Zealand was initially approved in 2015 with Middlemore Hospital being the sponsor for this study. As recruitment was slow, the study was extended to Westmead Hospital. Despite being the same protocol due to not having reciprocal agreement, another ethics application needed to be included with the Western Sydney Local Health District through Westmead Hospital. This approval was finalised in 2017 due to other logistical requirements, including operating room facilities that needed to be organised at Westmead Hospital prior to commencing the study. The recruitment with the involvement of two tertiary hospitals was slower than anticipated; the study was extended to Macquarie University Hospital, which required another ethics approval application that further delayed recruitment.

A centralised ethics approval system would improve participation from multiple sites, improve the practicality, and reduce time for recruitment in a multi-centre study, especially within the Australian system. Whilst larger multi-centred trials and industry sponsored studies would have dedicated administration staff to process these applications, smaller scale investigator driven studies surviving on smaller grants do not have this capability. Therefore, despite extending the study to other centres to improve generalisability and recruitment, the need for multiple full ethics approvals delayed commencement of the recruitment process from the study centres and hindered participation from other centres. Applying for ethics applications to all participating centres at the same time would have curtailed some of these issues.

Since there has only been one feasibility study (Schuhmann et al., 2014) published on cryobiopsy in PPLs at the time the study commenced, an interim analysis was planned to recalculate the sample size. The pre-planned interim analysis at the 25% recruitment stage occurred almost three years later (2018) due to the slow recruitment.

By this time, there has been an accumulation of published work of single arm studies in cryobiopsy Radial EBUS in PPLs demonstrating the safety and diagnostic utility, as well as the ability to combine with L-EBUS increasingly being used in clinical practice. The interim analysis also demonstrated that the cryo-radial had a superior safety profile over CT-TTB. This shed a

positive light on the cryo-radial procedure in the clinician's eyes and the pool of referring physicians reduced further, as clinicians did not want to randomise the patient to the CT-TTB arm.

All the above factors have affected the investigator's ability to recruit further, resulting in early termination of the study.

This early termination resulted in a loss of power and the Type 2 error. A more pragmatic randomisation protocol instead of a pure randomisation would have resulted in better recruitment numbers. As suggested by some clinicians, randomising by the MDT would be an option, but this would depend on the expertise and exposure of the panel that is present at the time the decision is made. This truly highlights the difficulty faced when deciding on a randomisation strategy for two markedly different interventions.

In 2015, there was a lack of exposure to Radial EBUS among the MDT panel, as well as MDT being a novel concept in New Zealand; it was a downside that has now been overcome. In fact, the ability to present at interventional pulmonology meetings to obtain expert opinion and for regional centres to participate via Zoom have improved the decision-making capability.

Hence, given that this is the first study to compare the novel cryo-radial against CT-TTB for PPLs despite the lower patient numbers, valuable data can be added to the knowledge to provide information on the cryo-radial procedure and to highlight the pitfalls to be avoided by future researchers regarding randomised multi-centre interventional studies.

Chapters 4 to 7 consist of four publications in this thesis. Following each published paper is an extended discussion to complement and broaden the discussion already conducted in the paper.

CHAPTER 4:

REBUST 2 STUDY – RESULTS AND DISCUSSION

4.1 Publication details and manuscript

Herath, S., Mahajan, H., Yap, E., Ing, A. (2022). Radial endobronchial ultrasound guided biopsy for lung lesions: Evaluating the role of the guide sheath size, addition of needle aspirate and multi-modal sampling, in improving the diagnostic yield: An exploratory randomized study. *Journal of Community Medicine and Public Health Reports*, 3(2). <https://doi.org/10.38207/JCMPHR/2022/FEB03020119>

Chapter 4 consists of the published paper and an extended discussion at the end, to complement and broaden the discussion already performed in the paper.

Radial Endobronchial Ultrasound guided biopsy for lung lesions: Evaluating the role of the guide sheath size, addition of needle aspirate and multi-modal sampling, in improving the diagnostic yield : An Exploratory randomized study.

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Abstract

Introduction:

Radial EBUS (R-EBUS) biopsy for lung lesions has a marked safety profile with less than 1.5% pneumothorax in comparison to CT-guided transthoracic biopsy of 20 %. However, the diagnostic yield of R-EBUS remains suboptimal at 73 % in comparison to CT -TTB at 92 %. The size of the Guide Sheath (GS), the addition of aspiration needle biopsy and multi-modal sampling may improve the diagnostic yield.

Methodology:

This is a multi-center randomized controlled study. All patients referred to R-EBUS for a biopsy were offered the study and randomized to large or small GS arms. An aspiration needle is added to the large GS arm to assess the additional diagnostic yield. Each patient had 1-3 biopsies of forceps and brush (+ needle aspirate in large GS arm). All procedures were done under sedation. CXR has taken 1-hour post-procedure. The study was terminated due to slow recruitment at 42 patients planned interim analysis.

Results:

The large GS tended to provide a better diagnostic yield (72 %) in comparison with small GS (60 %) OR 1.77 (95 % CI 0.44- 7.17), this was improved further with an addition of aspiration needle (86 %) in the large GS arm. Aspiration needle was the sole diagnostic specimen 13 % and able to provide EGFR analysis similar to forceps biopsy. Multimodal biopsies seem to improve diagnostic yield. No pneumothorax or bleeding was recorded.

Conclusion:

Despite the type 2 error and small sample size, the large GS seem to provide better results. We suggest utilising an aspiration needle to improve the diagnostic yield. A multimodal biopsy is encouraging with better diagnostic yield, EGFR capability without additional side effects.

Keywords: Cryo-biopsy, radial EBUS, CT-Guided transthoracic biopsy, Diagnostic yield, EGFR, Bleeding, pneumothorax, Biopsy technique.

Introduction

Peripheral pulmonary Lesions (PPL) suspected of lung cancer requires a safe and effective biopsy. Radial Endobronchial ultrasound (R-EBUS) is a widely used guided bronchoscopy method [1,2] for diagnosis of PPL, with marked safety profile. In five meta-analyses of over 50 studies with 7000 patients on R-EBUS, the overall side effect rate was 1.5 % with 0.7 % requiring a chest drain insertion [1-5] -guided transthoracic (CT-TTB) biopsy, which has a pneumothorax rate of 20 % with a chest drain rate of 7.3 % in a meta-analysis of 48 papers consisting of 10,383 biopsies.[6]. Yet, the diagnostic yield of R-

EBUS is suboptimal (69 %-73 %) [4,7-9], in comparison to the -guided transthoracic biopsy yield of 92 % [10,11]. Despite being recommended by the American College of Chest Physicians (ACCP) clinical guidelines as a mainstream diagnostic method of PPL, this lower diagnostic yield hinders R - EBUS being a more widely used biopsy technique in PPL [12]. R-EBUS is a flexible wire-like ultrasound probe that gives a 360-degree radial view of the airway. It can be used via the working channel of the bronchoscope and advances to the bronchial subsegments beyond the bronchoscopy vision. This R-EBUS is

introduced using a guide sheath (GS), which houses the ultrasound and has a snug fit to it. Once the PPL is located using the ultrasound, the ultrasound is withdrawn leaving the GS in situ which then acts as an extended working channel. Biopsy tools are introduced via this GS. There are two commercially available GS sizes. The small GS (K 201-Olympus) houses small instruments and accommodates biopsy equipment up to a diameter of 1.4 mm and the large GS (K203-Olympus) houses all conventional bronchoscopy biopsy equipment up to a diameter of 1.91 mm.

Despite the radial, ultrasound being able to visualise or the PPL the diagnostic yield seemed to lag by 10-20 %. This was first reported by Herth et al in 2006 with a visualisation success of 89 % yet the diagnostic yield was only 70 % [13]. Nearly all studies published since then had continued to report this gap between visualisation and diagnosis. [14-18]. One reason for this gap and a lower diagnostic yield, maybe the size of the GS used, that then determines the size of the biopsy tool utilised. If the biopsy tools used are too small, the resultant small biopsy samples may get destroyed whilst being processed.

The two sizes of the GS had been head-to-head compared only in a single study [19]. This limited data demonstrated overall diagnostic yield was no different (77 % for large GS and 83 % for the small GS), but the small GS was better at diagnosis of left upper lobar PPL due to the better reach (40 % for the large GS and 88 % for the small GS) [19]. Despite this lack of data, the small GS had been preferentially used in published studies, with only 2/14 studies in a review study using the large GS (2.6 mm outer diameter, Olympus K 203) [20].

Another reason for the lower diagnostic yield of R-EBUS maybe not customising the biopsy tool to suit the PPL location in relation to the bronch

bronchi, some are located adjacent to the bronchus. Forceps biopsy however, the forceps cannot pierce the bronchial wall hence a PPL situated adjacent to a bronchus will get a negative result. An excellent solution to this would be an aspiration needle biopsy that would pierce the bronchial wall and can obtain tissue from a PPL located adjacent

to the bronchi.

There is marked underutilisation of aspiration needles. The AQIRE Registry (ACCP Quality Improvement Registry, Evaluation, and Education) data demonstrate the use of aspiration needles is only 16.4 % [21]. At the time of this study, all available aspiration needles came in with a diameter of 1.8mm and could be used only via the large GS, hence the reluctance to use the large GS may be one reason for the low uptake of the aspiration needle as well.

Another reason for the low diagnostic yield of R-EBUS may be the reliance on a single type of biopsy mainly forceps biopsy for sampling. Using multiple biopsy tools in combination (multi-modal biopsy) may produce a better result due to the ability to biopsy both adjacent and PPL has not being explored. In a meta-analysis of 57 studies all 57 used forceps biopsies, 29/57 (51 %) used dual biopsies with forceps and cytology and only 5/57 had used aspiration needle together with forceps or brush as multi-modal biopsy [2]. Due to the reluctance of performing multi-modal biopsies in the literature, the safety profile in performing multiple biopsies in PPL is not well known.

In the era of personalised medicine for lung cancer, the ability to perform molecular testing including EGFR (Epidermal Growth Factor Receptor) status becomes important. In these small biopsy samples from R-EBUS, 80 % were suitable for EGFR analysis [22]. However, the available data is mostly with forceps biopsies. There are limited data demonstrating the ability of cytology brush to provide EGFR analysis [23]. There are no data on the ability of aspiration needles to perform EFGFR analysis nor comparison of aspiration needle to forceps biopsy in this regard.

Therefore, the impact of various biopsy tools on the diagnostic yield nor the impact of the size of the GS that then determines the size of the biopsy tools have not been well explored.

This randomized exploratory study was designed with the primary objective of comparing the diagnostic yield between the large and small-GS from all modalities of sampling tools used in R-EBUS. The secondary objective was assessing the side effect profile and the ability to perform EGFR testing.

Methodology

This is a single-blind, randomized, multicentre, interventional exploratory study.

Ethics approval: Australia (HREC 2019/ETH02599) and New Zealand (15/STH/3).

Patients over 18y, referred for R-EBUS were recruited. Pregnant and lactating patients, patients on anti-coagulation that cannot be stopped for medical reasons were excluded. Aspirin was permitted. Informed consent was taken from all patients and randomized to the large-GS (K -203 Olympus-outer diameter 2.6mm and the largest diameter of biopsy tool that can be accommodated 1.91mm) or small-GS (K-201 Olympus-Outer diameter 2mm and the largest diameter of biopsy tool

that can be accommodated 1.4mm) arms. A planning CT scan of the chest within one month of the procedure was needed with the slice thickness of at least 1mm for the bronchoscopist to perform a manual pathway to the segmental bronchi.

Patients in the large-GS arm was sampled with forceps, brush, and

21G: 8mm length); while patients in the small-GS arm were sampled with forceps and brush only as a small-sized aspiration needle was not available. For each sampling method, 1-3 samples were taken at the discretion of the proceduralist. For each GS type, the sequence of the sampling method was randomized. The small USS probe 1.7mm

Olympus UM-S20-17S was used for all procedures.

The type of needle aspirate used was not randomized and determined by availability. If both needle aspirates were available the Medtronic 8mm needle was selected over the 15 mm Boston scientific needle, due to the ease of use in the upper lobes.

All procedures were performed under conscious sedation. Once the PPL was detected on radial ultrasound, the ultrasound probe was removed leaving the GS in situ and sampling instruments were introduced via the GS. Fluoroscopy was not used. The GS position within the PPL was checked with an ultrasound between each sampling instrument. Rapid Onsite Cytology Examination (ROSE)

The sample size was calculated with a Type 1 error of 5 %, and power of 80 %, requiring a sample size of 58 subjects to detect a minimum difference of 25 % in yield between the arms and 88 patients to determine a difference between upper and lower lobar location. A planned analysis was carried out at 25 % recruitment to confirm the sample size, due to the above data being limited to a single published study. At this 25 % analysis point, the study was terminated due to a perceived clinical benefit with the large-GS and slow recruitment resulting from this referrer bias. The data were analysed using Stata Version 17.

Results

42 patients from two tertiary hospitals were recruited from January 2015 to December 2017. 24 were assigned to the large GS and 18 to the small GS. There were no statistically significant differences

between the two groups in any of these baseline parameters (**Table 1**).

Table 1: Comparing the patient demography and CT characteristics between the Large GS and small GS arms.

Comparison	Large-GS arm	Small-GS arm	P value
Mean age (years)	64.3 (SD14.18)	66.9 (14.02)	P<0.05
Mean FEV 1	2.04 L (74.7%)	1.92 L (77.8%),	P<0.05
Gender - Female	15/24 (62.5%)	9/18 (50%)	P<0.05
Lesion size	4.2 cm (SD 1.8)	Vs 3.7 cm (SD 2.3)	P<0.05
Lobar distribution	17/24 (70%)	14/18 (77%)	P<0.05

Two subjects in the small-GS arm and two in the large-GS arm had a diagnosis via Linear-EBUS prior to the R-EBUS procedure, performed as per institutional guidelines. One lesion in the thin-GS arm was not visible on ultrasound. After these three patients were excluded, a total of 22 patients from the large-GS group and 15 from the small GS group underwent R-EBUS. In the large-GS arm, 19/22 (86 %) had a diagnosis made on R-EBUS. (16 malignant, three benign). Of the three who were non-diagnostic, two proceeded with CT-guided biopsy which confirmed malignancy and one received radiotherapy based on high clinical probability of malignancy. In the small-GS arm, R-EBUS was diagnostic in 9/15 (60 %). (Five malignancies, four benign). Of the six who were non-diagnostic, four were confirmed malignancy (one lobectomy, three CT-guided biopsy), one was benign on follow-up, and one was lost to follow-up. The overall diagnostic yield, with only the forceps and brush, as sampling methods was 16/22 (72 %) in the large- GS arm and 9/15 (60 %) in the small-GS arm. OR 1.77 (95 % CI 0.44- 7.17). When the

aspiration needle was added to the large-GS arm, the diagnostic yield improved to 19/22 (86 %) Vs 9/15 (60 %) OR 4.2 (95 CI 0.85-20), P =0.118. 3/22 (13.6 %) were diagnosed only from the aspiration needle. In the large-GS arm, a diagnosis was made solely from forceps in 18.1 %, needle aspirate in 13.6 % and brush in 4.5 %.

In terms of ability to perform EGFR testing, 12/16 (75 %) had EGFR status diagnosed in the large-GS. The number of malignancies diagnosed and ability to perform EGFR were analysed: with the aspiration needle there were 13/16 (81 %) malignancies, with 62 % suitable for EGFR, Cytology brush had 11/16(69 %) malignancies with 54 % suitable for EGFR and Forceps had 9/16 (56 %) malignancies with 66 % suitable for EGFR.

In the small-GS, a diagnosis was made solely on forceps in 7 % and brush in 7 %. 40 % of the small-GS samples were suitable for EGFR. All patients had a chest-X ray one-hour post-procedure. No bleeding or pneumothorax was reported in both arms.

Discussion

Both large and small-GS arms had similar baseline patient demographics and lesion size.

In comparing the use of small biopsy forceps and brush through the small-GS, the larger biopsy forceps and brush, via the large-GS

demonstrated a tendency for a better diagnostic yield and better suitability for EGFR testing. This may be due to the larger samples obtained from the large biopsy tools that would survive the processing better. It may also be possible that the large GS arm had more

malignancies than the small GS producing better diagnostic yield. However, these results were not statistically significant in keeping with the only previously published study by Kurimato et al [19].

The addition of an aspiration needle improved the diagnostic yield of the large GS further. This may be due to the ability of the aspiration needle to sample PPL placed adjacent to bronchi as well as inside the bronchi. 13.6 % of patients had a diagnosis made solely from the aspiration needle. There is limited retrospective analysis published by Chen et al that had compared the aspiration needle against forceps biopsy with R-EBUS and demonstrated 80 % diagnostic yield with aspiration needle in comparison to 77 % with forceps biopsy [8]. Our data is also supported by the ACQUIRE registry data that demonstrate aspiration needle was able to provide a diagnosis 9.5 % of the time when forceps biopsy was negative [21]. The aspiration needle increased the diagnostic yield as the sole diagnostic sample and also complement the procedure by improving the overall diagnostic yield in our study and reported by previous limited studies as well [24-26]. The ability of aspiration needle sample to be examined at ROSE for immediate results and manoeuvre the GS to a better location, if needed, and the ability to perform the procedure under sedation are additional advantages [27].

Whilst the ability to perform EGFR had been reported to be 80 % for forceps biopsy with R-EBUS [22], studies have demonstrated cytology brush to be able to provide EGFR as well but less (66 %) than that of forceps biopsy [23], however in cytology samples with malignant cells, a 100 % ability for the cytology brush to provide EGFR analyse on the malignant samples had been demonstrated [28]. Studies have not prospectively compared the ability to perform EGFR in aspiration needles nor compared it with forceps biopsy in radial EBUS. In this study, aspiration needle was suitable for EGFR analysis

similar to the forceps biopsy; adding further value to the already improved diagnostic yield.

The addition of the aspiration needle to the large-GS, improved the diagnostic yield further, without an increase in complications, especially pneumothorax, this has been confirmed by one other study demonstrating no increase in adverse events with the use of needles aspirate even when the PPL was not visible on ultrasound [24].

Multi-modal biopsy techniques have not been well considered as a measure of improving diagnostic yield. The tendency has been on high reliance on forceps biopsy alone and addition of cytology brush on a few studies with a very limited combination of aspiration needle as a multi-modal biopsy tool². Although studies in R-EBUS had demonstrated that the addition of cytology brush had improved the diagnostic yield compared to forceps alone, the concept of multimodal biopsy was not well explored. In this study, using multi-modal sampling (forceps, brush +/- aspiration needle) has resulted in an improved overall diagnostic yield in both arms.

With the multimodal biopsy techniques, the patients in the large GS arm received a maximal of 3 types (Forceps biopsy, cytology brush and aspiration needle) of biopsies with a maximum of three passes in each biopsy leading to nine biopsies per patient, yet there were no reported pneumothorax and bleeding, and all patients were discharged home as planned. Whilst this is in keeping with the overall safety of the R-EBUS procedure, as well as the haemostasis achieved by using a GS, this may also be due to the larger (mean 3.7-4.2cm) PPL size in this cohort as well. Multi-modal biopsy needed to be performed in smaller PPL with further studies to confirm safety. Using the GS consistently may have led to better haemostasis and higher diagnosis in both arms as acknowledged by the previous meta-analysis. [3].

Limitations

The main limitation of this study is the early termination due to lack of recruitment. These results are not statistically significant due to the small sample size with the resultant type 2 error. In this study all procedures were performed using the GS therefore, appreciating that some centres do not use the GS due to the extra expense or the inability to reach certain locations, the result of this study is not

applicable when a GS is not used.

We were not able to perform a needle aspirate in the small GS arm due to the lack of availability of a small needle which is a future development requirement. We are unable to provide guidance on the best needle aspirate to use as there was no randomisation of the different types of needle aspirate and further studies are needed.

Conclusion

We suggest including an aspiration needle and using multi-modal biopsy tools with R-EBUS via a large GS to improve the diagnostic yield of R-EBUS. The ability to perform EGFR testing with aspiration needle samples further strengthens the argument. We noted utilising a GS consistently improved the side effect profile and the diagnostic yield as noted in the previous meta-analysis.

Multi-modal sampling is able to provide better diagnostic results and

the tools used need to be tailored to PPL characteristics for the optimum results. Despite the higher number of biopsies performed the safety profile of R-EBUS was well preserved.

The R-EBUS procedure can be performed under sedation and require fluoroscopy. If biopsy tools can be improved to provide better diagnostic yield, this extremely safe method would be widely utilised and form an integral pathway of a lung cancer diagnosis.

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Abbreviations List:

R-EBUS: Radial Endobronchial Ultrasound-guided biopsy CT: Computed Tomography

EGFR: Epidermal Growth Factor Receptor GS: Guide Sheath

Guarantor: Dr. Samantha Herath is the Guarantor of the content of the manuscript including the data and analysis.

Author contribution

1. Dr. Samantha Herath (Respiratory Physician) is the principal

investigator and designed the study protocol, designed consent forms, and data entry forms, obtained ethics approval for ethics committees in Australia and New Zealand. Dr. Herath applied and secured grant funding and equipment funding for the Cryo-Radial procedure. Dr. Herath performed the Cryo-Radial procedure and collected data. She was responsible for patient follow-up, data entry, and data analysis as well as the write-up of this paper.

2. Dr. Alvin Ing (Respiratory Physician) is a co-investigator and assisted with designing the study protocol, performed the Cryo-Radial procedure, and collected data. He was responsible for patient follow-up and the write-up of this paper.

3. Dr. Elaine Yap (Respiratory Physician) assisted with designing the study protocol, assisted in obtaining ethics approval for ethics committees in New Zealand. She performed the Cryo-Radial procedure and collected the data. She was responsible for patient follow-up, data entry, and analysis. She assisted in the write-up of this paper.

4. Dr. Hema Mahajan (Pathologist) is a co-investigator. She assisted in the design of the study protocol and assisted in the analysis of pathological specimens.

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4.2 Discussion of the REBUST 2 study

4.2.1 Summary of existing evidence on use of GS and needle aspirate technique

The Radial EBUS GS is a pivotal instrument used for diagnostic success as it acts as an extended working channel by directing instruments to the correct location. Unlike CT-TTB, with guided bronchoscopy techniques, there is no real-time confirmation of the biopsy tool being in the PPL. Therefore, using a GS and frequent assessment of the position of the GS with the Radial EBUS ultrasound probe needs to be done to secure the correct position. Apart from increasing the diagnostic yield, there are additional benefits in using the GS, such as reducing the bleeding risk due to the tamponading effect (Kurimoto et al., 2004).

The of GS was introduced by Kurimoto et al. (2004). Chung et al. (2007) also demonstrated the “measured distance” method, where Radial EBUS was used without the GS. After a PPL is located, the distance is measured from the PPL to the end of the working channel. The conventional instruments from the GS kit are then matched to the distance and the “measured distance”. This assumes that the conventional instruments when reintroduced without a GS would reach the same location (S. Zhang et al., 2016). Whilst this may work for a larger PPL, it would not work for a smaller PPL, nor for a more peripherally located PPL. In the meta-analysis of Radial EBUS in 2011, Steinfert et al. (2011) noted 31% of diagnoses had not used the GS. This may have contributed to the lower diagnostic yield in this literature review.

Even when the GS was used, the literature has suggested a favoured use of the small GS. In a 2017 meta-analysis on guided bronchoscopy, with a diagnostic yield of 58.3% to 84.4%, in 14 studies (L. Zhang et al., 2017), only two studies using the large GS, which recorded better yields of 77% (Kurimoto et al., 2004) and 84.2% (Oshige et al., 2011). This predominant use of the small GS with its accompanying small tools may be a reason for the lower diagnostic yield of Radial EBUS.

In terms of comparing the large GS with the small GS, there is only a single publication present. This demonstrated that the large GS had a visualisation of 93% and a diagnostic yield of 77%; and the small GS had a visualisation of 97% and diagnostic yield of 83%. In this abstract, there was a difference in the upper lobar diagnostic from 88% in the small GS to 40% in the large GS. Further studies have been sorely lacking in comparing the two sizes of the GS and their accompanying instruments, therefore, the REBUST 2 study is aimed at providing additional information to this area of minimal data.

The aspiration needle is an underutilised diagnostic tool that has been used in only five out of 57 studies in a meta-analysis (Ali et al., 2018) despite previous data demonstrating that the aspiration needle has a good diagnostic yield in both concentric and eccentric PPLs (Chao et al., 2009).

In 2023, a meta-analysis of needle aspirate use in PPL in 2000-2021 demonstrated only seven studies involving 510 patients had been published; the research demonstrated the sparse use of needle aspirates in PPL (Lou et al., 2023).

Due to sparse data available, the REBUST 2 study was designed to determine whether the size of the GS, which in turn decides the size of the biopsy tools, would be relevant to the diagnostic yield. In the large GS arm as a secondary end point, an aspiration biopsy needle was added to the large GS. This could not be extended to the smaller GS because of the lack of a smaller calibre aspiration needle that could fit into the small GS at the time of the REBUST 2 study in 2015.

4.2.2 Significance of REBUST 2 and salient results

At the time of commencing the REBUST 2 study in New Zealand in 2015, the use of aspiration needles for PPL was not performed. Data on the safe use of an aspiration needle was sparse and the uptake of aspiration needles or PPL globally was low.

Comparing and contrasting findings with existing studies

These findings were compared and contrasted with published literature to understand the relevance and significance of this study, as well as discuss in terms of (i) diagnostic yield in comparing small and large GS; (ii) addition of aspiration needle to the large GS; (iii) ability to perform EGFR; (iv) multi-modal biopsy; and (v) safety.

Diagnostic yield in comparing small GS and large GS

This study has demonstrated that with the use of conventional forceps and a brush, the large GS arm had a diagnostic yield of 72% compared to the small GS with forceps and a brush (60%). This finding was not statistically significant. This finding is similar to the abstract published by Kurimoto et al. (2007) where he demonstrated a similar diagnostic yield with the large GS arm at 77% and the small GS arm at 83%. Yet, at the end of publication, Kurimoto et al. (2007) had commented that the small GS increases the visualisation of the PPL. However, the visualisation does not equate to a diagnostic yield; the diagnostic yield in effect was similar between the small and large GS and this conclusion appears to be partial to the small GS. The small GS was only favourable in this study when it relates to a specific area in the left upper lobe, the apicoposterior

segment, therefore, demonstrating that the small GS had a higher yield in the LUL (LB 1+2) (88%) compared to the large GS (40%).

This is the only comparison study published and hence the data is sparse.

The REBUST 2 study demonstrated an overall diagnostic yield of 86% for the large GS when all biopsy tools were combined (cytology brush, forceps biopsy and needle aspirate); the small GS arm demonstrated an overall diagnostic yield of 60% when all modalities of biopsy (cytology brush and forceps) were used. These results are not statistically significant and they had a large confidence interval (95% CI 0.44-7.17). With the study being underpowered, a conclusion cannot be made based on these results.

However, Table 4.1 summarises the diagnostic yield of each biopsy tool, demonstrating that the larger GS may have a better yield even when compared with conventional biopsy tools. For example, forceps with a large GS resulted in a diagnostic yield of 63% compared to small GS forceps of 33% and similar brush of the large GS (63%) and small GS (26%).

A higher diagnostic yield of 72% was present with forceps and brush alone in the large GS compared to 60% in the small GS. This difference was further accentuated when the TBNA needle was used increasing to 86% vs 60%. Therefore, the large GS appeared to be particularly useful when using a TBNA needle.

Table 4.1 Summary of the diagnostic yield of each of the biopsy tools

	Brush	Forceps	Needle aspirate	Brush and forceps only	Overall
Small GS n=15	4/15 (26%)	5/15 (33%)	0	9/15 (60%)	9/15 (60%)
Large GS n=19	12/19 (63%)	12/19 (63%)	13/19 (68%)	16/22(72%)	19/22 (86%)

There may be other reasons why the small GS had lower results. The visualisation was 15/16 for the small GS and 22/22 for the large GS, however, it did not seem to have any impact on the end result. The Olympus bronchoscope with an outer diameter of >4.9mm and a working channel of >2.8mm was also used for large and small GS procedures, as mentioned in the Methodology section of this research. In 2015-2018, this was the only bronchoscope specification that was available in the two centres where recruitment was performed. Therefore, using a 2.8mm working channel bronchoscope combined with a small GS may reduce its assumed reach to obtain more peripheral PPL.

Another reason for the lower diagnostic yield in the small GS may be due to the lower number of malignancies that were randomized to it (Table 4.2), as well as specimens being processed by a pathologist.

Table 4.2 Summary of the final diagnosis

Total randomised n=42	Large GS n=24	Small GS n=18
Procedure completed	n=22	n=16
USS visibility	22/22	15/16
Diagnostic yield	19/22 (86%)	9/15 (60%)
	Malignancy – 16/19 n=11 adeno n=4 n=1 melanoma	Malignancy – 5/9 n=4 adeno n=1 SCC
	Non-malignant – 3/19 n=2 chronic inflammation n=1 eosinophilic inflammation	Non-malignant – 4/9 n=1 cryptococcus n=1 TB n=2 chronic inflammation

As mentioned previously, this study was the first of its kind to investigate Radial EBUS being used in New Zealand. The pathology team was not accustomed to using smaller forceps and brush samples, and some samples were not viable for post-processing. A steep learning curve was experienced from the pathology and procedural teams during this study, which may have reduced the results of the small-sized samples obtained. Therefore, this innovative processing procedure may have also added to the destruction of small samples taken from the small GS arm.

It must be noted that whilst some studies have not used the GS with Radial EBUS, many published papers that had used the GS have not specified the size of the GS used.

Additional of aspiration needle to large GS arm

In the REBUST 2 study, the introduction of the needle aspirate via the large GS arm improved the diagnostic yield of the large GS arm by 13%, compared with using forceps biopsy and brush. With the large GS, an additional aspiration needle increased the total diagnostic yield from all modalities of the sampling success rate to 86%. Chao et al. (2009) demonstrated similar results with their randomised control trial that showed that the addition of needle aspirate to forceps biopsy and wash increased the diagnostic yield by 17.8%.

Ability to perform EGFR testing

In the REBUST 2 large GS arm, 12/16 (75%) had the EGFR status diagnosed from all modalities of sampling. In comparing this with previous studies in Radial EBUS, Guisier et al. (2016) reported that 80% of sampling from conventional Radial EBUS forceps and brush was suitable for EGFR testing. This is in keeping with the REBUST 2 study data.

In the large GS arm of the REBUST 2 study, the diagnostic success of a malignancy and the ability to perform EGFR is listed as the aspiration needle with 62% suitable for EGFR, brush with 54% suitable for EGFR and forceps with 66% suitable for EGFR. Yu et al. (2018) demonstrated that 60.3% brushings had malignancies detected and 96.5% of these were suitable for EGFR testing, which also aligns with our study data. Yu et al. (2018) further demonstrated that when forceps failed to obtain a result for EGFR testing (39% of patients), the brush was able to produce a result for EGFR testing. This proves the importance of using multi-modal biopsy in improving the diagnostic yield in EGFR testing.

There are many publications on the use of the L-EBUS aspiration needle in performing EGFR testing successfully. Labarca et al. (2018) reporting on 33 studies incorporating 2698 patients demonstrated a diagnostic yield for EGFR mutation of 94.5% in L-EBUS. However, no study has demonstrated the ability to perform EGFR mutation analysis data on needle aspirate with Radial EBUS biopsy of PPL. This REBUST 2 study demonstrates that the aspiration needle has a similar capacity to forceps biopsy in performing EGFR testing. Although this is a small dataset, it is an original contribution to the current literature on aspiration need use in PPL.

Use of multi-modality biopsy techniques

In the REBUST 2 study of the large GS arm, a diagnosis was made solely on forceps (18.1%), needle aspirate (13.6%) and brush (4.5%). In the small GS arm, the diagnosis was made solely on forceps (7%) and brush (7%). When analysing all biopsy modalities used, combining multi-modal biopsies resulted in improved diagnostic yield as opposed to the use of a single biopsy. This was the case with both large and small GS equipment, not only for the general diagnostic yield, but as mentioned above when performing EGFR analysis using sampling from multi-modal biopsy. This was in keeping with the RCT published by Chao et al. (2009) with 202 patients randomised to two arms. One arm with a combined aspiration needle, forceps and wash had a diagnostic yield of 78.4% from all modalities of sampling compared to the other arm with only forceps and wash (60.6%). In this study by Chao et al. (2009) the combined diagnostic yield was much higher than individual diagnostic yields of each sampling method which included the diagnostic yields of aspiration needle (62.5%) forceps biopsy (48.9%) and bronchial wash (19.8%). The recently

published analysis of the NAVIGATE study data also confirms the use of multi-modal biopsy in improving the overall diagnostic yield (Gildea et al., 2021).

Yet at the time of performing this study, multi-modal biopsy was not a concept assigned to Radial EBUS biopsies. The REBUST 2 study is an early study that commenced in 2015; it demonstrated the improvement in overall diagnostic yield, as well as the diagnostic ability for EGFR testing when using multi-modal biopsies with Radial EBUS.

4.2.3 Safety profile

Despite combining multi-modal biopsy techniques in the REBUST 2 study which comprised of a minimum of two biopsies (one single pass from forceps biopsy and one from brush) to a maximum of nine passes (three passes each from aspiration needle, brush and forceps), there were no pneumothorax and bleeding. All patients were discharged on the same day of the procedure as planned. The side effect profile did not change from that published in the meta-analysis (Ali et al., 2017; McGuire et al., 2020; Sainz Zuniga et al., 2020; Steinfort et al., 2011). Using an aspiration needle did not incur additional risk to the procedure, similar to that reported by the RCT performed by Chao et al. (2009) using an aspiration needle and forceps biopsy.

Demonstrating the safety of the procedure is being preserved, despite using multi-modal biopsy techniques; it added to the sparse data in the multi-modal biopsy used in the Radial EBUS literature.

4.2.4 Additional findings of the REBUST 2 study

Usefulness of rapid onsite examination (ROSE) during Radial EBUS procedure

Using ROSE with the L-EBUS procedure is commonplace, however, only a few studies in Radial EBUS have used ROSE. During the REBUST 2 study, the New Zealand centre did not have the capability to perform ROSE with either L-EBUS or Radial EBUS procedures. The Australian centre did have the capability of ROSE, but in order not to amend the protocol, ROSE was used as per the proceduralist's preference. Sometimes, ROSE was available when the patient had a L-EBUS prior to their planned Radial EBUS procedure.

ROSE was markedly efficient in obtaining a quick response as to whether the aspiration needle was placed in the correct location when performing aspiration needle biopsies, and if not, the position could be changed. If ROSE was consistently used, it may have provided an even higher diagnostic yield with an aspiration needle. Previous studies had demonstrated the use of ROSE associated with a high diagnostic yield in Radial EBUS (Xu et al., 2020); ROSE also reduced the

time of the procedure (Leoncini et al., 2020), as well as the number of biopsy tools that were used (Gildea et al., 2021). This data on the use of ROSE was not collected in the REBUST 2 study.

4.2.5 Limitations of the REBUST 2 study

As mentioned above, the main drawback to the study was its early termination, which resulted in not being able to demonstrate a significant difference between the small and large GS arms. The study was also planned to consider lobar differences between upper and lower lobes with a sample size of 88, but the early termination did not allow for the outcome to be analysed with adequate power to detect a difference.

As mentioned in the methodology section as a main limitation, the study terminated because most referring respiratory physicians noted that patients randomized to the large GS arm received better results and patients randomised to the small GS arm needed repeated procedures. This perceived clinical benefit resulted in referrer bias. Coupled with this, there were two other competing studies on Radial EBUS with the same inclusion/exclusion criteria, which also hindered recruitment.

Another drawback of the REBUST 2 study at the time of commencement in 2015 was the lack of dedicated aspiration needles for PPLs to be used via Radial EBUS. Therefore, the Boston Scientific aspiration needle 21G with a needle length of 15mm and the Medtronic aspiration needle 21G with a length of 8mm were used. The choice of aspiration needle was based on availability. The Medtronic needle was the preferred choice when both needles were available, due to its shorter length of 8mm that made it easier to navigate the upper lobes of the bronchi. Whilst all New Zealand patients had eXcelon needle aspirate, the Australian unit had both aspiration needle types of available. However, the type of needle aspirate was not randomly allocated. Therefore, there was insufficient data to suggest the utility of one aspiration needle over the other.



Figure 4.1 Needle aspirates used: (A) eXcelon 21G 15mm length; and (B) Medtronic 21G 8mm length

Another limitation in the study was the unavailability of a small aspiration needle fitting inside the small GS that prevented a comparison of the two aspiration needle sizes. As the majority of clinicians prefer the use of the small GS, this would explain the reluctance among clinicians in using an aspiration needle that needs to be used via the large GS. This limitation has now been overcome somewhat by the introduction of the PeriView FLEX 21G, 1.5mm outer diameter and 20mm in length aspiration needle by Olympus as this is designed as a dedicated aspiration needle for Radial EBUS procedures with extreme flexibility. Further studies using this smaller aspiration needle are eagerly awaited.

4.2.6 Implications of the REBUST 2 study

The REBUST 2 study adds to the safety data of all investigations into Radial EBUS by demonstrating an extremely low adverse event and pneumothorax rate. Even with the use of multi-modal biopsies and aspiration needles, the safety data did not change. This study has demonstrated that a routine 1-hour post-procedure chest X-ray may not be required, resulting in reduced radiation exposure and cost to the patient and healthcare system. A chest X-ray may be limited if the patient was symptomatic, therefore, a suggestion was made to the hospital administrative committees.

The aspiration needle can be readily used with the sedation bronchoscopy without the need for extra precautions against pneumothorax or bleeding and can be included as an integral biopsy tool into any unit performing Radial EBUS with minimal cost.

4.3 Future direction

This study used the eXcelon 21G needle and 21G Medtronic needle, which were not Radial EBUS specific tools. The recently developed Olympus 21G needle is an aspiration needle to be used specifically for Radial EBUS. The PeriView FLEX needle well-known for its flexibility and ability to reach the upper lobes.

While the Olympus 21G needle is flexible, it carries a needle length of 20mm. The shorter needle length of 8mm and similar flexibility of the Medtronic needle should be compared with the Olympus 21G PeriView FLEX needle to determine if a shorter needle is more beneficial so that consideration can be given to the development of a shorter needle that is specific to Radial EBUS and a needle that can be accommodated via the small GS. Incorporating a ROSE to Radial EBUS procedures with the use of a needle or cytology brush in accurately locating PPLs needs to be evaluated. Therefore, ROSE should play a vital role in Radial EBUS with the use of an aspiration needle or cytology brush that confirms the diagnosis immediately.

CHAPTER 5:

CRYO-FEASIBILITY STUDY – RESULTS AND DISCUSSION

5.1 Publication details and manuscript

Herath, S., & Yap, E. (2018). Novel hybrid cryo-radial method: An emerging alternative to CT-guided biopsy in suspected lung cancer. A prospective case series and description of technique. *Respirology Case Reports*, 6(2) (e00287).

Chapter 5 consists of the published paper and an extended discussion at the end, to complement and broaden the discussion already identified in the paper.

Novel hybrid cryo-radial method: an emerging alternative to CT-guided biopsy in suspected lung cancer. A prospective case series and description of technique

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Abstract

In diagnosing peripheral pulmonary lesions (PPL), radial endobronchial ultrasound (R-EBUS) is emerging as a safer method in comparison to CT-guided biopsy. Despite the better safety profile, the yield of R-EBUS remains lower (73%) than CT-guided biopsy (90%) due to the smaller size of samples. We adopted a hybrid method by adding cryobiopsy via the R-EBUS Guide Sheath (GS) to produce larger, non-crushed samples to improve diagnostic capability and enhance molecular testing. We report six prospective patients who underwent this procedure in our institution. R-EBUS samples were obtained via conventional sampling methods (needle aspiration, forceps biopsy, and cytology brush), followed by a cryobiopsy. An endobronchial blocker was placed near the planned area of biopsy in advance and inflated post-biopsy to minimize the risk of bleeding in all patients. A chest X-ray was performed 1 h post-procedure. All the PPLs were visualized with R-EBUS. The mean diameter of cryobiopsy samples was twice the size of forceps biopsy samples. In four patients, cryobiopsy samples were superior in size and the number of malignant cells per high power field and was the preferred sample selected for mutation analysis and molecular testing. There was no pneumothorax or significant bleeding to report. Cryobiopsy samples were consistently larger and were the preferred samples for molecular testing, with an increase in the diagnostic yield and reduction in the need for repeat procedures, without hindering the marked safety profile of R-EBUS. Using an endobronchial blocker improves the safety of this procedure.

Introduction

Peripheral pulmonary lesions (PPL) are common findings on computed tomography (CT) lung imaging and are on the rise [1]. With the advancement of new immunotherapies, there is an ever-increasing demand for not only diagnostic samples but for adequate-sized samples to facilitate numerous molecular testing. The current gold standard for diagnosing a PPL is by CT-guided core biopsy [2], which carries a high diagnostic yield (90%) [2–4] but at the expense of an increased side effect profile, predominantly pneumothorax (up to 34%) [2–8], pulmonary

haemorrhage (27%), and haemoptysis (4%) [2–8]. Furthermore, lesions close to major vessels or close to the mediastinum carry unacceptable risks of bleeding with CT-guided biopsy. Some patients are unable to maintain the required body position, for example, unable to raise the arms due to shoulder arthritis or perform breath holding due to anxiety, to enable a safe CT-guided biopsy.

Radial EBUS (R-EBUS) is emerging as a safer alternative to CT-guided biopsy, with a reduced pneumothorax risk of 1% and with haemothorax and haemoptysis being extremely rare [9,10]. However, the diagnostic yield of R-EBUS is only 73% (CI 0.70–0.76), which compares unfavourably with

CT-guided biopsy [10]. This is mainly due to the smaller size of the biopsy samples that can be obtained in Radial EndoBronchial Ultrasound Guide Sheath (R-EBUS). Studies examining factors associated with diagnostic yield in R-EBUS GS have found PPL size, location of PPL in relation to the radial probe (concentric versus eccentric), and sampling instruments to be influential as well [9,10].

Cryobiopsy or “cold biopsy” has been in use for bronchoscopic procedures for many years [11,12]. However, the use of cryobiopsy in sampling PPL is a new utility of this old tool. A recent pilot study demonstrated higher diagnostic yield with cryobiopsy in this context in comparison to R-EBUS forceps biopsies [13]. This study did not compare the diagnostic capability of other R-EBUS sampling methods, like the cytology brush or needle aspiration. This study did not utilize an endobronchial blocker as a prophylactic measure to prevent bleeding either.

We hypothesize that the addition of cryobiopsy to R-EBUS GS increases the diagnostic yield compared to all the conventional sampling tools of R-EBUS that are currently in use, including biopsy forceps, cytology brush, and aspiration needle usage. We also hypothesize that placing an endobronchial blocker pre-procedure and inflating this after the biopsy improves the safety of this hybrid technique.

Case Series

Methodology

This is a prospective case series of the first six consecutive adult patients who underwent R-EBUS to locate PPL, followed by sampling with conventional R-EBUS tools and cryobiopsy at our institution. All the procedures were undertaken at Middlemore Hospital, Auckland, New Zealand, between March and October 2015. Institutional and ethics approval were obtained, and all patients provided written informed consent.

Patients were offered this hybrid technique if they had a PPL 1 cm or above on CT scan of the lungs. All procedures were conducted as day cases and patients received either conscious sedation or a general anaesthesia depending on comorbidities. Neither navigation bronchoscopy nor fluoroscopy was used in this case series.

Description of technique

Prior to the procedure, the sub-segmental locations of the PPL are identified, and one to two bronchioles are marked as the point of placement of the radial probe within the lesion.

