Respirology Case Reports

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 filed 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 cryo-probe 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 | | | Cryobiopsy | Preferred sample for EGFR analysis | Final diagnosis |
				Needle aspiration	Brush	Forceps			
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.
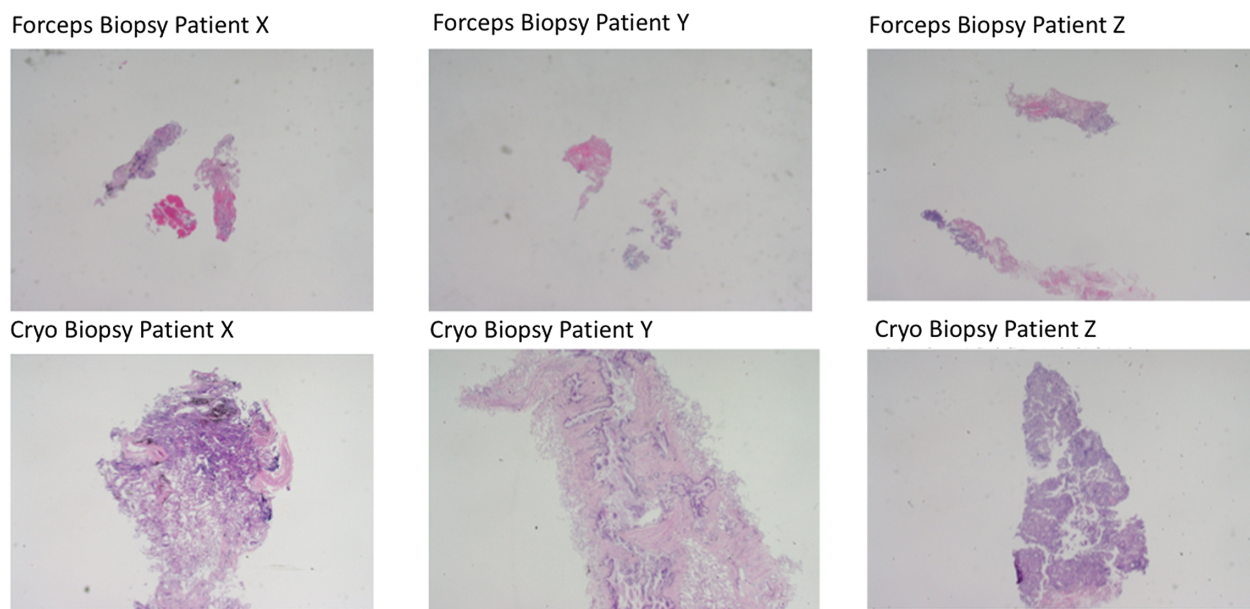
In this study, one aspect we were assessing was the feasibility of performing cryobiopsy and the ability to tolerate a bronchial blocker with sedation only. We noted that, with sedation only, the patients tolerated the procedure with variable discomfort due to the bronchial blocker causing irritation 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
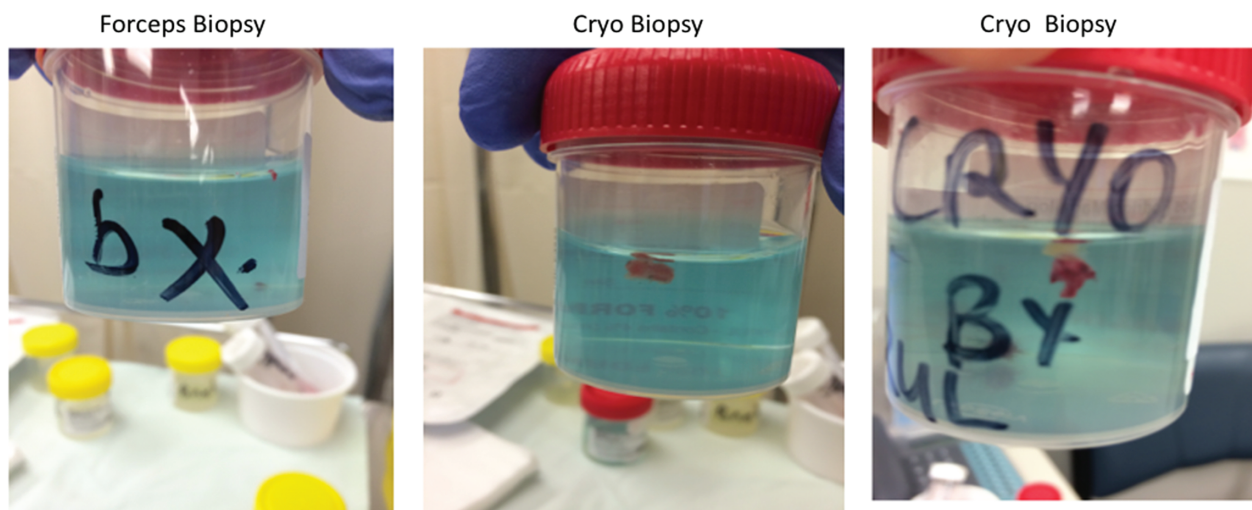
Figure 2. Macroscopic comparison of forceps biopsy (left) with the cryobiopsy (two samples on the right).

Forceps Biopsy Patient X

Forceps Biopsy Patient Y

Forceps Biopsy Patient Z

Cryo Biopsy Patient X

Cryo Biopsy Patient Y

Cryo Biopsy Patient Z

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

Acknowledgments

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