Following this, all R-EBUS instruments are measured. The R-EBUS GS is 2 cm longer than the 1050 mm, 1.9 mm flexible cryobiopsy probe. Therefore, to enable



Figure 1. Comparison of lengths of the R-EBUS GS and cryobiopsy probe (1050 mm).

contact with the lesion, the GS needs to be trimmed by 3 cm from the distal end (Fig. 1). All other instruments should be measured only after the GS has been trimmed.

Flexible bronchoscopy is performed with a 2.8 mm working-channel flexible bronchoscope. The radial probe (mini-USS probe 20 MHz, UM-BS20-20R- Olympus, Tokyo, Japan), which is housed within the 2.6 mm guide sheath (GS), is extended into the lung periphery through the bronchoscope. Once the PPL is identified on ultrasound, the radial probe is retracted while the GS is left in place to serve as an extended working channel to allow sampling tools to pass directly back into the lesion. Fluoroscopy was not used in any of these procedures.

An endobronchial blocker (Arndt size 7) is placed in close proximity to the chosen segment of the lung at the start of the procedure to manage any potential bleeding. The blocker is secured at the angle of the mouth of the patient with tape, and a nurse assistant is assigned to hold the blocker firmly in place to avoid dislodgement during pulling of the cryobiopsy. If an endotracheal tube (ETT) is used, the blocker is placed outside the ETT, and the cuff is inflated to hold this position in addition to the nurse assistant holding the blocker at the angle of the mouth as mentioned before.

Once the lesion is located on ultrasound, sampling was performed in a pre-determined random order using the forceps, cytology brush, and aspirating needle. Cryobiopsy was always performed last (ERBE Cryoprobe 1.9 mm diameter, length 1050 mm). A uniform freezing time of

4 s was used for each cryobiopsy, after which the cryoprobe and bronchoscope were removed en-bloc. Immediately on the removal of the cryoprobe from the airway, the endobronchial blocker was inflated with air (maximum 5 mL) for 2 min before gradual deflation under direct vision. Each patient had one to three cryobiopsies. Cryobiopsy samples are thawed in saline and fixed in formalin. Cytology specimens from the brush and needle are placed in cytolyt solution. Rapid on-site evaluation (ROSE) was not available at this institution. Two pathologists independently assessed all samples from forceps biopsy, cytology brush, aspiration needle, and cryobiopsy to determine the best sample (defined as the number of cells per high power field) for diagnosis and molecular testing.

A chest X-ray (CXR) was taken within 1 h of the procedure to assess for pneumothorax. Adverse events during the procedure were recorded.

Results

Four of the six patients were female (67%), with a mean age of 56 years (42–76 years). Five (83%) PPLs were in the upper lobes, and the mean size was 4.1 cm (1.9–6.6 cm). All the PPLs were visualized as concentric lesions on the radial ultrasound. In this series, five patients had conscious sedation, and one patient (patient 6) had a general anaesthetic. Four patients had a single cryobiopsy, whereas the other two patients had two (patient 6) and three (patient 1) cryobiopsies, respectively.

Five of the six (83%) patients received a diagnosis from this procedure; all were malignant, with the predominant histology being adenocarcinoma (Table 1). The remaining patient underwent surgical resection, and histology confirmed a hamartoma. Amongst the five patients with positive R-EBUS yields, four had positive samples from both cryobiopsy, and either one or more of the R-EBUS samples. The remaining patient (Patient 6) had a negative result from cryobiopsy, and the diagnosis was derived from the brush and needle aspirate cytology samples. We believe that a technical error contributed to the negative cryobiopsy findings in this patient. In this patient, the GS was not trimmed, which meant that the cryoprobe, which is shorter in length to the GS, was not extended beyond the GS into the PPL. This would have led to a sampling error (Table 1).

In the four patients where comparison between cryobiopsy and conventional samples were available, the cryobiopsy samples were superior in terms of size (mean 6.4 mm) compared with forceps biopsy (mean 3.4 mm) and the number of malignant cells per high power field. This difference would be even more exaggerated if tissue volume had been calculated. Independent analysis from two pathologists found that the cryobiopsy samples were

the preferred samples selected for mutation analysis and molecular testing in all four cases. One patient (Patient 1), who had a large cell neuroendocrine tumour, required complex immunohistochemistry analyses, which included multiple staining methods and transfer of samples for a second opinion. We feel that the large cryobiopsy sample (8 mm) facilitated this and saved the patient from a repeat procedure (Figs. 2–3).

There was no pneumothorax or bleeding requiring additional airway blockade or any intervention greater than suctioning.

Discussion

There is limited literature on using cryobiopsy in peripheral lung lesions suspicious for lung malignancy, particularly one that compares the yield with three conventional R-EBUS sampling tools in the same population. The use of an endobronchial blocker was not reported previously in this type of sampling.

Our results have shown that cryobiopsy samples were consistently larger and contained more preserved architecture and less crush artefacts, and were therefore superior to conventional samples for diagnosis and molecular analysis. This represents a step forward in the utility of R-EBUS in evaluating PPL as the rate-limiting step so far has been the need to use tools that fit into the 2 mm GS which in turn limits the size of the biopsy. Additionally, it is well recognized that small forceps create significant crush artefact because of the tearing motion of the forceps “teeth,” whereas the freezing process does not appear to cause any sustained freezing damage to the tissue. The use of cryobiopsy via the GS appears to overcome both these issues without incurring significant cost or adverse effects on the patients.

The adverse event rate in this population of lung malignancies was very low, which contrasts the use of cryobiopsy in interstitial lung disease (ILD) where the pneumothorax rate has been described to be up to 28% [14]. This could be because of the very peripheral biopsies performed in the ILD series, which was not a requirement in this population. Additionally, fibrotic lung tissue also appears to be a risk factor for pneumothorax when cryobiopsy is employed in ILD [14].

The cryobiopsy patients in this PPL group also had a lower rate of bleeding than that observed in the ILD patients. One possible explanation is that this patient population was less likely to have pulmonary hypertension in contrast to the ILD patient group, but a direct comparison was not possible. The number of biopsies employed in diagnosing ILD usually ranges from 4 to 6 biopsies, whereas diagnosis of lung masses on average is performed

Table 1. Characteristics of Radial EBUS and Cryobiopsy samples.

Index	Gender/ Age	PPL size (cm)	Location	Radial EBUS sampling				Preferred sample for EGFR analysis	Final diagnosis
				Needle aspiration	Brush	Forceps	Cryobiopsy		
1	F-54	3.88	LUL	Negative	Positive	Negative 1 mm	Positive 8 mm	Cryobiopsy	Large cell, high-grade neuroendocrine
2	F-47	4	RML	Positive	Positive	Positive 5 mm	Positive 6 mm	Cryobiopsy	Adenocarcinoma
3	F-76	5.3	LLL	Positive	Positive	Negative 2 mm	Positive 6 mm	Cryobiopsy	Squamous cell carcinoma
4	M-52	3	RUL	Positive	Positive	Positive 5 mm	Positive 7 mm	Cryobiopsy	Adenocarcinoma
5	F-42	1.9	LUL	Negative	Negative	Not done	Negative 5 mm	Surgery	Hamartoma (surgical resection)
6	M-69	6.6	LUL	Positive	Positive	Negative 4 mm	Error in measurement	Radial brush	Adenocarcinoma

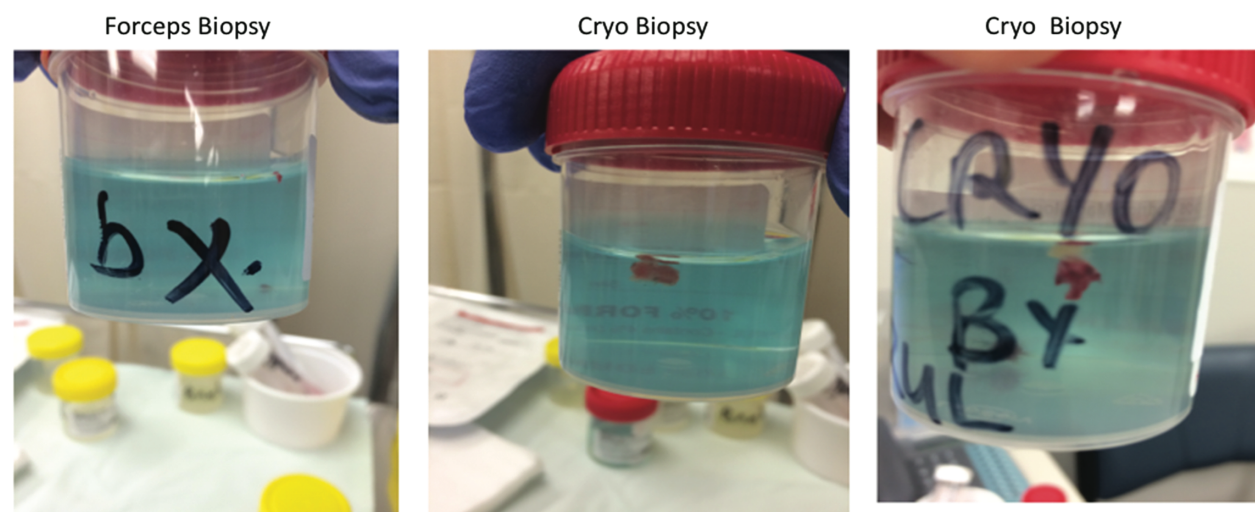
with 1–2 samples, which may also be another factor for this lower rate of bleeding for PPL.

The presence of the endobronchial blockers is likely to have also aided in reducing any bleeding and prevented blood spillage into the other bronchial segments, thereby preserving visibility for subsequent sampling. This highlights the advantage of using endobronchial blockers in procedures such as these.

In this study, one aspect we were assessing was the feasibility of performing cryobiopsy and the ability to tolerate a bronchial blocker with sedation only. We noted that, with sedation only, the patients tolerated the procedure with

variable discomfort due to the bronchial blocker causing irritation at the vocal chords. Hence, the first five patients in this series had sedation only, and from the sixth patient onwards, we opted to use general anaesthetic for this procedure.

We did not use fluoroscopy for this case series. This essentially meant that we had to frequently assess the position of the GS after every biopsy modality, and on occasions, if the patient had coughed, the position was assessed after every single biopsy. This increased the time of the procedure. This did not affect the safety of our patient cohort considering that their lesion size was 4.1 cm, but

**Figure 2.** Macroscopic comparison of forceps biopsy (left) with the cryobiopsy (two samples on the right).

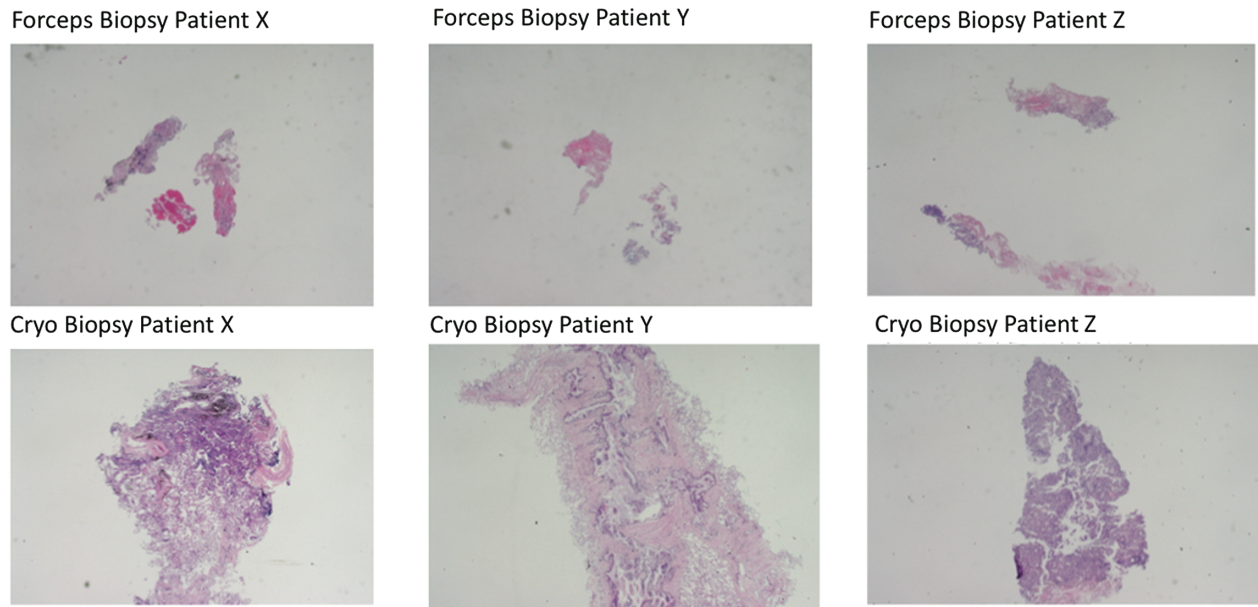


Figure 3. Microscopic comparison of the biopsy techniques in three patients in this study.

such an increased time for the procedure would have a greater impact on the safety for lesions of a smaller size. As a consequence of this feasibility study, we now opt to use fluoroscopy at all possible times, and especially for smaller lesions, in our routine practice to improve time efficiency and safety of this procedure.

Our study also highlights the importance of accurate instrument measurement pre-operatively, especially if multiple instruments were to be used. As the shortest instrument is the cryoprobe, it is essential that this is measured and marked first before any of the other instruments. Even though most of the PPLs were in the upper lobes, which are notoriously more difficult to access with larger bronchoscopes, we did not encounter such difficulties with the 2.8 mm working channel bronchoscope.

We have demonstrated the safety and utility of this hybrid cryobiopsy/R-EBUS technique in evaluating PPL, where the yield is at least as good, if not superior, to conventional sampling instruments in this case series. The major limitation in this study is the small number of patients involved. Consequently, strong conclusions cannot be drawn from the findings, but the authors feel that the results are encouraging enough for this technique to potentially evolve into an invaluable bronchoscopic biopsy tool in lung cancer management. Further larger trials are required to establish not only efficacy and safety but also diagnostic yield relative to CT-guided biopsy. A multicentre study [15] (CT CROP clinical trials.gov registration: (NCT02395939) is in the recruitment stage, and may provide answers to these questions.

Disclosure Statements

Appropriate written informed consent was obtained for the publication of this case series and accompanying images.

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5.2 Discussion of the Cryo-feasibility study

5.2.1 Significance of the study

This is the first study to use cryobiopsy for lung lesions in New Zealand. At the time of conceptualising and planning this study in 2015, there was only a single published feasibility study available (Schuhmann et al., 2014) in using cryobiopsy with Radial EBUS for PPL. This German study had a markedly different bronchoscopy unit setup to that of the New Zealand unit with different anaesthetic and sedation facilities, and different airway safety facilities. The German unit had anaesthetic support for all procedures performed under a general anaesthetic. The airway support was performed by using rigid bronchoscopy and high frequency jet ventilation. At this point in New Zealand, all bronchoscopy procedures were performed in an endoscopy unit under sedation. There was no anaesthetic support for bronchoscopy or theatre facilities to perform these procedures. The rigid bronchoscopy was sparingly used for major airway obstruction. Therefore, a feasibility study was performed to assess the efficacy and safety in using cryobiopsy with Radial EBUS for PPLs in the New Zealand setting, as well as the need to assess safety procedures.

The ethics approval process was rigorous as this was the first human study in New Zealand to use a cryobiopsy probe in the lung. Apart from the usual ethics application, a face-to-face meeting with the ethics committee was necessary to answer the multitude of questions from committee members who required clarification about the procedure and the training of the proceduralist as well as safety measures taken and information that was required in the patient information sheet to provide the patient with this increased risk of bleeding.

This was a prospective study that recruited six consecutive patients for Radial EBUS biopsies followed by at least one cryobiopsy. The aim was to ensure efficacy and safety of the study and to assess the safety parameters that needed to be addressed in the New Zealand setting.

Additional safety procedures: In New Zealand, due to the lack of operating rooms and rigid bronchoscopy facilities, instead of a rigid bronchoscopy, a prophylactic bronchial blocker with ETT tubes were considered. The bronchial blocker was placed outside the ETT, but adjacent to the ETT and held in place by inflating the ETT cuff once the blocker was in place. This study reported the use of a bronchial blocker in this manner for cryobiopsy in the PPL for the first time in 2017, and it is an original contribution to the literature.

5.2.2 Salient results and comparison to existing evidence

Once the feasibility study was completed in New Zealand, it was evident that cryobiopsy via the large GS produced a yield of up to 83%. This was in keeping with diagnostic studies published by Schuhmann et al. in 2014 (Schuhmann et al., 2014) with improved yield of 74.2% and by Imabayashi et al. who published a similar diagnostic yield (Imabayashi et al., 2019) of 86.1% in 2019. Schuhmann demonstrated that in 4/31 (13%) diagnosis was made on cryobiopsy alone, which was similar to the Cryo-feasibility study resulting in 1/6 (16%) diagnosis made solely on cryobiopsy. The Cryo-feasibility study is also comparable to the Schuhmann study, which produced a freezing time of four seconds.

5.2.3 Ability to perform EGFR testing

The ability to perform EGFR testing was not assessed in the Cryo-feasibility study or the Schuhmann et al. (2014) feasibility study. However, the ability to perform molecular testing was commented on by Schuhmann et al. (2014) who reported that cryobiopsy produced three times larger sample sizes compared to forceps. In the Cryo-feasibility study, cryobiopsy samples were only twice as large as indicated in Schuhmann et al. (2014) despite using the same freezing time. This may be due to a difference in the compressed gas used with the cryobiopsy procedure in Germany compared to New Zealand. Whilst Schuhmann et al. (2014) used nitrous oxide as the cryogen, which cools the surrounding tissue to -89° Celsius, the New Zealand Cryo-feasibility study used liquid carbon dioxide as the cryogen, which only freezes to -59° Celsius, which may explain the size difference of the sample to some degree.

5.2.4 Use of multi-modality biopsy techniques

In the Cryo-feasibility study, each patient received a forceps biopsy, radial brush and needle aspirate followed by a cryobiopsy. In one patient, the diagnosis was made on the radial needle and brush alone but not on cryobiopsy or forceps. Therefore, using multiple biopsy tools in combination improved the diagnostic yield as opposed to being limited to a single tool alone. Schuhmann et al. (2014) used forceps and cryobiopsy, demonstrating that the addition of cryobiopsy improved the diagnostic yield, similar to the Cryo-feasibility study.

The Cryo-feasibility study published in 2017 was the first study that demonstrated the advantage of the addition of needle aspirate and cytology brush in combination of cryobiopsy in PPLs as multi-modal diagnostic methods to improve the diagnostic yield. Hence, this is an original contribution to the current literature. The only other study in cryobiopsy in PPLs at the time was published by Schuhmann et al. (2014), which had used only forceps biopsy and cryobiopsy alone.

5.2.5 Safety profile

This was the first New Zealand study to use a cryobiopsy procedure in the lung. It assisted the understanding of the requirements to perform the procedure safely in the New Zealand setting.

During the Cryo-feasibility study, the technique was continually refined to maximise safety. The initial safety plan for bleeding was to keep a bronchial blocker placed in the lobar airway. Two bronchoscopists remained in the procedure room with the second bronchoscopists advancing a separate bronchoscope to the area of the biopsy as soon as the biopsy was taken (the two-scope approach), to suction the blood around the inflated bronchial blocker. The inflated blocker seemed to irritate patients while they were under sedation, therefore, requiring deepening of the sedation. However, in some patients, the irritation of the blocker caused marked coughing and displacement of the bronchial blocker.

The bronchial blocker was not placed inside the lumen of the ETT as this would have limited the space available to manoeuvre the bronchoscope. Unlike the rigid bronchoscopy (diameter 12.5mm), the largest diameter of ETT used was approximately 8mm. Using the bronchial blocker outside the ETT meant there was no competition for space within the ETT tube lumen. However, placing the blocker in the correct segment caused difficulty when the bronchial blocker was placed outside the ETT, in comparison to rigid bronchoscopy. The placement of a bronchial blocker required extra time, and on occasions it was not possible to direct the blocker to the correct position, especially to the upper lobes. This method of using a bronchial blocker in cryobiopsy for PPLs was first published in the Cryo-feasibility study in 2017, as well as the technique of securing a bronchial blocker outside the ETT due to the unavailability of rigid bronchoscopy. An explanation of these techniques mentioned in the paper is an original contribution to the current literature.

There were no pneumothorax or moderate/major bleeds in the Cryo-feasibility study. The Schuhmann et al. (2014) study of 31 patients demonstrated one case of moderate bleed. This study did not use a bronchial blocker. The better safety profile of the Cryo-feasibility study may be due to the consistent use of the bronchial blocker and assigning a nurse to hold the blocker in place and inflating it. The bronchial blocker was prophylactically inflated after each biopsy. All patients in the Cryo-feasibility study were noted to have had a mild bleed and the blocker was able to successfully contain the bleed.

5.2.6 Implications of the Cryo-feasibility study

The Cryo-feasibility study recorded the first lung cryobiopsy procedure performed in New Zealand. It was a steep learning curve for the proceduralists, nursing staff and anaesthetists involved who had to share the airway. This study established the first cryobiopsy and Radial EBUS service in New Zealand. Policies and procedures were developed during this study for the safety of patients and the author trained all staff involved in the procedure, including nurses and proceduralist. After completion of the Cryo-feasibility study, a cryo-radial service was established as a novel diagnostic service at Middlemore Hospital, Auckland.

The safety profile of the cryobiopsy technique for PPLs was not well known at the time of this study. Modifications had to be made to the Cryo-feasibility study to accommodate the New Zealand setting. One important and successful change was the addition of using the prophylactic Arndt 7 bronchial blocker to contain bleeding and stop soiling of the airways. This study was the first to demonstrate the use of a prophylactic blocker in a cryo-radial procedure for a PPL with good effect, when used outside the ETT. The use of a prophylactic blocker is now incorporated in most published papers on cryo-radial procedures for PPLs (Imabayashi et al., 2019; Taton et al., 2018).

The cryobiopsy technique required the purchase of a cryobiopsy machine and probes, which were funded and secured by a grant. Grant applications were made to a multitude of grant bodies, from which two grants were approved: (i) Ko Awatea seed funding; and (ii) Asser trust fund. Both enabled the purchase of much needed cryobiopsy equipment which continued to be used in the clinical practice after the trial was completed.

This study has raised the awareness of hospital management of the need for operating room facilities and anaesthetic support for complex bronchoscopies, which has resulted in Middlemore Hospital now operating a dedicated general anaesthetic list for complex bronchoscopy procedures.

5.2.7 Limitations of the Cryo-feasibility study

Practical aspect

New Zealand units perform bronchoscopy in endoscopy suites under sedation. It was difficult to obtain operating room facilities and support for bronchoscopy procedures, as anaesthetists were hesitant in performing this type of shared airway procedure, therefore, the number of anaesthetists who took up the challenge was limited. Although all proceduralists were trained bronchoscopists,

cryobiopsy was new to New Zealand, therefore, training and upskilling of proceduralists, pathologists, anaesthetists and nursing staff were required during the study.

Technical aspect

The reusable 1.9mm cryoprobe was the only cryobiopsy tool available in New Zealand at the time of this study. These earlier cryoprobes were shorter at 950mm compared with the Radial EBUS GS measuring 1050mm which meant the GS had to be trimmed by 30mm in order to expose the freezing tip of the cryoprobe. This resulted in the loss of the radio-opaque tip of the Radial EBUS GS. In one patient, the GS was not trimmed due to human error, resulting in a negative sample for cryobiopsy as the GS was longer than the cryoprobe even when the cryoprobe was advanced maximally as noted in the Cryo-feasibility paper. Furthermore, due to the tight fit of the cryobiopsy probe when applied through the large GS, only a few passes could be performed. Due to the constant pushing of the stiff cryoprobe, through the tight fitting GS, the proximal end of the cryoprobe became twisted and resulted in breakages after a few procedures.

Due to the novel procedure, training was required for assisting proceduralists and nursing staff, which added additional time requirement to the procedural time. This longer procedure limited the patient's tolerance with sedation only and through the Cryo-feasibility study, the importance of general anaesthesia to improve patient comfort and diagnostic yield was well noted.

Pathology data collection

The Cryo-feasibility study was predominantly performed to explore the safety of this novel method, but data was not collected on the comparison of the forceps biopsy architecture versus the cryobiopsy architecture to detect if there were less crush artefacts in the cryobiopsy samples.

5.3 Future directions

5.3.1 Practical aspect

The Cryo-feasibility study demonstrated that cryobiopsy can be performed with a bronchial blocker placed outside the ETT, and does not necessarily require rigid bronchoscopy equipment. Having a dedicated assistant holding the bronchial blocker in place and inflating the blocker was vital to control any bleeding and to ensure the blocker did not get displaced with the pulling of the cryoprobe and bronchoscope.

Although any bleeding risk was well controlled, there was more mild oozing than anticipated with Radial EBUS alone. Therefore, the unit was equipped with tranexamic acid, cold saline and extra

bronchial blockers in case of a dislodgement or rupture of a bronchial blocker. Prior to commencing the procedure, the team was informed of the appropriate patient positioning and which lung to intubate in case of an uncontrollable bleed. The entire team, including the proceduralist, anaesthetist and nurses, required upskilling and training due to multiple equipment being used and the requirement for a shared airway.

Inserting the blocker, performing a cryobiopsy via the Radial EBUS GS and repositioning the second and third cryobiopsy, required more time than performing the usual Radial EBUS procedure. This extra time was not recorded during the study, but was noted it as an interesting finding by the research team. Repeatedly, time ran out with the study patients during the bronchoscopy list. However, this measurement of time was included as a measured parameter in the following CT-CROP study (Chapter 6).

The combination of Radial EBUS biopsies with cryobiopsy (multi-modal biopsies) improved the success rate. Due to the mild ooze that cryobiopsy produced, it was recommended for the cryobiopsy to be done as the final biopsy after performing the Radial EBUS biopsies.

5.3.2 Leading to the next study

Given the ability to establish safety in this procedure and the higher yield of 83%, cryo-radial hybrid procedures could be an alternative for CT-TTB, but this study was limited by the small number of patients to enable a conclusion to be drawn. The Cryo-feasibility study has demonstrated an adequate safety profile and yield for the ethics committee to proceed towards the planned RCT. The safety measures discovered during this study have been invaluable for planning the RCT and ensuring safety in the usual clinical practice.

In Chapter 6, a RCT was performed to compare the diagnostic yield between cryo-radial and CT-TTB (Chapter 6).

CHAPTER 6:

CT-CROP STUDY – RESULTS AND DISCUSSION

6.1 Publication details and manuscripts

Herath, S., Wong, C., Dawkins, P., Veale, A., Yap, E., Williamson, J., Low, I., Mahajan, H., Prvan, T., Barnard, S., Hawkins, S., Cookson, D., Singh, T., & Ing. A. (2002). Cryobiopsy with radial-endobronchial ultrasound (Cryo-Radial) has comparable diagnostic yield with higher safety in comparison to computed tomography-guided transthoracic biopsy for peripheral pulmonary lesions: An exploratory randomised study. *Internal Medicine Journal*. Jun 8. <https://doi.org/10.1111/imj.15833>. Epub ahead of print. PMID: 35675149.xt

An extended discussion is at the end to complement and broaden the discussion already performed in the paper.

ORIGINAL ARTICLE

Cryobiopsy with radial-endobronchial ultrasound (Cryo-Radial) has comparable diagnostic yield with higher safety in comparison with computed tomography-guided transthoracic biopsy for peripheral pulmonary lesions: an exploratory randomised study

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Key words

cryo-biopsy, radial EBUS, CT-guided biopsy, diagnostic yield, EGFR, bleeding.

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Abstract

Background: Computed tomography-guided transthoracic biopsy (CT-TTB) is the 'gold standard' biopsy for lung nodules. Radial-endobronchial ultrasound (R-EBUS) bronchoscopy is another recommended biopsy but carries a lower diagnostic yield. Addition of cryobiopsy with R-EBUS (Cryo-Radial) has shown promising results. There are no studies comparing CT-TTB with Cryo-Radial biopsy.

Aim: The co-primary aims were the diagnostic yield and safety. The secondary aim: ability to test epidermal growth factor receptor (EGFR).

Methods: A randomised controlled, multicentre exploratory study was conducted at three tertiary hospitals. Patients with nodules >1 cm on CT of the chest were randomised to CT-TTB or Cryo-Radial. With Cryo-Radial, patients had 1–3 cryo-biopsies in addition to at least one R-EBUS biopsy through the 2.6 mm guide sheath.

Results: Forty-eight patients were randomised: 22 to CT-TTB and 26 to Cryo-Radial. Sixteen in the CT-TTB and 20 in the Cryo-Radial received the allocated biopsy. The diagnostic yield was CT-TTB 93.8% (15/16) versus Cryo-Radial 85% (17/20) $P = 0.61$ and the odds ratio was 0.37. For 5/13 (38%), a diagnosis was solely made on cryobiopsy. Eleven (78%) of 14 in CT-TTB versus 7/10 (70%) Cryo-Radial were suitable for EGFR testing $P = 0.66$, with odds ratio 0.63. Pneumothorax occurrence was 44% (7/16) in CT-TTB versus 4.2% (1/24) in Cryo-Radial. Two (12.5%) of 16 CT-TTB required chest drain insertion.

Conclusion: Cryo-Radial is comparable in diagnostic yield and ability to perform EGFR testing with a significantly lower risk of pneumothorax, compared with CT-TTB. Cryo-Radial has the additional advantage of mediastinal staging during the same procedure with Linear-EBUS and is a promising first-line tool in the diagnostic method of lung cancer.

Abbreviations: Cryo-Radial, Cryobiopsy and Radial EBUS; CT, computed tomography; CT-TTB, CT-guided transthoracic biopsy; EGFR, epidermal growth factor receptor; GS, guide sheath; ICT, intercostal chest tube; ILD, interstitial lung disease; R-EBUS, radial endobronchial ultrasound-guided biopsy.

Funding: Arnott Early Career Research Entry Grant from RACP Foundation (Australia): 2018; Basser Research Entry Scholarship from RACP Foundation grants (Australia): 2017; ASSER Trust grant funding for the multicentre RCT (New Zealand) 2015.

Conflict of interest: None.

Prior Abstract Presentation: Herath S, Yap E, Dawkins P, Veale A, Williamson J, Ing A. Multi centre randomised control trial of the diagnostic yield and safety of radial EBUS guided cryobiopsy (Cryo-Radial) versus CT guided transthoracic biopsy for lung masses demonstrate similar diagnostic success (CT CROP), Presentation at TSNZ Queensland 2019. *Respirology* 2019; 24: 93.

Introduction

Lung cancer is the leading cause of cancer death worldwide with a poor 5-year survival of 15%. Early diagnosis and timely treatment of peripheral pulmonary lesion (PPL) improves survival.¹ In 18% of patients, delays in lung cancer treatment were due to repeated investigations and the need for a further staging procedure after diagnosis.² The challenge for clinicians is to select the optimum investigation based on high-yield and low complication rates to obtain samples sufficient for immunohistochemistry and molecular testing,³ with a preference for combined diagnostic and staging procedure.

Computed tomography-guided transthoracic biopsy (CT-TTB) has been the most utilised technique for obtaining tissue in PPL, with a meta-analysis in 2015 of 48 studies with 10 383 biopsies demonstrating a diagnostic yield of 92.1% (67–97%).⁴ The major drawback in CT-TTB is the high pneumothorax rate of 20.5% (8.2–47.3%) with intercostal chest tube (ICT) placement of 7.3% (0–31.1%).⁵ In comparison, guided bronchoscopy techniques, including Radial Endo-Bronchial Ultrasound (R-EBUS) have a better side-effect profile.

Five meta-analyses in guided-bronchoscopy studies demonstrated a pneumothorax rate less than 1.5% and ICT less than 0.6%.^{6–10} However, R-EBUS is associated with a lower diagnostic yield 70–73%.^{6,10} This may be due to an insufficiency in sampling tools with frequent use of forceps, often as the sole biopsy method, resulting in smaller samples and ‘crush’ artefact and inability to reach PPL located eccentrically to the bronchi.

Cryobiopsy has been added to the armamentarium of R-EBUS sampling to improve yield (Cryo-Radial), with the reported diagnostic yield up to 86%. In these studies, cryobiopsy specimens provided the sole diagnosis in up to 40% of cases.¹¹ The single arm data on the Cryo-Radial procedure for PPL is still sparse, limited to a single meta-analysis of nine studies totalling 300 patients¹² demonstrating a favourable low, severe complication rate of only 1.8%.

A recent meta-analysis comparing CT-TTB versus R-EBUS involving 2025 patients noted higher yield in CT-TTB (odds ratio (OR) 0.23; $P < 0.0001$), as well as much higher complication rates (OR 7.27; $P < 0.00001$).¹³ These R-EBUS studies had not included cryo-biopsy as a sampling method.

Comparative studies between Cryo-Radial EBUS procedure and the widely used CT-TTB are sorely lacking and to our knowledge this is the first comparison exploratory randomised control trial between these two methods.

Methodology

This was a multicentre, open-label, parallel arm, randomised controlled interventional study, registered at

clinicaltrials.gov: NCT02395939. The protocol was approved by the respective institutional ethics committees (National Health and Disability Commission (HEDC) NZ for an interventional diagnostic study, Ethics Approval Number: New Zealand: 15/NTA/48; Human Research and Ethics Committee (HREC), NSW, Australia, approval number HREC/16/WMEAD/337) and informed written consent was obtained from all patients. Patients with a PPL greater than 1 cm (both benign and malignant radiological appearance) on axial computed tomography (CT) chest imaging, requiring a diagnosis, were offered the study, and recruited from three tertiary hospitals across Australia and New Zealand between January 2015 and August 2018. Patients were excluded if they had size significant (>1 cm) mediastinal adenopathy on CT of the chest, had a more accessible site for biopsy or had comorbidities preventing either procedure. Patients with pregnancy, anticoagulation, which cannot be withheld for more than 5 days, and severe congestive cardiac failure with ejection fraction <40 were excluded. Aspirin use was permitted. Pulmonary hypertension was not assessed. For patients without size significant mediastinal adenopathy, Linear EBUS was performed first, if PPL >3 cm or centrally located, with onsite cytologist, and proceeded to R-EBUS if negative.

The co-primary end-points were the diagnostic yield of combined sampling from the Cryo-Radial procedure with guide sheath (GS) use (combined yield of cryobiopsy, forceps, brush, and aspiration needle), against the CT-TTB for the diagnosis of PPL and the safety profile between the two arms. The secondary objective was to compare the suitability for epidermal growth factor receptor (EGFR) testing.

Procedure details: bronchoscopic Cryo-Radial arm

All procedures were performed by experienced interventional bronchoscopists. The choice of airway support (endotracheal tube or rigid bronchoscopy Storz size 8.5 or 12 mm), use of fluoroscopy and use of a prophylactic bronchial blocker (Fogarty size 4 Fr) for prevention of bleeding was at the discretion of the bronchoscopist. All patients had airway inspection, followed by R-EBUS (20 MHz, UM-BS20-17R; Olympus, Tokyo, Japan) introduced with a large guide sheath (GS) (K-203 2.6 mm).

Once the PPL was located on ultrasound, the GS was left *in situ* serving as an extended working channel. A dedicated assistant anchored the bronchoscope and GS to avoid displacement. At least one conventional R-EBUS sample with either brush, forceps or needle aspiration through the GS was performed at the discretion of the bronchoscopist. Cryobiopsy was taken as the

last sample. The cryoprobe (1.9 mm diameter, 1050 mm length; Erbecryo2, Germany) was used through the GS. Freezing time was 4 s for each biopsy. Up to three cryobiopsies were taken for each patient.

Following each cryobiopsy, the cryoprobe was removed en-bloc with the bronchoscope and the bronchial blocker was inflated immediately. The cryobiopsy tissue was thawed in saline and fixed in formalin.

Procedure detail: CT-TTB

All procedures were performed by trained interventional radiologists using local anaesthetic. CT-TTB core biopsies were obtained using an introducing needle (17-19G) with a core needle size above the introducing needle. A minimum of two passes were required for each PPL with further passes performed at the discretion of the radiologist. Samples were fixed in formalin.

Safety data

The Common Terminology Criteria for Adverse Events (CTCAE) was used to define pneumothorax, bronchial haemorrhage and other (hypoxia, mediastinal haemorrhage and respiratory failure). A chest X-ray 1-h post-procedure was performed. A nurse call out was made at 24 h and at 7 days post procedure to capture delayed side-effects.

Histological/cytological analysis of tissue specimens

Each sample was analysed by two independent pathologists who concurred on the diagnosis (Appendix S1).

Follow up

The patient was followed up for 12 months with repeat CT imaging 6 monthly to confirm a benign diagnosis.

Statistical analysis

An assumed CT-TTB sensitivity of 90% and Cryo-Radial sensitivity of 70¹⁴ was accepted from the existing studies. To detect a difference of 20% in the sensitivity, with a statistical power of 0.85 at a significance level of 0.05, a total of 158 patients was required. Permuted block randomisation was performed with 1:1 ratio and a block size of four. A *P*-value was set at 0.05.

Sample size calibration was planned at 25% recruitment due to the sparsity of studies on the Cryo-Radial procedure in PPL. The Internal Data Safety Monitoring Board reviewed the adverse events.

All parametric tests were reported using paired *t* test and smaller sample sizes were reported with Chi-squared test

and Fisher's exact test. A significant *P*-value was set at 0.05.

Closure of study

This study had slow recruitment from initiation, due to a multitude of reasons. Despite selecting study centres with a high incidence of lung cancer, due to the lower socioeconomic background the presentations were late and patients had high co-morbidities, resulting in a much smaller number of patients without nodal or metastatic disease who were eligible for the study as per protocol. Second, the study carried out randomisation without taking into consideration the characteristics of the PPL (central vs peripheral or air bronchograms) or patient characteristic (emphysema). This pure randomisation resulted in some clinicians deciding not to send patients to the study. This resulted in a smaller pool of clinicians referring their patients to the study. Third, the pre-planned interim analysis at the 25% recruitment occurred almost 3 years after trial commencement. During this time growing body of publications of single arm studies in Cryo-Radial in PPL demonstrating safety and diagnostic utility, affected the investigators ability to recruit even further. The steering committee decided to terminate the study and publish the results as a further delay would dampen the relevance of the results of this study. To our knowledge, at the time of publication, this is the only study that has compared the Cryo-Radial procedure with CT-TTB. Thus, the resulting smaller sample size and the underpowering of the study to detect a true difference had become the major drawback of this study.

Results

A total of 48 patients was recruited; 22 were randomised to CT-TTB and 26 to the Cryo-Radial arm. Six from the CT-TTB arm and six from the Cryo-Radial arm did not undergo their allocated biopsy procedure following randomisation. Sixteen patients underwent CT-TTB. Twenty-five underwent bronchoscopy, with only 20 proceeding to the allocated Cryo-Radial biopsy (Fig. 1). There was no significant difference in patient characteristics, co-morbidities, functional scores (Table 1) or CT characteristics of the PPL (Table 2) between the two groups. The mean PPL size was 36.1 mm (33.6–38.6 mm). Fluoroscopy was used in four of the Cryo-Radial procedures.

CT-TTB

The size of co-axial needles ranged from 17G to 19G, with the 17G used most (81%). Core needle size ranged from 18G to 20G with the 18G used most (81%). The mean

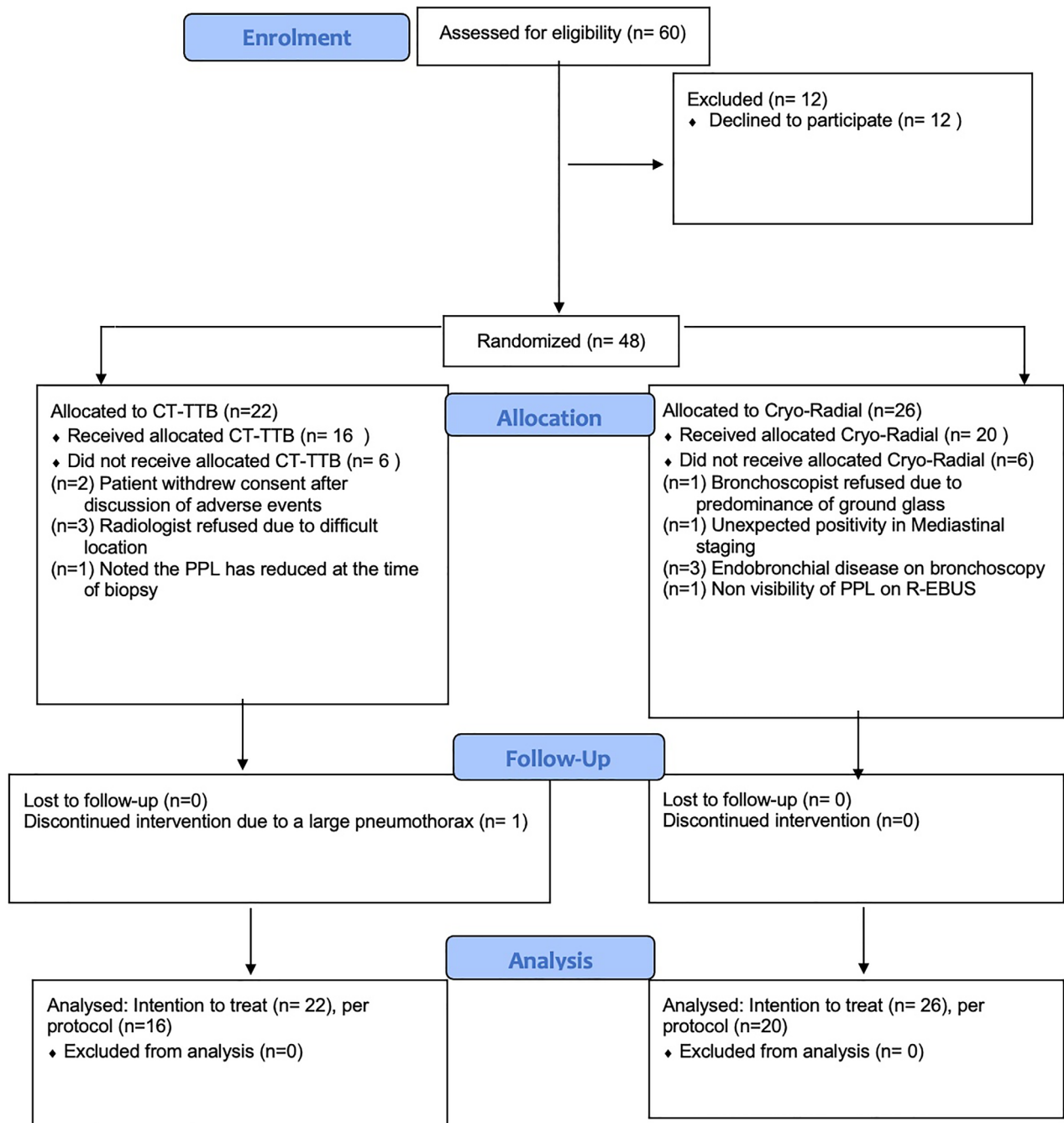


Figure 1 The patient recruitment consort flow diagram

number of passes was three. The mean time taken to complete the CT-TBB procedure was 16.1 min (SD 17.6 min) (Table 3). The diagnostic yield for the CT-TBB group was 15/16 (93.8%). There were 15 malignancies and one benign lesion (Table 4). The one non-diagnostic patient had an adenocarcinoma that was diagnosed through Radial EBUS outside the study.

Cryo-Radial biopsy

Of the 25 patients who underwent bronchoscopy, three had unexpected malignant endobronchial disease. Seven (28%) of 25 patients had concurrent Linear EBUS prior to R-EBUS and one patient had malignancy identified during the staging procedure. In one patient, the PPL

Table 1 Demographic data between the two arms

Characteristic	Radial-Cryo arm (n = 26)	CT-TTB arm (n = 22)	P-value
Sex			
Female	11 (42.3%)	10 (45.5%)	0.827
Male	15 (57.7%)	12 (54.4%)	
Age	63.5 (SD 10.5)	Mean 65.6 (SD 14.4)	0.572
COPD	14 (53.8%)	10 (45.5%)	0.562
FEV1	2.27 (SD 0.79)	1.9 (SD 0.93)	0.266
Non-smoker	7 (27%)	8 (36%)	0.76
Current smoker	8 (30.8%)	4 (4.5%)	
Ex-smoker	11 (42.3%)	9 (40.9%)	0.158
HTN	9 (34.6%)	3 (15.3%)	
DM	4 (15%)	3 (14%)	0.832
OSA	3 (11.5%)	2 (9.1%)	0.812
IHD	5 (19.2%)	2 (9.1%)	0.323
Anti-coagulation/anti platelet use	7 (26.9%)	4 (18.2%)	0.833
ASA 1	20 (76.9%)	20 (90.9%)	0.216
ASA 2	7.7% (4%)	2 (9.1%)	
ASA 3	4 (15.4%)	0 (0%)	0.580
ECOG 0	19 (73.1%)	16 (73%)	
ECOG 1	5 (19.2%)	3 (13.6%)	0.580
ECOG 2	1 (3.8%)	3 (13.6%)	
ECOG 3	1 (3.8%)	0 (0%)	

COPD, chronic obstructive airways Disease; FEV1, forced expiratory Volume in 1 second; HTN, hypertension; DM, Diabetes Mellitus; OSA, obstructive sleep apnoea; IHD, ischaemic heart disease; ASA, American Society of Anaesthesiology; ECOG, Eastern Cooperative Oncology Group.

Table 2 CT characteristics of the pulmonary lesion between the two arms

	Radial-Cryo arm (n = 26)	CT-TTB arm (n = 22)	P-values
Size (largest diameter in axial images) (mm)	33.6 mm (SD 21.6)	38.6 mm (SD 24.2)	0.456
Location of lesion on axial CT			
Inner 1/3	7 (26.9%)	2 (9.1%)	0.193
Middle 1/3	5 (19.2%)	8 (36.4%)	
Outer 1/3	14 (53.8%)	12 (54.5%)	
Lobar location: UL	16 (61.5%)	14 (63.6%)	0.994
Lobar location: LL/RML	10 (38.5%)	8 (36.4%)	
Presence of air bronchograms	13 (50.0%)	6 (27.3%)	0.083
No air bronchogram	12 (46.2%)	11 (50%)	
Not recorded	1 (3.8%)	5 (22.7%)	

LL, lower lobe; RML, right middle lobe

was not detected on ultrasound. Twenty patients underwent the allocated Cryo-Radial procedure and all 20 PPL were located by ultrasound; 14 were concentric and six were eccentric. The mean time to detect the PPL was 7 min (range 1–20 min). The mean time taken for the

Table 3 Comparing the logistics and resources required in both arms

	Cryo-Radial	CT Bx
Place of procedure	Operating rooms, 19/25 (76%) Endoscopy suite, 6/25 (24%)	Radiology department, 16/16 (100%)
Airway usage	13/25 (52%) had a rigid bronchoscopy in place, 7/25 (28%) Laryngeal Mask Airway (LMA), 1/25 (4%) Endotracheal tube (ETT) and 4/25 (16%) had their own airway	Own airway, 16/16 (100%)
Imaging used to detect the lesion	4/26 (15%) of patients undergoing Cryo-Radial procedure had fluoroscopy	16/16 (100%) had a repeat CT of the chest
No passes done of the lesion	No. biopsies done using cryo-probe: 1 pass (6/16), 2 passes (9/16) and 3 passes (1/16) for cryo-biopsy Out of the six patients who had one pass, four did not yield diagnostic material. Only 2/6 (33%) had a successful diagnosis All patients having two or more passes had a successful diagnosis 10/10 (100%)	No. passes done: CT-guided biopsy, 2 passes (1/15), 3 passes (10/15), 4 passes (2/15) and 5 passes (1/15).
Time taken for the procedure	49.8 (SD 17.9) min, $P < 0.01$ Paired <i>t</i> test	16.1 (SD 17.6) min

Cryo-Radial procedure was 49.8 min (SD 17.9 min) (Table 3). The mean number of passes were: radial forceps, 4 ($n = 11$); brush, 3 ($n = 16$); needle aspirate, 3 ($n = 7$); and cryo-biopsy, 2 ($n = 13$) (Table 5).

Of the 20 patients who had a PPL located during the R-EBUS procedure, cryobiopsy could not be performed in four due to the inability to advance the stiff cryo-probe into tight-fitting GS, but a diagnosis was made on conventional R-EBUS sampling (forceps, $n = 1$; brush, $n = 1$; or needle aspirate, $n = 2$). The Cryo-Radial procedure was diagnostic in 17/20 (85%). There were 10 malignancies and seven benign PPL (Table 4).

The PPL in the three non-diagnostic patients were eccentrically placed and were smaller in size (mean 21 mm). Following PET-CT, one was treated on suspicion for organising pneumonia and one proceeded to target radiotherapy on high suspicion of malignancy without further biopsy. One had a confirmed adenocarcinoma on CT-TTB.

Five (38%) of 16 patients obtained their diagnosis from cryobiopsy alone. In this group, three had malignancies and two were non-malignant (vasculitis and eosinophilic inflammation). The size of the PPL was smaller (mean 27 mm) in this group. Two out of five were located eccentrically and 3/5 were in the outer third of the lung parenchyma.

Comparing diagnostic yield between Cryo-Radial and CT-TTB

The overall diagnostic yield from Cryo-Radial group was 17/20 (85%), in comparison with 15/16 (93.8%) in the CT-TTB arm. The difference was not statistically significant: $P = 0.61$; OR 0.37 (95% CI 0.006–5.4). In comparing a diagnosis of malignancy, the Cryo-Radial group had 10/12 (83%) malignancies diagnosed in comparison with 13/14 (93%) in CT-TTB ($P = 0.33$).

Ability to perform EGFR between Cryo-Radial and CT-TTB

Seven (70%) out of 10 malignancies in the Cryo-Radial and 11/14 (78%) in the CT-TTB arm were suitable for EGFR testing. Odds ratio 0.63 (95% CI 0.06–6.2), $P = 0.66$. All patients with a diagnosis of malignancy made on cryobiopsy had adequate tissue for EGFR testing.

Adverse events

Pneumothorax

Seven CT-TTB patients sustained a pneumothorax with two requiring ICT and unplanned hospital admissions (Grade 3), with an average of 2.5 days in hospital. One

Table 4 Comparing the diagnostic yield between Cryo-Radial and CT Bx arms

	Cryo-Radial	CT BX	P-value
Making a diagnosis per protocol	17/20 (85%)	15/16 (93.8%)	$P = 0.61$ Odds ratio 0.37 (95% CI 0.006–5.4)
Diagnosis (ITT) made	21/26 (81%)	15/22 (68.2%)	$P = 0.056$
No	4/26 (15.4%)	1/22 (4.5%)	Odds ratio
Not done	1/26 (3.8%)	6/22 (27.3%)	(95% CI) 0.5–7.3
Suitability for EGFR-suitable	7/10 (70%)	11/14 (78%)	$P = 0.66$
Borderline/not suitable	3/10 (30%)	3/14 (22%)	Odds ratio (95% CI 0.06–6.2)
Diagnosis made	Malignancy, $n = 10$ (adenocarcinoma 7, squamous cell cancer 1, carcinoid 2) Benign, $n = 7$ (vasculitis 1, organising pneumonia 3, eosinophilic and neutrophilic inflammation 3)		Malignancy, $n = 14$ (adenocarcinoma 9, squamous cell cancer 5) Benign, $n = 1$ (granuloma)

Table 5 Diagnostic yield from individual biopsy tools in the Cryo-Radial arm

	Cryobiopsy	Forceps biopsy	Cytology brush	Needle aspirate
Diagnostic yield	13/16 (81.2%)	7/11 (63%)	9/16 (56.2%)	5/7 (71%)
No. passes, mean (range)	2 (1–3)	4 (2–5)	3 (1–3)	3 (1–3)
Overall diagnostic yield of the Cryo-Radial arm with multimodal biopsy: 17/20 (85%)				

Table 6 Adverse events

Adverse events (CTCAE criteria)	Cryo-Radial Arm	CT arm	P-value
Pneumothorax: total (Grades 1–3)	1/24 (4.2%)	7/16 (44%)	0.004*
Pneumothorax: Grade 3	0/24	2/16 (12.5%)	0.154
Haemorrhage: Grade 1	4/24 (16%)	5/16 (31%)	0.126
Haemorrhage: Grade 2	1/20 (5%)	0/16	0.56

*indicates significant P-value

The Common Terminology Criteria for Adverse events (CTCAE) was used to define adverse events.

Pneumothorax was defined as Grade 1 Asymptomatic: Clinical or diagnostic observation; no intervention required), Grade 2 (Symptomatic intervention required), or Grade 3 (Symptomatic intervention and hospital admission), Grade 4 (Life threatening urgent treatment), Grade 5 (Death).

Haemorrhage was defined as Grade 1 (Mild symptoms, intervention not required), Grade 2 (Symptomatic, requiring medical intervention), Grade 3, Transfusion and operative (endoscopic or operative), Grade 4 Life-threatening, Grade 5 (Death).

patient in CT-TTB who sustained a pneumothorax developed subcutaneous emphysema (Table 6).

The Cryo-Radial arm had one patient with a small pneumothorax managed conservatively (Grade 1). The pneumothorax rate between CT-TTB versus Cryo-Radial was 7/16 (44%) versus 1/25 (4.4%) ($P = 0.004$). In total, 2/16 (12.5%) patients with pneumothorax in the CT-TTB arm needed an ICT vs 0% (Cryo-Radial), $P = 0.154$.

Haemorrhage

Five (31%) of 16 patients in the CT-TTB arm and 4/18 (22%) in the bronchoscopic arm had Grade 1 bleeding, $P = 0.126$. In the Cryo-Radial arm, one patient had a Grade 2 haemorrhage, and did not have a prophylactic bronchial blocker. Nineteen (79%) of 24 patients had a prophylactic bronchial blocker placed in the Cryo-Radial arm. No other adverse events were recorded in either arm.

Discussion

While CT-TTB is the most widely employed diagnostic tool for PPL due to the high diagnostic yield, the associated high pneumothorax rate and inability to diagnose and stage lung cancer in a single procedure have set clinicians on the path to investigate alternative diagnostic modalities that can overcome these limitations.

In the present study, the combined diagnostic yield from R-EBUS using cryobiopsy with conventional sampling was 85%, compared with 93.8% in the CT-TTB arm. The difference was not statistically significant, suggesting the diagnostic yield of Cryo-Radial is comparable with CT-TTB. However, our study was terminated early due to poor recruitment and was underpowered to draw definite conclusions and demonstrate exploratory data of this favourable outcome.

The PPL size in the present study had a mean of 36.1 mm. This larger PPL size is a reflection of the

patients presenting late, as the recruitment centres were located in markedly lower socioeconomic areas with a large migrant population.

The number of R-EBUS cryobiopsy studies within the literature remains small. Our diagnostic yield for Cryo-Radial is comparable with international studies, with the diagnostic yield between 74.2%¹⁴ and 86.1%¹¹ in similar PPL size (29 and 37 mm) to ours (36 mm). A recent meta-analysis of nine studies demonstrated a yield of 77% (CI 71%–84%), which is lower than our study and that by Imabayashi *et al.*¹¹ Included in the meta-analysis were studies that did not use GS,¹⁵ which may partly explain the lower diagnostic yield.

In our study, cryobiopsy produced the sole diagnosis in 31% patients, in keeping with other studies demonstrating similar yield.¹¹ The additional diagnostic yield from cryobiopsy maybe due to the larger size of the samples, less crush artefact¹⁴ and the high integrity of deoxyribonucleic acid enabling better mutation analysis¹⁵ in comparison with forceps biopsies. The ability to successfully biopsy eccentric lesions is another advantage.¹⁴

Our study demonstrated a higher diagnostic yield with the combined Cryo-Radial sampling in comparison with cryobiopsy alone (85% vs 81%), which was also demonstrated by Udagwa *et al.*, strengthening the rationale for combining cryobiopsy with R-EBUS standard sampling to improve the diagnostic yield.

In published studies, R-EBUS conventional biopsies have demonstrated an 80% success rate in performing molecular testing¹⁶ and CT-TTB, 18–20G core needle sample was capable of EGFR mutation analysis in 16/18 (88%) cases.¹⁷ This was similar in our study and both the Cryo-Radial (70%) and CCT-TTB groups (78%) were not statistically significant. All positive cryobiopsy samples for malignancy in our study were capable of EGFR testing.

The American College of Chest Physicians guidelines recommend R-EBUS³ as an initial diagnostic procedure for PPL due to its safety profile. This safety profile is seen in

Cryo-Radial for PPL as well, with both Schumann *et al.* ($n = 31$)¹⁴ and Imbayashi *et al.* ($n = 35$)¹¹ demonstrating no pneumothorax and 3% moderate bleeding. Both studies used a bronchial blocker and freezing times (2–5 s), similar to our study of 4 s freeze time. The Cryo-Radial data in our study demonstrated a 4.2% Grade 1 pneumothorax and 4.2% Grade 2 haemorrhage without any major side-effects and were similar to the published studies.

The potential for haemorrhage was a major concern for the Cryo-Radial procedure, extrapolating from the interstitial lung disease (ILD) cryobiopsy data where ILD cryobiopsies record a moderate/severe bleeding of 12% and pneumothorax of 39% in a meta-analysis of 11 studies, including 731 patients.¹⁸ This study adds to the growing body of safety data in Cryo-Radial for PPL that demonstrates a pivotal difference from the ILD cryobiopsy data demonstrating improved safety in Cryo-Radial for PPL. The consistent use of the GS and a bronchial blocker improved the safety in our study. The only published meta-analysis on the Cryo-Radial procedure demonstrated a major side-effect rate of 1.8%,¹² highlighting the favourable safety profile. Interestingly, in Radial-Cryo for PPL, studies demonstrated a higher bleeding risk with higher freezing times,¹¹ while the number of biopsies (up to three biopsies) has not been associated with increased bleeding risk.¹¹

The pneumothorax rate was significantly higher in the CT-TTB group in our study at 44%. This high rate was within the confidence interval of published meta-analysis⁴ and may be due to capturing all Grade 1 pneumothoraxes on chest X-ray as well as detecting delayed pneumothorax diagnosed during the nurse call out. The overall chest drain requirement (Grade 3) was 2/16 (12.5%), in keeping with the published meta-analysis,⁵ as well as the study data from one participating institution.¹⁹

There is a significant difference in the rate of pneumothorax and the unexpected admission rates between the two arms favouring Cryo-Radial arm.

Delay in staging procedures is a well known factor for treatment delays in lung cancer.² In this study, 7/25 (28%) had a concurrent linear EBUS performed for staging, prior to the Cryo-Radial procedure, and 1/7 (14.2%) had a malignancy diagnosed, despite not having size significant lymphadenopathy. Therefore, concurrent procedures may reduce treatment delays.²⁰

Limitations

Protocol limitations

The early termination of the study reduced the power of this study to detect a true difference between the study arms.

This study used randomisation to allocate the biopsy method to each PPL, without considering the PPL or patient characteristics to avoid bias in decision-making, which may have contributed to the poor recruitment. A multidisciplinary discussion may have aided in a more pragmatic allocation, but could add significance bias, depending on the skill set and experience of the team.

Our protocol excluding patients with size significant mediastinal adenopathy, which automatically excluded centrally located PPL. Over >50% of PPL in our study were in the outer 1/3 of the CT chest and only 60% had air bronchograms. While this is favourable for CT-TTB,²¹ it was unfavourable for Cryo-Radial, as more central lesions with air bronchograms have higher yield with R-EBUS.⁶

At the time of designing this study in the New Zealand setting, EGFR mutation was the only available biomarker and this study is limited to EGFR analysis.

Technical limitations

The major difficulty during the Cryo-Radial procedure was the stiff reusable 1.9 mm cryoprobe that was a tight fit to the 2.6 mm R-EBUS GS and difficult manoeuvrability, especially in the upper lobes. This has been cited as a major drawback in other studies as well.¹¹ Due to the same reason, the number of cryobiopsy passes were limited to a mean of two in our study. Schuhmann *et al.*¹⁴ demonstrated the yield of cryobiopsy improves with the number of passes with the first pass 61% to second pass 71% and third pass 74%. The newly available single use flexible cryoprobe had eliminated the technical difficulties encountered during this study with resultant higher diagnostic yield.²²

This exploratory study, while demonstrating comparable diagnostic yield, better safety profile and added advantage of staging concurrently with a Cryo-Radial bronchoscopy compared with CT-TTB, also provides valuable information on recruitability and pitfalls in study design, especially randomisation, to aid future studies.

Conclusion

The main limitation as explained above in this exploratory study, was the slow recruitment resulting in early termination of the study that lead to the smaller sample size. Within these limitations, compared to CT-TTB, Cryo-Radial is able to provide a satisfactory diagnostic yield and ability to test for EGFR. Addition of cryobiopsy improves the diagnostic yield of R-EBUS conventional sampling alone.

We recommend at least two cryobiopsy passes and addition of at least one R-EBUS biopsy methods through the GS to improve the Cryo-Radial diagnostic yield. With the use of GS, Fogarty size four bronchial blocker, and

rigid bronchoscopy by an experienced interventional bronchoscopist, the control of bleeding was excellent. A 4-s freezing time developed a balance between adequacy of tissue and side effect profile.

The Cryo-Radial Procedure is more resource and skill intensive, compared to the CT-TTB method. However, this was offset by the markedly reduced side effect profile. Being able to perform a combined Linear and Radial-EBUS study as a single procedure, which may expedite treatment is another advantage.²⁰

Cryo-Radial procedure is a promising tool as a first line diagnostic method for a PPL suspected of lung cancer.³

Clinical trial registration

[clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02395939): NCT02395939.

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Data availability statement

Data collected during this study will be available through email: info@sydneyrespiratoryspecialist.com.au, after approval of the proposal with signed data access agreement for investigator-initiated studies, on deidentified participant data.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Appendix S1 Data collection sheet for pathology.

6.2 Discussion of CT-CROP study

6.2.1 Significance of the study

In the study results, CT-TTB had a diagnostic yield of 93.8% and the cryo-radial arm had a diagnostic yield of 85%. The cryo-radial arm did not have a higher yield than CT-TTB as expected despite optimising biopsy tools and the addition of cryobiopsy. This was not statistically significant, keeping in mind the early termination of the study, resultant loss of power and the Type 2 error. Although there were multiple RCT comparing the Radial EBUS procedure (with conventional biopsy only) to CT-TTB (Fielding et al., 2012; Fu et al., 2021; Steinfort et al., 2011), no study had compared CT-TTB with the cryobiopsy Radial EBUS procedure, therefore, this is an original contribution to existing knowledge.

6.2.2 Salient results and comparison to existing evidence

The cryo-radial procedure for PPL has limited data with only 12 studies (Sryma et al., 2021; Tanaka et al., 2021). Each single arm study in this meta-analysis consisted of up to 55 patients. Of recent, a retrospective study of 1024 patients published by Herth et al. (2021) is the largest patient cohort on the use of cryobiopsy in PPL. There were only five prospective studies on cryobiopsy in PPL. The CT-CROP study adds to the growing body of data prospectively in this novel procedure for PPLs.

The combined cryo-radial procedure demonstrated an improved diagnostic yield of 85% (PPL size 36mm) with freezing time of four seconds in this CT-CROP study, compared to using Radial EBUS alone with forceps and brush, as demonstrated by Steinfort et al. in their 2011 meta-analysis research for PPL >20mm, which resulted in a diagnostic yield of 77.7%. This was in keeping with papers published by Schuhmann et al. (2014) demonstrating a diagnostic yield of 74%, Imabayashi et al. (2019) diagnostic yield of 86%, Arimura et al. (2019) diagnostic yield of 87%, and Nasu et al. (2019) diagnostic yield of 81% with similar PPL sizes of 29-37mm and freezing time of 2-5 seconds, using the similar 1.9mm, 1150mm reusable cryoprobe. Herth et al. (2021) had a diagnostic yield of 91% with a freeze time of 3-5 seconds with 3-5 passes.

However, other studies have produced a worse diagnostic yield than the CT-CROP study. The likely reason for this lower diagnostic yield is explained below. Hibare et al. (2017) who also used a 4-second freezing time, resulted in a lower yield of 67.3%. In the Hibare et al. study, the size of PPL was not recorded in 14/55 patients due to the CT chest performed in another institution, which may have indicated inadequate data for the planning process, therefore, demonstrating the pivotal role of planning a bronchoscopic pathway to the PPL in this procedure.

Another study with a lower diagnostic yield was Kho et al. (2019) who also used a 3-4 second freezing time with a 34.8mm PPL similar to CT-CROP, however, the diagnostic yield was 67.5%. This group used the large Radial EBUS GS only in 57.9% of cases. However, only 36.8% of PPL were concentrically located in comparison to the CT-CROP study of 50%. These factors may explain the lower yield (Kho et al., 2019). The higher diagnostic yield associated with the use of the GS was well documented by Nasu et al. (2019) who demonstrated a 95.8% diagnostic yield with the GS compared to 69% without. However, the group had a positive bronchus sign in 92% of cases, which may also contribute to this diagnostic yield.

One of the major drawbacks in the CT-CROP study was the stiff cryoprobe that resulted in difficulty in manoeuvring to the upper lobes. This may have reduced the diagnostic yield of the CT-CROP study. Imabayashi et al. (2019) and Arimura et al. (2019) also documented the stiff cryoprobe causing a major technical difficulty and a challenge in manoeuvring to the upper lobes, similar to the CT-CROP study. In the CT-CROP study, the stiff cryoprobe could not be advanced through the GS to upper lobar locations in four patients, resulting in a lower diagnostic yield. With the most recent development of the flexible single use cryoprobe, this technical difficulty was overcome. As expected, the first published paper by Tanaka et al. (2021) on using this reusable 1.7mm cryobiopsy probe in 50 patients, resulting in a diagnostic yield of 94% (83.5-98.8%) in PPLs sized <30mm with a freezing time of three seconds. This introduced a remarkable step towards improving the diagnostic yield of the cryo-radial procedure. The study used Radial EBUS and fluoroscopy alone and did not require expensive equipment, such as EMN or VBN (Tanaka et al., 2022).

The number of cryobiopsy passes also improves the diagnostic yield. Schuhmann et al. (2014) demonstrated an increased yield of up to 74% with three passes. In the CT-CROP study, due to the stiff cryoprobe, the mean number of cryobiopsy passes was two; this may have reduced the diagnostic yield.

Cryobiopsy use in benign PPL: The ability to diagnose benign lesions was also high with cryobiopsy with all benign PPLs in the CT-CROP study having a diagnosis made on cryobiopsy. The ability for cryobiopsy to diagnose benign PPLs via Radial EBUS has not been widely published.

Use of cryobiopsy in small PPL: The use of cryobiopsy in small PPLs is limited. Triton et al. (2018) demonstrated that in small PPLs <20mm (16mm+/3mm) the diagnostic yield of cryobiopsy was 69%. This study used EMN with the cryoprobe and fluoroscopy, with five forceps biopsies and two cryobiopsies used for each patient. It was noted that cryobiopsy had twice as large

samples compared to forceps biopsy with a freezing time of 7-8 seconds. This promising result presents cryobiopsy to be useful in small lung nodules.

6.2.3 Ability to perform EGFR testing

In the CT-CROP study, the ability to perform EGFR testing from all modalities of sampling was comparable, particularly for cryo-radial (70%) and CT-TTB (78%). These results were in keeping with published data on CT-TTB, demonstrating up to 88% success in EGFR testing (Solomon et al., 2010) and up to 80% in Radial EBUS data (Guisier et al., 2016). Only a few studies have reported on the ability of molecular testing in cryobiopsy samples for PPLs. In terms of EGFR testing for cryobiopsy, in the CT-CROP study, all malignant samples in the cryobiopsy arm were suitable for EGFR testing. This was similar to Imabayashi et al. (2019) who noted that all 26 patients diagnosed with lung cancer with cryobiopsy had the required molecular and IHC tests.

Udagawa et al. (2020) compared the ability to perform molecular testing with cryobiopsy samples with Radial EBUS forceps samples, noting that the forceps sample size was 2mm and the cryobiopsy size was 15mm. They also commented that cryobiopsy had a better intact DNA and RNA for sequencing and hence was a better sample for molecular testing. The Udagawa study supports our study in that cryobiopsies are well suited for molecular testing.

Arimura et al. (2019) reported on 23 patients with a PPL mean size of 36mm, demonstrating a diagnostic yield of 87%. They also indicated a much larger volume with cryobiopsy samples compared to forceps biopsy. With cryobiopsy, DNA analysis was performed on all cancer patients in this study, including EGFR and PD-L1.

6.2.4 Use of multi-modality biopsy techniques

In the CT-CROP study, five patients from a total of 20 who had the cryo-radial procedure received a diagnosis from cryobiopsy only. Four that did not have a diagnosis made on cryobiopsy had the diagnosis made on various Radial EBUS biopsies (n=1 forceps; n=1 brush; n=2 needle aspirate). In a retrospective analysis of 35 patients, Imabayashi et al. (2019) demonstrated that three cases of malignancy were diagnosed with forceps and brush that were negative in the cryobiopsy sample, therefore, confirming the utility of multi-modal biopsies, similar to the CT-CROP study. Hibare et al. (2017) also had 2/55 patients diagnosed with cryobiopsy alone whilst 4/55 were diagnosed via Radial EBUS forceps alone. The CT-CROP study adds to this growing body of evidence, demonstrating the benefit of combining different biopsy tools via Radial EBUS as a “multi-modal” biopsy.

6.2.5 Safety profile

Pneumothorax: In the CT-CROP study, the pneumothorax rate of CT-TTB was 44% and the chest drain rate was 12.5%. There were 2/16 unanticipated admissions to hospital, averaging 2.5 days length of stay.

In comparison, the safety of the cryo-radial arm was excellent with only one moderate bleed controlled with tranexamic acid; there was only one pneumothorax (4%) and hospital admission was not required. All patients were discharged on the same day as planned. However, although high, the recorded pneumothorax rate was within the parameters of the confidence interval for the CT-TTB meta-analysis (DiBardino et al., 2015). The high rate recorded may have been due to identifying pneumothorax in asymptomatic patients, as all patients received a CXR irrespective of symptomatology at the one-hour post-procedure. The chest drain insertion rate was similar to the previous published literature from one of the participating institutions (Barnard, 2013).

The occurrence of pneumothorax was infrequent (4.4%) with the cryo-radial procedure in the CT-CROP study. Schuhmann et al. (2014), Taton et al. (2018), Arimura et al. (2019) and Kho et al. (2019) all experienced no pneumothorax; Imabayashi et al. had a 2/36 (5.5%) pneumothorax, which was managed conservatively. Therefore, the CT-CROP study data is in keeping with others and confirms the low risk of pneumothorax, and adds to the body of data, demonstrating the safety of cryo-radial procedures in PPLs.

Bleeding risk: With correct equipment (bronchial blocker, ETT or rigid bronchoscopy, general anaesthesia and GS), an experienced team and a trained interventionalist, the cryo-radial procedure was performed safely. In the CT-CROP study, using a bronchial blocker prophylactically was the main factor preventing a significant bleed and containing the bleed to a segment. There was one moderate bleed recorded that required tranexamic acid where the bronchial blocker was not used due to the patient's excessive coughing. A second bronchoscopist was on standby to attend to the patient as soon as the biopsy was performed in a two scope approach. In the event of a bronchial blocker becoming dislodged or failing to inflate, the second bronchoscopist would suction the blood and control the bleed without delay. This technique was also adapted by Tanaka et al. (2021) who used a second bronchoscopist, similar to the CT-CROP study, but without the use of a bronchial blocker. Naki et al. (2022) adopted a similar two-scope approach as another method that has gained popularity due to the ease of use when compared with the bronchial blocker method for PPLs.

Using a prophylactic blocker is a predictable method in controlling bleed. For a biopsy of a PPL, a small bronchial blocker could be placed in the adjacent segment to which the PPL is located.

This is adopted to prevent the dislodging of the bronchial blocker that would occur if the bronchial blocker and the cryoprobe are both inserted to the same segment. In the CT-CROP study, the overall bleeding risk was 4.4%.

Currently, there is no data in the literature about the use of cryobiopsy for tumours with a high risk of bleeding and this is an original contribution to knowledge.

6.2.6 Discussion on managing bleeding risk in PPL during cryobiopsy

As mentioned in the methodology section of this thesis, the routine practice during biopsy of PPL was to position the radial ultrasound probe in an optimal position away from vascularity. With cryobiopsy procedures, this aspect was paid more attention; the ultrasound was deliberately placed away from blood vessels that were noted as 'hypoechoic areas'. The resultant safe biopsy was maybe partly due to the localisation of a safer avascular area to biopsy using the radial ultrasound.

During the research in 2015-2018, placement of the ultrasound probe in the least vascular area was employed routinely based on knowledge obtained from linear EBUS imaging, however, the degree of importance of an ultrasound to avoid vascularity was not emphasised. This was appreciated during the study after increased experience with the techniques of Radial EBUS and cryobiopsy. Therefore, this ultrasound feature can be utilised as a safety mechanism for future procedures.

Other factors involved included the use of a bronchial blocker and the choice of freezing time, as discussed below. It must be noted that the freezing time is dependent on the probe size. For the CT-CROP study, a 1.9mm probe with a 4-second freezing time is a good balance between an adequate sample and the risk of bleeding. With the newer 1.7mm and 1.1mm probes, their respective best freezing times need to be explored in future studies.

However, there are studies reporting higher bleeding risk with the cryo-radial procedure, compared to the CT-CROP study. Therefore, an analysis was performed to determine the reasons.

In Naki et al. (2022), a PPL measuring 28mm with 3-6 second freezing time using a 2.4mm cryoprobe produced a moderate bleeding risk of 18%, which was much higher than in the CT-CROP study. Another reason for the higher bleeding risk may be due to the smaller sized PPL (28mm) compared to the PPL in the CT-CROP study (36mm), as the high freeze time of six seconds could also freeze beyond the small PPL and damage the adjacent lung parenchyma (Naki,

et al., 2022). Furthermore, the cryobiopsy probe was larger at 2.4mm compared to the 1.9mm cryoprobe used in the CT-CROP study.

During the CT-CROP study, after the freezing time of four seconds, the GS and cryoprobe had to be removed at the same time because the larger size tissue sample could not be withdrawn through the GS. If the GS could be kept in place to obtain a sample, for example, by using the smaller 1.1mm cryoprobe, then the bleeding risk may be reduced. With case studies already published on this method (Herath, 2023), this is an area that requires further investigation.

General anaesthesia is useful as it enables the use of ETT or a rigid bronchoscopy to protect the airway of the patient, as well as assists in keeping the bronchial blocker in place to enable adequate control of the patient's cough. In the CT-CROP study, the bronchial blocker was used in 18/20 (90%) patients. Imabayashi et al. (2019) also used a blocker with satisfactory success with only a single moderate bleed, similar to the CT-CROP study 1/35 (3%). However, Imabayashi et al. (2019) had 24/36 (66%) mild bleeds, possibly due to not using the bronchial blocker constantly and only used in 7/36 (19.4%). Taton et al. (2018) also used a bronchial blocker, rigid bronchoscopy, general anaesthesia and the GS, similar to the CT-CROP study; there was no severe bleeding. However, there was moderate bleed which occurred in 14% of patients, which was much higher than the CT-CROP study. It could be explained by the higher freezing time of 7-8 seconds for the small sized PPL at 20mm in Taton et al. (2018). Kho et al. (2019) also used a prophylactic bronchial blocker with 3-4 second freeze time but did not demonstrate any moderate or severe bleeding. In comparison, a more recent study by Tanaka et al. (2021) demonstrated the use of the new flexible cryoprobe without using a bronchial blocker. There was a much higher rate of moderate bleed at 62%, therefore, the bronchial blocker was a highly recommended method used in the CT-CROP study.

Freezing time also affects bleeding risk, which was previously demonstrated by Imabayashi et al. (2019). Their research indicated a significant increase in the bleeding risk when the freezing time was increased from two to five seconds. This was also noted in the Taton et al. (2018) study, comprising of 32 patients with a freeze time of 7-8 seconds. The result was moderate 4/32 (12.5%) bleed, higher than CT-CROP study and that of Imabayashi et al. (2019) (mean freeze time 3 seconds) and Schuhmann et al. (2014) (mean freeze time 4 seconds); all studies had a freezing time below four seconds.

A longer freezing time may not be required for malignant lesions, as opposed to biopsies in interstitial lung disease patients where larger tissue samples are required to ascertain the tissue architecture.

This safety aspect is highlighted again in a meta-analysis of nine studies of cryobiopsy demonstrating a major complication rate of only 1.8% (Sryma et al., 2020). These findings were also confirmed by the retrospective analysis by Herth et al. (2021) demonstrating a 3.5% bleeding risk and 6.3% pneumothorax risk in 1024 patients.

6.2.7 Implications of the CT-CROP study

This is the first study that compares CT-TTB with the cryo-radial procedure and is an original contribution to knowledge. It demonstrates a satisfactory diagnostic yield, although not higher than that of CT-TTB, as well as a better safety profile that will provide clinicians with greater confidence in allocating a cryo-radial procedure as the first diagnostic technique to patients who present with a PPL. Other researchers will be able build on the study's methodology and refine the procedure. The novel arrival of the flexible cryoprobe used in a study by Tanaka et al. (2021) demonstrated a higher success rate by removing the technical error of the stiffness of the cryoprobe, identified in this study. This study has demonstrated the usefulness of multi-modal biopsies, demonstrating that when four patients had a negative cryobiopsy, they received a diagnosis from a different Radial EBUS biopsy, and five patients received a diagnosis from cryobiopsy alone.

The study demonstrates that the safety profile of Radial EBUS continues to be present even when cryobiopsy is added to the procedure. Thus giving confidence to the clinicians that cryo-radial is a safe procedure, even in high-bleeding risk tumours when the correct safeguards are used and performed by a skilled proceduralist.

The duration of performing the cryobiopsy procedure was longer than a Radial EBUS with conventional biopsy alone. In the CT-CROP study, 49.8 (SD 17.9) minutes were reported, similar to the Kho et al. (2019) time of 45min (35-60min). Schuhmann et al. (2014) also commented on this longer time required for cryobiopsy, due to the need to reposition the blocker, GS and Radial EBUS after each biopsy. Yet, despite the procedure being prolonged, the high safety profile and the ability to combine with L-EBUS for staging as a concurrent procedure improve treatment delays, as well reduce the number of procedures required for the diagnosis and staging.

To add cryobiopsy to the Radial EBUS procedure without losing the high safety profile of Radial EBUS requires preparation. Any clinician planning on introducing cryo-radial in their unit will need to understand the additional equipment required if bleeding occurs, such as a bronchial blocker and rigid bronchoscopy or ETT. There is additional cost involved, as identified in this study, including the need for anaesthetic support including anaesthetic nurse and equipment as

well as consumables like bronchial blocker to name a few. The importance of the proceduralist skill, is highlighted. Although the proceduralists involved in this study were veterans in bronchoscopy, the cryobiopsy procedure was new to Australia and New Zealand, and proceduralist required upskilling. Therefore, there is a longer learning curve due to multiple equipment required.

6.2.8 Limitations of the CT-CROP study

The main limitation of this study is the Type 2 error that was caused by early termination of the study. This study was originally planned for 158 patients to be recruited with a pre-planned analysis at one-quarter, due to having only a single previously published feasibility study on cryobiopsy in PPL (Schuhmann et al., 2014).

From the initiation stage of the study, the referral rate was slow, as detailed in Chapter 3 (Methodology) and in the *Internal Medical Journal* paper (above). This was due to a combination of factors. The study protocol excluded patients with nodal disease, and since the study was recruiting patients from low socioeconomic background and late presentations, the number of suitable patients were limited. Furthermore, the protocol mandated randomisation of patients irrespective of PPL location or patient characteristics (e.g. presenting with emphysema). Adhering to the strong randomisation principal was the strength of this study. Yet, one could say it was also the demise of the study as a group of referring clinicians believed the randomisation should have included an MDT discussion and therefore, did not refer to the study, which meant that only a smaller number of clinicians were referring their patients.

Another drawback was the selection bias that occurred after randomisation, which resulted on patients not undergoing the procedure they were randomised to. As mentioned previously, following a lengthy debate, randomisation was performed without considering the PPL or patient characteristics.

Therefore, once allocated to the CT-TTB arm, for 3/22 patients in the CT-guided biopsy arm, the radiologist decided that the biopsy was technically difficult. This difficulty could be caused by a host of factors, including: (i) PPL is located close to fissures; (ii) CT-TTB needle requiring traverse multiple fissures to reach the biopsy; (iii) PPL is in close proximity to the blood vessel; (iv) patient characteristics; (v) PPL is located in the emphysematous lungs; (vi) patient presents with joint/mobility issues therefore cannot be placed in an optimal position; and (vii) patient cannot follow commands to hold their breath for the biopsy.

Similarly, 1/26 in the cryo-radial arm did not undergo their allocated biopsy because the PPL was a semi-solid lesion located in the outer 1/3 of the lung parenchyma. Compared to CT-TTB, more patients can proceed to cryo-radial biopsy, yet upper lobar locations and peripherally placed PPL are more challenging for cryo-radial biopsies, therefore, decisions need to be made on a case-by-case basis.

This selection bias post-randomisation would have been avoided if the initial randomisation was performed more pragmatically by taking into account patient and PPL characteristics via an MDT approach. However, at the time of this study design in 2015, the MDT concept in New Zealand was novel and Radial EBUS was a new tool that many were unfamiliar with. However, in the present-day environment, discussions at interventional bronchoscopy meetings would be an excellent way to facilitate this type of decision-making and to provide pragmatic randomisation protocols.

Therefore, an intention-to-treat analysis (although planned) was not performed due to this phenomenon of patients falling into a biopsy procedure that they were clinically not indicated. Only a per-protocol analysis was performed that compared patients who had actually undergone the procedure.

Added to this, during this time in New Zealand, all bronchoscopy procedures were performed under sedation. As the CT-CROP study required operating theatres and anaesthetic support, some clinicians believed that waiting for a theatre facility may delay the patient unnecessarily, resulting in not referring patients. In essence, the study was innovative, but the infrastructure was not available. In noting the low recruitment, an attempt was made to involve more sites to alleviate the issue. This resulted only in a minor improvement as each participating centre required submission of a full ethics approval which delayed commencement of the study and hindered other centres from participating as they did not wish to pursue a full ethics application process.

Therefore, the interim analysis at 25% recruitment took almost three years. During these three years, multiple single arm studies on cryobiopsy had emerged, demonstrating the successful use of cryobiopsy in PPL in clinical practice (Taton et al., 2018).

Despite the early termination and lower number of patients, this is the only study to date that compares the gold standard CT-TTB with the cryo-radial procedure. Whilst the CT-CROP study did not show the diagnostic yield to be as high as CT-TTB, the procedure was significantly safer with fewer pneumothoraxes and bleeding. Therefore, it allows the clinician an alternative diagnostic path to CT-TTB for PPLs, especially in a high risk location (near a vessel, or when

there is significant emphysema present); it also allows the clinician to weigh up the most appropriate diagnostic path to be taken.

A pragmatic MDT approach would have been better suited to take into context the PPL, patient, and resources of the institution when choosing a biopsy method. Yet, even this method would have not provided a perfect result as MDT decisions vary vastly depending on the skill and exposure of the group that convenes.

There was a major technical difficulty associated with the stiff cryoprobe and tight-fitting GS, which resulted in a reduced yield as 4/20 patients in our study could not have their cryobiopsy performed. The new cryoprobe introduced into clinical practice alleviated the problem by using 1.1mm and 1.7mm probes (Tanaka et al., 2021). Using ROSE constantly with imprint cytology for cryobiopsy may have improved the diagnostic yield, as demonstrated by Imabayashi et al. (2019). Arimura et al. (2019) demonstrated a 70% sensitivity in the diagnostic yield when using ROSE. Not using ROSE for all cryo-radial procedures was a drawback to the CT-CROP study.

Schuhmann et al. (2014) used a freezing time of four seconds, demonstrating that cryobiopsy when compared with forceps biopsy had three times larger tissue samples. Taton et al. (2018) demonstrated cryobiopsy to be twice as large as forceps biopsy with a 7-8 second freezing time in small PPLs <20mm. In the CT-CROP study, measuring the sizes of the forceps and cryobiopsy was not in the protocol, therefore, this drawback resulted in valuable loss of comparative data.

One major obstacle in the CT-CROP study to perform the cryo-radial procedure was the availability of equipment and trained staff. This lack of generalisability and limitation on tertiary centres was a drawback. Despite being able to substitute the rigid bronchoscopy for an ETT, the cryo-radial still required large number of equipment and consumables in comparison to Radial EBUS biopsy. Also, many equipment and consumables were required to ensure the safety of the procedure, which was also not generalisable to all units.

6.3 Future directions

6.3.1 Practical aspect

Well-trained staff, airway protection and skilled interventionalists would be an absolute must to ensure the safe performance of a cryo-radial procedure. The use of a rigid bronchoscopy was my preference, also shared by Taton et al. (2018) and Schuhmann et al. (2014). The procedure can also be performed using an ETT and inserting a bronchial blocker outside the ETT, however, it becomes difficult to manoeuvre the bronchial blocker to the required location compared to using

the blocker with rigid bronchoscopy. Using a prophylactic blocker is highly recommended and a dedicated assistant is needed to hold the blocker in place. In terms of safety, having a unit stocked with emergency equipment for bleeding and pneumothorax is essential, as well as role play and advance life support skill training.

In considering the type of bronchial blocker for use in the CT-CROP study, a Fogarty blocker size 4-5 was used whereas the Cryo-feasibility study used the Arndt 7. The Fogarty blocker can be better tolerated because it is smaller in size. Imabayashi et al. (2019) used the Arndt 5 or Arndt 7 French, therefore, this option is also acceptable. Taton et al. (2018) used a Fogarty blocker when performing cryobiopsies for PPLs <20mm. The smaller size of the Fogarty blocker is useful in securing the blocker at a segmental level, which contains a bleed effectively. The freezing time of 3-4 seconds seemed to balance the bleeding with the effective sample, which is in keeping with Schuhmann et al. (2014), the CT-CROP study (4 seconds) and the more recent Tanaka et al. (2021) study (3 seconds).

The single use cryoprobe is now available and studies have already demonstrated a high diagnostic yield of the flexible 1.7mm cryoprobe (Tanaka et al., 2021). A successful application of the smaller 1.1mm cryoprobe with the small GS is eagerly awaited in future studies. In the era of lung cancer screening programmes, the need to biopsy smaller PPLs will be important and a smaller cryoprobe may assist in this regard.

As previously discussed, the freezing time and bleeding risk varies with the size of the cryoprobe and length of the freezing time. The current data available is mainly on the reusable 1.7mm cryoprobe, with most studies presenting a freezing time of 3-5 seconds with mild or moderate levels of bleeding managed by rigid bronchoscopy, bronchial blocker or a two-scope approach. In these studies, the cryoprobe and bronchoscope have to be removed all together, which was the main reason to have another method available to control the bleeding.

The dislodgement of the bronchial blocker following a cryobiopsy was an unwanted and unpredictable issue to occur, due to the rigid bronchoscope being pulled out during the biopsy. Until the proceduralist returns after separating the biopsy from the cryoprobe (which would take 1-2 minutes) or until a second bronchoscopist return to the biopsy site (30-60 seconds), it is not possible to demonstrate if the bronchial blocker is safely inflated and containing the bleed. One method to overcome this issue, is using a contrast medium instead of air in the bronchial blocker to inflate and observe the placement under fluoroscopy as soon as the biopsy is taken, which will only take a few seconds. This can be assessed in future studies.

Using ROSE will improve the diagnostic yield of the cryo-radial procedure. With the use of ROSE, an initial needle aspirate or brush performed was viewed via ROSE. If diagnostic material is present, then further biopsies could be performed from the same location; if there are no diagnostic material, then the biopsy tool can be redirected and sampled again until the correct location is reached. Thereby, ROSE ensures the correct placement of the biopsy tool and allows cryobiopsy to be performed with confidence, as noted by Taton et al. (2018), Imabayashi et al. (2019) and Gildea et al. (2021) who demonstrated that using ROSE reduces the number of biopsy tools used, thus adding value as an integral component in future Radial EBUS procedures where the multi-modal biopsies can be rationalised.

The CT-CROP study assessed PPLs of more than 10mm in size, however, further studies are essential to evaluate successful diagnoses of small PPLs to add to the body of data being further developed by Taton et al. (2018). Studies exploring the use of the small GS and the smaller 1.1mm single use cryoprobe are eagerly awaited.

This may all change the need for the vast amount of equipment if the smaller 1.1mm cryoprobe could be used with a reduced freezing time of 3-4 seconds (due to its small size) via the GS without removing the scope. The GS will tamponade the bleeding and the bronchoscope that remains will provide continuous monitoring in case of a bleed, as well as its ability to suction immediately (Herath, 2023). Further data on this concept is awaited to ensure this cryobiopsy procedure is a safe procedure to be used in a generalised setting in a multitude of centres.

6.3.2 Leading to the next study

Despite the efficacy of the cryo-radial procedure, it is highly dependent on equipment and facilities to ensure safety. These include a rigid bronchoscopy or ETT, anaesthetic support, theatre facilities, bronchial blockers, extra assistance in holding the blockers in place, and possibly a second bronchoscopist or a fellow. This level of resources is not available in all centres and the generalisability of the technique is poor. Yet patients require PD-L1 and molecular testing more than ever, therefore, finding a biopsy technique that can provide a core biopsy with the ability to perform PD-L1 testing via sedation bronchoscopy using Radial EBUS is discussed in Chapter 7.

CHAPTER 7:

R-CORE STUDY – RESULTS AND DISCUSSION

7.1 Publication details and manuscript

Herath, S., Bashirzadeh, F., Mahajan, H., Ing, A., & Fielding, D. (2022). Using the GenCut core biopsy tool with the radial endobronchial ultrasound guide sheath enables a high-quality histology sample capable of programmed cell death ligand 1 (PD-L1) testing. *Respirology Case Reports*, 10(5), e093. <https://doi.org/10.1002/rcr2.93>

Chapter 7 consists of the published paper and an extended discussion at the end to complement and broaden the discussion already performed in the paper.

Using the GenCut core biopsy tool with the radial endobronchial ultrasound guide sheath enables a high-quality histology sample capable of programmed cell death ligand 1 (PD-L1) testing

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Abstract

Radial EndoBronchial UltraSound (R-EBUS)-guided biopsies are a promising biopsy technique for pulmonary nodules suspected of lung cancer with great safety profile. Programmed cell death ligand 1 (PD-L1) testing is increasingly demanded from lung biopsies. GenCut is a novel blunt tool that can be used to obtain core biopsies. This case series explores prospective performance of the GenCut core biopsy with R-EBUS. Once Peripheral Pulmonary Lesion was located, GenCut biopsy was performed followed by conventional (forceps $\pm$ cytology brush) R-EBUS biopsies. The overall diagnostic yield for the 16 patients with a mean peripheral pulmonary lesion (PPL) size of 4.1 cm was 100% from multi-modal R-EBUS sampling. The diagnostic yield for GenCut tool alone was 13/16 (81.2%) and the ability to perform PD-L1 from GenCut was 10/16 (62.5%). There were no adverse events recorded. GenCut tool is a novel blunt instrument that can be used safely to obtain a core biopsy suitable for PD-L1 in combination with R-EBUS without compromising the high safety profile.

KEYWORDS

GenCut core biopsy, lung cancer, PD-L1, radial EBUS, safety

INTRODUCTION

With the advancement of lung cancer treatment with novel immunotherapies, including programmed cell death ligand 1 (PD-L1), a core biopsy sample is preferred to make a diagnosis. Despite the increased safety of radial EBUS (R-EBUS), diagnostic yield is sub-optimal (74%)^{1,2} and the ability to perform PD-L1 was not explored. The

smaller size and crush artefacts from R-EBUS forceps³ maybe overcome by the addition of cryobiopsy but requires expensive facilities⁴⁻⁶ including general anaesthesia, fluoroscopy and additional measures to improve safety⁵ (i.e., bronchial blockers $\pm$ rigid bronchoscopy for haemostasis). This study explores the ability of the novel GenCut biopsy to obtain a histology sample suitable for PD-L1 testing under sedation.

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METHODS/DESCRIPTION

This is a prospective, interventional, pilot case series performed at two tertiary hospitals in Australia from February 2018 to January 2019 (Ethics approval: AU RED HREC/17/WMEAD/461 and trial registration number: ANCTR-U1111-1204-2333). The co-primary end points were the ability to obtain a histology sample suitable for PDL-1 from GenCut biopsy (Medtronic Pty Ltd) and the safety of the procedure.

All patients referred to R-EBUS were offered GenCut in addition to at least one conventional R-EBUS biopsy (forceps, brush and needle aspiration). Lesions >1.5 cm in size, located in the outer half of the lung parenchyma, were included. Patients unsuitable for flexible bronchoscopy were excluded. The procedure was performed in endoscopy suit in accordance with the institutional guidelines. The R-EBUS ultrasound (USS) probe (20 MHz, UM-BS20-20R, Olympus, Tokyo, Japan) was used within the large guide sheath (GS) (Olympus K-203). The GenCut was introduced through the GS as the first mode of biopsy, followed by conventional R-EBUS biopsies.

GenCut (Covidien-GenCut-core-biopsy-system) had demonstrated satisfactory histology samples in porcine models.⁷ It is a flexible (1140 mm length, 1.9 mm diameter)

hollow tube with a 1-cm blunt metal tip comprising of inwardly placed sharp blades (Figure 1). Once in the lesion, suction is applied using a lockable 20-ml syringe. The suction ensures that the tumour is 'anchored' into the biopsy tool. The tool was then rotated clockwise 45° at a time, whilst maintaining the suction, to complete a 360° rotation, ensuring ongoing acquisition of material via suction into the tool (Figure 1). The specimen was pushed out using air via a 10-ml syringe to Hanks's solution, followed by a cytology brush insertion and a 10-ml saline push to expel the residual tissue. Samples were then placed in formalin.

Suitability of PD-L1 testing was assessed by an independent pathologist from each institution for all samples and documented in a standardized format. The procedures were performed in the endoscopy suit with either sedation alone or with general anaesthesia and with laryngeal mask airway. A chest x-ray was performed 1-h post-procedure. Fluoroscopy was used if the proceduralist requested it. Routine follow-up was executed. Data collection forms for patient demography, adverse events, pathology sampling and bronchoscopy were completed.

RESULTS

Eighteen patients were recruited during the study period: nine males (50%), mean age 67 years (SD 11.8). Thirteen (72%) were current or ex-smokers. Seven of 18 (35%) had chronic obstructive airways disease, 5/18 (27%) had cardiac disease and 3/18 (16%) had type 2 diabetes mellitus. No one recorded pulmonary hypertension.

The mean lesion size was 4.1 cm (SD 1.3 cm). Eight were in the left upper lobe (LUL), seven in the right upper lobe (RUL) and three in lower lobes. Eleven of 18 (61%) had sedation and seven had general anaesthesia for the procedure. Two of 18 did not proceed with the study; one had resolution of peripheral pulmonary lesion (PPL) confirmed on chest computed tomography and one had a positive paratracheal lymph node on linear EBUS.

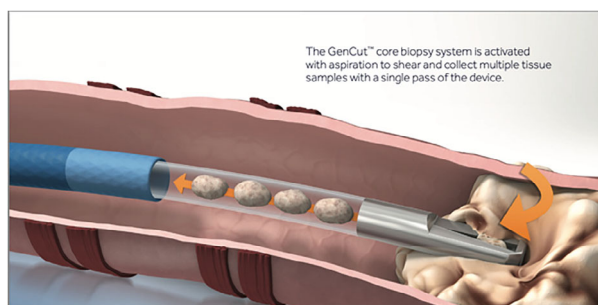


FIGURE 1 GenCut core biopsy tool demonstrating 'anchoring' to the tissue via suction and the samples drawn into the tool (courtesy of Medtronic's Pty Ltd)

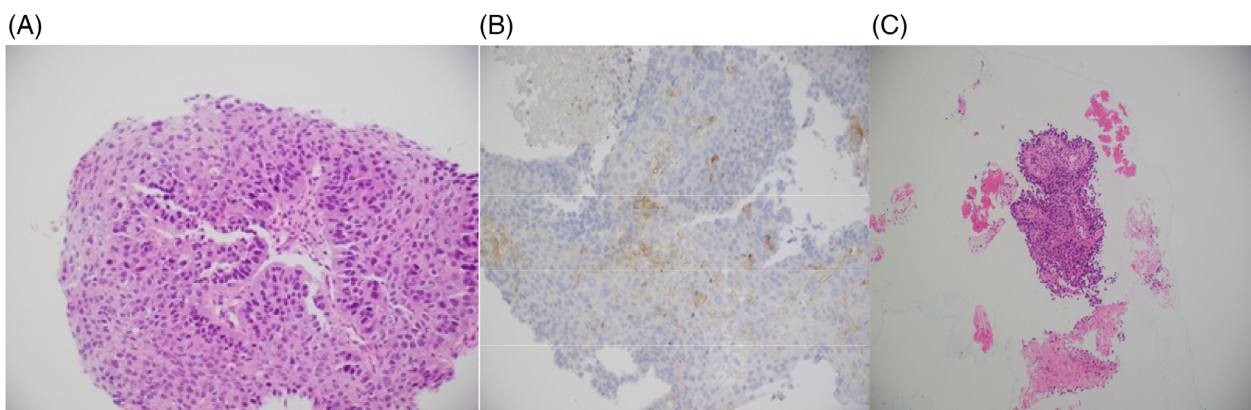


FIGURE 2 Well-preserved histology sample of a GenCut biopsy demonstrating squamous cell carcinoma, haematoxylin and eosin (H&E) staining $\times 20$ (A) and weak programmed cell death ligand 1 (PD-L1) staining 8% of the same core biopsy sample (B), compared to forceps biopsy with H&E staining $\times 20$ (C)

Sixteen completed the R-EBUS. Fifteen (93%) had a concentric USS visibility. The mean procedure time was 41 min (SD 13.9 min). The mean time to detect the lesion was 1.47 min (SD 1.5 min). Fluoroscopy was used for 9/16 (53%). The anaesthetic agents used and mean values were 1% lignocaine (14 mL, SD 4.9 mL), midazolam (3.6 mg, SD 1.9 mg), fentanyl (85 mcg, SD 20.8 mcg) and propofol (50 mcg, SD 57.9 mcg).

All 16/16 (100%) had a successful diagnosis made from combined R-EBUS sampling. GenCut was not performed for 2/16 due to technical difficulties. One had excessive secretions and difficulty in accessing the RUL, and one had a highly friable mucosa in the LUL and the GenCut tool could not be 'anchored' to the airway to obtain adequate suction to draw the tissue into the tool. Both these PPL were diagnosed using a cytology brush and forceps biopsy, respectively.

The overall diagnostic yield from GenCut alone was 13/16 (81.2%) and the ability to perform PD-L1 from GenCut alone was 76% (10/13). Overall diagnostic yield from forceps biopsy alone was 75% (12/16) with 8/12 (66%) suitable for PD-L1 testing. However, in samples where both GenCut and forceps biopsies were suitable for PD-L1 testing, PD-L1 testing was performed using the GenCut core biopsy due to the preserved architecture (Figure 2A,B) in comparison to radial forceps (Figure 2C). The mean biopsy size of GenCut was 6.8 mm (2–18 mm) and forceps biopsy was 2.8 mm (1–7 mm).

Four of 13 had a single pass and 9/13 had two passes with GenCut. Other R-EBUS instruments used and the median number of passes were radial forceps ($n = 14$, mean 3.6, median 3), radial brush ($n = 11$, mean 1.5, median 2) and aspiration needle ($n = 5$, mean 1.4, median 2). There were 18 malignant PPL (66% adenocarcinoma, 33% squamous cell carcinoma) and two benign PPL (type 2 pneumocyte hyperplasia, eosinophilic hyperplasia).

There were no pneumothorax, pulmonary haemorrhage or unexpected representation to the hospital. All patients were discharged as planned.

DISCUSSION

Our study demonstrates that the ability of GenCut biopsy to make a diagnosis and perform PD-L1 was high. Despite the large sample sizes, there were no adverse events.

Compared to cryobiopsy for PPL, which requires rigid bronchoscopy, bronchial blockers and anaesthetic support to obtain a core biopsy, GenCut does not require extra equipment apart from the GenCut tool and it is logistically easy to incorporate into any unit that has R-EBUS services. The procedure can be performed with sedation in endoscopy suite. Fluoroscopy was not essential, although it may be more helpful in smaller and eccentric lesions. Accurate instrument measurement with the GS prior to the procedure is mandatory to obtain excellent results.

GenCut is used in a similar manner to conventional radial instruments. The tool easily slides into the large GS of the R-EBUS. It is flexible and bends upon itself enabling easy access to upper lobes. The study lesions were in the outer half of the lung and GenCut can be used in small tapering airways.

Few studies had demonstrated the ability of small R-EBUS samples to obtain satisfactory PD-L1 testing⁸; however, the published literature on GenCut core biopsy is sparse. A procaine model study demonstrated better samples with GenCut core biopsy compared to transbronchial biopsies.⁷ In a recent publication by Lea et al.⁹ on using GenCut tool and transbronchial biopsies in 324 lung nodules, GenCut tool had a diagnostic yield of 37.3% and transbronchial biopsy had a diagnostic yield of 43.2%. However, when PPL > 3 cm was analysed separately, the diagnostic yield was 65.2% with both GenCut and transbronchial biopsy. In our study, the diagnostic yield may have been higher due to the large mean size of the PPL in comparison to Orr et al. They used R-EBUS in 96% of patients and electromagnetic navigation (EMN) bronchoscopy in 49%. EMN was used in more peripheral PPL. In our study, all PPL were in the outer half of the lung parenchyma and did not require the EMN, which may be again due to the larger mean size of the PPL in our study.

The main limitation of the study is the inclusion of only larger lesion size (>1.5). Smaller lesions are not represented here in efficacy or safety. 'Anchoring' to the lesion was not successful in all lesions and using GenCut as the sole biopsy method is not encouraged. The small sample size of this series is another limitation. Other molecular testing including EGFR and ROS1 were not analysed during this study.

In conclusion, the novel GenCut biopsy with R-EBUS via the large GS is successful in obtaining a histology sample suitable for PD-L1. GenCut can be used without additional equipment, safely with sedation and is applicable to most centres.

CONFLICT OF INTEREST

None declared.

No financial disclosures for all authors. There were no commercial interest or trial subsidies offered for this study.

AUTHOR CONTRIBUTION

Dr Samantha Herath had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. Dr Samantha Herath, Dr Farzad Bashirzadeh, Dr Hema Mahajan, Prof. Alvin Ing and A/Prof David Fielding contributed substantially to the study design, data analysis and interpretation, and the writing of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved with ethics approval: AU RED HREC/17/WMEAD/461 and trial registration number: ANCTR-U1111-1204-2333.

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7.2 Discussion of the study

7.2.1 Significance of the study

The GenCut biopsy tool can simply be incorporated into the Radial EBUS procedure; it is inexpensive and requires minimal training. Furthermore, it is a generalisable simple method that can be used under sedation alone in regional centres. GenCut is an effective tool for improving the diagnostic yield as it has capability to obtain a core sample suitable for PD-L1 testing. Whilst all previous studies in GenCut have used EMN, this study proves its utility with Radial EBUS only, which is inexpensive, even for peripherally located PPL

The use of GenCut with only Radial EBUS and sedation bronchoscopy is an original contribution to knowledge.

The sample extracted from GenCut has proven to be better for PD-L1 analysis than forceps, radial brush and aspiration needle. GenCut is a blunt tool that can be used with sedation bronchoscopy for PPLs with no significant side effects. It can also be incorporated with Radial EBUS biopsies as a multi-modal biopsy technique.

7.2.2 Summary of salient results and comparison to existing evidence

The R-CORE study was designed to discover a tool that could perform a core biopsy without the need for extensive equipment or wide-ranging training of personnel. With R-CORE, the only equipment required was the GenCut biopsy tool itself. The procedure was predominantly performed with sedation and there was no difference of yield or side effects. Therefore, the GenCut tool was an alternative for cryobiopsy in obtaining a core biopsy using Radial EBUS without the expense and additional equipment or EMN. This is an original contribution to knowledge.

To date, studies on GenCut biopsy have been extremely limited, but favourable. Khandhar et al. (2015) used a porcine study performing 60 biopsies on six animals. They used conventional Radial EBUS tools; forceps and aspiration needle and compared them to the novel GenCut tool. In this study, the GenCut tool had 30/60 biopsies done. There was no bleeding with the GenCut tool (0/30), and pneumothorax was minimal at 2/30. In comparing the aspiration needle and forceps, the investigators found the manoeuvrability of GenCut to be higher. The cytologist rated the adequacy of tissues similar to forceps and the GenCut tool to be more superior than a needle aspirate (Khandhar et al., 2015).

The use of GenCut biopsy with Radial EBUS was recently published by Orr et al. (2021), who investigated 324 lung nodules. This is the only comparable evidence on GenCut tool used with Radial EBUS that is published. The PPL sizes in Orr et al. (2021) ranged from <10mm (8%), 10-20mm (28%), 20-30mm (19%) and over 30mm in 45%. They used forceps biopsy (up to five passes) and GenCut biopsy (up to three passes).

There were similarities and contraries when the R-CORE study was compared with the Orr et al. study. Overall, the R-CORE study consisted of 89% malignant PPLs whilst Orr et al. consisted of 84.2% malignancy, with distribution of malignancy in PPLs for both studies being similar.

Similar to the R-CORE study results, Orr et al. (2021) demonstrated an increase in the overall diagnostic yield when combining forceps biopsy and GenCut biopsy with a 14.6% diagnosis made only on the GenCut biopsy. Also comparable to the R-CORE study, Orr et al. (2021) noted that the volume of biopsy was larger with the GenCut tool than with forceps. Orr et al. (2021) also commented that the GenCut tool was easy to use and added only a few extra minutes to the procedure, another point similar to the R-CORE study. Therefore, this study strengthens the argument that the R-CORE study makes in using GenCut as an additional tool in a multi-modal biopsy of Radial EBUS to improve the diagnostic yield, particularly as GenCut is an easy-to-use tool.

The difference between the studies above was the diagnostic yield. The R-CORE study had a 100% diagnostic yield in PPL >1.5mm. Orr et al. (2021) identified the overall diagnostic yield to be lower at 55.6%. The diagnostic yield of GenCut alone was only 37.3% which was much lower than the R-CORE study which had 76% diagnostic yield. Orr et al. (2021) recorded the PPL size >20mm in 64% of patients and only 8% were central tumours, whereas in the R-CORE study, all PPLs were >15mm and all PPL were in the outer one-half of the lung parenchyma as per protocol. Therefore, the location was not dissimilar in both studies yet the lower diagnostic yield may be due to the smaller size of the PPL overall (Orr et al., 2021). The difference in diagnostic yield may be due to the R-CORE study using PPLs with air bronchograms, whereas in Orr et al. (2021) there was no protocolised selection for air bronchogram. The presence of air bronchogram and the increase in PPL size were positively correlated with the yield in both studies, which was similar to the PPL characteristics for a high yield with conventional Radial EBUS biopsies.

Despite the higher volume of tissues noted in the GenCut biopsy in the Orr et al. (2021) study, only 38% were used for molecular testing. The study authors concluded that despite the higher volume, the cellularity of GenCut was lower than forceps biopsy. This was in contrast with the R-CORE study. All positive GenCut biopsies (76%) were suitable for molecular testing and the

preferred sample for PD-L1 testing, in comparison to forceps biopsy, needle aspirate and cytology brush.

Compared to the R-CORE study, the overall complication rate for Orr et al. (2021) was higher at 5.5% with 1.5% requiring intervention. The pneumothorax rate was 4.9%. Again, this difference may be due to having smaller PPLs.

Orr et al. (2021) used Radial EBUS for 96% of PPLs and EMN for 49% of PPLs. EMN was mostly used when the PPL was peripherally located, whereas the R-CORE study only used Radial EBUS alone. Given that the R-CORE study had exclusively peripherally located PPLs, Radial EBUS seemed adequate in making a diagnosis without the requirement of expensive EMN technology. This is an original contribution to knowledge. However, no study publication has exclusively explored the ability of the GenCut biopsy tool to obtain core biopsy samples that can be used for PD-L1 testing; the R-CORE study has provided an answer to this timely question and is another original contribution to knowledge.

7.2.3 Safety profile

The procedure was predominantly performed under sedation bronchoscopy without fluoroscopy in this R-CORE study. Despite the large size of samples obtained, there was no pneumothorax or bleeding. This confirms that additional equipment, such as a bronchial blocker or rigid bronchoscopy was not required for this procedure, resulting in a straightforward, low-cost, sedation bronchoscopy procedure.

A blunt tool such as GenCut was extremely useful when added to other equipment used for Radial EBUS, particularly as the blunt tip markedly reduced the risk of pneumothorax and may be useful for COPD patients. This type of a blunt tool could be added to existing Radial EBUS tools; therefore, this study adds to the growing body of evidence for utilising the GenCut tool (Orr et al., 2021; Khandhar et al., 2015) and using GenCut as part of a multi-modal biopsy for Radial EBUS.

7.2.4 Limitations of the study

This study used PPLs >15mm and PPLs located in the outer one-half of the parenchyma with air bronchograms. Therefore, smaller lesions or eccentric lesions were not represented, and their applicability were not explored. With data from Orr et al. (2021), it appears that the GenCut tool like all other biopsy tools performs better with PPLs >30mm that had resulted in a 64% diagnostic yield.

7.3 Future directions

7.3.1 Practical aspect

During the GenCut biopsy procedure, samples were obtained via suction with rotation, therefore, it was important to “anchor” the tool to the PPL with adequate suction and also continue to maintain the suction; sometimes this “anchoring” was unsuccessful due to friable mucosa present. Occasionally, the nursing staff holding the suction would “let go” prior to completing the procedure resulting in loss of sample. Hence, education on this aspect was important prior to the commencement of the procedure.

Again, multi-modal methods have enabled a better diagnostic yield, with GenCut not recommended to be used as the only biopsy method. Combining GenCut to Radial EBUS conventional biopsies increased the ability of a diagnostic yield of the procedure. It is an effective tool for use in a sedation bronchoscopy unit due to its better side effect profile.

7.3.2 Future directions

More studies exploring the utility of the GenCut tool with Radial EBUS is essential to provide confidence for clinicians to use this method with sedation bronchoscopy in peripheral centres. This would reduce the delays experienced by regional patients who have had to be transferred to tertiary centres for biopsies of PPLs.

CHAPTER 8:

CONCLUSION

8.1 Chapter overview

This chapter integrates the main findings of this thesis and their implications in improving the diagnostic yield of Radial EBUS-guided biopsy methods, as well as maintaining the safety of the procedures. This thesis outlines the original contribution of the studies and their significance and impact within the Australian and New Zealand setting, as well as to the discipline of interventional bronchoscopy. The problems encountered when performing the studies during this thesis are summarised. Finally, based on the findings of this thesis, the future direction in interventional bronchoscopy pertaining to the diagnosis and treatment of PPL is discussed.

8.2 Importance of increasing the diagnostic yield in PPL

With the increased detection of PPLs suspected of lung cancer, due to the increased use of imaging modalities, the aging population with its higher incidence of cancer, and lung cancer screening programmes on the horizon, a safe and effective biopsy technique is important to improve survival and to reduce treatment delays and healthcare costs to the patient and the health system. An adequate biopsy is required for the numerous biomarkers, in the era of personalised medicine. Safety is increasingly important in the wake of lung cancer screening programmes that result in high numbers of false positive results. Given that over 70% of primary lung cancer patients have mediastinal spread at the time of presentation, a biopsy technique for the PPL that can combine linear EBUS, during the same procedure will save time and reduce the number of procedures for the patient.

In many Northern American countries, with the establishment of lung cancer screening pathways and robotic bronchoscopy, guided bronchoscopy biopsy has become the forefront of PPL diagnosis. Although there are no prospective studies, a retrospective study had already demonstrated an equal diagnostic yield in robotic bronchoscopy comparative to CT-TTB (Yu et al, 2023). This is still not the case with Australian and New Zealand settings. To date, the commonly used lung biopsy method for PPL is still CT-TTB.

Despite the high diagnostic yield of 92%, drawbacks of the CT-TTB method are, the inability to perform mediastinal staging and evaluate the airway prior to surgery, as well as the high side effect profile of pneumothorax with an average pneumothorax risk of 20%, which can be as high as 54% in emphysematous lungs, is concerning. (DiBardino et al., 2015). Yet, despite these

drawbacks, CT-TTB continues to be used, in Australia, and New Zealand as the “gold standard” biopsy technique for PPL. This is mainly due to the ready availability of CT-TTB, and the lack of a reliable alternative bronchoscopic biopsy method for PPL.

Radial EBUS is still a developing field in Australia and New Zealand with only tertiary centres having the capability to perform Radial EBUS and even fewer centres, that can provide satisfactory results. Added to this is the cost of navigational technology, which is prohibitive for Australian and New Zealand centres, however, it is not necessarily required for the successful use of Radial EBUS, as noted in these studies, which exclusively used manual readings of the CT chest and Kurimoto’s method.

Radial EBUS is the only guided bronchoscopy method available or affordable in Australian and New Zealand centres. Therefore, this thesis focuses on Radial EBUS as the main guided bronchoscopy method. The side effect profile of Radial EBUS-guided biopsies is highly favourable with a 1% risk of pneumothorax and less than 0.6% risk of a chest drain insertion. This makes it an excellent diagnostic modality from a safety point of view. Despite this excellent safety profile, the diagnostic yields of Radial EBUS in five meta-analyses have reported yields only up to 73% (67-84%) (Ali et al., 2017; McGuire et al., 2020; Wang-Memoli et al., 2012; Sainz Zuniga et al., 2020; Steinfort et al., 2011).

When it comes to guided bronchoscopy techniques, The focus globally has been directed heavily on improving navigation techniques. Essentially trying to improve the visualisation of the PPL, as means of increasing the diagnostic yield. Whilst improving the visualisation of a PPL does improve the diagnostic yield, the diagnostic yield always seems to be 10-20% below the visualisation capability.

8.3 Aims of the thesis and questions answered

This thesis comprises of four chapters (Chapter 4-7) and four published papers to answer five main questions, as listed below:

1. Does the addition of an aspiration needle to the Radial EBUS sampling via the large GS improve the diagnostic yield? Will the aspiration needle be useful for EGFR testing as well? Does the size of GS matter?
2. How could the novel cryo-radial method be introduced to the New Zealand setting without general anaesthetic and operating room facilities? What techniques can be

developed to improve the diagnostic yield without compromising safety? How can we adopt adaptation these techniques to the New Zealand setting?

3. How does the novel cryo-radial procedure with the multi-modal biopsy technique compare with the widely used CT-TTB in the diagnostic yield, and its ability to perform EGFR testing and safety?
4. Is the GenCut tool safe and effective in obtaining a core biopsy with sedation Radial EBUS in centres where general anaesthesia is not readily available?
5. Can multi-modal biopsy improve diagnostic yield? Does the multi-modal biopsy also increase the ability to perform EGFR better?

These questions were answered in the thesis as below:

The GS in Radial EBUS plays a pivotal role in acting as an extended working channel from the bronchoscope to the PPL. By correctly placing the GS, within, or as close in proximity, to the PPL, the accuracy with which the tools are reaching the PPL is increased. In the absence of real-time capability to observe the biopsy procedure, the GS is one of the best surrogate methods or semi-real-time modalities to ensure that the biopsy is taken from the correct position. Using the GS has been demonstrated over time with multiple studies in improving the diagnostic yield, which becomes even more important in smaller and more peripherally placed PPL (Bo et al., 2019; Kurimoto et al., 2004; Oki et al., 2021). Using the GS with novel biopsy tools such as cryobiopsy has also demonstrated increased yield and the additional independent diagnostic yield when using the GS (Nasu et al., 2019).

The size of the GS may also have an impact on the diagnostic yield, yet only sparse data is available comparing the large and small GS. The GS size determines the size of the biopsy instruments. Whilst a small GS can travel to a more peripherally located PPL, a larger GS houses larger equipment. Many studies using a Radial EBUS GS preferred the small GS because it increased the diagnostic yield. Without using a GS, the diagnostic yield remained low, which may be due to the use of the small GS and smaller biopsy tools. An RCT by Oki et al. (2021) demonstrated a 55.5% diagnostic yield using the small GS and 46.6% without. Yet, both diagnostic yields were low. In fact, data on comparing the size of the GS was limited and only a single conference abstract exists (Kurimoto et al., 2004). Therefore, when designing the REBUST 2 study, due to a lack of reliable data to calculate the sample size, a predefined analysis was planned for recalculating the sample size at 25% recruitment.

The REBUST 2 study was designed to answer the two questions mentioned above. Recruitment commenced in early 2016 with satisfactory acceptance rates. However, a slowing down in recruitment towards the end of the year was observed by research investigators. This was due to referring clinicians noting that patients randomised to the large GS received better results and those randomised to the smaller GS required a repeat procedure. The referring physicians commented on the lower diagnostic yield with the small GS arm. therefore, there seemed to be a reluctance to refer patients to be randomised for the study.

Another reason for the slowing of recruitment was the commencement of concurrent studies on the same topic, at the same hospitals. Whilst the recruitment for REBUST 2 was being undertaken at Westmead and Middlemore Hospitals, there were two other competing studies (CT-CROP/GenCut) starting in 2016, which also resulted in a further reduction in recruitment.

Although planned for 88 patients to be recruited, the REBUST 2 study was terminated early at 42 patient recruitment due to clinician concern about the tendency to display a lower diagnostic yield with the small GS. Early termination of the study resulted in a Type 2 error due to the low sample size and loss of power to detect a difference. This was a major drawback of this study.

8.4 Comparing small and large GS

Keeping this drawback in mind, The REBUST 2 study data compared the large GS biopsies versus small GS biopsies (by using conventional biopsy techniques of forceps and brush alone); a 60% diagnostic yield for the small GS and 71% for the large GS was demonstrated, however, this was not statistically significant. This finding in REBUST 2 study of similar diagnostic yield in large and small GS is in keeping with Kurimoto et al. (2007) which demonstrated no significant difference between the small and large GS. Also, the diagnostic yield demonstrated by Oki et al. (2021) with a small GS of 55.3% was similar to the small GS results in the REBUST 2 study of 60%.

Therefore, a difference between the large GS and small GS cannot be conclusively made from this data although a clinical trend for a better yield from the large GS can be argued and this study adds to the limited data that is available on this subject.

Due to the advantage of the large GS with the 2.6mm outer diameter and its ability to house a wide variety of standard bronchoscopic biopsy tools, the 1.9mm cryoprobe (Chapters 5 and 6) and the 1.9mm GenCut tool (Chapter 7) the large GS were used for all studies in this thesis from this point onwards.

8.5 Addition of the aspiration needle to the large GS arm

At the time of the REBUST 2 study (chapter 4) needle aspirate was not used at all, for peripheral pulmonary lesions via bronchoscopy in New Zealand in 2015. In fact, there seems to be a reluctance among respiratory physicians to use the needle aspirate in PPL. In a meta-analysis of Radial EBUS (Ali et al., 2017) only five out of 57 studies used the aspiration needle as a biopsy method. The ACCP Quality Improvement Tool for Chest Physicians by Chest Physicians (AQuIRE registry data) acknowledged this reduced usage of the aspiration needle among pulmonologists, demonstrating that 16.4% of PPL had been biopsied with the aspiration needle.

The REBUST 2 study was performed to explore whether adding a needle aspirate would improve the diagnostic yield and ability to perform EGFR status in PPL. There was no dedicated needle to be used in PPL and the Boston Scientific 21G and Medtronic 21G needles were used. In Chapter 4 (REBUST 2 study), when the 21G aspirate needle was added to the conventional biopsy tools of forceps and cytology brush in the large GS arm, the diagnostic yield improved from 71% to 86%, which is a 15% improvement in the diagnostic yield. The aspiration needle alone was the sole diagnostic tool in 13.6% of patients.

8.6 Using an aspiration needle for EGFR analysis

The ability of an aspiration needle to provide EGFR status was explored by the REBUST 2 study. 62% of aspiration needle samples were capable of EGFR testing, compared to 66% of forceps. Therefore, demonstrating the aspiration needle was as beneficial as forceps for the EGFR analysis. The aspiration needle was more successful at EGFR than the cytology brush (62% versus 54%).

Despite multiple studies demonstrating that the aspiration needle used in an L-EBUS biopsy is capable of EGFR analyses up to 94.5% (in a meta-analysis of 33 papers including 2698 patients), there are no studies that had explored the ability to perform EGFR testing in PPL when using an aspiration needle (Labarca et al., 2018). Therefore, this study provides important data to the literature despite being a small study.

REBUST 2 strengthens and adds to the data that demonstrate the value of adding an aspiration needle to Radial EBUS biopsies which could encourage clinicians to use the aspiration needle liberally. As this aspiration can be used under sedation, it can be easily applied to an Australian or New Zealand setting. No extra equipment is required as most units would have a TBNA aspiration needle available.

A systematic review by Lou et al. in 2023 on the use of needle aspirate on PPL in 2000-2021 demonstrated only seven published studies involving 510 patients. Therefore, data from the REBUST 2 study will increase the current body of literature on utilising the aspiration needle.

The PeriView flex needle that came to the market in 2018, is specially designed to be used with Radial EBUs and is available but is expensive around 400 AUD and needs to be purchased separately. Whilst this is a much-desired addition to the armoury of the toolbox, even for units that cannot afford this, a TBNA needle can be used in the same manner as performed in the REBUST 2 study safely, with good results.

8.7 Multi-modal biopsy for diagnostic yield

As mentioned above, in the REBUST 2 study large GS arm, the aspiration needle was the sole diagnostic modality in 13.6%. It is interesting to note that the brush was also a stand-alone diagnostic tool in 4.5% and forceps were the stand-alone diagnostic method in 18.1%. This combination of biopsy modalities resulted in a high yield of 86% with all sampling methods. In the large GS arm each patient had undergone at least two and up to nine biopsies. Despite the number of biopsies, there was no pneumothorax or bleeding. All patients were discharged as planned from the day procedure centre. Despite the addition of an aspiration needle and using multiple biopsies, the excellent safety profile of Radial EBUS was not lost.

Chao et al. demonstrated that the addition of forceps biopsy and aspiration needle improved results. (Chao et al., 2009). Although studies have mentioned combining biopsy tools, and also reported on higher yields when more biopsy tools were used. However, the concept of multi-modal biopsy on the diagnostic yield had not been appreciated. The term “multi-modal biopsy” has been only recently coined for the first time to demonstrate this phenomenon on a retrospective data analysis of the NAVIGATE study (Gildea et al., 2021).

The 2015-2019 studies mentioned in this thesis prospectively demonstrate the added advantage of multi-modal biopsies, as well as add to the existing sparse literature on this subject

8.8 Multi-modal biopsy for EGFR testing

The REBUST 2 study also demonstrated that the increased diagnostic yield with multi-modal biopsy sampling had extended itself to performing EGFR status. The overall ability to perform EGFR testing in the large GS arm was 12/16 (75%); 62% suitable for EGFR with the aspiration needle, 54% suitable for EGFR with the cytology brush, 66% suitable for EGFR with forceps,

and in combination, improving the yield. This is also an important contribution to knowledge demonstrating that multi-modal biopsy improves the ability to perform EGFR testing in PPL.

8.9 Feasibility of introducing novel cryobiopsy service to New Zealand

At the time of commencing the Cryo-feasibility study in 2015, there had only been one single-arm feasibility study on cryobiopsy for PPLs, published by Schuhmann et al. (2014), demonstrating for the first time the divergence in the safety of cryobiopsy for PPL from ILD cryobiopsy. A four-second freezing time was used by Schuhmann et al. (2014) who demonstrated a low side effect profile with no pneumothorax and 1/31 moderate bleed. For this study, a 1.9mm reusable cryoprobe was used.

Cryobiopsy was thought to be a high-risk procedure with high bleeding and pneumothorax risk based on solely the ILD literature. The divergence in the safety profile between cryo-radial and cryo for ILD was not known in 2015.

This cryobiopsy in PPL seemed an excellent technique to employ and master to obtain better tissue samples from PPL. However, there were many logistical and facility differences between Germany Heidelberg and Middlemore, New Zealand, where the Cryo-feasibility study was planned as a pilot study.

In the Schuhmann et al. (2014) feasibility study conducted in Germany, a general anaesthetic was used for all procedures. All procedures were done using rigid bronchoscopy and high-frequency jet ventilation. However, in the New Zealand setting, these facilities were not available, not just in 2015, but even now, bronchoscopy is a sedation procedure done in an endoscopy suite. There was no anaesthetic support and no theatre time available to perform complex bronchoscopy. Interventional bronchoscopy was in its infancy at the time in Australia and New Zealand, and performing a bronchoscopy procedure in theatre was not only rare but was also a major conundrum. The proceduralist would have to relocate all equipment from the bronchoscopy suite to the theatres and work with theatre nurses who had never seen a bronchoscopy procedure, together with an anaesthetist usually very anxious about sharing the airway.

Therefore, a different approach had to be designed to improve the safety of the procedure in the New Zealand setting. It was important to have the same trained bronchoscopy nursing staff to do the procedure as well as to design and develop a technique to perform the procedure safely in the bronchoscopy suite.

The Cryo-feasibility study explored using sedation for cryobiopsy and methods to improve safety that can be used without rigid bronchoscopy. A special ethics approval had to be obtained with an in-person ethics meeting to explain the use of cryobiopsy and the safety measures, as well as the adequacy of proceduralist training, as this was the first time cryobiopsy was used in the lungs in New Zealand. Following strict safety measures the ethics committee gave permission for this novel cryobiopsy in the lungs to be performed in New Zealand. One mandatory requirement was to have two respiratory physicians in the room to perform the procedure. In essence, during this study, there were usually three and sometimes four respiratory physicians present, due to the novelty of the procedure and to marvel at the large size of the cryobiopsy.

Novel methods had to be invented to improve the safety of the procedure to suit the New Zealand setting as below. The procedures were performed in the endoscopy suite. A bronchial blocker (ardent) was used to contain bleeding at the lobar level. When a patient required intubation, an ETT was used. The bronchial blocker was then placed outside the ETT. Once the bronchial blocker was placed in the desired position the ETT cuff was inflated to secure and prevent dislodgement. A dedicated assistant was assigned to hold the bronchial blocker in place. A second bronchoscopist was employed to assist with the control of bleeding (two-scope approach). As soon as the cryobiopsy was taken, the bronchial blocker was inflated every time, and a second bronchoscopist entered the airway to examine for the position of the inflated bronchial blocker and to suction any spillage of blood. These measures improved the safety of the procedure despite not using a rigid bronchoscope and were an original contribution to knowledge at a time when only a single study on cryobiopsy use in PPL has been published (Schuhmann et al., 2014).

The Cryo-feasibility pilot study consisted of six consecutive patients, exploring the addition of cryobiopsy to Radial EBUS conventional biopsy tools (at least one sample of forceps, brush and aspiration needle). The freezing time used was four seconds for cryobiopsy similar to the Schuman et al. (2014) study. This demonstrated an improved diagnostic yield of 83%. One patient out of six obtained the diagnosis from cryobiopsy only. In the Cryo-feasibility, study, there were no pneumothorax or bleeding episodes, demonstrating that the above measures were successful. The Cryo-feasibility study published in 2017 was the first paper to demonstrate the use of a bronchial blocker for PPL, as well as the first to explain the technique of using a bronchial blocker alongside an ETT, that would be applicable to the New Zealand setting.

Another observation during the Cryo-feasibility study is that patients under sedation tended to cough more, causing the bronchial blocker to be displaced. Therefore, future research on cryobiopsy, including the protocol for the CT-CROP study was designed to use sedation or GA

with an anaesthetist, to secure the location of the bronchial blocker and ensure the patient's comfort as well as enable the proceduralist to focus on the procedure.

8.10 Comparing the novel cryo-radial procedure with the multi-modal biopsy technique with the widely used CT-TTB

With encouraging results from the Cryo-feasibility study, the randomised control CT-CROP study was performed to compare the cryo-radial multi-modal biopsy procedure with CT-TTB. The CT-CROP study explored how the Radial EBUS method, armed with its multi-modal biopsy technique including cryobiopsy, would stand up to its direct competitor, CT-TTB. There was a diagnostic difference with the CT-TTB yield of 93.8%, and cryo-radial of 85%. This was not statistically significant however, can be argued that CT-TTB has a higher yield in a clinical context.

Despite the addition of the novel cryobiopsy, and combining multi-modal biopsies of forceps, brush, aspiration needle and cryobiopsy, the desired diagnostic yield similar to CT-TTB was not reached.

There may be a variety of reasons why the Cryo-radial arm had lower results than anticipated. Selection bias may also have had a part to play, as the randomisation sequence didn't take into consideration PPL or patient characteristics. Only 13/26 (50%) of patients had air bronchograms in the cryo-radial arm. Previous studies have shown that when CT bronchus sign is present the diagnostic yield was 74.1% and 49.6% for patients without the sign (Yarmus et al., 2017).

Added to that 14/26 (53.8%) of the Cryo arm had PPL in the outer 1/3 whilst 12/22 (54.5%) of CT-TTB were located in the outer 1/3. These are not favourable parameters for the Cryo-radial procedure, but much more favourable for the CT-TTB procedure. Previous studies have demonstrated that the CT-guided biopsy yield better results when the lesion is in the outer 1/2 of the lung parenchyma (Wang et al., 2018).

Accessibility to the PPL by some of the biopsy tools accounted for the lower yield. The stiff cryoprobe was not able to be advanced to the PPL in 4/20 cases. Difficulty was encumbered in accessing the PPL with the cryobiopsy due to the stiffness of the probe, despite locating it well with the ultrasound.

The lower-than-expected diagnostic yield from the Cryo-radial arm in the CT-CROP study could also be due to the learning curve of respiratory physicians. Both the Radial EBUS procedure and the cryobiopsy procedures were new to the interventional bronchoscopy field when it was commenced in 2015. The proceduralists had to learn two new procedures. These procedures were

performed by proceduralists already trained in rigid bronchoscopy as well as linear EBUS as a baseline but at the initial stages of performing the procedure time was a lot longer than anticipated due to the proceduralist and the whole team learning a new method with the multitude of equipment. This is in comparison to CT biopsy which was done as a routine procedure over decades by Radiologists.

This may also explain the much longer procedural time taken for the Cryo-radial procedure compared to CT-TTB as well. The time taken for the procedure was significantly higher in the Radial-Cryo arm in comparison to the CT-TTB arm (49.8 versus 16.1 min $P < 0.01$ Paired T test)

In the CT-CROP study, The pneumothorax was 4% for the cryo-radial biopsy and 44% for CT-TTB producing statistically significant results, demonstrating a much higher side effect profile for CT-TTB (Fu et al., 2021).

A high percentage of patients (90%; 18/20) in the CT-CROP study Cryo-radial arm used a prophylactic bronchial blocker. Despite the high number of samples taken, there was only a single moderate bleed occurring in a patient who did not have a bronchial blocker due to excessive coughing. There was only a single small pneumothorax managed conservatively. The use of a bronchial blocker constantly seemed to improve the safety of the cryo-radial procedure.

This is an original contribution to the literature and the first published paper that compares the widely-used gold standard to the cryo-radial procedure. All published literature on cryobiopsy in PPLs was limited to single-arm studies.

Despite not demonstrating a higher yield with cryo-radial procedure compared to CT-TTB, the safety of the Cryo-radial procedure and the very low pneumothorax rate compared to CT-TTB provides respiratory physicians with an alternate biopsy option that can be considered especially for patients with a high-risk biopsy with CT-TTB including emphysema or proximity to major vessels.

In this study, all consented participants were randomised to either CT-TTB or the cryo-radial arm without considering other patients or PPL factors. Agreement on the best randomisation pathway was a major debate faced throughout the design phase of this study. This randomisation choice was discussed and debated heavily at the time when the study was presented at the Australia/New Zealand interventional pulmonary group, as well as the investigator meeting to find the best randomisation method for this study.

Whilst some respiratory physicians suggested randomising according to patient and PPL characteristics others suggested randomising all comers without consideration of any other characteristic. Whilst using a lung cancer multidisciplinary meeting discussion to decide on the best biopsy method for a given PPL was discussed, many argued that in a multicentre setting, there will be no uniformity due to different skill sets and different facilities available. Certainly, at this point in 2015, lung cancer MDT had just started at Middlemore Hospital in New Zealand and was a novel concept. The Radial EBUS service had only just started as well and only a handful of respiratory physicians even knew of the existence of this method. At this time period, there were no interventional pulmonary group meetings for expert opinion either.

Therefore, after approximately six months of debating between investigators to agree on an unbiased division of patients into two entirely different biopsy techniques of Radial Cryo or CT-TTB, the decision was made to randomise all patients presenting with PPL to use the computer-generated system without considering any other patient or PPL characteristic. Whilst this may not have been the perfect solution, it seemed to be most appropriate pathway at the time to overcome this selection bias. Due to this choice of randomisation, some patients who were randomised to the CT-guided biopsy arm did not undergo the randomised procedure as it was not the best clinically indicated biopsy method for that patient, for example, close to fissures or marked emphysema. Hence, in hindsight, more pragmatic randomisation according to clinical characteristics would have given a real-world allocation of patients and possibly a better diagnostic yield for the cryo-radial procedure.

This study protocol was extremely selective and in retrospect resulted in low recruitment. According to the protocol, only patients that didn't have mediastinal adenopathy were randomised. This meant only 25-30% of all PPL referrals for lung cancer were anticipated to have localised disease and would be randomised. Yet the recruitment centres were located in lower socioeconomic areas with higher than anticipated late presentations and patients presenting with a localised disease burden were even fewer.

The participating study centres also did not have a regular GA list or anaesthetic support list that can be used for the study in 2015-2018. This usually meant the randomised patient to be placed on the acute theatre list and inevitably experience a delay in the procedure. Whilst the CT-TTB is a mainstream technique in Australia and New Zealand and also in Australia patients can readily obtain a biopsy from a private Radiology company, keeping the patient waiting for a bronchoscopy procedure on a trial, was not ethical and many patients were not enrolled in the study due to lack of appropriate bronchoscopy list availability.

Geographically Australia and New Zealand have a very small population compared to globally. Therefore, even when multi-centre trials are undertaken, it was difficult to recruit 158 patients during the planned time frame of three years, compared to the North American and Chinese units where the large population allowed larger recruitment in a smaller timeframe. International collaboration, especially in the context of interventional studies may be a consideration, although the quality of data and the ethical rigour need to be enforced. The high cost required with international collaboration wasn't feasible at this point for the CT-CROP study as it was funded solely by an investigator's early career grants.

Therefore, there was slow recruitment from initiation due to all the above-mentioned reasons and the study was terminated at 25% of the planned recruitment stage. This resulted in a Type 2 error and loss of power to detect a true difference. Lessons learnt here would be used in future research to avoid these pitfalls.

Within the CT-CROP study, the cryo-radial arm demonstrated an excellent safety profile. Each patient in the study had at least one cryobiopsy and one Radial EBUS biopsy (forceps, brush or aspiration needle), and some patients had three cryobiopsies, three radial aspiration needles, three forceps biopsies and three brushings, which could have resulted in a maximum of 12 samples in a single patient.

In the CT-CROP study, cryo-radial arm results demonstrated that the addition of cryobiopsy improved the diagnostic yield of Radial EBUS biopsy alone with a total diagnostic yield of 85% from all modalities of biopsies. Cryobiopsy was the only diagnostic biopsy in 5/20 (25%) of patients.

In the CT-CROP study, a multi-modal biopsy was carried out and demonstrated an increase in diagnostic yield. Individual biopsy tools provided the sole diagnostic tool with an aspiration needle presenting a 2/20 (10%) diagnosis, forceps 1/20 (5%), cytology brush 1/20 (5%) and cryobiopsy 5/20 (25%). In combination, this increased the diagnostic yield to 85% overall, demonstrating that multi-modal sampling increases the yield. Despite multiple biopsy tools with multiple passes being made, adverse events did not increase.

In this study, all cryobiopsy samples provided a positive result for malignancies suitable for EGFR testing. This is in keeping with Udagawa et al. (2020) who concluded that cryobiopsy provides large amounts of preserved DNA suitable for molecular testing compared to forceps alone, which again compliments our study findings. From all sampling methods in CT- CROP, the cryo-radial

arm (70%) was suitable for EGFR, compared with CT-TTB (78%), which was not statistically significant ($P=0.66$).

Cryobiopsy in high bleeding risk tumours: During the CT-CROP study, the radial ultrasound was deliberately placed to avoid vascularity noted on ultrasound, as a general precaution. During the study, four patients had cryobiopsies of PPL that are traditionally deemed high risk. The procedure was carried out with standard protocol without the knowledge of the tumour's origin. Therefore, together with the rigid bronchoscopy use, bronchial blockers the simple step of avoiding vascularity with use of radial ultrasound had aided in a safe biopsy. The use of Radial EBUS USS to avoid vascularity and its use in high-risk bleeding tumours was not reported previously and is a contribution to the literature.

When performing the cryobiopsy, additional procedures had to be introduced to improve the safety of the biopsy. In an operating room well equipped with rigid bronchoscopy, the bronchial blocker was well placed and held by a dedicated assistant and the cryobiopsy performed by an expert interventional bronchoscopist with a 4-second freezing time gave excellent control of bleeding. In New Zealand and Australia, the use of the bronchial blocker enabled centres without rigid bronchoscopy facilities to use cryobiopsy in PPLs.

To attain this level of excellent safety profile, immense resources and equipment was required. All bronchoscopy units in New Zealand and many in Australia perform bronchoscopy and Radial EBUS under sedation. Therefore, the generalisability of the cryo-radial procedure in this sense was extremely poor. To overcome the issue of resources and skill intensity, the utilisation of another biopsy tool that can provide a core histology sample but also can be used with sedation bronchoscopy was explored.

8.11 Use of the GenCut tool in obtaining a core biopsy with sedation Radial EBUS in centres where general anaesthesia is not readily available

At the time of these studies in 2016, PD-L1 testing became the forefront of immunotherapy. PD-L1 was only tested on histology specimens and it became an important task and a frequent request from the oncologist to provide a core biopsy from PPL to perform PD-L1.

As discussed above, obtaining a core biopsy was possible with cryobiopsy via Radial EBUS but was highly resource intensive and not attainable by most centres in New Zealand, therefore, an alternative way to use core biopsy was explored. The GenCut core biopsy system which was originally developed to be used with EMN was explored to determine if it could be used with

Radial EBUS alone (without EMN) with the aim of providing a safe and effective core biopsy whilst using Radial EBUS under sedation for use outside tertiary centres was explored (Chapter 7). In the R-CORE study, this GenCut tool was used with Radial EBUS GS without any other navigation.

The R-CORE study (Chapter 7) demonstrates the GenCut core biopsy system as a simple addition to Radial EBUS that can be used with sedation bronchoscopy. GenCut biopsies were taken via the Radial EBUS GS that contains the bleeding. In the R-CORE study, the diagnostic yield with combined multi-modal sampling including conventional Radial EBUS and GenCut tool biopsies was 100% in 16 consecutive patients. The procedure was performed by skilled interventionalists and all PPLs were >15mm located in the outer half of the lung parenchyma. The ability to perform PD-L1 was 76%. There was no pneumothorax or bleeding despite using at least one other Radial EBUS biopsy tool and often three other tools (forceps, brush and aspiration needle) as multi-modal biopsies.

A recently published paper has demonstrated the use of GenCut biopsy with EMN navigation and Radial EBUS. This study demonstrated that GenCut was the sole diagnostic sample in 14.6%. However, the overall diagnostic yield was lower at 50.6% (Orr et al., 2021) compared with the R-CORE study which demonstrated a better diagnostic yield of 76% with GenCut. This may be due to the larger PPL size selected in the GenCut study compared to Orr et al. (2021). The R-CORE study is an original contribution to knowledge; it demonstrates that the GenCut tool can be used effectively with Radial EBUS without expensive navigation technology such as EMN. The procedure is performed under sedation without the requirement for general anaesthesia or theatre facilities.

The R-CORE study also demonstrated that biopsy techniques in combination (multi-modal) improved the diagnostic yield of Radial EBUS. In the R-CORE study, adding the forceps biopsy to the GenCut core biopsy resulted in 100% diagnostic yield when GenCut biopsies for two patients were not performed due to technical issues. All studies in this thesis were designed to demonstrate the use of multi-modal biopsies to improve the diagnostic yield with only Radial EBUS without the addition of expensive navigation methods and is an original contribution to knowledge, demonstrating the improved diagnostic yield with a multi-modal biopsy technique.

8.12 Difficulties faced during the study

There were multiple technical, protocol and logistical difficulties faced during the study.

8.12.1 Technical difficulties

The main technical difficulty was caused by the stiff 1.9mm reusable cryoprobe and the unavailability of an Aspiration needle that was specially designed to traverse the lung parenchyma.

Cryobiopsy probe: Length

The length of the cryoprobe caused difficulties. Initially, in 2015, the cryoprobe was 950mm in length, which was 20mm shorter than the 1050mm GS. This difference necessitated the GS to be trimmed by 30mm so that the tip of the cryoprobe would be longer than the GS to enable contact with the tissue, as explained in the Cryo-feasibility study paper in Chapter 5. By trimming the GS, the radio-opaque tip was lost, resulting in the loss of the ability to visualise fluoroscopy. This requirement to trim the tip also necessitated additional steps in instrument measurement. Staff needed to remember to trim the GS prior to measuring the biopsy tools. Human error tended to occur, which resulted in one patient in the Cryo-feasibility study having a negative biopsy as staff overlooked trimming the GS. During the CT-CROP study, this issue was overcome as the length of the cryoprobe was addressed by ERBO CRYO Germany by making available a longer probe of 1150mm, thus, eliminating the need to trim the GS.

Cryobiopsy probe: Stiffness

The stiffness of the reusable cryoprobe was another issue. This caused major difficulty in its manoeuvrability, especially in dealing with the upper lobes. Both Imabayashi et al. (2019) and Arimura et al. (2019) used the cryoprobe via the large GS, commenting that the stiff cryoprobe resulted in reduced diagnostic yield. Arimura et al. (2019) used alcohol wipes prior to the biopsy to overcome this stiffness. This difficulty was shared in the CT-CROP study. One quarter (4/16: 25%) of cryobiopsies in the cryo-radial arm could not be performed as the cryoprobe could not advance to the upper lobes due to stiffness.

Cryobiopsy probe: Tight fit to the GS

Added to the stiffness of the cryoprobe was the tight fit of the cryoprobe to the large GS. The outer diameter of the large GS at 2.6mm diameter and the largest equipment it can house was a maximum diameter of 1.91mm. The reusable cryoprobe with a 1.9mm outer diameter resulted in an extremely tight fit. Therefore, the cryoprobe had to be pushed through the GS with force; this

was a challenge. This excessive force resulted in the proximal end of the cryoprobe becoming twisted and then eventually breaking off. In the CT-CROP study, the mean number of passes per patient was two. This limitation may have reduced the diagnostic yield in the CT-CROP study as Schuhmann et al. (2014) demonstrated increased yield in up to three cryobiopsy passes, leading to the recommendation for five passes for maximum diagnostic yield.

Flexible single-use 1.7mm and 1.1mm cryoprobes became available for clinical use in Australia after the CT-CROP study completed recruitment in 2018. This new probe was expected to revolutionise the way in which cryobiopsies were being used for PPLs. As the smaller cryoprobe could be used via the smaller GS, additional centres are likely to employ this technique. This first study demonstrating the use of the 1.7mm probe resulted in an encouraging 95% diagnostic yield (Tanaka et al., 2021).

Aspiration needle: Unavailability of aspiration needle designed for use in PPLs

At the time these studies were performed, there was no dedicated aspiration needle to biopsy PPL. The aspiration needles that were available were ones used to perform blind mediastinal nodal biopsies (of the pre-EBUS era). The Wang needle and eXcelon 21G needle were available and used as a substitute. The Cryo-feasibility study used the Wang and eXcelon 21G needle aspirate. Since these were not designed for PPL, the needles were stiff and long. This lack of a dedicated lung parenchymal biopsy needle may be the reason for the low uptake of aspiration needles in PPLs, as commented by Tanner et al. (2018).

Medtronic had developed a lung-specific 8mm length, flexible 21G needle, which was designed to be used with EMN. This was used with most of the CT-CROP and R-CORE patients in Australia. However, the Medtronic needle aspirate was not available in New Zealand, therefore, New Zealand continued to use the eXcelon needle aspirate. However, due to the lack of a well-designed needle aspirate for the lung and various availability across centres, the utility and diagnostic yield of the studies may have been affected.

The newly-developed lung parenchyma-specific Olympus PeriView FLEX aspiration needle 21G is 1.5mm in diameter with high flexibility; it was designed to be used in PPL is a welcome development.

8.12.2 Logistical difficulties specific to New Zealand and Australian setting

Unavailability of anaesthetic support and operating room facilities

In Australia and New Zealand, bronchoscopy procedures were routinely performed in endoscopy suites under sedation. Therefore, for the CT-CROP study that required operating room facilities, this poor availability affected the study, mainly during the early phase. This reduced access resulted in a long wait time for the patient to have his/her procedure. Therefore, many clinicians did not refer the patients for the CT-CROP study, knowing of this drawback, particularly as it was unethical to have a prolonged wait via the study when the patient can readily have a CT-TTB biopsy under local anaesthetic use. This was a major concern at the initiation stage of the study in New Zealand. Added to this issue, anaesthetists were not familiar with bronchoscopic procedures that require sharing the airway. Only a few anaesthetists were up for the challenge, which then caused further delays. This problem was overcome by convincing management of the need for anaesthetic support and operating theatre time for complex bronchoscopies based on the data from the Cryo-feasibility study.

8.12.3 Unavailability of ROSE

During this study, ROSE was not available in the New Zealand centre. Therefore, the usage of ROSE was not included in the protocol for the REBUST 2 and CT-CROP studies. Study centres that had the capability of using ROSE were utilised, as per the proceduralist's request. If ROSE was consistently used, it may have assisted in assessing the sample from the aspiration needle or cytology brush immediately, and manoeuvring the GS to a better location if there was a negative response in ROSE.

Emerging data demonstrate that not only ROSE is valuable in obtaining a diagnosis in 71% of patients. By using ROSE, the number of biopsy tools used would also be reduced (Gildea et al., 2021). Therefore, ROSE became a useful adjunct to rationalising multi-modal biopsies.

ROSE was noted to have high sensitivity of 88.6% to 98.2% on malignant tumours (C. H. Chen et al., 2015; Wan et al., 2020). ROSE had also been demonstrated to improve the diagnostic yield when forceps biopsies were performed only with fluoroscopy, thus demonstrating the individual increase in the diagnostic yield that ROSE adds to the procedure, even in the absence of navigation methods (Leoncini et al., 2020). An RCT using ROSE in 58 patients demonstrated an increased diagnostic yield of 85.7% with ROSE compared to the non-ROSE group at 70.3%. The biopsy tool used was forceps. In addition, the ROSE group reduced the procedure time to 24.6 minutes

compared to the non-ROSE group at 31.5 minutes. Not having ROSE may have reduced the overall diagnostic yield of the studies.

Reduced availability of fluoroscopy

Both centres in Middlemore, New Zealand and Westmead, Australia used fluoroscopy sparingly. The usual room where the bronchoscopies were performed was not lead lined in New Zealand, which meant that if fluoroscopy was to be used, a different room needed to be used, therefore, causing a longer wait time for the procedure to occur. Only 4/26 patients in the CT-CROP study had fluoroscopy. Although fluoroscopy was not used due to the lack of availability, it was noted that the diagnostic yield did not suffer significantly, and an attempt was not made to organise fluoroscopy for the Radial EBUS procedures.

Instead, more emphasis was placed on improving the accuracy of pre-planning by obtaining thin slices of CT chest, ensuring the CT scan was recent (less than one month) and of good quality.

Competing studies

Middlemore Hospital in New Zealand had two competing studies (R-CORE and CT-CROP) whereas Westmead Hospital Sydney in Australia had three competing studies (R-CORE, CT-CROP and REBUST 2), all being recruited at the same time with similar selection criteria. This resulted in a lower capability for each individual study to recruit.

8.12.4 Protocol drawbacks

The slow recruitment process was caused by protocol drawbacks of the CT-CROP study. The resultant early termination reduced the validity of the results due to the loss of power to detect a difference due to the smaller sample size.

Randomisation

Deciding on a randomisation criterion between the CT-TTB and cryo-radial was a major problem encountered with the CT-CROP study as discussed above. A drawback of the CT-CROP protocol design was the use of pure randomisation to assign the biopsy technique to the PPL without considering the characteristics of the PPL (PPL location, air bronchogram) or the patient characteristics. Using randomisation as the only method of deciding the biopsy technique resulted in a proportion of patients, in both arms, not undergoing the randomised procedure, because they were randomised to an arm that would not provide the best benefit to the patient.

Therefore, a more pragmatic approach with the MDT in matching the best diagnostic technique in accordance with the patient and PPL characteristics and expertise and equipment available may be beneficial. However, the MDT approach in itself may not be an ideal solution because MDT decisions are governed by the skill and expertise of the participating group. However, this pure randomisation resulted in only a small pool of clinicians referring patients to the study and slow recruitment from initiation. Furthermore, with an improved understanding of techniques and more widespread knowledge of the techniques among Respiratory Physicians, Interventional Pulmonology meetings (IP) meetings are now available in most major centres. The regional centres could zoom into these meetings and thereby provide a better consensus agreement for studies like this where an agreed decision can be made for randomisation, which is a major strength compared to eight years ago when no such service existed.

Strict exclusion criteria

The next problem was the protocol having strict exclusion criteria. This was also an issue with the CT-CROP study. The protocol excluded patients with positive mediastinal lymphadenopathy. This was included as most patients with mediastinal lymph nodes will have central tumours and if not excluded, will favour the cryo-radial arm, while being disadvantaged to the CT-TTB arm.

In the CT-CROP study, over half the patients had PPLs in the outer one-third of the lung parenchyma and 50% had air bronchograms. Hence, some patients randomised to the cryo-radial arm were not well suited for the procedure despite undergoing the biopsy procedure which may have resulted in a lower diagnostic yield.

The REBUST 2 and R-CORE studies did not have the above strict criteria in the protocol and were easier to recruit.

Study centres in low socioeconomic areas

This study was carried out at Middlemore Hospital in New Zealand and Westmead Hospital Sydney in Australia, with over 95% recruitment in both hospitals. These hospitals are tertiary referral centres and situated in very low socioeconomic areas. The lung cancer prevalence was high due to the high prevalence of smoking which is combined with a low socioeconomic standing. Due to the high incidence of lung cancer, it was expected that the CT-CROP study requiring 158 patients would be completed in three years. However, this was not the case.

Patients in these areas presented late and the majority of them had metastatic disease or nodal disease, therefore, they were ineligible to participate in the study. Due to the lower level of income and health literacy, more patients had severe co-morbidities and were not suitable for anaesthesia.

In effect, a smaller number of patients presenting with the early-stage disease were eligible for the study. It is possible that the low socioeconomic group may not have been the best demographic group for the CT-CROP study. Therefore, it took three years to recruit 48 patients, which is 25% of the sample size.

Due to the extremely slow recruitment over the three-year period and the knowledge on cryobiopsy on PPL expanding with 10 publications added to the growing body of literature within three years, the steering committee decided to close the study as the relevance of the study would dampen if continued. This led to a Type 2 error and reduced power to detect the difference in the primary outcome.

This slow recruitment also affected the REBUST 2 study requiring early termination, yet the reasons were different from the CT-CROP study. The REBUST 2 study experienced acceptable recruitment at the initiation stage. With time, the referring clinicians became aware of better diagnostic yields with the large GS, as well as repeated procedures with the small GS, therefore, they were reluctant to refer patients to the study after a period of adequate recruitment. During the pre-planned interim analysis, the sample size was recalculated. There was a tendency for the large GS to prove a better diagnostic yield, although not clinically significant, was in keeping with the experience of the clinicians, which resulted in the early closure of the study.

8.13 Significance and implications of the finding

This thesis explores GS and the use of needle aspirate, which were known modalities of Radial EBUS but an underutilised method. The thesis also explores the use of novel cryobiopsy and adopting the technique to suit the local facilities and the use of a novel GenCut tool in sedation bronchoscopy to obtain a core biopsy. The thesis aimed at modifying and introducing methods to adapt the cryobiopsy techniques to New Zealand facilities.

The below findings were noted to be significant contributions to the literature either as new findings or as strengthening the sparse data available.

This thesis explores the modification of existing methods.

Adapting the cryobiopsy technique to the New Zealand setting, using a bronchial blocker for the first time in cryobiopsy for PPL instead of rigid bronchoscopy and publishing the feasibility of doing the cryo-radial procedure under sedation, and then demonstrating safety in the procedure under sedation, outside operating rooms, demonstrate the modification of the cryobiopsy technique used in European centres to adapt the New Zealand setting.

This thesis discovered new information.

Cryo-radial procedure in PPL has sparse data limited to 12 studies and most of these studies were retrospective analyses. All these studies were single-arm studies. The CT-CROP study is the first randomised control study that demonstrates the safety of the cryo-radial procedure for PPL in comparison to CT-guided biopsy.

Demonstrating that the use of a bronchial blocker to contain the bleeding effectively and recommended usage of this for cryo-radial biopsies to improve safety instead of rigid bronchoscopy was also a novel concept published.

Demonstrating and publishing that needle aspirate can produce EGFR mutation analysis-which had not been published previously, despite a smaller data set.

Demonstrating the successful use of the GenCut tool to obtain a core biopsy with sedation bronchoscopy without any navigation support is another novel concept.

This thesis provides new solutions to problems.

Most centres in Australia and New Zealand cannot use rigid bronchoscopy cryobiopsy to obtain a core biopsy sample, due to resource intensiveness and a lack of trained personnel. Therefore, a different solution, the GenCut tool was substituted to provide core biopsy instead of cryobiopsy under sedation and demonstrated efficacy and safety.

This thesis devises new investigative methods.

To date, the comparison of the novel cryo-radial method with the CT biopsy “gold standard” is the only study published in Australia and New Zealand.

This thesis provides additional support for existing theories, models and interpretations.

This thesis provides additional data to the growing body of literature on the ability of needle aspirate to increase the yield of PPL, as well as highlights the need for a PPL-specific flexible aspiration needle. (The study was completed prior to the preview flex needle becoming available.) The thesis also adds to the body of data by demonstrating that a multi-modal biopsy may improve the yield overall, as well as exhibit its ability to perform EGFR analysis.

This thesis also demonstrates that cryobiopsies provide additional diagnostic yields to forceps biopsies with will provide further evidence to the existing literature. In the field of cryobiopsy in

PPL so far, only 10 published papers with 300 patients with only five papers are prospectively done.

Using the Radial EBUS ultrasound to assess for vascularity and redirecting the ultrasound to a better location had been noted in increasing the safety profile even in high-risk bleeding tumours in addition to the measures discussed above. The use of cryobiopsy in PPL with a high risk of bleeding was not previously published.

8.14 Significance to the discipline of respiratory medicine/ interventional bronchoscopy

These studies in the thesis, publications and presentations, exposed Australian and New Zealand clinicians to the concept of Radial EBUS as an achievable and simple diagnostic method, they can employ in their units. Noting the high safety profile would enable physicians to use these methods with confidence. Having knowledge of multiple biopsy techniques will assist respiratory physicians in choosing a modality to suit the PPL and available facilities and not just depend on forceps as many had previously done so.

Lack of navigation method was thought to be a drawback in Radial EBUS as most North American centres used EMN. However, these studies demonstrated that this is not the case as none of the studies used an expensive navigation method and relied mostly on Kurimoto's method.

These studies have demonstrated that each biopsy tool requires different skills and equipment. Therefore, incorporating multi-modal biopsy techniques, including novel techniques such as cryobiopsy, GenCut biopsy and the frequent use of the aspiration needle, should be part of the training for future interventional bronchoscopy fellows who would be employed at various clinical locations where facilities would differ. The knowledge of using different biopsy tools based on the resources available will enhance the biopsy process to diagnose a PPL with confidence.

The comparison of cryo-radial biopsy with CT-TTB is novel; it will encourage more respiratory physicians, in particular non-interventional physicians, to consider cryo-radial as another biopsy technique for PPL, in combination with L-EBUS for an all-in-one staging and diagnosis process. The low side effect profile in all Radial EBUS biopsy techniques, including the cryo-radial arm, will encourage the MDT to decide favourably to use the cryo-radial procedure as the first biopsy method for PPLs.

The presentation of this study's results was encouraging for the oncologist to refer more patients to Respiratory for bronchoscopic/EBUS biopsies as opposed to CT-guided biopsies.

Historically The data available for cryobiopsy in the lung was taken from the body of literature available for ILD cryobiopsy which had a significant pneumothorax (39%) and bleeding risk (12%); the bleeding risk with cryobiopsy was always feared (Johannson et al., 2016). With the growing concern for the use of cryobiopsy in ILD, position papers were released, and the competence of the proceduralist and the standardisation of the procedure were demanded by experts in the ILD cryobiopsy (Hetzl et al., 2018). There has been global recognition of the higher safety requirements and standardisation of the ILD-cryobiopsy procedure with a recent release of CHEST guidelines and expert panel opinions (Maldonado et al., 2020).

Data on cryobiopsy in PPL is limited to 12 studies and available data demonstrate an excellent safety profile compared with the safety profile for cryobiopsy in ILD. This CT-CROP data, therefore, adds to the body of evidence emerging on the safety of cryobiopsy in PPLs that sets it apart from cryobiopsy in ILD.

Much benefit has resulted in the hospitals these studies were carried in. The acceptable results and the excellent safety of the procedure convinced the formation of a regular operating list for interventional bronchoscopy procedures. Middlemore Auckland hospital now is in possession of a regular list based on the experience and the drawbacks highlighted during the cryo-feasibility study.

During this process of these studies, publications and pilot studies, many registrars, advanced trainees and fellows were involved as investigators and benefited from being exposed to this safe technique of Radial EBUS. Many of these junior doctors who became Respiratory consultants recently had used the Radial EBUS business case and these published papers from these studies to convince their local hospitals to commence Radial EBUS services and use cryobiopsy as a biopsy method.

Although robotic bronchoscopy is advancing in leaps and bounds, the prohibitive cost will not see this service established in Australia or New Zealand as a mainstream diagnostic technique for another decade. Even if Robotic bronchoscopy is established most robotic procedures require Radial EBUS to be used via the GS. Therefore, Radial EBUS and its guided biopsy methods are a cost-effective method to invest in, with a Radial EBUS processor which costs around AUD \$35,000, a fraction when it comes to medical expenditure. The studies in this thesis would add to the existing data on Radial EBUS and cryobiopsy to assist in policy making in the future.

8.15 Significant impacts in Australian and New Zealand

The studies in this thesis were carried out in Australia and New Zealand from 2015 to 2019 in four academic centres. Radial EBUS is a novel method used in New Zealand; it is the only guided bronchoscopy method available since 2014 and little has changed since.

Establishing the first Radial EBUS service as well as the first cryobiopsy service in New Zealand was a major breakthrough that occurred due to these studies in the thesis.

As this was an innovative cryobiopsy service used for lung biopsy in New Zealand, all equipment, including the cryobiopsy machine and probes, needed to be purchased. For this, the author applied to multiple funding bodies. As a part-time staff specialist respiratory physician, most applications were rejected. However, the ongoing effort resulted in four separate grants being awarded, which amounted to AUD\$100,000+, thus enabling the purchase of the cryobiopsy machine and cryobiopsy probes for Middlemore Hospital in New Zealand. The purchase of this equipment resulted in patients receiving ongoing medical services after the study was completed.

Another major clinical impact is that training respiratory physicians on new methods of the aspiration needle, GenCut tool and cryobiopsy with its safety procedures in Middlemore Hospital, New Zealand and Westmead Hospital, Australia. Nurses and doctors learned about equipment measurements, identification of PPLs via USS, policies, and the procedures manual for safety and resuscitation. This was new knowledge imparted and a new service commenced on the back of these studies.

Through these studies and accompanying training, the interventional bronchoscopy unit at Middlemore Hospital, Auckland now claims multiple publications in the area (Good et al., 2018; Herath & Yap, 2018; Yap & Low, 2017). Through these studies, a seamless service was established with many respiratory physicians in Middlemore Hospital and Westmead Hospital developing interest and engaging in providing a new service. With service development and the addition of the cryo-radial procedure as a routine procedure to the armoury of biopsies. These hospitals now offer these services as the standard clinical practice.

Bronchoscopy units in New Zealand do not have anaesthetic support, rigid bronchoscopy facilities and theatre availability. This holds true today, even in tertiary centres. Only a few Australian and New Zealand centres have rigid bronchoscopy capability, and even if it is available, it is used sparingly. Currently, most Australian and New Zealand centres perform bronchoscopy under sedation. The REBUST 2 and R-CORE studies provide these much-needed tools that can be used in sedation bronchoscopy in regional and peripheral centres to improve the

diagnostic yield of the Radial EBUS procedure, where delays and the transference of patients to major centres can be avoided. By demonstrating the ability for EGFR testing with an aspiration needle and PD-L1 with GenCut, additional units will be able to adopt these tools with confidence due to their safety.

In carrying out these studies in New Zealand from 2014 to 2018 when interventional bronchoscopy was in its infancy, the Radial EBUS method became established based on these studies. Today, Radial EBUS and cryobiopsy is a mainstream biopsy method for PPL.

8.16 Future directions

8.16.1 Diagnosing malignancy without a biopsy

Bronchoscopic imaging

The ability to visualise exceeded the ability to obtain a diagnostic biopsy sample, therefore, it would be beneficial to determine if a diagnosis could be made by visualising the image without a biopsy. Imaging can be used to determine the malignant or benign nature of a PPL, as well as used to avoid adverse effects of biopsies. The gap between visualisation and diagnosis can be bridged, particularly when a diagnostic biopsy is not required and only visualisation is necessary. Two techniques currently in development are: (i) confocal laser endomicroscopy (CLE); and (ii) Raman spectroscopy.

CLE is an imaging technique that uses laser and autofluorescence by applying fluorescein to stain the PPL endoscopically. This technique identifies malignant characteristics in PPLs. A recent concept study on CLE demonstrated that after a short educational session, 95% of raters could accurately identify a lung cancer from a benign parenchyma (Fuchs et al., 2013).

Robotic bronchoscopy has also been combined with needle-based confocal laser microscopy, demonstrating additional feedback of the tool in PPL, especially in difficult-to-reach and smaller lung nodules. Development in this area is eagerly awaited (Manley et al., 2022).

Raman spectrometry is an imaging system that uses inelastic scattering of light to identify normal, inflamed and malignant patterns endoscopically. Both these methods are in the early stages of development with promising possibilities (Kramer et al., 2021).

Non-bronchoscopic techniques to identify early lung cancer

Whilst the focus has been on interventional bronchoscopic methods on diagnosing PPLs suspected of lung cancer, it must be appreciated that there is a large array of non-bronchoscopic techniques that are emerging in the diagnosis of early lung cancer. These include biomarkers, liquid biopsy, biosensor, radio genomics and artificial intelligence (Prabhakar et al., 2018).

8.16.2 Moving towards therapeutic bronchoscopy

Over the last decade, interventional bronchoscopy has expanded from being considered a diagnostic modality to an area of therapeutic procedures. As the localisation of PPL has improved, attempts have been made to treat PPL with bronchoscopic procedures. Newly developed therapeutic bronchoscopic methods to treat malignant PPL include radiofrequency ablation, microwave ablation and steam ablation.

Therapeutic use of GS and aspiration needle

The Radial EBUS GS has been used to drain lung abscesses. Yaguchi et al. (2018) reported on using the Radial EBUS GS to apply suction after placing the GS to draw up 4-5 millilitres of fluid.

The author of this research has also reported on using the aspiration needle via the large GS to drain a large cyst filled with fluid in a patient suspected of lung cancer. The abstract was presented to TSANZ in 2019 (Herath, 2019, Poster presentation – Appendix 4).

Using the GS to mark segmental/subsegmental airways with Radial EBUS has been popularised over the last few years, especially in combination with robotic cardiothoracic surgery where subsegmental resections have been attempted, including dye-marking a small pulmonary nodule with the Radial EBUS GS. Lachkar et al. (2017) demonstrated the dye becoming visible in 24/25 cases with the surgeon explaining that if dye-marking was not used in 21/25 cases, it would not have been possible to locate the PPL. The result published by Xu et al. (2016) showed a similar success rate. Multiple small PPLs detected during lung screening requires a similar intervention to locate the PPL for patients needing surgical involvement.

A surgical resection is the preferred benchmark for Stage 1 NSCLC as increased numbers of early cancers are detected within an aging population, however, patients may not be suitable for surgical intervention due to their age and comorbidities. For these patients, therapeutic bronchoscopic methods need to be developed to offer minimally invasive therapeutic procedures to obtain remission or local control for a greater time.

Bronchoscopic guided radiofrequency ablation (bRFA), bronchoscopic thermal vapour ablation (BTVA) and bronchoscopic microwave ablation (bMWA) are three promising tools that are in development.

Bronchoscopic guided radiofrequency ablation (bRFA)

Percutaneous CT-guided RFA has been used for over a decade. In 2012, the five-year early-stage survival of non-operable NSCLC was 47% with stereotactic body radiotherapy and 20.1-27% with percutaneous CT-guided RFA (Palma et al., 2012). The problem with percutaneous RFA were Grade 1 and Grade 2 radiation pneumonitis and frequent occurrence of pneumothorax that required a chest drain. There was also a high local recurrence rate with RFA (up to 50%) (Koizumi et al., 2015).

To overcome these issues, bronchoscopic RFA has been explored, which resulted in the introduction of a new internal cooled tip electrode for RFA via the bronchoscopic working channel in 2013. The length of the tip was 10mm with five beads located. The electrode was attached to a monopolar radiofrequency generator with a maximum output of 50 watts. The patient was grounded using steel mesh dispersive electrodes. Cooled water was circulated via the electrode to prevent highly heated tissue from becoming attached to the electrode, called the “pop” phenomenon. The generator automatically switched the electrode off if elevation of temperature occurred. All procedures were done with sedation and local anaesthetic. The RFA was done for 50 seconds under spontaneous breathing. Safety and repeatability were the most advantageous benefits with this method.

A case study by Koizumi, et al., (2013) of non-respectable NSCLC patients aged 80 years gained four years of stability while 70-year-old patients achieved three-and-a-half years of stability. No adverse reactions were recorded. The median progression-free survival rate was 35 months (95% CI 36-87%). Koizumi et al. (2015) demonstrated a control of nearly three years.

Bronchoscopic microwave ablation (bMWA)

Percutaneous MWA has been used for unresectable lung tumours; a high rate of pneumothorax (63% with 19% requiring an ICT) and centrally located lesions may be technically difficult (Tsakok et al., 2019). Additional complications have included pleural effusions (6-19%), haemothorax, empyema and skin burn.

Recent interest has occurred in using bMWA. With the bronchoscopic approach and efficient heating of the tissue, bMWA may provide a safe and highly effective method with minimal invasiveness. The goal of any ablation is to have a circumferential ablation margin of at least

5-10mm. Furthermore, cooling methods (water cooled versus CO₂ cooled) may influence the ablation zone created, and CBCT guidance for intra- and post-procedures are considered invaluable for the accurate placement of the ablation probe.

More recently, a 30-patient retrospective data of bMWA demonstrated 25 patient's nodule size of 15.1mm with 50% bronchus sign positivity, and technical success was reported as 100%. The mean minimal ablation margin was 5.51mm, and the mean hospital stay was 1.73 days. Complications included pain (13.3%), pneumothorax requiring drainage (6.67%) post-ablation reaction (6.67%), pleural effusion (3.3%) and haemoptysis (3.3%). With the use of CBCT and EMN (Covidien), after a median flow of up of 12 months, no progression was seen (Chan et al., 2021).

Bronchoscopic thermal vapour ablation (BTVA)

The use of BTVA for lung volume reduction in COPD has been used for over a decade now. The potential for this technology to be used for lung cancer was explored by Steinfert et al. (2021). In a single arm pilot study of six patients who had their surgical resection planned for Stage 1 NSCLC, BTVA for lung cancer was performed via thermal vapour energy through a bronchoscope to the target lesion. A pre-surgical CT chest was also performed. The median procedure time was 12 minutes. All results were partly solid adenocarcinomas; a near complete necrosis was seen in two patients with large uniform ablation zones noted in four patients. There were no side effects. The technique appeared to be promising, however, further evaluation is required.

These novel therapeutic modalities are still in the early stages of development, but they hold promise for use in combination with CBCT and robotic bronchoscopy, therefore, this fascinating development of therapeutic interventional bronchoscopy is eagerly awaited.

8.17 Recommendations

In this thesis, the hypothesis that optimising biopsy tools and using multi-modal biopsy techniques improve the gap between visualisation and diagnostic yield was only partially proven.

Several drawbacks with the biopsy tools that occurred during these studies may have had a negative effect on the diagnostic yield. The cryobiopsy probe available at the time of the study was stiff and a tight fit to the GS. Aspiration needles specifically designed for Radial EBUS biopsy of PPL were not available. ROSE was not available for most biopsies. If these barriers

were addressed, further improvement of the diagnostic yield would be expected by optimising the biopsy tools.

With the arrival of the new 1.1mm and 1.7mm flexible cryoprobes, the drawbacks encountered with the stiff and large 1.9mm cryoprobe are eliminated. A study had already demonstrated the 1.7mm single-use flexible cryobiopsy probe being used to improve the diagnostic yield rapidly to 94% in PPLs with a mean size of 20.8 (Tanaka et al., 2021). This study used Radial EBUS without the expensive EMN or VBN. With this improvement, further studies are eagerly awaited in reproducing these results with the use of novel single-use flexible cryoprobes.

Further, the 1.1mm cryoprobe that has been used with ultrathin bronchoscopy, as well as robotic GS, may provide a more controlled smaller biopsy that can be extracted through the Radial EBUS GS. There are already published case reports (Herath, 2023) demonstrating the ability of a 3-4 second cryobiopsy to be successfully extracted through both the small and large GS, negating the need for a bronchial blocker. Further studies would be a much welcome development that would enable Cryo-radial to be used without the expensive rigid bronchoscopy or bronchial blocker usage, without compromising safety.

Given the encouraging results of the recently published study that demonstrated the utility of ROSE in reducing the number of biopsy tools, the consistent use of ROSE will assist in rationalised usage of multi-modal biopsy to suit the PPL and the type of malignancy. This will reduce the time and cost of the procedure (Gildea et al., 2021).

Further studies now need to be done to start rationalising the tools in the toolbox to suit the PPL location and patient characteristics to obtain maximal results. In addition to this, the ability to visualise the biopsy tool in the PPL real-time during biopsy will also assist in bridging this gap from visualisation to diagnostic yield. To this effect, studies on Robotic bronchoscopy and cone beam CT are underway and are eagerly awaited.

Proceduralist training is another important aspect that needs to be improved as interventional bronchoscopy in Australia and New Zealand is becoming a subspecialty of its own. There is a real gap that exists in the diagnostic yield between the single-centre tertiary hospital trials and the real-life experiences in Radial EBUS with the diagnostic yield in some multi-centre trials being less than a toss of a coin (Ost et al., 2016; Tanner et al., 2018). To acquire knowledge of planning a biopsy of a PPL, to use multiple biopsy tools safely and to improve real-life results, proceduralist training becomes of paramount importance. Learning to read CT chest scans well and having advanced knowledge of the bronchial tree is essential in mapping a pathway to the PPL. Now

having published literature and material on Kurimoto's method that can be used anytime without expensive equipment is a major advantage (Kurimoto, 2020). This procedural training should not be limited to tertiary centres and should be available more widely so that these simple techniques can be well used. For this, more hands-on training workshops and online material need to be available as well as the ability to do observership rotations in tertiary centres.

Accreditation and maintenance of competence is other important aspect. Whilst the Radial EBUS procedure is simple and the biopsy tools can be learnt easily, the rigour with which the procedure is done shapes the diagnostic yield, hence the wide discrepancy in the diagnostic yield. Accreditation guidelines for interventional bronchoscopy fellowships in North America have been well established (Mullon et al., 2017). However, the Australia/New Zealand Thoracic Society accreditation of advanced procedures, including rigid bronchoscopy and cryobiopsy are still in their infancy. Australian and New Zealand Respiratory Advance training does not incorporate complex bronchoscopy training and is limited to flexible bronchoscopy. Furthermore, Australian and New Zealand trainees are not required to perform mandatory training in intensive care. Currently, in Australia, there are only a few centres that offer a 12-month interventional bronchoscopy fellowship. Therefore, the opportunities are minimal. More fellowship training opportunities and better competency assessment and competency maintenance are required to improve the knowledge and mastery of these tools and techniques. To improve the safety of the industry, accreditation and standardisation are of utmost importance.

Whilst the CT-CROP study does not show diagnostic yield to be as high as CT-TTB, the procedure is significantly safer with fewer pneumothoraxes and less bleeding. It, therefore, allows the clinician an alternative diagnostic path besides CT-TTB for PPLs, especially when a CT-TTB is considered high risk (near a vessel, or when there is significant emphysema), and allows the clinician to weigh up the most appropriate diagnostic path especially via an interventional bronchoscopy multidisciplinary meeting, given the availability of alternatives with various tools that have been shown to be safe and effective.

These studies and a multitude of abstract presentations at Thoracic Society meetings from 2016 to 2019 have helped to introduce Radial EBUS-based diagnostic options in New Zealand where Radial EBUS and cryobiopsies are now mainstream in the field of Interventional bronchoscopy. Demonstrating safety in using Radial EBUS tools that can be used in sedation enables more centres to take up this valuable tool.

With the advancement of biopsy tools with smaller cryoprobes and smaller needle aspirates coupled with robotic bronchoscopy, diagnostic accuracy is expected to be very high. Due to this

reason in the future, interventional bronchoscopy will no longer be limited to diagnosis. With an aging population with increasing comorbidities and a higher detection of smaller nodules, most patients may not be able to undergo surgery and may benefit from bronchoscopic treatment methods for their lung malignancy.

Ultimately, we envisage an improved diagnostic yield with robotic bronchoscopy with the use of rationalised multi-modal biopsy tools, whilst using ROSE and obtaining a larger non-crushed tissue using cryobiopsy with an immediate diagnosis of the PPL, followed by staging the mediastinum. This could then follow up with the best therapeutic modality to suit the PPL to treat the malignancy.

The paradigm of the diagnosis to treat lung cancer in one single bronchoscopic procedure is the ultimate target of improving the timeframe from diagnosis to treatment and patient survival.

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APPENDICES

Appendix 1: Arnott Research Entry Scholarship in Cancer Research dated 8 December 2017



8 December 2017

Dr Samantha Chandani Herath
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via email: scherath@yahoo.com

Dear Dr Herath

Project title: *Randomised controlled trial comparing the diagnostic yield of CT guided transthoracic biopsy vs. cryobiopsy (via the radial endobronchial ultrasound guide sheath) in parenchymal pulmonary lesions suspected of lung cancer. (CT CROP)*

As Chair of the Grants Advisory Committee and on behalf of the RACP Board, it is with pleasure that I write to advise that you have been awarded the *Arnott Research Entry Scholarship in Cancer Research* for 2018 for the amount of \$45,000.00.

Please email foundation@racp.edu.au by Monday 8 January 2018 advising whether or not you intend to accept the award under the conditions set out in the accompanying documents. This includes supplying the Foundation with a progress and or summary report, as applicable to your award.

If it is your intention to accept the award, please sign and return the award acceptance form together with the other required documentation to foundation@racp.edu.au.

I also request that you provide notification if there have been any changes to the content of your original application, including timeframes and research scope.

Please note that the actual amount to be awarded is subject to your final budget and your compliance with the Terms and Conditions governing the award and will depend upon any other non-College Fellowships or support that you may be awarded.

Please do not hesitate to contact the Senior Administration Officer, Nicola Price, with any questions or concerns regarding the award or acceptance documentation on 02 9256 9639 or foundation@racp.edu.au.

Yours sincerely

Professor Michael Horowitz
Chair
Grants Advisory Committee

145 Macquarie Street, Sydney NSW 2000, Australia • Tel: +61 2 9256 5444 • Fax: +61 2 9252 3310
Email: racp@racp.edu.au • Web: www.racp.edu.au • ACN 000 039 047 • ABN 90 270 343 237

Appendix 2: Basser Research Entry Scholarship dated 1 December 2016



RACP
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1 December 2016

Dr Samantha Mudiyansele Herath
16 Warrimoo Avenue
ST IVES NSW 2075

via email: scherath@yahoo.com

Dear Dr Herath

Project title: Randomised controlled trial comparing the diagnostic yield of CT guided transthoracic biopsy vs cryobiopsy (via the radial endobronchial ultrasound guide sheath) in parenchymal pulmonary lesions suspected of lung cancer

As Chair of the Grants Advisory Committee and on behalf of the Board of The Royal Australasian College of Physicians (RACP), it is with pleasure that I write to advise that you have been awarded the 2017 Basser Research Entry Scholarship for \$45,000.

Please email foundation@racp.edu.au by 16 Dec 2016 advising whether or not you intend to accept the award under the conditions set out in the accompanying documents. This includes supplying the Foundation with a progress and or summary report, as applicable to your award.

If it is your intention to accept the award, please sign and return the award acceptance form together with the other required documentation to foundation@racp.edu.au.

I also request that you provide notification if there have been any changes to the content of your original application, including timeframes and research scope.

Please note that the actual amount to be awarded is subject to your final budget and your compliance with the Terms and Conditions governing the award and will depend upon any other non-College Fellowships or support that you may be awarded.

Please do not hesitate to contact the Senior Administration Officer, Nicola Price, with any questions or concerns regarding the award or acceptance documentation on 02 9256 9639 or foundation@racp.edu.au.

Yours sincerely

Professor Michael Horowitz
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Email: racp@racp.edu.au • Web: www.racp.edu.au • ACN 000 039 047 • ABN 90 270 343 237

Appendix 3: TSANZ 2019 poster presentation: Using cryobiopsy safety in high bleeding risk PPL

Herath, S. (2019). Safe and effective use of cryobiopsy with Radial EBUS for lung masses with a high bleeding risk: Case series and description of technique. *Respirology*, 24(1), 151-152.



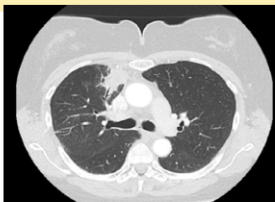
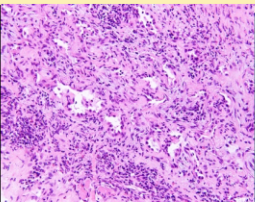
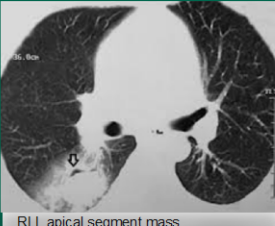
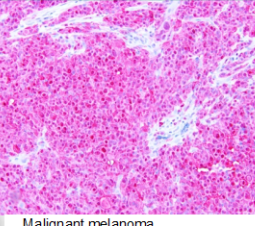
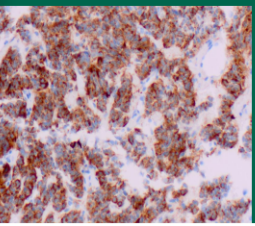
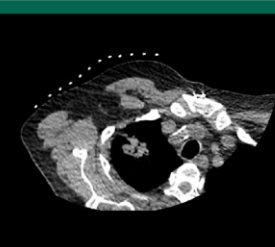
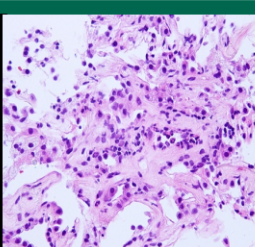
SAFE AND EFFECTIVE USE OF CRYOBIOPSY WITH RADIAL EBUS FOR LUNG MASSES WITH A HIGH BLEEDING RISK

CASE SERIES AND DESCRIPTION OF TECHNIQUE

Samantha Herath^{1,2,3}

¹Department of Respiratory and Sleep Medicine, Westmead Hospital, NSW 2145
²Macquarie University Hospital, 3, Technology Place, NSW 2109
³University of Sydney, Sydney Adventist Hospital Medical School, NSW 2075



Background	Tumor type	CT appearance	Cryobiopsy
Cryo-biopsy is an emerging tool in the diagnostic armory for suspected lung cancer (1,2). We describe our experience in using cryo-biopsy with radial EBUS (R-EBUS) in tumours of high bleeding risk and describe the technique we adopted.	Case 1 54y, Female, non-smoker with incidentally detected spiculated opacity in Right upper lobe. Cryo-radial biopsy (CR-BX): Organising pneumonia	 RUL anterior segment mass	 Organizing Pneumonia
Description of technique All cases were done under general anaesthesia with three cases using a 12mm storz rigid bronchoscopy. A 5F fogherty catheter was placed prophylactically adjacent to the lesion and inflated post biopsy. The radial EBUS with large Guide sheath(GS) was used to locate lesion and the cryoprobe was inserted through the GS. Once in place activated for 4 seconds. Cold saline, adrenaline and tranexamic acid was drawn and kept but was not required.	Case 2 57y, Male, current smoker with Right lower lobe mass. CR-BX: malignant melanoma	 RLL apical segment mass	 Malignant melanoma
Conclusions All patients underwent CR BX for lung cancer but ended up with diagnosis of tumours that are usually categorised as higher bleeding risk. Correct use of cryo-radial can give excellent diagnosis in benign as well as malignant tumours and can be used safely even in tumours carrying a higher risk of bleeding with the use of correct technique in units with adequate resuscitation facilities	Case 3 77Y, Male, lifetime non-smoker presented with a diagnosis of atypical cells suggestive of small cell lung cancer on a trans-bronchial biopsy of a Right middle-lobe. CR-BX: typical carcinoid tumour	 RML medial segment star bronchus mass	 Chromogranin positive for carcinoid
	Case 4 60y, Male, heavy smoker presented with Right upper-lobe mass. He had high C-ANCA levels. Mass resolved with high dose immunosuppression CR-BX: Vasculitis (Wagener's granulomatosis)	 RUL apical segment mass	 Acute capillaritis : Vasculitis
References Schumann M BK, Bugalho A, Warth A, Schnabel PA, Herth FJ, Eberhardt R. Endobronchial ultrasound-guided cryobiopsies in peripheral pulmonary lesions: a feasibility study. <i>The European respiratory journal</i>. 2013;43(1):233-9 Herath S, Yap E. Novel hybrid cryo-radial method: an emerging alternative to CT-guided biopsy in suspected lung cancer. A prospective case series and description of technique. <i>Respirology case reports</i> 2018;6 (2); e00267			

TP 085

TP 086

PLEURAL EFFUSION MANAGEMENT AT AUSTIN HEALTH: A SINGLE-CENTRE AUDIT

GUO H¹, LEONG T¹, KHOR Y¹, AHMED N¹, MCDONALD C¹

¹Austin Health, Melbourne, Australia

Introduction: Pleural effusions are common presentations with a wide range of differential diagnoses. A structured approach for thoracentesis is essential for timely diagnosis and appropriate management. To maximise procedural safety and diagnostic yield, current guidelines recommend the use of bedside ultrasound guidance in addition to appropriate sample collection and testing.

Aim: To assess the standard of care provided for patients presenting with pleural effusions at Austin Health between 2015 and 2016. In particular, to evaluate the timing and method of thoracentesis and appropriateness of pleural fluid testing.

Methods: A retrospective audit was performed of all patients who presented to Austin Health with pleural effusions between 2015 and 2016. Data was extracted from scanned medical records relating to patient demographics; aetiology of pleural effusions; timing and method of thoracentesis; and pleural fluid testing.

Results: Over 700 patients were included in the audit, with median age of 69 and a slight male predominance. These patients were admitted under a range of medical and surgical specialty units. The majority of patients presented with simple unilateral effusions and underwent thoracentesis within 24 hours of admission. In most cases, the procedure was performed by the admitting unit after remote sonographic localisation. Most episodes of thoracentesis resulted in appropriate pleural fluid testing. However, surgical teams performed less biochemical analysis of the pleural fluid and the median volume of fluid sent for cytological analysis was less than 10 mL.

Conclusion: Most patients presenting with pleural effusions underwent timely thoracentesis with appropriate pleural fluid analysis. However, the majority of procedures were performed with remote ultrasound localisation without consistent biochemical testing. This highlights areas of improvement for the inpatient management of pleural effusions, and forms the basis of a prospective study into the use of bedside ultrasound for thoracentesis by dedicated respiratory proceduralists.

Grant Support: Nil.

SAFE AND EFFECTIVE USE OF CRYOBIOPSY WITH RADIAL EBUS FOR LUNG MASSES WITH A HIGH BLEEDING RISK: CASE SERIES AND DESCRIPTION OF TECHNIQUE

HERATH S^{1,2}

¹Staff Specialist, Department of Respiratory and Sleep Medicine, Westmead Hospital, Sydney, Australia, ²Clinical Lecturer, Sydney Medical School, the University of Sydney, Sydney, Australia

Introduction/Aim: Cryo-biopsy is an emerging tool in the diagnostic armory for suspected lung cancer (1,2). We describe our experience in using cryo-biopsy with radial EBUS (R-EBUS) in tumours of high bleeding risk and describe the technique we adopted.

Case series: (Table 1)

Case 1: 54 years, Female, non-smoker with incidentally detected sub-pleural opacity in Right upper lobe. Cryo-radial biopsy (CR-BX): Organising pneumonia.

Case 2: 57 years, male, current smoker with Right lower lobe mass. CR-BX: malignant melanoma.

Case 3: 77 years, male, lifetime non-smoker presented with a diagnosis of atypical cells suggestive of small cell lung cancer on a trans-bronchial biopsy of a Right middle-lobe.

CR-BX: typical carcinoid tumour.

Case 4: 60years, Male, heavy smoker presented with Right upper-lobe mass. CR-BX: vasculitis. He had high C-ANCA levels. Mass resolved with high dose immunosuppression. All cases required Radial EBUS for visualisation of the lesion. Mean of two CR-BX were done per patient. The sample size of 7 mm were noted without significant bleeding or pneumothorax.

Description of technique: All cases were done under general anaesthesia using a 12 mm storz rigid bronchoscopy. A 5F fogarty catheter was placed prophylactically adjacent to the lesion and inflated post biopsy. The radial EBUS with Guide sheath(GS) (Olympus 203) was used to locate lesion and the cryoprobe was inserted through the GS. Once in place cryo-probe was activated for 4 seconds. Cold saline, adrenaline and tranexamic acid was drawn and kept but was not required.

Discussion: All patients underwent CR BX for lung cancer but ended up with diagnosis of tumours that are usually categorised as higher bleeding risk.

Correct use of cryo-radial can give excellent diagnosis in benign as well as malignant tumours and can be used safely even in tumours carrying a higher risk of bleeding with the use of correct technique in units with adequate resuscitation facilities.

Grant Support: None

Conflicts of Interests: None

REFERENCES

- Schuhmann M BK, Bugalho A, Warth A, Schnabel PA, Herth FJ, Eberhardt R. Endobronchial ultrasound-guided cryobiopsies in peripheral pulmonary lesions: a feasibility study. *The European respiratory journal*. 2013;43(1):233-9
- Herath S, Yap E. Novel hybrid cryo-radial Method: an emerging alternative to CT-guided biopsy in suspected lung cancer. A prospective case series and description of technique: *Respirology case reports* 2018;6 (2); e00287.

Key words:

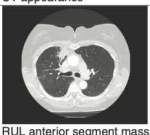
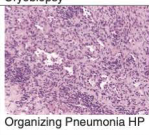
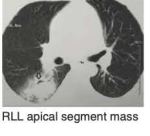
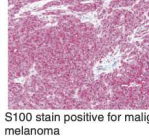
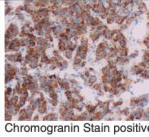
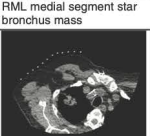
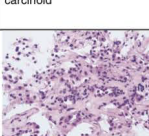
Lung cancer, cryo-radial biopsy, safety, large samples, bleeding risk

Appendix 4: Abstract – Herath, S. (2019). TSANZ. Therapeutic use of Radial EBUS guide sheath

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Abstracts

Table 1: comparing the Radiological appearances to the Histological appearances from cryobiopsy showing well preserved large samples.

Tumour type	CT appearance	Cryobiopsy
Case 1 Pneumonia	 RUL anterior segment mass	 Organizing Pneumonia HP
Case 2 Malignant melanoma	 RLL apical segment mass	 S100 stain positive for malignant melanoma
Case 3 Carcinoid Tumour	 RML medial segment star bronchus mass	 Chromogranin Stain positive for carcinoid
Case 4 Vasculitis (Wagener's granulomatosis)	 RUL apical segment mass	 Acute capillaritis on biopsy; Vasculitis

TP 087

THERAPEUTIC USE OF RADIAL EBUS WITH ASPIRATION NEEDLE VIA THE GUIDE SHEATH. CASE REPORT AND DESCRIPTION OF TECHNIQUE.

HERATH S¹

¹Westmead Hospital, Sydney, Australia

Introduction/Aim: Radial Endobronchial USS guided biopsy (R-EBUS) is becoming popular diagnostic tool (1). Therapeutic use of R-EBUS is not reported. We report a case of a cystic lung mass that was drained using a 21G aspiration needle via the R-EBUS GS.

Case report: A 67-year-old, non-smoking female was referred for Respiratory opinion for a Left upper lobe (LUL) mass with a background history of a recently diagnosed locally advanced cervical squamous cell cancer as a malignancy was suspected.

Patient was asymptomatic and afebrile with normal biomarkers. She had no other co-morbidities. She had history of fully-sensitive tuberculosis treated in the Philippines in 2002.

A R-EBUS procedure was planned under sedation using BF TH190 rotatable bronchoscope and large GS (Olympus 203). The lesion was concentrically visualised. Initial biopsy with the forceps and brush met with wall like resistance and did not yield diagnostic material.

Additional 21G Medtronic aspiration needle (21G, 8mm length 1.9mm diameter) was used via the GS and the thick wall of the cyst was pierced. Initial aim was only to obtain a diagnostic sample, however, despite stopping the suction, continuous oozing of brown thick fluid occurred under pressure through the needle sheath. A 150ml of turbid fluid was drained. GS was removed without any complications of bleeding or pneumothorax.

A post procedure CT chest demonstrated complete drainage of the cyst.

12-month post follow-up, the cyst remained dry and stable and deemed to be a previous tuberculous cavity.

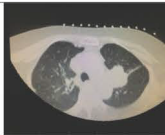
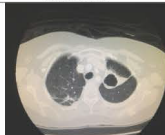
Conclusion: R-EBUS with a GS and aspiration needle was used safely to completely drain this large cyst without any adverse events under sedation, highlighting not only the diagnostic but also the therapeutic utility of using an aspiration needle via the GS.

Further studies will be required to explore the use in bronchoscopic aspiration of cavities and even introduction of medications to the cavity.

Grant Support: None.

Conflicts of interest: None.

Table 1 A: Pre-aspiration CT chest LUL mass B: Post aspiration CT chest LUL cavity with a thick wall C: Aspirated purulent material

1. Pre-aspiration CT chest LUL mass	A. Post aspiration CT chest LUL cavity with a thick wall	B. Aspirated purulent material
		

REFERENCE

Steinfurt D P, Khor Y,H, Manser R,L, Irving L,B. Radial probe endobronchial ultrasound for the diagnosis of peripheal lung cancer: Systematic review and meta-analysis. European Respiratory Journal. 2011;37 (4):902-10.

Appendix 5: Abstract – Herath, S. (2019). TSANZ. REBUST 2

Abstracts

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TO 130

TO 131

MULTI CENTRE RANDOMISED CONTROLLED TRIAL WITH RADIAL EBUS (R-EBUS) COMPARING THIN VS THICK GUIDE SHEATH (GS) WITH THE ADDITION OF AN ASPIRATION NEEDLE BIOPSY, IN THE DIAGNOSIS OF PERIPHERAL LUNG LESIONS (REBUST 2)

HERATH S^{1,6}, YAP E², LOW I³, MAHJAN H⁴, ING A^{5,6}

¹Westmead Hospital, Sydney, Australia, ²Department of Respiratory and Sleep Medicine, Middlemore Hospital, Auckland, New Zealand., ³Department of Pathology, Middlemore Hospital, Auckland, New Zealand, ⁴Western Sydney University and NSW Health Pathology, Tissue Pathology & Diagnostic Oncology, ICPMR, Westmead Hospital, CNR Hawkesbury and Darcy Road, Westmead, Australia, ⁵Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia, ⁶Faculty of Medicine and Health Sciences, University of Sydney, Sydney, Australia

Introduction/Aim: R-EBUS of peripheral lung lesions has been increasingly used, but overall diagnostic yield remains suboptimal (1). The size of the biopsy instruments may impact the yield. We aim to compare the 2 available guide GS sizes (2mm and 2.6mm) to assess diagnostic yield and safety and the utility of an additional aspiration needle biopsy.

Methods: In a multi-centre randomised control trial, all patients referred for R-EBUS were randomised to thick or thin GS. All underwent conventional R-EBUS biopsies and sampling (forceps, brush, lavage). The thick GS arm had an additional aspiration needle biopsy in a pre-defined random order. All samples were assessed by blinded pathologists and adequacy of samples was agreed upon.

Results: 52 patients were recruited from March 2015 – June 2018. 20 were randomised to the thin GS and 32 to the thick. 4 patients with mediastinal LN positivity excluded. 48 patients were analysed. Mean age was 68.4 y (range 39-86; male 21 [44%]). Mean FEV1 was 1.54L (61%) (range 0.75 -3.45L). 72% of lesions were in the upper lobes.

The overall diagnostic of R-EBUS was 69% (79% for malignant lesions and 43% for benign).

The biopsy specimen with best yield was the aspiration needle in 13/33, followed by forceps 12/33, brush 6/33 and wash 2/33.

In thin GS arm (n = 19) the diagnostic yield was 47% (67 % for malignant lesions, 25% for benign).

In the thick GS arm (n = 29), the diagnostic yield was 82% (87% for malignant lesions, 66% for benign).

There was one pneumothorax (thin GS) and one minor bleed (thick GS).

Conclusion: Use of the thick GS resulted in statistically higher diagnostic yield for malignancy (87% vs 63% $P < 0.05$). The use of an aspiration needle in the thick GS arm allowed an additional 17% patients to be diagnosed which would have been missed with conventional techniques. Adverse events were similar, and addition of a needle did not increase the pneumothorax rate.

Grant Support: None.

Conflicts of interest: None.

REFERENCE:

- Steinfort D P, Khor YH, Manser J, Irving LB. Radial probe endobronchial ultrasound for the diagnosis of peripheral lung cancer: Systematic review and meta-analysis. *European Respiratory Journal*. 2011;37(4):902-10.

COMPARISON OF LINEAR ENDOBRONCHIAL ULTRASOUND – TRANSBRONCHIAL NEEDLE ASPIRATION (EBUS-TBNA) USING 22G PROCORE NEEDLE VS 22G OLYMPUS NEEDLE

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¹The Department of Thoracic Medicine Royal Adelaide Hospital, Adelaide, Australia, ²The University of Adelaide, Adelaide, Australia, ³The Department of Anatomical Pathology, SA Pathology, Adelaide, Australia, ⁴The Department of Surgical Pathology/Cytopathology, SA Pathology, Adelaide, Australia

Introduction/Aim: EBUS-TBNA is the definitive investigation for mediastinal lymphadenopathy. Failure to obtain adequate tissue for diagnosis and ancillary tests results in repeat procedures. Both the 22G Olympus needle and the Echotip 22G ProCore needles have a bevelled tip, but the ProCore needle also has a reverse lateral bevel. This feature is designed to collect tissue during retrograde withdrawal of the needle. This study aims to determine if there is a difference between the needles in diagnostic rate or sample suitability for ancillary tests.

Methods: Patients undergoing linear EBUS were randomised to either the 22G Olympus needle or the 22G ProCore needle. The result of each TBNA pass was documented as sufficient or insufficient at rapid onsite examination (ROSE). Diagnosis and suitability of samples for ancillary tests were recorded. Fisher's exact test was used for statistical analysis.

Results: 80 patients were recruited. There were 34 benign cases, 34 malignant cases and 12 insufficient cases. Median number of passes was 5 for both needles. The diagnostic rate was higher for the Olympus needle (45/49 [92%]) than the Procore needle (23/31 [75%]) but this failed to reach statistical significance (Fisher exact statistic =0.0514 [$P = < 0.5$]). For procedures with confirmed adequate sampling at ROSE, the average number of passes to reach adequacy was 0.9 for the Olympus needle and 1.7 for the Procore needle. Cell block yield for diagnosis was similar (Olympus 57%, Procore 51%). For malignant cases, there was no significant difference in obtaining sufficient samples for immunohistochemistry testing (Olympus 80%, Procore 79%). Due to the small number of malignant cases we were unable to assess for a difference in molecular testing.

Conclusion: While the difference between the 22G Procore needle and 22G Olympus needle was not statistically significant, there was a trend favouring diagnostic accuracy for the 22G Olympus needle.

Grant Support: Australian Postgraduate Award, University of Adelaide Dawe's Scholarship (top-up), Royal Adelaide Hospital.

USE OF INDWELLING PLEURAL CATHETER IN THE MANAGEMENT OF MALIGNANT ASCITES: A RETROSPECTIVE AUDIT OF 46 PATIENTS

CHAN K¹, BADIEI A¹, TAN C¹, FITZGERALD D¹, STANLEY C², FYSH E³, SHRESTHA R⁴, MURUGANANDAN S¹, THOMAS R¹, LEE Y¹

¹Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Perth, Australia, ²Department of Respiratory Medicine, Royal Perth Hospital, Perth, Australia, ³Department of Respiratory Medicine, St John of God Midland Public Hospital, Perth, Australia, ⁴Department of Respiratory Medicine, Fiona Stanley Hospital, Perth, Australia

Introduction/Aim: Patients suffering from malignant ascites often require repeated large volume paracentesis (LVP) for symptomatic relief. This typically requires hospital admission. The use of tunnelled indwelling pleural catheters for malignant pleural effusion is well established. Here we report the first Australian experience of placing indwelling pleural catheters peritoneally (IPeC) for management of recurrent malignant ascites.

Methods: A retrospective audit of IPeC use in patients with recurrent, symptomatic MA between 2010-2018 in three hospitals in Western Australia. Procedure data were collected from a database. Outcomes were procedure success rate and safety profile.

Results: Forty-six patients (median age 65; female 57%) underwent 48 catheter insertion procedures performed mostly by specialist pleural pulmonologists. The underlying cancers included gynaecological ones (33%), mesothelioma (25%) and gastrointestinal (20%) malignancies. The majority of patients (93%) had prior LVP (median 2.0 drainages) before IPeC insertion. All IPeC were inserted successfully under ultrasound guidance. Most patients (85%) were discharged within 3 days of the procedure. Adverse events were mild and most were self-limiting, including pain (27%), transient hypotension after initial fluid drainage (8%), bacterial peritonitis (8%), wound oozing (8%), fluid loculation (2%) and catheter dislodgement (2%). Most patients (98%) did not require further paracentesis after IPeC insertion. A total of five patients had IPeC removed because of dislodgement, suboptimal catheter tip position, peritonitis, fluid loculation and cessation of fluid production during chemotherapy. The former two patients had IPeC re-inserted shortly afterwards. Patients with bacterial peritonitis responded to antibiotics and only one required catheter removal.

Conclusions: Use of tunnelled IPeC can minimise further invasive drainage procedures and potentially reduce hospitalization in patients with symptomatic malignant ascites. Placement of IPeC was associated with a low rate of adverse events and most of them can be managed conservatively.

Grant Support: NHMRC Fellowships (RT, YCGL), WA Cancer Council (RT, YCGL), WACPCN Fellowship (SM, DF), ERS Long-term Research Fellowship (DF)

Declaration of Interest Statement: Rocket Med Ltd has provided free IPeC drainage kits and a unrestricted educational grant for previous trials led by YCGL. YCGL has served on the advisory boards of Carefusion/BD and Sequana Med Ltd.

MULTICENTRE RANDOMIZED CONTROL TRIAL OF THE DIAGNOSTIC YIELD AND SAFETY OF RADIAL EBUS GUIDED CRYO-BIOPSY (CRYO-RADIAL) VERSUS CT GUIDED TRANSTHORACIC BIOPSY FOR LUNG MASSES DEMONSTRATE SIMILAR DIAGNOSTIC SUCCESS. (CT CROP)

HERATH S^{1,4}, YAP E², DAWKINS P², VEALE A², WILLIAMSON J³, ING A^{3,4}

¹Department of Respiratory and Sleep Medicine, Westmead Hospital, Sydney, Australia, ²Department of Respiratory and Sleep Medicine, Middlemore Hospital, Auckland, New Zealand, ³Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia, ⁴Faculty of Medicine and Health Sciences, University of Sydney, Sydney, Australia

Aim: The addition of cryo-biopsy to R-EBUS procedures (Cryo-Radial) is increasingly utilised as a means of obtaining larger biopsy samples suitable for molecular and PDL-1 testing (1,2). We aimed to compare Cryo-Radial with the current gold standard CT guided transthoracic biopsy (CT-Bx) for diagnostic yield, adequacy for molecular and immune testing, and safety.

Methods: Multi-centre, non-blinded, Randomised Control Trial. In the Cryo-Radial arm, at least one conventional R-EBUS sample (brush+/- forceps) was performed. Two pathologists independently analysed the biopsy samples.

Results: 44 patients (61% male) in three centres were consented between March 2015 and Sept 2018. 19 were randomised to CT-Bx and 25 to Cryo-Radial. Mean age 70yrs (48-81). Mean FEV1 1.9L (69% [45%-105%]).

In the CT-Bx arm 4 patients did not undergo the procedure. 13/15 who underwent the CT-Bx had a successful biopsy (87%) with one pneumothorax (1/15) requiring intercostal catheter insertion (7%).

In the Cryo-Radial arm 3/25, the procedure was abandoned due to technical difficulty in reaching upper lobes. In 19/22 (86%) patients, the bronchoscopy procedure was successful. Further 3/25 did not progress to the cryo-radial biopsy as a positive diagnosis was obtained prior during the same bronchoscopy procedure. 16/25 had a successful cryo-biopsy (73%) of which 6/16 were benign with excellent diagnostic accuracy.

There was no significant bleeding or pneumothorax. The diagnostic success of CT-Bx compared with bronchoscopic Cryo-Radial for lung masses was not significantly different (87% Vs 86%). Patients with successful biopsies in both arms had adequate testing for molecular sampling.

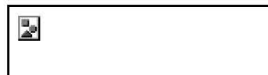
Conclusion: The novel Cryo-Radial method appears to provide samples of sufficient size for a multitude of testing in peripheral lung masses. The two procedures have similar diagnostic accuracy compared with a possible superior safety profile for Cryo-Radial. Cryo-radial also has the advantage of being able to stage the mediastinum during the same procedure. A smaller cryoprobe will help navigate the technical difficulties faced during this study.

Grant Support: This study was supported by the below funding bodies:

- Asser Trust Research Grant, Auckland New Zealand.
- 1. RACP foundation Grant 1: Better research grant 20
- 2. RACP Foundation grant 2: Amott's Early career researcher in lung cancer. (RACP foundation)

Appendix 7: Patient information and consent (REBUST 2)

Participant Information Sheet/Consent Form



Participant Information Sheet/Consent Form

Interventional Study - Adult providing own consent

Westmead Hospital

Title Randomised controlled trial comparing the diagnostic yield of Radial Endo-Bronchial Ultra-Sound (R-EBUS) guided biopsy when using a thick USS probe vs. a thin USS probe, in patients with parenchymal lung lesion. (R-EBUST2)

Short Title *REBUST 2*
Protocol Number *2*

Principal Investigator *Prof John Wheatley*

Associate Investigator(s) *Dr Samantha Herath, Dr Peter Wu*

Location *Westmead Hospital*

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project. This is because you have an abnormal area in your lung and your doctor would like this to be investigated.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

It is important to obtain a sample of tissue from the abnormal area in your lung so we can make a diagnosis. This can be done by various methods.

The Respiratory Specialist in charge of your care has decided the best option for you would be by using a procedure called Radial EBUS.

Radial EBUS is a bronchoscopy procedure or commonly known as a "camera test" that is introduced through the mouth to look at the windpipes and the lungs. There is an additional small ultra sound (USS) probe that is inserted through the bronchoscope that can perform an "internal scan", to locate the abnormal area in the lung accurately, to take a sample.

The USS probes come in 2 different sizes. One is 1.7mm in diameter called a thick probe and one is 1.4 mm diameter called a thin probe. Westmead Hospital has access to both probes.

These USS probes are small and look like thin wires. Each probe has a covering called a guide sheath. It is through this guide sheath that the doctor will introduce equipment to take samples of the abnormal area in the lung. Guide sheath (GS) makes the sampling very accurate.

The purpose of this study is to determine the effectiveness and safety of the thick probe versus the thin probe method.

The procedure itself is a part of routine management of your clinical condition. Some centres that do this procedure use only one type of probe to examine the lesion while some centres use two types of probes. In our centre, usually we perform examination either with one type or both types of probes according to the type and location of lesion. Some lesions are served better with one type of probe than the other.

If you wish to participate in the study, the only change that will occur, will be allocating you to be examined by one type of probe, using a random allocation chart. You have equal chance of being selected to either the thick probe or the thin probe method.

The test will not take any longer than the routine examination.

The study doctor Samantha Herath will use these results to obtain a PhD degree. Her PhD studies are looking at improving the ability to diagnose abnormal areas in the lungs, suspected of lung cancer, effectively: whilst improving the safety of these procedures.

3 What does participation in this research involve?

This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

Once your Respiratory Specialist decides on referring you to the radial EBUS procedure, one of the study doctors who will be performing the procedure will talk to you about the study and give you a copy of this information sheet.

Please take your time to read the sheet carefully and discuss with anyone you feel comfortable. If you are willing to participate in the study, you will be asked to sign an Informed Consent Form. This form outlines that you have been given all the information about the study and that you understand what the study involves and are happy to participate. If you have questions at any stage, please ask one of the study doctors. You will be included in the study only once you have signed the Informed Consent Form.

Regardless of whether you decide to participate in the study or not, you will have routine visits for the management of your clinical condition. These visits are routine and are part of standard

medical care that you would receive regardless of whether you are participating in this research study or not.

If the Respiratory Specialist looking after you also happens to be one of the doctors performing the radial EBUS procedure, decisions regarding your ongoing care will be jointly made by two respiratory physicians. This is done to improve your clinical care as one of the doctors is involved with the study.

If you decided to take part in the study, you do not need to do any additional tests. The results of your biopsy sample will be sent to your Respiratory Specialist as per usual practice.

Participation in the study does not alter any risks that you may encounter during the planned bronchoscopy procedure.

There are no additional costs associated with participating in this research project, nor will you be paid.

4 What do I have to do?

You do not have to do anything different for this study. Your specialist has recommended you undergo this procedure as part of your clinical care.

5 Other relevant information about the research project

This study has been performed in New Zealand and so far 20 patients have participated. We are undertaking this study here at Westmead and hope to recruit 60 patients. The results from both centres will be used for this research.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with Westmead Hospital.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this hospital. Other options are available; these include having a standard CT guided biopsy of the lung. Your study doctor will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include a safer way of getting a biopsy of the abnormal area in your lung.

9 What are the possible risks and disadvantages of taking part?

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.

Your Specialist will discuss the risks of the procedure with you. The procedure you will undergo is no different to the standard procedure and the risks for this procedure are:

Side Effect	How often is it likely to occur?	How severe might it be?	How long might it last?
Air leak from the lung	1 in 100	moderate	1- 7 days
Bleeding from biopsy site	1 in a 1000	mild	1- 2 hrs

These days, whilst anaesthesia is generally very safe there are some risks associated with anaesthesia. The most common problems associated with anaesthesia are feeling unwell or vomiting, bruising at the site of injections, sore throat or hoarse voice. Most patients do not have these problems. If these problems do happen, they usually get better very quickly. Damage to teeth may occur, but this is rare. The risk of brain damage or death due to anaesthesia is very rare.

The risk of problems from anaesthesia increases for patients who are having more major surgery, those with medical problems and those that require difficult anaesthetic procedures. If you have any concerns about these issues, you should discuss them with the study team.

Using the two probes instead of one does not have any risks involved.

10 What will happen to my test samples?

The biopsies taken during the procedure will be sent to the hospital laboratories for assessment as per hospital policy. Once analysed and the results sent to your Specialist the samples will be destroyed.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

All medications that you are currently taking will continue during the study.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as unacceptable side effects

15 What happens when the research project ends?

You will be able to find out the results of the study when it is completed. We intend to publish the results of the study in medical journals and present them to colleagues at medical meetings. This will allow medical professionals to learn from the results of the study.

If you would like the results sent to you at the conclusion of the study (3 years) please let the study doctor know.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Only the study staff will have access to this information and it will be kept securely in the Department of Respiratory Medicine at Westmead Hospital. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

In accordance with relevant Australian and/ or NSW privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

18 Who is organising and funding the research?

This research project is being conducted by Dr Samantha Herath

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Western Sydney Local Health District.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor on 9845 6797 or any of the following people:

Clinical contact person

Name	Prof John Wheatley
Position	Staff Specialist
Telephone	9845 6797 after hours please call 9845 5555 then ask to page Prof John Wheatley
Email	john.wheatley@sydney.edu.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Name	Westmead Hospital Patient Advice and Liaison Service
Telephone	9845 7014
Email	wsldh-pals-mail@health.nsw.gov.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	Western Sydney Local Health District HREC
HREC Executive Officer	Kellie Hansen
Telephone	9845 8183
Email	WSLHD-researchoffice@health.nsw.gov.au

Local HREC Office contact (Single Site -Research Governance Officer)

Name	Margaret Piper
Position	Research Governance Officer
Telephone	9845 9634
Email	WSLHD-RGO@health.nsw.gov.au

Consent Form - Adult providing own consent

Title Randomised controlled trial comparing the diagnostic yield of Radial Endo-Bronchial Ultra-Sound (R-EBUS) guided biopsy when using a thick USS probe vs. a thin USS probe, in patients with parenchymal lung lesion. (R-EBUST2)

Short Title *REBUST 2*
Protocol Number 2

Principal Investigator Prof John Wheatley

Associate Investigator(s) *Dr Samantha Herath, Dr Peter Wu*

Location *Westmead Hospital*

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Westmead Hospital concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I acknowledge that any regulatory authorities may have access to my medical records **specifically related** to this project to monitor the research in which I am agreeing to participate. However, I understand my identity will not be disclosed to anyone else or in publications or presentations.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Under certain circumstances (see Note for Guidance on Good Clinical Practice CPMP/ICH/135/95 at 4.8.9) a witness to informed consent is required.*

Name of Witness* to
Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher[†] (please print) _____
Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

By signing this consent section, I agree to the use of tissue samples obtained previously from my routine biopsy or surgery for the purposes of additional testing for (no additional testing will be performed. Only routine testing)

Name of Participant (please print) _____
Signature _____ Date _____

Name of Witness* to
Participant's Signature (please print) _____
Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Name of Study Doctor/
Senior Researcher[†] (please print) _____
Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of and information concerning the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title Randomised controlled trial comparing the diagnostic yield of Radial Endo-Bronchial Ultra-Sound (R-EBUS) guided biopsy when using a thick USS probe vs. a thin USS probe, in patients with parenchymal lung lesion. (R-EBUST2)

Short Title *REBUST 2*
Protocol Number *2*

Principal Investigator Prof John Wheatley

Associate Investigator(s) *Dr Samantha Herath, Dr Peter Wu*

Location *Westmead Hospital*

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Westmead Hospital.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

Appendix 8: Patient information and consent (Cryo-feasibility)



PARTICIPANT INFORMATION SHEET

STUDY TITLE:

Assessing the feasibility of performing a novel Cryo-biopsy procedure for diagnosis of Peripheral Pulmonary Lesions (PPL), suspected of lung cancer at Middlemore Hospital; with the view of commencing a multi centre RCT

PROTOCOL NO: Version 1.2 ; dated 15/01/2015

STUDY DOCTORS:

Dr.Samantha Herath, Dr.Andrew Veale

TELEPHONE:

09 267 4949 ext 6617

0211298979

Introduction

The Respiratory department of Middlemore Hospital invites you to participate in a medical research study.

This information sheet gives you information about the study, what you need to do if you decide to participate and explains how your medical information may be used and who may see it.

Please take as much time as you need to read the information carefully and decide if the study is of interest to you. You do not have to decide today whether or not you will participate in this research. Before you decide, you can talk to anyone you feel comfortable about the research. This may be friend, family/whanau or anyone you choose for you, to better understand the research study.

The information sheet may contain words that you do not understand. You can request your study doctor to explain any words or procedures that you do not clearly understand. Please feel free to ask us if you have any questions regarding this study.

Please note that your participation in this research study is **entirely voluntary**. It is your choice whether to participate or not. Whether you choose to participate or not, the management you receive, will continue and nothing will change.

Why have you been invited to this research?

You are being asked to participate in this medical research study because you have an abnormal area in your lung that is suspicious of cancer and is planned to have a "camera test with an Ultrasound" called Radial EBUS.

Once you are diagnosed with an abnormal area in the lungs, it is important to obtain a sample of tissue from this abnormal area to ascertain the diagnosis. This can be done by various methods.

The Respiratory Specialist looking after you has decided the best option for you to obtain a sample would be by an investigation called Radial EBUS. This is the standard procedure done in our unit to

Participant Information Sheet version 1.1 dated 15/01/2015

CMDHB Cryobiopsy Feasibility Study Protocol ; Version 1.2- dated 13/01/2015

safely obtain a tissue sample from an abnormal area. This is done by using a needle through a “camera test”. This camera also has a special ultrasound that can do a scan from inside your windpipes to locate the abnormal area in the lungs. Therefore the chance of getting a successful result is high.

This method of biopsy is much safer. Rate of air leak around the lung after the procedure is around one in 100. The rate of bleeding in the lung is even less at a rate of one in 250. However, the chance of getting a positive result is around 73%.

We aim to increase the success rate of this Radial EBUS technique by introducing another biopsy method called cryo biopsy while using the same “camera test” with the ultrasound. Therefore, if you take part in the study, during your scheduled standard Radial EBUS test, you will also have this new biopsy method as an additional test.

This cryobiopsy method gives larger biopsy samples without increase in side effects. This method has been explored in patients like yourself before and found to be safe very safe.

Cryo biopsy is

What is the purpose of this study?

The purpose of this study is to determine the effectiveness and safety of the cryo biopsy method in Middlemore Hospital

What will the study involve?

Once your Respiratory specialist decides on referring you for a Radial EBUS biopsy to diagnose the abnormal area in the lung, he /she will take your permission to refer you to the study doctors.

One of the study doctors who will be performing the procedure will talk to you about the study and give you a copy of this information sheet. After reading the information and discussing with the study doctors, If you agree to participate in this study you will be requested to give your consent or agreement to participation. This participation is entirely your choice.

You will be scheduled for a standard Radial EBUS “Camera test with Ultrasound” procedure. This is the procedure your Respiratory Specialist wishes you to have and is the standard method used to obtain a sample of tissue from abnormal lung areas. If you participate in the study, you will be having an additional type of biopsy, while undergoing the same test, at the same time called a cryo biopsy. You will not feel or see any difference from your routine Radial EBUS procedure.

The cryo-biopsy is done by introducing a small (1.9mm) probe through the camera or “bronchoscope” to the abnormal lung area already detected by the Radial EBUS procedure. Then a liquidized cold gas is sent via the probe to the abnormal area for a short time, usually 4 seconds. When the gas touches the abnormal lung area it freezes the tissue. This frozen tissue attaches itself to the probe. Then the bronchoscopist can take this sample out. You will not feel the coldness as the windpipes will be numb with anaesthetic. This technique is very similar to what is being used when freezing skin abnormalities like “skin warts”.

Participant Information Sheet version 1.1 dated 19/02/2015

CMDHB CT-CROP study protocol, Version 1.1 - dated 06/01/2015

The only additional safeguard we use with cryobiopsy method is using a tube called endotracheal tube when we take the samples out from your windpipes during cryo-biopsy. This tube is used to make the retrieval of the biopsy sample easy as well as to improve comfort to you.

You do not need to do additional preparations or come for follow up appointments. This is only a single one time test. You will likely to spend 20 minutes longer in the hospital.

After the procedure, you will have a Chest X ray. Once this is checked by your study doctor you will be allowed to go home.

A study doctor will call you in 1 week to find out if you have experienced any side effects.

At the end of the procedure the samples that were taken will be given to the pathology lab in Middlemore Hospital to analyse and reach a diagnosis. However, the results will not be available to you for another 1 week and the results will go directly to the respiratory specialist looking after you and you may discuss the diagnosis with him.

You will require someone to accompany you home and you will not be able to drive or operate heavy machinery for 24 hours after the procedure. This is routine procedure for any biopsy technique.

The risks to look out for after the procedure are an air leak outside the lung called a "pneumothorax" and bleeding into the lungs.

There is about 1 in 100 risk of air leak and half of patients with air leak will require a tube placed in the chest to drain the air out. The other half only requires watchful waiting. If you develop a pneumothorax you will feel shortness of breath and sometimes pain in the chest. This is an early complication and in 99% of patients we will know whether this complication had occurred before you leave the hospital.

There is a risk of bleeding in about 4% of patients and we would like you to observe for coughing up blood. After the procedure you will cough up blood mixed with spit and this is normal and expected. However as the day goes it should get minimal and none after 24 hours. If the coughing up of blood worsens or if you cough up more than five tablespoons of fresh red blood we request you come back to the emergency department of Middlemore Hospital for assessment.

All above mentioned complications rarely occur with Radial EBUS procedure. Using cryo biopsy does not increase the complication rates according to previous studies.

Please take your time to read the sheet carefully and discuss with anyone you feel comfortable with. If you are willing to participate in the study, you will be asked to sign an Informed Consent Form. This form outlines that you have been given all the information about the study and that you understand what the study involves. If you have questions at any stage, please ask one of the study doctors (contact details at the end of this information sheet). You will be included in the study only once you have signed the Informed Consent Form.

Regardless of whether you decide to participate in the study or not, you will have routine visits for the management of your clinical condition as planned.

If the Respiratory specialist looking after you also happens to be one of the doctors performing the cryo biopsy procedure, you will be jointly looked after by two respiratory specialists. This is done to

improve your clinical care as one of the doctors is involved with the study and we do not want your clinical care to be affected.

If you decided to take part in the study, you do not need to do any additional tests. There is requirement for any additional time of 20 minutes from you for the procedure.

The results of your biopsy sample will be available to your Respiratory specialist as per usual practice and will not be available to you directly, whether or not you participate in the study.

Participation in the study does not hasten or delay your diagnostic process.

Will taking part cost anything?

There will be no costs to you as a result of being involved in the study.

What are the possible benefits?

This is an opportunity to contribute to important research - the information we gather from this study may help us to treat future patients with cancer related lung abnormalities more effectively and safely.

Confidentiality and Data Privacy

If you decide to participate in the study, medical and personal information will be collected about you as part of the research study. The information that we collect from this research study will be kept confidential. Information about you that will be collected will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it (i.e. coded) instead of your name. Only the researchers will know what your number is and the information will be stored securely in locked offices.

If we encounter important information regarding the diagnosis that need prompt medical action, we request your consent for us to relay this information to your respiratory specialist urgently and if he/she is not available to your GP.

The biopsy samples taken from you are very small and usually all the sample is required to detect the abnormality. We do not plan to store any tissue samples. However, in the event you would like to take the unused sample home, (if residual sample is available) please inform us and we can make necessary arrangements.

Right to Refuse or Withdraw

Participation in this study is entirely voluntary. You do not have to take part in this study if you do not wish to do so and refusing to participate will not affect your treatment in any way. You will still have all the benefits that you would otherwise have.

You may stop participating in the research at any time to you wish without losing any of your rights as a patient at Middlemore Hospital.

Will I be able to know the study results?

You will be able to find out the results of the study when it is completed. We intend to publish the results of the study in medical journals and present them to colleagues at medical meetings. This will allow medical professionals to learn from the results of the study.

If you require the published paper or presentation to be posted/ emailed to you, please clearly convey this information to the study doctors at enrolment. Please also note the study will take 6 months to be completed.

Where can I get more information about the study?

You can call the research doctor whose details are at the bottom of this information sheet.

Statement of Approval

Ethics approval was granted from the Health and Disability commission: for an independent investigator initiated interventional study.

approval number:.....

Patient's Rights

1. If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact an independent Health and Disability advocate:
 - Free phone: 0800 555 050
 - Free fax: 0800 2 SUPPORT (0800 2787 7678)
 - E-mail: advocacy@hdc.org.nz
2. Please remember, you may withdraw your consent and withdraw from the study at any point of time.

Contact Details of Study Doctors

Dr Samantha Herath

Ph: 09 276 0044 ext 6617

Cell phone: 0211298979

Participant Information Sheet version 1.1 dated 19/02/2015
CMDHB CT-CROP study protocol ,Version 1.1 - dated 06/01/2015

Informed Consent Form

STUDY TITLE: Assessing the feasibility of performing a novel Cryo-biopsy procedure for diagnosis of Peripheral Pulmonary Lesions (PPL), suspected of lung cancer at Middlemore Hospital; with the view of commencing a multi centre RCT

PROTOCOL NO: Version 1.2; dated 13/01/2015

Subject Name: _____ **Date of Birth:** _____

1. I have read the Participant Information sheet, or it has been read to me. I have been given sufficient time to consider whether or not to participate.
2. I have had the opportunity to use whanau / family support, a friend or legal representative to help me ask questions and understand the study. All questions have been answered satisfactorily.
3. I understand that my involvement in this research study is voluntary (my choice) and may not be of any direct benefit to me. I understand that I can withdraw at any time during the study without affecting my future medical care.
4. I consent to the collection and processing of my personal information (including information about my health) by the research staff.
5. I agree to take part in this research study and for the data obtained to be published provided my name or other identifying information is not used.
6. I understand that the biopsy samples obtained would be processed to obtain a diagnosis for my condition and on request if excess sample is available will be given back to myself/whanau.

Declaration by participant:

I hereby consent to take part in this study.

Subject Signature: _____ **Date:** _____

Declaration by investigator:

Declaration by investigator: I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Investigator Name: _____

Investigator Signature: _____ **Date:** _____

Appendix 9: Patient information and consent (CT-CROP)



Health
Western Sydney
Local Health District

Participant Information Sheet/Consent Form

Interventional Study - Adult providing own consent

Westmead Hospital

Title	Randomised controlled trial comparing the diagnostic yield of CT-guided transthoracic biopsy vs cryobiopsy (via the radial endobronchial ultrasound guide sheath) in parenchymal pulmonary lesions suspected of lung cancer
Short Title	CT- CROP Study
Protocol Number	Version 1
Coordinating Principal Investigator/ Principal Investigator	Prof John Wheatley
Location	Westmead Hospital

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project. This is because you have an abnormal area in your lung that requires further investigation.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

Once you are diagnosed with an abnormal area in the lungs, it is important to obtain a sample of tissue from this abnormal area to ascertain the diagnosis. This can be done by various methods.

In Westmead Hospital we have two biopsy methods currently in use:

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1. CT guided biopsy which has been used for many years and
2. A bronchoscopy or "camera test" guided biopsy method called Radial EBUS

1. CT guided biopsy method:

The most common method used to obtain a diagnosis is called CT guided biopsy. This is done by using a needle that goes through the skin of the chest wall through to the abnormal area inside the lung. The success rate (obtaining an adequate sample) of this method is over 90%. However, there is about 30% or more risk of air leak outside the lung called a pneumothorax during the procedure and one quarter of these patients will require hospital admission due to a complication. The rate of bleeding is around 4% or one in 25.

2. Bronchoscopy or "camera test guided biopsy" method:

The other method is called Radial EBUS. This is done by using a needle through a "camera test" that is introduced through your mouth under sedation. This camera also has a special ultrasound that can do an internal ultrasound scan from inside your lungs to locate the abnormal area. This method of biopsy is very safe. Rate of air leak around the lung after the procedure is 1% or around one in 100. The rate of bleeding in the lung is around 4%, at a rate of one in 25. The chance of obtaining an adequate sample is around 73%.

Sometimes due to the small size of the samples obtained by both of these methods we are unable to determine what the diagnosis is.

We aim to increase the success rate of this Radial EBUS technique even further by introducing another biopsy method called cryobiopsy while using the same "camera test" with the ultrasound. This cryobiopsy method gives larger biopsy samples without increase in side effects. This method has been explored in patients like yourself before and found to be very safe. However, this cryobiopsy method is still a new procedure, therefore before we introduce this method to our routine clinical practice, we would like to offer this procedure as a clinical trial to our patients to confirm that this method is equally effective to the current gold standard biopsy method, the CT guided biopsy mentioned above, whilst having the added advantage of being a much safer method of biopsy.

The cryo-biopsy is essentially a "cold biopsy". It is done by introducing a small probe through the camera or "bronchoscope" to the abnormal lung area already detected by the internal ultrasound. Then a liquidized cold gas is sent via the probe to the abnormal area for a short time; 4 seconds. When the gas touches the abnormal lung area it freezes the tissue. This frozen tissue attaches itself to the probe. Then the doctor can take this sample out. You will not feel the coldness or pain at the time of procedure or later on as the windpipes will be numb with anaesthetic. You will have a general anaesthetic for this procedure and will be completely asleep throughout the procedure. This technique is very similar to what is being used when freezing skin abnormalities like "skin warts or sun cancers".

If you participate in this study we will first make sure that the abnormal area in your lung can be biopsied by either the CT guided biopsy method or the R EBUS method with equal success rates.

This will be decided by the Respiratory Physicians and the radiologists involved in this study.

The results of this research will be used by the study doctor Dr Samantha Herath to obtain a PhD degree.

3 What does participation in this research involve?

Once you have decided to participate in this study and have signed the consent form, you will be randomly assigned to one of the two methods of biopsy. This is because sometimes we do not know which treatment is best for treating a condition. To find out we need to compare

different treatments. To do this, we put people into groups and give each group a different treatment. The results are compared to see if one is better. To try to make sure the groups are the same, each participant is put into a group by chance (random). You will be randomly assigned to either a biopsy through the chest wall or an internal biopsy taken from inside the lung. You will have an equal chance of being assigned to one or the other, much like tossing a coin.

This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

If you are assigned to the CT guided biopsy group:

You will be asked to attend the CT scanning department at Westmead Hospital. There is no additional cost for you. You will need to have nothing to eat for 6 hours prior to the procedure.

The CT staff will ask you to change into a hospital gown and lay down on the CT bed on your side. The doctor will then clean the skin using antiseptic liquid and they will insert local anaesthetic into the skin. They will then wait a few minutes for the local anaesthetic to take effect and numb the area.

The CT Scan will then be performed, this will be exactly the same as the CT scan you have had previously. Whilst you are having the CT scan when they identify the position of the lung abnormality they will mark this area on the skin surface. The Doctor will then insert a needle into the area and pass it through the skin and into the lung aiming for the area of abnormality. They will then withdraw the needle and remove the sample they obtain from inside the needle. This process may need to be repeated several times to get sufficient sample.

Once the procedure is completed the doctor will place a small adhesive dressing over the area and you will be allowed to sit up.

You will be required to stay in CT scanning for one hour after the procedure. After one hour you will need to have a standard chest Xray to check for any leakage from your lung.

If the results of the chest Xray show no leakage, you may go home.

If the results show any leakage you will be seen by a respiratory doctor at the hospital and whatever treatment you require will be provided. This may involve you having to be admitted and stay overnight, it also may require a drain be inserted into your lung to remove the leakage from your lung.

The sample will be sent for analysis and the results will be sent to your treating Respiratory Specialist. You will need to discuss these results with them at your next appointment.

If you are assigned to the Bronchoscopy/Camera test biopsy group:

Prior to the booking for the biopsy because this method requires a general anaesthetic you will be required to attend the pre- admission clinic at Westmead Hospital. You will be seen by the anaesthetist that will perform the anaesthetic, he/she will ask you questions about your general health and past anaesthetic experiences. At this visit the anaesthetist will discuss the risks of undergoing the procedure with you. These risks will be different for every person and depend on your other health problems.

You will be asked to attend the Bronchoscopy Unit at Westmead Hospital. You will be asked to have had nothing to eat for 6 hours prior and nothing to drink for 2 hours prior. The anaesthetist will insert a "drip" into a vein in your arm. This will be used to deliver the anaesthetic to put you to sleep.

Once you have changed into a hospital gown, you will be asked to lie down on a bed and will be taken in to the bronchoscopy unit.

During the procedure you will be given some oxygen therapy, this will be via a thin piece of tubing that will sit across your top lip and will deliver the oxygen via your nose.

The anaesthetist will put the anaesthetic drugs through the drip in your arm and you will go to sleep. You will be completely asleep until the entire procedure is completed which will take up to 1 hour.

The doctor will then introduce the camera through your mouth and down into your lungs, the biopsies will be taken through the bronchoscope and you will not feel anything whilst they are taken. Once the samples have been collected the bronchoscope will be removed and the anaesthetist will allow you to wake up.

You will remain in the recovery section until you are fully awake. After one hour you will need to have a standard chest Xray to check for any leakage from your lung.

If the results of the chest Xray show no leakage, and once the anaesthetist has reviewed your condition and is happy for you to leave, you may go home.

If the results show any leakage you will be seen by a respiratory doctor at the hospital and whatever treatment you require will be provided. This may involve you having to be admitted and stay overnight, it also may require a drain be inserted into your lung to remove the leakage from your lung.

The sample will be sent for analysis and the results will be sent to your treating Respiratory Specialist. You will need to discuss these results with them at your next appointment.

There are no additional costs associated with participating in this research project, nor will you be paid. All medication, tests and medical care required as part of the research project will be provided to you free of charge.

If you decide to participate in this research project, the study doctor will inform your local doctor and your respiratory specialist.

4 What do I have to do?

No matter which method you are assigned to you will also be required to sign a clinical consent form for the procedure you will undergo. This consent form is required by the hospital and will be kept with your medical records.

Regardless of which biopsy method you are randomised to, you will need to stop taking medications that thin the blood for 5 days prior to the procedure, this is routine practice before a lung biopsy even if you don't participate in this study. The study doctors will inform you of what drugs you need to stop prior to the procedure. If you do have to stop taking blood-thinning tablets like warfarin, you will need to have a blood test completed on the morning of the procedure to check that it is safe to biopsy.

Once you have been randomly assigned to the different methods of biopsy, the doctor will review your CT scan previously performed and decide if this method is suitable for your lung abnormality. The doctor will inform you if you are suitable, if you are not suitable they will discuss the best management for you with your respiratory specialist. Participating in this study will not cause any delay in your diagnosis of your condition.

5 Other relevant information about the research project

There will be 158 participants in this research study from both Australia and New Zealand. The hospitals involved in this research in Australia are Westmead, Liverpool and Concord Hospital. We anticipate there will be 20 - 40 patients from Westmead Hospital.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with Westmead Hospital.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this hospital. Other options are available; these include proceeding to just the standard CT guided biopsy method or proceeding to Radial EBUS camera test without the new cryobiopsy method. Your study doctor will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include that we are able to obtain a larger sample of the abnormal area of your lung and hence be more likely to be able to diagnose what the abnormal area is. By being able to test this new method of biopsy it may improve diagnosis of future patients with abnormal areas in the lung. Therefore we may be able to offer a safer biopsy method to the current gold standard.

9 What are the possible risks and disadvantages of taking part?

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.

The side effect of each of the methods of biopsy are listed below:

CT Biopsy method

Side Effect	How often is it likely to occur?	How severe might it be?	How long might it last?
Air leakage out of the lung	3 - 4 out of 10 patients (30-40%)	Mild to moderate	4 – 7 days
Bleeding into the lung	4 out of 100 patients (4%)	Mild to severe	7 days
Bleeding outside the lung	1 out of 1000 (0.1%)	Mild	7 days

Bronchoscopy/Camera Biopsy method

Side Effect	How often is it likely to occur?	How severe might it be?	How long might it last?
Air leakage out of the lung	1 out of 100 patients (1%)	Mild	4 – 7 days
Bleeding into the lung (coughing up blood)	4 out of 100 patients (4%)	Mild to moderate	7 days
Bleeding outside the lung	1 out of 1000 (0.1%)	Mild	7 days

Any side effect experienced should be reported immediately to study staff, Study staff will check your chest xray to ensure that you do not have any air leakage or bleeding before you leave the hospital. If you suffer any side effects as a result of this you will be provided with medical care in the hospital free of charge.

If you become upset or distressed as a result of your participation in the research, the study doctor will be able to arrange for counselling or other appropriate support. Any counselling or support will be provided by qualified staff who are not members of the research project team. This counselling will be provided free of charge.

These days, whilst anaesthesia is generally very safe there are some risks associated with anaesthesia. The most common problems associated with anaesthesia are feeling unwell or vomiting, bruising at the site of injections, sore throat or hoarse voice. Most patients do not have these problems. If these problems do happen, they usually get better very quickly. Damage to teeth may occur, but this is rare. The risk of brain damage or death due to anaesthesia is extremely rare.

The risk of problems from anaesthesia increases for patients who have underlying major medical problems like heart disease. Your anaesthetist will discuss the possible side effects of you undergoing an anaesthetist depending on your personal circumstances, with you, at your pre-operative clinic visit. If you have any concerns about these issues, you should discuss them with the anaesthetist.

This biopsy method requires a good quality CT scan to accurately locate the abnormal area in your lung. If your previous CT scans are not adequate for the biopsy to be taken, they will need to be repeated. We don't anticipate this will be required very often but if you do have to undergo a repeat CT scan you will be exposed to some degree of radiation.

This research study involves exposure to a significant amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is about 6 mSv if you have a High Resolution CT scan as part of this trial. The dose from this study is comparable to that received from many diagnostic medical x-ray and nuclear medicine procedures. At this Westmead Hospital version 1 dated 22nd November 2016 based on Master Participant Information Sheet/Consent Form Version 1 dated 31st July 2016 Page 6 of 12

dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be low and theoretically is approximately equivalent to dying in a water boat accident.

10 What will happen to my test samples?

The samples collected from the biopsy will be sent to the pathology unit at Westmead Hospital. These samples will be labelled with your name and record number so the pathologist can identify your sample and send the report to your doctor. Once the samples have been analysed they will be stored and then destroyed as per the laboratory protocol. At Westmead Hospital samples will be destroyed after 5-15 years.

Your samples will not be used for any other purpose other than to attempt to diagnose your lung abnormality.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project. Your study doctor should also explain to you which treatments or medications need to be stopped for the time you are involved in the research project.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the study investigators up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:

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- Unacceptable side effects
- The drug/treatment/device being shown not to be effective
- The drug/treatment/device being shown to work and not need further testing

15 What happens when the research project ends?

Once this study is completed we will send a report on the study findings to participants. If you would like to receive this report please let the study staff know.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Information collected will be stored in the Department of Respiratory Medicine at Westmead Hospital and will only be accessible to study staff. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. All information collected for this study will have your name replaced with a study number that cannot identify you.

In accordance with relevant Australian and/or NSW privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree, be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

18 Who is organising and funding the research?

This research project is being conducted by Prof John Wheatley and Dr Samantha Herath as well as the investigators listed on page 1 of this information sheet.

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Western Sydney Local Health District.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor on 9845 6797 or any of the following people:

Clinical contact person

Name	Prof John Wheatley
Position	Department Head
Telephone	9845 6797 or after hours call 9845 5555 then ask to page Prof Wheatley
Email	john.wheatley@sydney.edu.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Position	Patient Advice and Liaison Service
Telephone	9845 7214
Email	wsldh-pals-mail@health.nsw.gov.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	Western Sydney Local Health District
HREC Executive Officer	Kellie Hansen
Telephone	9845 8183
Email	wsldh-researchoffice@health.nsw.gov.au

Local HREC Office contact (Single Site -Research Governance Officer)

Name	Margaret Piper
Position	Research Governance Officer
Telephone	9845 9634
Email	wsldh-rgo@health.nsw.gov.au

Consent Form - Adult providing own consent

Title

Randomised controlled trial comparing the diagnostic yield of CT-guided transthoracic biopsy vs cryobiopsy (via the radial endobronchial ultrasound guide sheath) in parenchymal pulmonary lesions suspected of lung cancer

Short Title

CT-CROP Study

Protocol Number

Version 1

Coordinating Principal Investigator/
Principal Investigator

Prof John Wheatley

Location

Westmead Hospital

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Westmead Hospital concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I acknowledge that any regulatory authorities may have access to my medical records specifically related to this project to monitor the research in which I am agreeing to participate. However, I understand my identity will not be disclosed to anyone else or in publications or presentations.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Under certain circumstances (see Note for Guidance on Good Clinical Practice CPMP/ICH/135/95 at 4.8.9) a witness to informed consent is required.*

Name of Witness* to

Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/

Senior Researcher† (please print) _____

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Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Optional paragraph:

I understand that, if I decide to discontinue the study treatment, I may be asked to attend follow-up visits to allow collection of information regarding my health status. Alternatively, a member of the research team may request my permission to obtain access to my medical records for collection of follow-up information for the purposes of research and analysis.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	Randomised controlled trial comparing the diagnostic yield of CT-guided transthoracic biopsy vs cryobiopsy (via the radial endobronchial ultrasound guide sheath) in parenchymal pulmonary lesions suspected of lung cancer
Protocol Number	Version 1
Coordinating Principal Investigator/ Principal Investigator	Prof John Wheatley
Location	Westmead Hospital

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Westmead Hospital.

Name of Participant (please print) _____
Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

--

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher [†] (please print) _____
Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

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Appendix 10: Patient information and consent (R-CORE)



Participant Information Sheet/Consent Form

Interventional Study - Adult providing own consent

Westmead Hospital

Title	Assessing the ability to obtain a core biopsy using the novel Gen Cut tool with Radial EBUS for Peripheral Lung lesions (R-CORE)
Short Title	R-CORE
Protocol Number	1.0
Principal Investigator	Prof John Wheatley
Associate Investigator(s)	<i>Dr Samantha Herath</i>
Location	<i>Westmead Hospital</i>

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project. This is because you have an abnormal area in your lung and your doctor would like this to be investigated as this is suspicious of cancer.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

It is important to obtain a sample of tissue from the abnormal area in your lung so we can make a diagnosis. This can be done by various methods.

The Respiratory Specialist in charge of your care has decided the best option for you would be by using a procedure called Radial EBUS.

Radial EBUS is a bronchoscopy procedure or commonly known as a "camera test" that is introduced through the mouth to look at the windpipes and the lungs. There is an additional small ultrasound (USS) probe that is inserted through the bronchoscope that can perform an "internal scan", to locate the abnormal area in the lung accurately, to take a sample.

The USS probes are small and look like thin wires. The outer diameter of the probe is 1.7mm. Each probe has a covering called a guide sheath. The outer diameter of the guide sheath is 2mm. It is through this guide sheath that the doctor will introduce equipment to take samples of the abnormal area in the lung. Guide sheath makes the sampling very accurate and safe.

Many types of biopsy instruments can be introduced with accuracy to the exact location of the abnormal lung mass using this guide sheath. The currently used instruments have been effective in obtaining a diagnosis but due to the small size of the equipment the biopsy samples have been small as well. Therefore, sometimes these biopsies are not suitable to test for advance testing for different tumour markers. Tumour markers are proteins that are found in blood, urine and other tissues and bodily fluids. These proteins can be elevated by the presence of one or more types of cancer.

To overcome this hurdle and to obtain a larger sample without increase in side effects, a new biopsy tool had been developed called a Gen cut tool. This is like a thin wire containing a small thin blade at the tip of the wire that can perform slicing of the tissue.

The purpose of this study is to determine the effectiveness and safety of this Gen cut biopsy tool in comparison to the conventional Radial EBUS biopsy tools. The procedure itself is a part of routine management of your clinical condition. Studies published on this tool are only a few but demonstrates promising results in obtaining larger samples and no increase in side effects.

If you wish to participate in the study, the only change that will occur, will be performing one extra biopsy with this new Gen cut tool. Your Radial EBUS procedure biopsies will take place as per routine planning.

The test will take 10 minutes longer than the routine examination.

The results of this research will be used by the study doctor Samantha Herath to obtain a PhD degree. Her PhD studies are looking at improving the ability to diagnose abnormal areas in the lungs, effectively: whilst improving the safety of these procedures.

3 What does participation in this research involve?

This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

Once your Respiratory Specialist decides on referring you to the radial EBUS procedure, one of the study doctors who will be performing the procedure will talk to you about the study and give you a copy of this information sheet.

Please take your time to read the sheet carefully and discuss with anyone you feel comfortable. If you are willing to participate in the study, you will be asked to sign an Informed Consent Form. This form outlines that you have been given all the information about the study and that you understand what the study involves and are happy to participate. If you have questions at any

stage, please ask one of the study doctors. You will be included in the study only once you have signed the Informed Consent Form.

Regardless of whether you decide to participate in the study or not, you will have routine visits for the management of your clinical condition. These visits are routine and are part of standard medical care that you would receive regardless of whether you are participating in this research study or not.

If the Respiratory Specialist looking after you also happens to be one of the doctors performing the radial EBUS procedure, decisions regarding your ongoing care will be jointly made by two respiratory physicians. This is done to improve your clinical care as one of the doctors is involved with the study.

If you decided to take part in the study, you do not need to do any additional tests and there will not be any new procedures being done. The results of your biopsy sample will be sent to your Respiratory Specialist as per usual practice.

There are no additional costs associated with participating in this research project, nor will you be paid.

4 What do I have to do?

You do not have to do anything different for this study. Your specialist has recommended you undergo this procedure as part of your clinical care.

5 Other relevant information about the research project

This study is a multi-centre study. There will be multiple centres across Australia and New Zealand that will recruit patients for this study. We are undertaking this study here at Westmead and hope to recruit 10 patients. The results from all centres will be used for this research.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with Westmead Hospital.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this hospital. Other options are available; these include having a standard CT guided biopsy of the lung. Your study doctor will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research.

9 What are the possible risks and disadvantages of taking part?

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.

Your Specialist will discuss the risks of the procedure with you. The procedure you will undergo is no different to the standard procedure and the risks for this procedure are:

Side Effect	How often is it likely to occur?	How severe might it be?	How long might it last?
Air leak from the lung	1 in 100	moderate	1- 7 days
Bleeding from biopsy site	1 in a 1000	mild	1- 2 hrs

Using the Gen Cut tool: studies have found the side effects to be similar to above mentioned Radial EBUS side effects. This additional biopsy will add 10 minutes to the procedure, therefore, 10 minutes more anaesthesia will be given to you.

If we are unable to obtain a sample we will refer you back to your doctor who will decide on future investigations and follow up.

Participation in the study does not alter any risks that you may encounter during the planned bronchoscopy procedure.

10 What will happen to my test samples?

The biopsies taken during the procedure will be sent to the hospital laboratories for assessment as per hospital policy. Once analysed and the results sent to your Specialist the samples will be destroyed.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

All medications that you are currently taking will continue during the study apart from some types of blood thinners. The study doctor who will discuss this study will inform if you need to stop the blood thinners and for how long.

Westmead Hospital Participant Information Sheet/Consent Form Version 2 dated 01 Dec 2017 based on WSLHD Master Participant Information Sheet/Consent Form Version 2 dated 15 Nov 2017

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13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as unacceptable side effects

15 What happens when the research project ends?

You will be able to find out the results of the study when it is completed. We intend to publish the results of the study in medical journals and present them to colleagues at medical meetings. This will allow medical professionals to learn from the results of the study.

If you would like the results sent to you at the conclusion of the study (3 years) please let the study doctor know.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Only the study staff will have access to this information and it will be kept in a locked office within the department. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

In accordance with relevant Australian and NSW privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

18 Who is organising and funding the research?

This research project is being conducted by Dr Samantha Herath who will received funding support from Medtronics.

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Western Sydney Local Health District.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor on (02) 8890 5555 then page Dr Samantha Herath or any of the following people:

Clinical contact person

Name	Prof John Wheatley
Position	Staff Specialist
Telephone	8890 6797 after hours please call 8890 5555 then ask to page Prof John Wheatley
Email	john.wheatley@health.nsw.gov.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Name	Westmead Hospital Patient Advice and Liaison Service
Telephone	8890 7014
Email	wslhd-pals-mail@health.nsw.gov.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	Western Sydney Local Health District HREC
HREC Executive Officer	Kellie Hansen
Telephone	8890 8183
Email	WSLHD-researchoffice@health.nsw.gov.au

Local HREC Office contact (Single Site -Research Governance Officer)

Position	Research Governance Officer and quote research study number 5363
Telephone	8890 9634
Email	WSLHD-RGO@health.nsw.gov.au

Consent Form - Adult providing own consent

Title	Assessing the ability to obtain a core biopsy using the novel Gen Cut tool with Radial EBUS for Peripheral Lung lesions (R-CORE)
Short Title	R-CORE
Protocol Number	1.0
Principal Investigator	Prof John Wheatley
Associate Investigator(s)	Dr Samantha Herath
Location	Westmead Hospital

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Westmead Hospital concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I acknowledge that any regulatory authorities may have access to my medical records **specifically related** to this project to monitor the research in which I am agreeing to participate. However, I understand my identity will not be disclosed to anyone else or in publications or presentations.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.
I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Name of Witness* to
Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/

Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title Assessing the ability to obtain a core biopsy using the novel Gen Cut tool with Radial EBUS for Peripheral Lung lesions (R-CORE)

Short Title R-CORE

Protocol Number 1.0

/ Principal Investigator Prof John Wheatley

Associate Investigator(s) Dr Samantha Herath

Location Westmead Hospital

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Westmead Hospital.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher[†] (please print) _____

Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

Appendix 11: Data collection (Patient demography and medical/radiology)

Data collection sheet:

Patient demography and Medical/Radiological details

Patient demographic characteristics

Study ID	
Date of procedure	
Site	
Study arm	
Age	
Sex	
Employment	

Patient medical details

Presenting complaint	
COPD	
COPD X (Mild/moderate/sever)	
FEV1 in L	
FEV1 %	
DLCO	
Hypertension	
Chronic Renal failure	
Ejection Fraction in ECHO	
Obstructive Sleep Apnoea	
Ischemic Heart disease	
American Society of Anaesthesiologists classification of Physical Health (ASA) score	
Eastern Cooperative Oncology Group (ECOG) Performance score	

Radiological characteristic of the PPL

Size of PPL on axial CT-largest diameter	
Location of PPL (Outer 1/3, middle 1/3 or inner 1/3)	
Lobar location	
Presence of Air bronchogram on CT chest imaging	

Appendix 12: Data collection (Pneumothorax and bleeding)

Data collection sheet for pneumothorax and bleeding:

Study:

Study ID:

Date:

The Common Terminology Criteria for Adverse events (CTCAE) was used to define adverse events

Pneumothorax

Grade 1 Asymptomatic: Clinical or diagnostic observation; no intervention required,)	
Grade 2 (Symptomatic intervention required),or	
Grade 3 (Symptomatic intervention and hospital admission)	
Grade 4 (Life threatening urgent treatment)	
Grade 5 (Death) .	

Haemorrhage

Grade 1 (Mild symptoms, intervention not required),	
Grade 2 (Symptomatic, requiring medical intervention),	
Grade 3, Transfusion and operative (endoscopic or operative)	
Grade 4 Life-threatening	
Grade 5 (Death).	

Appendix 13: Data collection – Bronchoscopy (REBUST 2)

DATA COLLECTION SHEET- Attach bronchoscopy report

Title:

Chart review to identify factors influencing the improved diagnostic yield with use of WANG aspiration needle during RADIAL EBUS procedure for peripheral lung mass.

	Medical record number/		Replace this with study no as soon as chart is identified.								
1	Study number										
2	Date of radial EBUS										
	Location of the mass		Indicate the sub segment of the bronchial tree according to the radiology report								
3	Does a bronchus run directly to the lesion		Yes or no according to clinical noted of the interventional fellow/physician								
3	Size of the mass		In cm								
4	Distance of the centre of the mass to main carina		In cm								
5	Visualisation of the mass via radial EBUS		Indicate the percentage from 0-100%								
6	Sampling method and diagnostic yield	<table border="1"> <tr> <td>WANG</td><td></td></tr> <tr> <td>Brush</td><td></td></tr> <tr> <td>Lavage</td><td></td></tr> <tr> <td>Forceps</td><td></td></tr> </table>	WANG		Brush		Lavage		Forceps		
WANG											
Brush											
Lavage											
Forceps											
7	Side effects	<table border="1"> <tr> <td>Haemoptysis</td><td></td></tr> <tr> <td>Pneumothorax</td><td></td></tr> <tr> <td>Other</td><td></td></tr> <tr> <td></td><td></td></tr> </table>	Haemoptysis		Pneumothorax		Other				Indicate if patient had emphysema according to CT report.
Haemoptysis											
Pneumothorax											
Other											

Appendix 14: Data collection – Bronchoscopy (Cryo-feasibility)

Assessing the feasibility of performing a novel Cryo-biopsy procedure for diagnosis of Peripheral Pulmonary Lesions (PPL), suspected of lung cancer at Middlemore Hospital; with the view of commencing a multi centre RCT

Data collection sheet: Bronchoscopy Procedure

Study ID	Date of procedure	
Procedural time	Start time of Radial EBUS Start time of cryo-biopsy Finish time of cryo-biopsy Finish time of Radial EBUS	
Sedation:	Midazolam dose Fentanyl dose 2% lignocaine spray	
Patient reported discomfort	- Better than expected - As expected, - Worse than expected	
Nurse reported discomfort	1 (now discomfort) to 10 (maximal discomfort) 1 2 3 4 5 6 7 8 9 10	
Procedural difficulty in taking cryo-biopsy	- Easy - Moderate difficulty (needing sedation top up) - Difficult: abandoned procedure	
Final diagnosis reached by	WANG/Excelon aspiration needle Forceps biopsy Cytology brush Bronchial Wash Cryobiopsy Ability to do EGDR on cryo-biopsy (yes/no)	
Side effects The Common Terminology Criteria for Adverse events (CTCAE) was used to define adverse events	Pneumothorax Grade 1 Asymptomatic: Clinical or diagnostic observation;no intervention required,) Grade 2 (Symptomatic intervention required) Grade 3 (Symptomatic intervention and hospital admission) Grade 4 (Life threatening urgent treatment) Grade 5 (Death) . Haemorrhage Grade 1 (Mild symptoms, intervention not required), Grade 2 (Symptomatic, requiring medical intervention), Grade 3, Transfusion and operative (endoscopic or operative) Grade 4 Life-threatening Grade 5 (Death)	

Appendix 15: Data collection – Bronchoscopy (CT-CROP)

CT CROP: Data Collection Sheet for Bronchoscopy

Procedure: Radial EBUS guided cryo biopsy for Peripheral Pulmonary Lesion

Patient sticker:

Or Patient name and NHI

Date of procedure								
Name of proceduralist								
Type of sedation	Conscious sedation				GA			
Procedure taken place in:	Bronch suit				Operating theatre			
Was airway protection used?	no		ETT				LMA	
Was a bronchial blocker used?	yes				no			
Total time taken for the procedure								
Total time taken to locate the PPL from USS in minutes								
Total amount of lignocaine used in mg (20mg in 1 ml of 2% lignocaine)								
Total amount of midazolam used								
Total amount of fentanyl used								
Total amount of propofol used								
Total amount of dexmedetomidine used								
Was fluoroscopy used?								
Dose of fluoroscopy in millisuvs								
Radial USS image view	eccentric			Full circumference				
Number of cryo biopsies performed	0	1	2		3			
Radial EBUS: number of passes with brush	0	1	2		3			
Radial EBUS: number of passes with TBNA	0	1	2		3			
Radial EBUS: number of biopsies with forceps	1	2	3		4		5	
CXR 1 hour of post- procedure	Done				Not done			
Procedural Complications	Pneumothorax				yes		no	
	Intra-op bleeding				yes		no	
	Other							
Was the patient discharged from hospital post procedure as planned?	yes				no			
If not discharged per plan number of extra hours of monitoring required?								

Did procedural complications require ED or ward admission?	yes	no
If admitted number of days in hospital?		
If admitted reason for admission?		
Complications: did the patient receive blood transfusions: if yes the number of units	Yes/no	No of units:
Complications: Did the patient receive ICT post procedure?		
Complications: Hypoxia		
Complications: Bleeding		

Name of bronchoscopist/registrar:.....

Signature..... Date:.....

Once completed and signed please scan and e mail this form to:

Dr.Samantha Herath

scherath@yahoo.com 0418343787

Appendix 16: Data collection – Bronchoscopy (R-CORE)

Subject Study Code: _____

R CORE: Data Collection Sheet for Bronchoscopy

Procedure: Radial EBUS guided Gen cut and conventional biopsy for Peripheral Pulmonary Lesion

Patient sticker:

Or Patient name and NHI

Date of procedure					
Name of proceduralist					
Type of sedation	Conscious sedation		GA		
Procedure taken place in:	Bronch suit		Operating theatre		
Was airway protection used?	no	ETT		LMA	
Total time taken for the procedure					
Total time taken to locate the PPL from USS in minutes					
Total amount of lignocaine used in mg (20mg in 1 ml of 2% lignocaine)					
Total amount of midazolam used					
Total amount of fentanyl used					
Total amount of propofol used					
Total amount of dexmedetomidine used					
Was fluoroscopy used?					
Dose of fluoroscopy in millisuvs					
Radial USS image view	eccentric		Full circumference		
Number of Gen cut biopsies performed	0	1			
Radial EBUS: number of passes with brush	0	1	2	3	
Radial EBUS: number of passes with TBNA	0	1	2	3	
Radial EBUS: number of biopsies with forceps	1	2	3	4	5
CXR 1 hour of post- procedure	Done			Not done	
Procedural Complications	Pneumothorax		yes	no	
	Intra-op bleeding		yes	no	
	Other				
Was the patient discharged from hospital post procedure as planned?	yes			no	
If not discharged per plan number of extra hours of monitoring required?					

Subject Study Code: _____

Did procedural complications require ED or ward admission?	yes	no
If admitted number of days in hospital?		
If admitted reason for admission?		
Complications: did the patient receive blood transfusions: if yes the number of units	Yes/no	No of units:
Complications: Did the patient receive ICT post procedure?		
Complications: Hypoxia		
Complications: Bleeding		

Name of bronchoscopist/registrar:.....

Signature..... Date:.....

Appendix 17: Data collection – Radiology (CT-CROP)

CT CROP: Data Collection Sheet for Radiology

Procedure: CT guided core biopsy for Peripheral Pulmonary Lesion (PPL)

Patient sticker:

Or Patient name and NHI

Date of procedure						
Name of proceduralist						
Total time taken for the procedure (From administering of local to needle removed)						
Total amount of lignocaine used						
Other sedation/IV analgesia used						
Size of co axial needle						
Size of core needle						
Number of passes done	1	2	3	4	5	6
CXR 1 hour of post- procedure	Done			Not done		
Procedural Complications	Pneumothorax on CXR		yes		no	
	Haemoptysis		no	minor	major	
	Pul.haemorrhage on CT		yes		no	
Was the patient discharged from hospital post procedure as planned?	yes			no		
Did procedural complications require ED or ward admission?	yes			no		
Please specify complication and action taken						

Name of Radiologist:

Signature..... Date:.....

Once completed and signed please scan and e mail this form to:

Dr.Samantha Herath

scherath@yahoo.com 0418343787

Appendix 18: Data collection – Pathology (CT-CROP)

CT CROP: Data Collection Sheet for Pathology

CT guided core biopsy or Radial EBUS guided biopsies + Cryo biopsy for Peripheral Pulmonary Lesion (PPL)

Study ID Code: _____

Type of biopsy	CT guided Core/FNA	Cryo biopsy
Pathologist who reviewed this sample		
Diagnosis		
Degree of confidence in the subtype of tumour <u>Describe subtype:</u> 1:Low 2:Moderate 3:High	1	2
Degree of confidence that the tumour is from suggested primary site <u>Suggested primary:</u> 1:Low 2:Moderate 3:High	Primary site:	
	1	2
Description of quantity of diagnostic material 1:Poor 2:Suboptimal 3:Adequate	1	2
Description of quality of diagnostic material 1:Poor 2:Suboptimal 3:Adequate	1	2
Measurement of tissue size		
% involved by tumour		
Suitability of sample for IHC 1: Not suitable 2: Borderline 3: Suitable	1	2
Suitability of sample for ancillary testing (EGFR) 1: Not suitable 2: Borderline 3: Suitable	1	2

This section to be filled only if patient had Radial EBUS sampling as well

Type of biopsy	Brushing	TBNA	Forceps
Pathologist who reviewed the sample	WV	HM	
Was a cell block performed?	Yes/no	Yes/no	
Diagnosis			
Degree of confidence in the subtype of tumour <u>Describe subtype:</u> 1:Low 2:Moderate 3:High	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Degree of confidence that the tumour is from suggested primary site <u>Suggested primary:</u> 1:Low 2:Moderate 3:High	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Description of quantity diagnostic material	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3

1:Poor 2:Suboptimal 3:Adequate			
Description of quality of diagnostic material 1:Poor 2:Suboptimal 3:Adequate	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Measurement of tissue size			
% involved by tumour			
Suitability of sample for IHC 1: Not suitable 2: Borderline 3: Suitable	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Suitability of sample for ancillary testing (EGFR) 1: Not suitable 2: Borderline 3: Suitable	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Best overall sample	CT guided biopsy	Cryo biopsy	Radial biopsy
Best Radial sample	Radial FNA	Radial brush	Radial forceps

Name of pathologist:.....

Signature..... Date:.....

Once completed and signed please scan and e mail this form to:

scherath@yahoo.com

0418343787 (CT CROP research co-ordinator)

Note:

Quantity and Quality of material

1. Poor : Unable to diagnose *provide reason
2. Suboptimal : Able to diagnose but with some limitations *provide reason
3. Adequate : Able to diagnose without limitation

Suitability of sample for IHC

1. Not suitable : *provide reason
2. Borderline : *provide reason
3. Suitable : Beyond borderline

Suitability of sample for ancillary testing

1. Not suitable : Below borderline *provide reason
2. Borderline : *provide reason
 - a. Approximately preserved 50-200 tumour cells with minimal non-tumour background
 - b. >200 tumour cells but abundant non-tumour background
3. Suitable : Beyond borderline

Appendix 19: Data collection – Pathology (R-CORE)

Subject Study Code: _____

R CORE : Data Collection Sheet for Pathology

Radial EBUS guided Gen cut biopsy and conventional biopsies for Peripheral Pulmonary Lesion (PPL)

Gen cut biopsy assessment

Pathologist who reviewed this sample			
Diagnosis			
Degree of confidence in the subtype of tumour <u>Describe subtype:</u> 1: Low 2: Moderate 3: High	1	2	3
Degree of confidence that the tumour is from suggested primary site <u>Suggested primary:</u> 1: Low 2: Moderate 3: High	Primary site:		
	1	2	3
Description of quantity of diagnostic material 1: Poor 2: Suboptimal 3: Adequate	1	2	3
Description of quality of diagnostic material 1: Poor 2: Suboptimal 3: Adequate	1	2	3
% involved by tumour			
Suitability of sample for IHC 1: Not suitable 2: Borderline 3: Suitable	1	2	3
Suitability of sample for PD L1 staining 1: Not suitable 2: Suitable	1	2	

Radial EBUS conventional sampling assessment

Type of biopsy	Brushing	TBNA	Forceps
Pathologist who reviewed the sample			
Was a cell block performed?	Yes/no	Yes/no	
Diagnosis			
Degree of confidence in the subtype of tumour <u>Describe subtype:</u> 1:Low 2:Moderate 3:High	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Degree of confidence that the tumour is from suggested primary site <u>Suggested primary:</u> 1:Low 2:Moderate 3:High	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Description of quantity diagnostic material 1:Poor 2:Suboptimal 3:Adequate	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Description of quality of diagnostic material 1:Poor 2:Suboptimal 3:Adequate	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Measurement of tissue size			
% involved by tumour			

Subject Study Code: _____

Suitability of sample for IHC 1: Not suitable 2: Borderline 3: Suitable	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Suitability of sample PD L 1 staining 1: Not suitable 2: Suitable	1 / 2	1 / 2	1 / 2
Best overall sample for PD L 1 testing	Gen cut biopsy	Radial forceps	

Name of pathologist:.....

Signature..... Date:.....

Note:

Quantity and Quality of material

1. Poor : Unable to diagnose *provide reason
2. Suboptimal : Able to diagnose but with some limitations *provide reason
3. Adequate : Able to diagnose without limitation

Suitability of sample for IHC

1. Not suitable : *provide reason
2. Borderline : *provide reason
3. Suitable : Beyond borderline

Suitability of sample for ancillary testing

1. Not suitable : Below borderline *provide reason
2. Borderline : *provide reason
 - a. Approximately preserved 50-200 tumour cells with minimal non-tumour background
 - b. >200 tumour cells but abundant non-tumour background
3. Suitable : Beyond borderline

Appendix 20: Ethics Approval – Middlemore (REBUST 2)



Health and Disability Ethics Committees

Ministry of Health
Freyberg House
20 Aitken Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdec@moh.govt.nz

23 January 2015

Dr Samantha Herath
Department of Respiratory Medicine
Level 3, Esme Green Building
CMDHB
Hospital Road, Otahuhu 1062

Dear Dr Herath

Re:	Ethics ref:	15/STH/3
	Study title:	Randomised controlled trial comparing the diagnostic yield of Radial Endo-Bronchial Ultra-Sound (R-EBUS) guided biopsy when using a thick USS probe Vs. a thin USS probe, in patients with parenchymal lung lesion (PPL), suspected of lung cancer. (R-EBUST2)

I am pleased to advise that this application has been approved by the Southern Health and Disability Ethics Committee. This decision was made through the HDEC-Expedited Review pathway.

Summary of Study

- Good application.
- The allocation of 2 treating doctors to prevent possible conflict of interest is a novel and simple solution.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Southern Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.
2. Before the study commences at *any* locality in New Zealand, it must be registered in a WHO-approved clinical trials registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au).
3. Before the study commences at a *given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that

the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

Summary of ethical issues (outstanding)

The main ethical issues considered by the Committee and which require addressing by the Researcher are as follows.

1. PISC could benefit from a lay title - Lung Biopsy Trial
2. Needs page numbers
3. Please add HDEC approval statement from the HDEC Template PIS/CF.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to HDEC before commencing your study.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

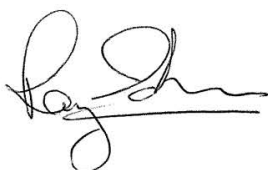
Your next progress report is due by 23 January 2015.

Participant access to ACC

The Southern Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,



Ms Raewyn Idoine
Chairperson
Southern Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering Letter: Covering letter	1.0	05 January 2015
CV for CI: CV for CI	1.0	05 January 2015
Protocol: Study protocol	1.1	11 November 2014
Evidence of scientific review: Letter stating that the protocol was reviewed by the Department of Respiratory Medicine.	1.0	05 January 2015
Evidence of scientific review: CMDHB research office application for research registration	1.0	05 January 2015
Participant information sheet	1.0	05 January 2015
Informed consent	1.0	05 January 2015
Application	1	-

Appendix B

Statement of compliance and list of members

Statement of compliance

The Southern Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008713) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	Category	Appointed	Term Expires
Ms Raewyn Idoine	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Mrs Angelika Frank-Alexander	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Dr Sarah Gunningham	Non-lay (intervention studies)	01/07/2012	01/07/2015
Assoc Prof Mira Harrison-Woolrych	Non-lay (intervention studies)	01/09/2014	01/09/2015
Dr Fiona McCrimmon	Lay (the law)	01/09/2014	01/09/2015
Dr Nicola Swain	Non-lay (observational studies)	01/07/2012	01/07/2015
Dr Devonie Waaka	Non-lay (intervention studies)	01/07/2013	01/07/2016
Dr Mathew Zacharias	Non-lay (health/disability service provision)	01/07/2012	01/07/2015

<http://www.ethics.health.govt.nz>

Appendix 21: Ethics Approval – Middlemore (Cryo-feasibility)



Health and Disability Ethics Committees

Ministry of Health
Freyberg House
20 Aitken Street
PO Box 5013
Wellington
6011

hdec@moh.govt.nz

03 February 2015

Dr Samantha Herath
Department of Respiratory Medicine
Level 3, Esme Green Building
CMDHB
Hospital Road
Otahuhu 1062

Dear Dr Herath

Re:	Ethics ref:	15/CEN/5
	Study title:	Assessing the feasibility of performing a novel Cryo-biopsy procedure for diagnosis of Peripheral Pulmonary Lesions (PPL), suspected of lung cancer at Middlemore Hospital; with the view of commencing a multi-centre RCT

This application was reviewed by the Central Health and Disability Ethics Committee and provisionally approved pending receipt of further information. This decision was made through the HDEC-Expedited Review pathway.

Summary of ethical issues (outstanding)

The main ethical issues considered by the Committee which require addressing by the Researcher are as follows.

- Please note that health information should be kept for ten years and not five.
- Please provide an explanation of the “cryo-biopsy” process so that the participant understands what is to happen.
- Please proof the information sheet as it is full of typos and spelling mistakes.
- Please clarify if Radial EBUS technique is standard of care in your unit, i.e. the patients would routinely have the Radial EBUS technique to biopsy PLL rather than say the gold standard procedure, so that the main difference in this study is simply the use of the cryo-biopsy technique.

Further information requested

The further information requested in order for the Central Health and Disability Ethics Committee to make a final decision is as follows.

1. Please address the points raised above.

Timeline for providing further information, and for giving a final opinion

You have 90 days to provide this further information. Your application will be considered to have been withdrawn if this information is not received on or before 04 May 2015. A new application would be required in this case.

The 15-day clock within which a final decision must be made on this study is suspended as of the date of this letter. This clock, on which 1 days remain, will restart on the date on which **all** of the further information requested above is received by the Central Health and Disability Ethics Committee.

Please remember to track changes made to new versions of documentation.

How to respond to a Provisional Approval

You will need to submit your new or amended documents through Online Forms.

New versions of existing documents:

Steps

Screenshots

1. Go to the Documents Tab to upload the revised documentation requested by the secretariat

2. To update versions of documents, go to the List tab. Select View/Manage to upload a newer version of the document.
 - For example you can upload new versions of the PIS/CF
 - Remember to track changes.

Document Type	Document Date	Description	Version	Size	Updated by	Tools
Declined letter for previous application in respect of the same (or substantially similar) study	(none)					
Consent letter	(none)					
CV for CI	(none)					
CV for other Investigators	(none)					
Evidence of CI indemnity	(none)					
Evidence of scientific review	(none)					
Evidence of sponsor insurance	(none)					
Investigator's Brochure	(none)					
Other	(none)					
PIS/CF	29/04/2014	Text	1	12 KB		View/Manage
PIS/CI for persons interested in welfare of non-consenting participants	(none)					
Protocol	(none)					
Summary/updates/minutes	(none)					

3. When you click View/Manage for a particular document it will take you to the upload tab for that document.

Document type: PIS/CF

Version: 1 Document date: 23/04/2014

Description: Text

Choose file to upload: [Browse...](#)

* Please note that the fields Version and Description will be visible by the assessing organisation

[Upload New Version](#) [Cancel](#) [Delete](#)

History

Upload Date	Document Date	Version	System Version	File Size	Uploaded by	Action
29/04/2014	23/04/2014	1	1 (current)	12 KB		View Uploaded File

History

Upload Date	Document Date	Version	System Version	File Size	Uploaded by	Action
29/04/2014	23/04/2014	2	2 (current)	12 KB		View Uploaded File
29/04/2014	23/04/2014	1	1	12 KB		View Uploaded File Delete

New documents:

Steps

Screenshots

- For New documents, go to the upload tab.

- For example you can upload a word document responding to questions raised by the Committee.

- Select the document type. Add a version number, document date and add a description if required.

Browse your computer to find the new file and select Upload File.

- The new document will now be uploaded and visible on the List Tab. Before submitting check to see all your documents are on the List tab and are displaying the correct version and document date.

To submit:

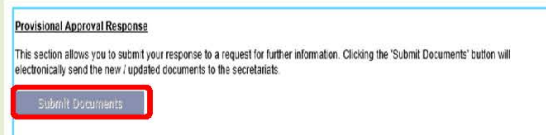
7. Once you have uploaded all new documents or updated all existing documents click the E-Submissions tab.



8. Scroll down until you see 'Provisional Approval Response'.

This button will only be able to be used when you have received a 'Provisional Approval' letter.

Please note: only click submit once.



Please don't hesitate to contact the HDEC secretariat if you have any queries. We look forward to receiving your response.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'H Walker'.

Mrs Helen Walker
Chairperson
Central Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

<i>Document</i>	<i>Version</i>	<i>Date</i>
Participant information sheet	1.1	15 January 2015
Protocol: Protocol for feasibility study	1.2	13 January 2015
Covering Letter: Covering letter	1.0	15 January 2015
Survey/questionnaire: Data collection sheet and questionnaire	1.0	15 January 2015
inormed consent	1.1	15 January 2015
Evidence of scientific review: Approval from the Co investigator and the head of the department	1.0	15 January 2015
CVs for other Investigators: CV of Dr. A. Veale	1.0	15 January 2015
CV for CI: CV of Dr.Samantha Herath	1.0	15 January 2015
Application		

Appendix B

Statement of compliance and list of members

Statement of compliance

The Central Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008712) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	Category	Appointed	Term Expires
Mrs Helen Walker	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Mr Paul Barnett	Lay (the law)	01/07/2012	01/07/2015
Dr Kay de Vries	Non-lay (observational studies)	19/05/2014	19/05/2017
Mrs Gael Donoghue	Non-lay (health/disability service provision)	01/07/2012	01/07/2015
Mrs Sandy Gill	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Dr Ptries Herst	Non-lay (intervention studies)	01/07/2012	01/07/2015
Dr Dean Quinn	Non-lay (intervention studies)	01/07/2012	01/07/2015
Dr Cordelia Thomas	Lay (ethical/moral reasoning)	19/05/2014	19/05/2017

<http://www.ethics.health.govt.nz>

Appendix 22: Ethics Approval – Middlemore (CT-CROP)



Health and Disability Ethics Committees

Ministry of Health
Freyberg Building
20 Aitken Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdec@moh.govt.nz

18 May 2015

Dr Samantha Herath
Department of Respiratory Medicine
Level 3, Esme Green Building
CMDHB
Hospital Road, Otahuhu 1062

Dear Dr Herath

Re: Ethics ref:	15/NTA/48
Study title:	Randomised controlled trial comparing the diagnostic yield of Radial Endobronchial Ultrasound (R-EBUS) guided Cryo-biopsy Vs. CT-guided transthoracic biopsy in patients with parenchymal lung lesion (PPL), suspected of lung cancer. (CT-CROP)

I am pleased to advise that this application has been approved by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Summary of Study

- The Committee stated the study is interesting.
- The study involves comparing a new 'cryo-biopsy' method that is used with the Radial EBUS navigation system against the current gold standard: CT-guided biopsy.
- The CT-guided method has a diagnostic yield of 90% but it also has a high rate of complications. Roughly 30% of participants risk pneumothorax of which half require a chest drain and hospital stay.
- The Radial EBUS procedure has a diagnosis yield up to 74% with less than 1% risk of pneumothorax. The usual biopsy method involved in Radial EBUS is by forceps biopsy and cytology brush.
- This study looks at using cryo-biopsy to improve the diagnostic capability of Radial EBUS.
- The cost to administer each method is the same, at around \$2,700 however this does not include the associated costs with side effects from the CT-guided method.
- The previous linked feasibility study will take one additional biopsy for participants who already need a biopsy as part of clinical care.
- The Committee commended the PIS/CF.

Summary of ethical issues (resolved)

The main ethical issues considered by the Committee and addressed by the Researcher are as follows.

- Is the cryo-biopsy method practiced anywhere else internationally? Dr Herath explained that a study had been completed in Germany with 39 patients.

- Is there a risk that a participant will go undiagnosed, noting that significant difference in diagnosis change between 74% compared to 90%? Dr Herath explained that while the cryo-biopsy is a new method it had already been studied and proven to be effective.
- The Committee asked if there is a risk due to patients who don't get the gold standard treatment if they are randomised to the cryo-biopsy arm. Dr Herath explained that the cryo-biopsy is an extra test, on top of a standard radial EBUS biopsy. There is no withholding of standard treatment involved.
- The Committee asked what the process was when a mass was identified. Dr Herath explained that it was roughly 60% malignant 40% benign (when there is a mass). If it is malignant treatment occurs. If benign then follow up will occur with further imaging – either CT guided or a repeat test of the radial EBUS. These processes will likely be reviewed on a case by case.
- Dr Herath confirmed that Maori consultation will occur as part of the locality review process.
- The Committee asked if the researchers were going to use surveys and questionnaires. Dr Herath noted that the follow up will be an informal phone call to seek self-reported GP visits, 'how did you feel etc.' No use of standardised forms or Quality of Life methodology. The call is to seek information that would not be in health records.
- Dr Herath confirmed she is trained in the cryo-biopsy technique.
- Dr Herath explained that a sample size recalculation is planned for mid-way through the study.
- The Committee noted the data safety monitoring arrangements.
- Dr Herath confirmed that a feasibility study has occurred. The study was to assess tolerability of the procedure, adding that every patient who undergoes the cryo-biopsy procedure needs an endotracheal tube inserted.
- Dr Herath confirmed there will be a total of 228 participants.
- The Committee queried what funding arrangements are in place. Dr Herath explained that they had approached the Auckland Medical research Foundation and ASSER trust for this study.
 - Explain what randomisation is for participants in lay language.
 - All patients have the right to have a lay language summary of results. Please offer this to participants.
 - Please note the right to withdraw is possible up until the point of sedation and after this they can no longer withdraw. Please make this clear.
 - Please proof read and review for jargon. Explain any jargon in lay language.
 - Amend statement of approval to reflect that study was approved by the Northern A HDEC, not the Health and Disability Commission.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern A Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.
2. Before the study commences at *any* locality in New Zealand, it must be registered in a WHO-approved clinical trials registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au).
3. Before the study commences at a *given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that

the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

The main ethical issues considered by the Committee and which require addressing by the Researcher are as follows.

The Committee requested the following changes to the Participant Information Sheet and Consent Form:

- Please add a lay language title.
- Add lay study title under what is the purpose of the study
- The Committee noted that there is a requirement for ACC information to be included. The Committee suggests the following:

If you were injured in this study, which is unlikely, you would be eligible for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

- Please add a separate sub-heading for 'Risks', within the 'what will the study involve' section.
- Please include Maori contact information.
- Please add more information about the post-procedural chest x-ray, as standard of care and for either procedure.
- The Committee suggested pictures could be included in the PIS to help potential participants understand the two different modalities. Particularly due to the lay association of biopsy from an external needle.
- Explain what randomisation is for participants in lay language.
- All patients have the right to have a lay language summary of results. Please offer this to participants.
- Please note the right to withdraw is possible up until the point of sedation and after this they can no longer withdraw. Please make this clear.
- Please proof read and review for jargon. Explain any jargon in lay language.
- Amend statement of approval to reflect that study was approved by the Northern A HDEC, not the Health and Disability Commission.

Please submit your non-standard conditions by email to HDECS@moh.govt.nz

Please note HDEC review is not required for non-standard conditions however they must be completed prior to commencing your study. Do not submit non-standard conditions as a post approval form (PAF).

For information on non-standard conditions please see section 128 and 129 of the Standard Operating Procedures at <http://ethics.health.govt.nz/home>.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 12 May 2016.

Participant access to ACC

The Northern A Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'B J Fergus', with a horizontal line underneath.

Dr Brian Fergus
Chairperson
Northern A Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

<i>Document</i>	<i>Version</i>	<i>Date</i>	
Participant information sheet	1.2	19 February 2015	
Informed consent form	1.0	19 January 2015	
Evidence of scientific review: Peer review conformation	1.0	23 April 2015	
Protocol: Protocol	1.2	19 January 2015	
CV for CI: CV for Sam Herath	1.0	30 April 2015	
CVs for other Investigators: Dr.Braden Te Ao	1.0	30 April 2015	
CVs for other Investigators: Dr.Irene Zeng	1.0	30 April 2015	
CVs for other Investigators: Dr.Irene Low (Pathologist)	1.0	30 April 2015	
CVs for other Investigators: Dr.Stewart Hawkins (Radiologist)	1.0	30 April 2015	
CVs for other Investigators: Dr.Stuart Barnard(Radiologist)	1.0	30 April 2015	
CVs for other Investigators: Dr.Dan Cookson (radiologist)	1.0	30 April 2015	
CVs for other Investigators: Dr.Andrew Veale (Respiratory Physician)	1.0	30 April 2015	
Covering Letter: Covering Letter	1.0	20 March 2015	
Evidence of scientific review	1	04 May 2015	
Evidence of scientific review	1	11 May 2015	

Appendix B
Statement of compliance and list of members

Statement of compliance

The Northern A Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008714) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

<i>Name</i>	<i>Category</i>	<i>Appointed</i>	<i>Term Expires</i>	<i>Present on 12/05/2015?</i>	<i>Declaration of interest?</i>
Dr Brian Fergus	Lay (consumer/community perspectives)	01/07/2012	01/07/2015	Yes	No
Dr Karen Bartholomew	Non-lay (intervention studies)	01/07/2013	01/07/2016	Yes	No
Ms Susan Buckland	Lay (consumer/community perspectives)	01/07/2012	01/07/2015	Yes	No
Ms Shamim Chagani	Non-lay (health/disability service provision)	01/07/2012	01/07/2015	Yes	No
Dr Christine Crooks	Non-lay (intervention studies)	01/07/2013	01/07/2015	No	No
Mr Kerry Hiini	Lay (consumer/community perspectives)	01/07/2012	01/07/2015	Yes	No
Mr Mark Smith	Non-lay (intervention studies)	01/09/2014	01/09/2015	Yes	No
Ms Michele Stanton	Lay (the law)	01/07/2012	01/07/2015	Yes	No

<http://www.ethics.health.govt.nz>

Appendix 23: Ethics Approval – Westmead (CT-CROP)

HREC Committee Secretariat:

A/Prof Clement Loy
Medical Graduate – Neurologist

Mrs Patricia Fa
Clinical Trials Pharmacist

HREC Committee Members:

Ms Narelle Bell
Lawyer

Sr Patricia Bolster RSM
Catholic Chaplain

Mrs Therese Burke
Clinical Trial Coordinator

Prof Angus Dawson
Professor of Bioethics

Mr John Fisher
Lawyer

Mr John McLeod
Layman

Mrs Janette Parry
Laywoman

Dr Christopher Ryan
Medical Graduate - Psychiatrist

Mrs Katherine Schaffarczyk
Nurse Educator

Mr John Shaw
Layman

Dr Geoff Sheed
Medical Graduate – Surgeon

Dr Tony Skapetis
Dental Graduate

Dr Howard Smith
Medical Graduate – Endocrinologist

Ms Shane Waterton
Laywoman

Dr Christine Wearne
Clinical Psychologist

Mrs Christina Whitehead
Research Co-Ordinator - RN

Research Office File No: **(4827)**

HREC Ref: AU RED HREC/16/WMEAD/337
SSA Ref: AU RED SSA/16/WMEAD/347

28 September 2016

Prof John Wheatley
Respiratory Medicine
Westmead Hospital

Dear Prof Wheatley

Project title: "Randomised controlled trial comparing the diagnostic yield of Cryo-biopsy via Radial Endo-Bronchial Ultra-Sound guide sheath Vs CT-guided transthoracic biopsy in patients with parenchymal pulmonary lesions (CT CROP)."

Thank you for submitting the above project which was considered by the WSLHD Human Research Ethics Committee at its meeting held on 27 September 2016.

In order to make a determination of the ethical acceptability of your project, please respond to the following request for additional information / clarification:

GENERAL (Ref National Statement 2.1):

- If randomly allocated are you risking not getting a diagnosis?
- It is noted that the letter from the Department of Medical Physics states that the effective radiation dose each participants will receive from this trial is above standard care levels (recommended dose per 5 years) and will place them in a risk category. The investigator should address this issue.

In order to facilitate the HREC's reconsideration of your project, please provide the requested information as soon as possible. Your response should be directed to The Secretary, WSLHD Human Research Ethics Committee, Research Office.

Please note that if the requested information is not received within 6 months the application will be cancelled and returned to you.

HUMAN RESEARCH ETHICS COMMITTEE

Research Office, Level 2, REN Building
Westmead Hospital, Hawkesbury & Darcy Roads, Westmead NSW 2145
Telephone 02 9845 8183 Facsimile 02 9845 8352
Email: WSLHD-ResearchOffice@health.nsw.gov.au

WESTERN SYDNEY LOCAL HEALTH DISTRICT
ABN 48 702 394 764

WSLHD Office, Westmead Hospital Campus
Institute Road, Westmead NSW 2145
PO Box 533, Wentworthville NSW 2145
Telephone 02 9845 5555

Should you have any queries about your project, please contact Kellie Hansen, HREC Executive Officer on 9845 8183.

Yours sincerely



Mrs Kellie Hansen
Research Office Manager
WSLHD Research & Education Network

Appendix 24: Ethics Approval – Westmead (R-CORE)

HREC Committee Secretariat:

A/Prof Clement Loy
Medical Graduate – Neurologist

Mrs Patricia Fe
Clinical Trials Pharmacist

HREC Committee Members:

Ms Narelle Bell
Lawyer

Ms Joy Bowen
Catholic Chaplain

Prof Angus Dawson
Professor of Bioethics

Mr John Fisher
Lawyer

Mr John McLeod
Layman

Mr Sean Murgovan
Physiotherapist

Mrs Janette Parry
Laywoman

Dr Christopher Ryan
Medical Graduate - Psychiatrist

Mrs Katherine Schaffarczyk
Nurse Educator

Mr John Shaw
Layman

Dr Geoff Shead
Medical Graduate – Surgeon

Dr Tony Skapetis
Dental Graduate

Dr Howard Smith
Medical Graduate – Endocrinologist

Ms Shane Waterton
Laywoman

Dr Christine Weame
Clinical Psychologist

Mrs Christina Whitehead
Research Co-Ordinator - RN

Research Office File No: **(5363)**

HREC Ref: SSA | AU RED HREC/17/WMEAD/461

Ref: | AU RED SSA/17/WMEAD/

1 December 2017

Dr Samantha Herath
Department of Respiratory Medicine
Westmead Hospital

Dear Dr Herath

Project title: Assessing the diagnostic ability and safety in obtaining a core biopsy from peripheral lung lesions using the novel Gen Cut tool with Radial EBUS (R-COR)

Thank you for Ms Tracey Burns' correspondence addressing the matters raised in the HREC's letter dated 3 November 2017 following single ethical review of the above project at its meeting held on 31 October 2017.

This HREC has been accredited by the NSW Department of Health as a lead HREC to provide the single ethical and scientific review of proposals to conduct research within the NSW public health system. This lead HREC is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research* and the *CPMP/ICH Note for Guidance on Good Clinical Practice*.

This proposal meets the requirements of the National Statement and I am pleased to advise that the HREC has now granted ethical approval of this Single site research project to be conducted at:

- Westmead Hospital – Prof John Wheatley
- Concord Hospital – Prof Alvin Ing
- Royal Brisbane and Women's Hospital – Dr David Fielding

The following documentation has been reviewed and approved by the HREC:

- NEAF submission code AU/1/C4F0313
- Protocol, version 1, dated 1 September 2017
- Master Participant Information Consent Form, version 2, dated 15 November 2017
- Data collection Sheet, R CORE: Patient demography, version 1, dated 30 November 2017
- Data Collection Sheet, R CORE: Pathology, version 1, dated 30 November 2017
- Data Collection Sheet, R CORE: Bronchoscopy, version 1, dated 30 November 2017
- Data Collection Sheet, Adverse Events, version 1, dated 1 September 2017
- Pain scale, version 1, dated 1 September 2017

HUMAN RESEARCH ETHICS COMMITTEE

Research Office, Level 2, REN Building
Westmead Hospital, Hawkesbury & Darcy Roads, Westmead NSW 2145
Telephone 02 9845 8183 Facsimile 02 9845 9636
Email: WSLHD-ResearchOffice@health.nsw.gov.au

WESTERN SYDNEY LOCAL HEALTH DISTRICT

A&N 48 702 394 764

WSLHD Office, Westmead Hospital Campus
Institute Road, Westmead NSW 2145
PO Box 533, Wentworthville NSW 2145
Telephone 02 9845 5555

Please note the following conditions of approval:

- The Chief Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project.
- **For clinical trials of implantable medical devices only** – The Chief Investigator will confirm to the HREC that a process has been established for tracking the participant, with consent, for the lifetime of the device and will immediately report any device incidents to the Therapeutic Goods Administration (TGA).
- The Chief Investigator will immediately report any protocol deviation / violation, together with details of the procedure put in place to ensure the deviation / violation does not recur.
- The Chief Investigator will provide to the HREC in the specific format, proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, must be provided to the HREC to review in the specific format. Copies of all amendments when approved by the HREC must also be provided to the Research Governance Officer.
- The Chief Investigator must notify the HREC, giving reasons, if the project is discontinued at a site before the expected date of completion.
- The Coordinating Chief Investigator must provide an annual report to the HREC and a final report at completion of the study, in the specified format. HREC approval is granted for a period of 12 months and ongoing approval is contingent upon annual submission. Annual Reports for all studies should be submitted in November, they will be processed and presented to the HREC at their January meeting. A copy of the Annual / Final Research Report Form can be obtained electronically from the Research Office on request.
- The HREC has the discretion to adopt other appropriate mechanisms for monitoring depending on the complexity, design and risk perceived including
 1. Discussion of relevant aspects of the project with investigators, at any time,
 2. Random inspection of research sites, data or consent documentation,
 3. Interview with research participants or other forms of feedback from them, and
 4. Request and review reports from independent agencies such as a Data Safety Monitoring Board.
- If your research project is an interventional trial, please ensure it is registered on one of the clinical trial registries, eg <http://www.actr.org.au>.
- It should be noted that compliance with the ethical guidelines is entirely the responsibility of the Chief Investigator.

You are reminded that this letter constitutes *ethical approval only*. You must not commence this research project at a site until separate authorisation from the Chief Executive or delegate of that site has been obtained. Copies of this letter, together with any approved documents as enumerated above, must be forwarded to all site investigators for submission to the relevant Research Governance Officer.

Should you have any queries about the HREC's Terms of Reference, Standard Operating Procedures or membership, please contact the Executive Officer through the Research Office on 8890 9007 or emailing wsldh-researchoffice@health.nsw.gov.au.

In all future correspondence concerning this study, please quote Research Office File Number **(5363)**

The HREC wishes you every success in your research.

Yours sincerely



Mrs Pat Fa
Secretary
WSLHD Human Research Ethics Committee

cc WSLHD Research Governance Officer

Appendix 25: CT-CROP study information (single page flyer)

CT CROP STUDY

Multi-Centre RCT comparing the diagnostic yield of CT-guided core biopsy vs. Cryo biopsy (via the radial EBUS) in parenchymal pulmonary lesions (PPL) suspected of lung cancer.

Lung cancer is fast becoming a chronic disease with an ever-increasing demand for larger biopsy samples. Cryobiopsy “cold biopsy” used with Radial EBUS is a novel emerging method that gives larger non-crushed histology samples in lung masses. **Aim:** Compare the emerging cryobiopsy method Vs the CT guided biopsy as a much safer, painless and cost effective procedure with non-inferior efficacy.

All patients will a PPL (> 1cm) that requires a biopsy

Computerised Randomisation

n= 158

CT guided BX

Performed at PRP Radiology Westmead

Cryo biopsy/Radial EBUS

Performed at Endoscopy unit Westmead Hospital

Comparison of the current data on two biopsy methods	CT guided biopsy	Cryo biopsy
Efficacy	65-95%	74-83%
Pneumothorax rate	15-20%	<1%
Pulmonary Hemorrhage/haemoptysis	4-27%	< 4%
Method of anesthesia	LA	GA
Second procedure for staging	Required	No

Only a single study visit is required.

Some additional information regarding the cryobiopsy/Radial EBUS procedure

1. Cryobiopsy is markedly safer and no discomfort during or post cryobiopsy procedure.
2. Your patient will only need a single procedure for staging and diagnosis (linear EBUS will be done at the same time if required).
3. Multiple lesions biopsied at the same time if needed.
4. Cryobiopsy gives larger non-crushed histology samples for analysis.

Inclusion criteria

- Age 18 or above
- Has a Lung mass that requires a biopsy.
- Lung mass is > 1 cm in size
- Able to give consent
- Air Bronchogram to the lesion is NOT required. Lung point navigation will be used to track the lesion.

Exclusion Criteria

- Pregnancy
- Not suitable for a Biopsy procedure including a Platelet count <150, INR >=1.5, Haemoglobin <100
- On anticoagulation/ anti platelet therapy (apart from aspirin) that cannot be stopped due to medical reasons.
- Mediastinal adenopathy that can be biopsied with linear EBUS should have this procedure first and enrol only if it fails to derive a diagnosis.

Your support is much appreciated

Please send all referrals to

Dr. Samantha Herath

Department of Respiratory and Sleep Medicine, Westmead Hospital

Fax: 02 98457286

For further information, please contact

Dr. Samantha Herath on 0418343787 or scherath@yahoo.com

We will contact your patient **urgently** to organize a clinic visit at Westmead Hospital to discuss the study and organize the appropriate biopsy **without delay**.

NEEDLE ASPIRATION USING A 21G NEEDLE (EXCELON OR WANG), VIA RADIAL EBUS GUIDE SHEATH, GIVES BETTER SAMPLES IN COMPARISON TO CYTOLOGY BRUSH OR FORCEPS BIOPSIES. A DESCRIPTIVE CASE SERIES

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Introduction/Aim: Despite the better safety profile, the yield of Radial Endobronchial Ultra Sound (R-EBUS) remains lower (73%) than CT-guided biopsy (90%) in diagnosing peripheral pulmonary lesions (PPL). An additional needle biopsy (Using Exelon 21 G or WANG 21G) was performed via the R-EBUS Guide Sheath to see if there is additional benefit to the conventional sampling with cytology brush and forceps.

Methods: All patients who had undergone R-EBUS and had a needle biopsy, in addition to forceps, and brushings via the GS (Olympus K-203, 1.7 mm) were prospectively included. Each patient had up to three attempted biopsies with each of the three sampling methods.

The ability to diagnose malignancy and the best sample out of the three modalities were recorded. Best sample was defined as 'the preferred sample for EGFR testing and carrying the highest number of malignant cells/hpf'. A Chest X-Ray was performed 1-h post procedure.

Results: Twelve consecutive patients had needle biopsy in addition to forceps and brush. There were nine women. Mean age was 64 years. (Range 47–85 years).

The mean size of the PPL was 4.7 cm (1.9 to 7.5 cm) with predominant upper lobe distribution 8/12 (66%).

Overall, R-EBUS success rate was 83% ($n = 10/12$). The diagnostic yield from each individual modality alone, was recorded with the highest success of 66% ($n = 8/12$) from the needle biopsy, followed by brush 58% (7/12) and forceps biopsy 42% ($n = 5/12$).

Overall, the preferred sample for EGFR testing was from needle aspiration in 6/12 (50%) with the brush being the sample of choice in 2/12 patients (16%). No pneumothorax or bleeding was recorded in this group.

Conclusion: These preliminary results indicate that when sampling a PPL, addition of a 21G needle biopsy improves the diagnostic yield of R-EBUS and is the better sampling modality in comparison to the cytology brush or forceps, without an increase in the side effect profile.

Grant Support: none

Appendix 27: Manuscript – Case series using cryobiopsy with Radial EBUS in high-bleeding-risk PPL



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CASE SERIES



WILEY

Using cryobiopsy with Radial EBUS in high-bleeding-risk, peripheral pulmonary lesions (PPL): description of cases and technique

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Associate Editor: David Lam

Abstract

Cryobiopsy is an emerging tool in the diagnosis of peripheral pulmonary lesions (PPL) and becoming an important tool in the toolbox. Anecdotally the data on cryobiopsy use in the lung was extrapolated from the use of transbronchial cryobiopsy (TBCB) in Interstitial Lung disease (ILD). Similar to ILD data, cryobiopsy in PPL also provided larger tissue compared to forceps biopsies. Yet, unlike TBCB in ILD, the safety profile for cryobiopsy in PPL seems much more favourable, yet the number of publications on cryobiopsy in PPL remains sparse. Some PPL, both malignant and non-malignant are considered to be of a high bleeding risk due to vascularity of the tumour and/or inflammation of the blood vessels and surrounding tissue. The use of cryobiopsy and the risk of bleeding in this type of PPL have not been described. This paper describes four patients with PPL, undergoing cryobiopsy with radial EBUS for suspected lung cancer, and later diagnosed to have a PPL, deemed to be of a high bleeding risk. The use of cryobiopsy with radial ultrasonic examination for the vasculature of the PPL, bronchial blocker use, and airway protection as well as an expert team preserved the safety of the procedure.

KEYWORDS

bleeding, Cryo-biopsy, lung cancer, PPL, radial EBUS

INTRODUCTION

Cryobiopsy is an emerging tool in the diagnostic armoury for peripheral pulmonary lesions (PPL) suspected of lung cancer. Most data on cryobiopsy in the lung was extrapolated from interstitial lung disease (ILD) data. A feared complication of transbronchial cryobiopsy (TBCB) in ILD was the high bleeding risk. A meta-analysis in TBCB-ILD demonstrated a moderate bleeding risk requiring an endoscopic procedure to be 16.9%¹–39%.² Life-threatening complications had been known when adequate precautions were not taken with 12% of serious haemorrhage.³ This high-risk profile led to heightened anxiety and the development of guidelines around TBCB in ILD.⁴

Cryobiopsy is now increasingly used in PPL suspected of lung cancer. Emerging data on cryobiopsy in PPL demonstrates a high diagnostic yield compared to the forceps biopsy.^{5,6}

The side effect profile for cryobiopsy in PPL seems to be far more favourable than TBCB in ILD. A meta-analysis of cryobiopsy in PPL by Sryma et al, in 2023 demonstrated that in 300 patients in nine studies, the occurrence of major adverse event was 1.8%.⁶ A further retrospective study by Herth et al noted clinically significant bleeding only in 3.5%.⁷ Therefore, a clear divergence is beginning to emerge with the cryobiopsy for PPL, from TBCB in ILD, demonstrating a much lower bleeding risk.

The ability to perform cryobiopsy in PPL safely was not reported specifically in tumours considered to be more vasculogenic due to tumour angiogenesis, thereby, having a higher bleeding risk. Melanoma,⁸ renal cell cancer⁹ and carcinoids are deemed vascular tumours of malignant nature that have high bleeding risk. Hypersensitivity pneumonitis and vasculitis,¹⁰ due to their highly inflammatory nature were thought to be of high bleeding risk despite being non-malignant conditions.

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CASE SERIES

Description of cases and technique

We describe our experience in using cryobiopsy with radial endobronchial (radial EBUS) and guide sheath (GS) within a study protocol with a uniform technique to biopsy PPL. The four patients outlined here were referred for a cryobiopsy for PPL for this study as suspected lung cancers, and the diagnosis was not known at the time of the procedure.

All patients were fit to undergo a flexible bronchoscopy and general anaesthesia. All patients had a CT scan of the chest within 1 month of the procedure.

In this study, all patients underwent general anaesthesia. Airway protection was obtained using a rigid bronchoscopy or Endotracheal Tube (ETT). All patients had Radial EBUS performed (Small ultrasound probe: 20 MHz, UM-BS20-17S outer diameter 1.4 mm) housed in the large GS (K-203, 2.6 mm outer diameter), requiring a 2.8 mm working channel in the bronchoscope.

Three out of the four patients had the procedure performed under general anaesthesia in the operating rooms. A 12 mm Storz rigid bronchoscope was used for airway protection. One procedure (Patient 4) was performed in the endoscopy suite, using an endotracheal tube (ETT) and general anaesthesia. Fluoroscopy was not used for any of the cases.

Locating the optimal ultrasound position

It is of utmost importance to place the Radial EBUS GS in the optimum biopsy location, in terms of safety and diagnostic yield. Once the PPL was located, based on the pre-planned pathway according to the CT scan of the chest, the Radial EBUS within the GS, was moved proximally and distally to find the edges of the PPL. The GS was then placed in

the best concentric ultrasonic view of the PPL as well as the least vascularity. Vascular areas are noted as hypoechoic circumscribed black areas (Figure 1A) and malignancy was noted as continuous hyperechoic margins with heterogeneous internal echoes (Figure 1B). In this study, if vascularity was noted, an attempt was made to move the GS to an acceptable non-vascular area and if vascular areas could not be avoided, a cryobiopsy was not performed. All four patients reported here did not have vascularity on the ultrasound image.

The GS is then left in situ and biopsy tools were inserted through the GS. A radial EBUS forceps biopsy (1–3 passes) or a brush (1–3 passes) was used initially, followed by 1–3 passes of cryobiopsy (CRYO2; ERBE, Tuebingen, Germany, 1.9 mm 1150 mm reusable probe) via the GS. Four-second freezing with 1.9 mm cryoprobe via the GS was used for every case. On average 2 cryobiopsies per patient with an average sample size of 7 mm were noted for the cryobiopsy.

A 5F Fogarty bronchial blocker was placed prophylactically prior to commencing the biopsies and inflated immediately post-biopsy. The blocker was placed at the segmental level, adjacent to the segment where the PPL is located. Once the bronchial blocker was placed, the stellate was removed. Having a stellate assisted in directing the blocker to the correct location. Bending the tip of the blocker was easier when the stellate was present and directing it to the upper lobe was easier. The blocker placement was checked by the proceduralist and taped to the rigid bronchoscope or to the ETT to secure the location. There was a dedicated assistant to inflate the blocker. The anaesthetic nurse was allocated the task of holding the rigid bronchoscope in situ, to avoid unanticipated extubation due to the pulling associated with the biopsy. The specimen was thawed in saline and fixed in formalin.

An anaesthetist, anaesthetic nurse, Interventional bronchoscopist, an interventional bronchoscopy fellow or a

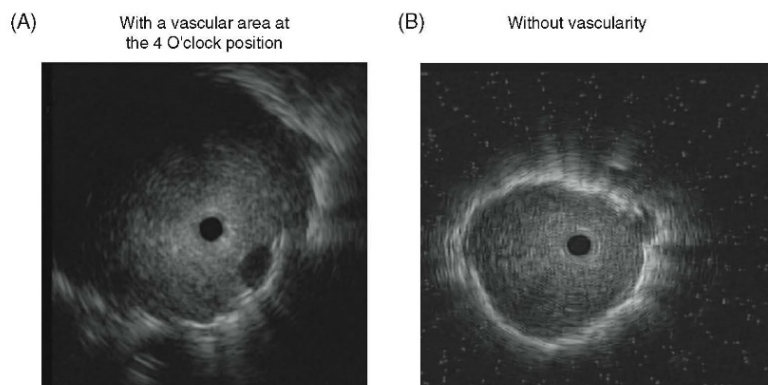
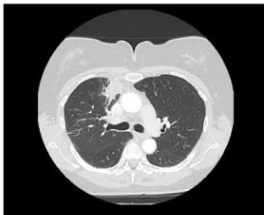
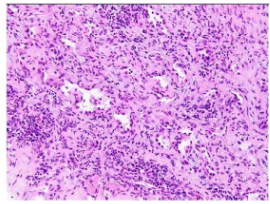
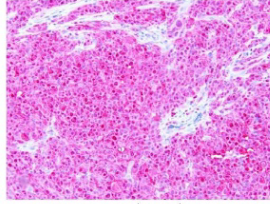
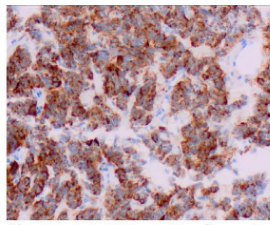
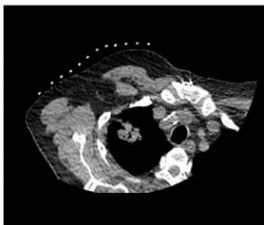
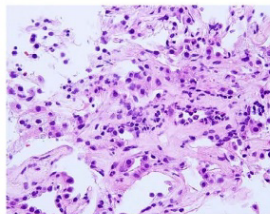


FIGURE 1 Radial EBUS image of a PPL. (a) With a vascular area at the 4 O'clock position and (b) without vascularity.

TABLE 1 Diagnosis, radiology, cryobiopsy histology and bleeding management in each case

Diagnosis of each case	Radiological appearance on CT	Histology from cryobiopsy	Bleeding according to adverse reactions
Case 1 Organising pneumonia 54 years, Female, non-smoker	 RUL anterior segment PPL (RB3)—26 mm	 Organizing pneumonia	Grade 1 Prophylactic inflation of size 5 Fogarty
Case 2 Malignant melanoma 57 years, Male, current smoker	 RLL apical segment PPL (RB6)—40mm	 S100 stain positive for malignant melanoma	Grade 1 Prophylactic inflation of size 5 Fogarty
Case 3 Carcinoid tumour 77 years, Male, non smoker	 RML medial segment small PPL(RB5) 20mm	 Chromogranin Stain positive for carcinoid	Grade 1 Prophylactic inflation of size 5 Fogarty
Case 4 Vasculitis (Wagener's granulomatosis) 60 years, Male, current smoker	 RUL apical segment PPL(RB1)—35mm	 Vascular invasion on biopsy	Grade 1 Prophylactic inflation of size 4 Fogarty

third-year respiratory advanced trainee and two trained nurses were present as a minimum for each procedure.

Cold saline, adrenaline and tranexamic acid were drawn and kept but were not required for any of these cases. All patients had grade 1 bleeding. There was no pneumothorax. The Common Terminology Criteria for Adverse Events (CTCAE) was used to define adverse events.

The final diagnosis was organizing pneumonia, melanoma, carcinoid, and vasculitis which were deemed of high bleeding risk PPL. Table 1 shows the diagnosis, radiology, cryobiopsy histology and bleeding management in each case. All the above cases were suspected lung cancers and the above high-risk bleeding tumours were surprising diagnoses. Despite this, there was no significant bleeding.

DISCUSSION

The first published paper on cryobiopsy in PPL was in 2014 by Schuhmann et al.⁵ demonstrating for the first time a marked divergence from TBCB in ILD with a much better safety profile. Out of the 34 patients in this feasibility study, only one had moderate bleeding. This group used a rigid bronchoscope for all patients.⁵ Encouraged by these results, authors of the New Zealand team¹¹ published a case series on cryobiopsy use in PPL in 2017, using sedation alone without rigid bronchoscopy. The most important introduction here was the use of a prophylactic bronchial blocker for containing the bleeding. There was no pneumothorax or bleeding despite using a similar freezing time to Schuhmann et al.

Since then, several studies have reported the use of cryobiopsy in PPL. These studies used a combination of rigid bronchoscopy and/or bronchial blocker (Fogarty or Arndt) as safety measures, either to contain the bleeding or have access to clear the bleeding quickly.⁶ Despite the better safety profile, the number of studies on cryobiopsy in PPL remains limited.⁶ Given the scarcity of data and extrapolation of most of the data from TBCB in ILD there remains a 'fear factor' around performing cryobiopsy for PPL.

Therefore, this case series will add to the existing data to demonstrate that with the use of the correct technique and facilities available to control bleeding, the 'high bleeding risk' tumours may not pose any higher risk and cryobiopsy for PPL continues to have a favourable safety profile.

A few explanations as to how the safety was maintained are highlighted below.

One is that the Radial EBUS probe was used with the GS and the cryoprobe was directly placed in the PPL at its maximal concentric circumference. With a freeze time of only 4 s, there was no damage to the lung parenchyma in contrast to the TBCB in ILD which directly freezes the lung parenchyma. Second, the bronchial blocker was placed at the segmental level adjacent to the segment where the biopsy is taken and immediately inflated post-biopsy. This prevents the blocker from getting displaced when the biopsy was pulled out. Thirdly, the bronchial blocker was inflated for 1 min and at deflation, no active, bleeding was noted with clotted blood seen distal to the blocker which wasn't suctioned out. This would cause a tamponading effect and an 'endobronchial blood patch' to cause haemostasis. Another reason may be during the Radial EBUS examination, the PPL was examined ultrasonically and the cryoprobe was placed where there is the least vascularity.

The radial EBUS ultrasound can be used to examine the PPL characteristics of size, shape, echogenicity, margin and blood vessels.¹² Despite lacking the ability to obtain a Doppler flow, the demonstration of hypoechoic areas, and noting pulsations within the PPL in keeping with vascularity is providing valuable information. Using Radial EBUS to guide the location of TBCB in ILD had been proposed as a method to reduce the bleeding risk with TBCB in ILD.¹³

In PPL biopsy with radial EBUS, the risk of bleeding has been minimal¹⁴ and not much focus has been placed on the ultrasonic examination of vascularity.

However, with the use of cryobiopsy in PPL, the risk of haemorrhage is higher and further measures need to be taken. This technique of using the radial ultrasonic image to detect vasculature to direct the ultrasound probe, maybe a simple solution, that can be added to the armoury of safety measures. Novel methods aimed at detecting Doppler flow in PPL including endoscopic doppler optical coherence tomography and autofluorescence imaging are underway and will provide useful information in the future.¹⁵

Having general anaesthesia, expert proceduralists and a team with experience all added to the safety of the procedure.

In conclusion, Radial cryobiopsy in high bleeding risk peripheral pulmonary lesions can be safely performed by experienced operators with the necessary precautions, including rigid intubation or ETT to secure the airway, and bronchial blocker. The use of ultrasound evaluation of vascularity using Radial EBUS, as well as other novel imaging methods to detect vascularity in PPL may be an added safety feature that needs further investigation.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study has signed patient consent and ethics committee approval. Ethics approval number: HREC/16/WMEAD/337.

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