# Exploring the optimal freeze time and passes of the ultrathin cryoprobe in transbronchial cryobiopsy of peripheral pulmonary lesions



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*To the Editor:*

Transbronchial cryobiopsy (TBCB) is an invaluable technique for obtaining high-quality lung tissue samples [1]. Its use in peripheral pulmonary lesions (PPLs) is increasing due to its potential to enhance diagnostic yield, especially in lesions that are adjacent to the airway [2, 3]. However, despite clear recommendations regarding freezing time and the number of passes using conventional 1.9- and 2.4-mm cryoprobes in interstitial lung disease (ILD) biopsies, PPL-TBCB remains unstandardised [4]. Furthermore, the introduction of the 1.7- and 1.1-mm single-use cryoprobes has necessitated further investigation to determine the optimal number of passes and freezing time for PPL-TBCB.

Herein, we conducted a 6-year retrospective analysis of our centre's radial endobronchial ultrasound (rEBUS)-guided PPL-TBCB. The study included PPL-TBCB procedures performed using either the reusable 1.9-mm or the single-use 1.1-mm cryoprobe. Artificial airway was employed in 61.3% (92 out of 150) of cases, consisting of endotracheal intubation (n=90), rigid bronchoscopy (n=1) or laryngeal mask airway (n=1). Total intravenous anaesthesia was employed for cases performed using artificial airway, while intermittent bolus doses of *i.v.* midazolam and fentanyl were used for cases performed using natural airway. The data were extracted from a study approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-ID-23-00520-LMM (IIR)). We compared the performance of the 1.1-mm cryoprobe to the 1.9-mm cryoprobe, and explored the optimal cryobiopsy passes and activation time for the 1.1-mm cryoprobe.

Both rEBUS and TBCB were performed according to previously described protocols [3]. Importantly, in this patient cohort, the 1.1-mm cryoprobe was removed *en bloc* with the bronchoscope rather than retrieval through the oversheath. The procedure was considered conclusive if histological, microbiological or cytological studies were consistent with the clinical presentation [5]. Patients with nondiagnostic procedures were referred for surgery or transthoracic computed tomography (CT)-guided biopsy when appropriate, or monitored with surveillance chest CT. If the biopsied lesion was found to have resolved or be stable on follow-up CT, it was deemed inflammatory [5]. The severity of bleeding was graded according to a standardised definition on a scale ranging from 1 to 4 [6].

SPSS (version 20) was used for data analysis. Results are presented as mean±SD for normally distributed variables and as median (interquartile range) for non-normally distributed variables. Categorical data are expressed as n (%). Independent-sample t-tests and Mann–Whitney or Wilcoxon signed rank tests were used to compare normally and non-normally distributed variables between groups, respectively, when appropriate. A p-value <0.05 was considered to be significant.

In our study, a total of 150 TBCBs were performed, with 81 cases using the 1.9-mm reusable cryoprobe and 69 cases using the 1.1-mm ultrathin cryoprobe. The overall diagnostic yield of cryobiopsy alone was 76.0% (114 out of 150). Concurrent forceps biopsy was performed in 99 out of 150 cases, with a diagnostic yield of 56.6%. When combining the results of TBCB and forceps biopsy, the overall combined diagnostic yield in our cohort reached 83.3%. Malignant cases accounted for 63.2%, while benign

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**In PPL-TBLC, quality of tissue matters more than quantity for accurate diagnosis. Comparable diagnostic yield with 1.1-mm cryoprobe can potentially be achieved in 6 s of freezing and three or more passes.** https://bit.ly/49cbmbW

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tumours and infectious aetiologies, including tuberculous nodules, comprised 9.6% and 27.2%, respectively. The overall pneumothorax rate was 1.3%, and bleeding (any grade) occurred in 42.7% (64 out of 150) of cases. Grade 2 bleeding occurred in 16.7% (25 out of 150), requiring only adrenaline saline flush. Grade 3 bleeding occurred in 9.3% (14 out of 150), requiring balloon blockade for <20 min. Grade 4 bleeding occurred in only 0.7% (one out of 150), requiring endobronchial Watanabe spigot insertion for 72 h; this was an anticipated complication as the nodule was suspected and later histologically confirmed to be carcinoid.

When comparing the specific performance of the 1.9- and 1.1-mm cryoprobes, the diagnostic yield for both probes was similar, with diagnostic yield of 72.8% and 79.7%, respectively (p=0.358). However, the 1.1-mm cryoprobe allowed biopsy of smaller targets (2.12±0.70 *versus* 2.99±1.25 cm, p<0.001) located more peripherally (target to visceral pleura distance 1.98±1.10 *versus* 2.61±1.16 cm, p<0.01), while also reducing procedural time (36.21±15.37 *versus* 47.87±13.38 min, p<0.001). Notably, more passes were performed using the 1.1- than the 1.9-mm cryoprobe (2.94±1.17 *versus* 1.84±0.76 passes, p<0.001). Longer mean activation time was deployed for the 1.1-mm cryoprobe (6.08±2.18 *versus* 1.59±1.12 s, p<0.001) compared to the 1.9-mm cryoprobe to achieve an equivalent mean total aggregate (gross) specimen size (7.72±3.99 *versus* 7.85±3.31 mm, p=0.832) and diagnostic yield (79.7% *versus* 72.8%, p=0.380). Notably, while the overall bleeding rate was similar between the two groups, the 1.1-mm cryoprobe exhibited significantly less Grade 3–4 bleeding (5.7% *versus* 13.6%, p<0.001).

In a subgroup analysis evaluating the 1.1-mm cryoprobe, we made several noteworthy observations. Firstly, a higher number of activations passes (three passes or more) was associated with a better diagnostic yield, while different freezing times did not affect diagnostic yield (table 1). There was no significant difference in bleeding rates (any grade) with respect to cryobiopsy passes or freezing time; nevertheless, there was a trend towards increased bleeding with freezing times ⩾10 s. Secondly, target size cut-off of 2 cm and the rEBUS orientation did not significantly impact the overall diagnostic yield (table 1), although this may be attributed to the small overall sample size. Additionally, the total mean specimen aggregate size did not significantly affect procedural outcomes.

The standardisation of freeze time and the number of passes in PPL-TBCB has yet to be established, leaving an important gap in the literature. Furthermore, the impact of specimen size on PPL-TBCB outcomes, as observed in ILD-TBCB, remains an area of uncertainty. In the context of ILD-TBCB, obtaining a sizable tissue specimen is crucial for an accurate representation of the underlying pathology [1]. It is recommended to aim for a gross TBCB specimen ⩾5 mm in diameter to allow proper histological analysis for ILD [4]. Previous animal studies have demonstrated that the 1.1-mm cryoprobe is capable of providing specimens comparable in size to those obtained with 1.7- or 1.9-mm cryoprobes with longer activation time [7]. However, in the case of PPL-TBCB, the necessity for large specimens may not be as significant, particularly considering that the majority of these lesions are malignant in nature, where achieving proper reach into the target site may hold greater importance [3, 8, 9]. This was reflected in our finding that specimen size does not differ significantly between conclusive and inconclusive cases in our PPL-TBCB cohort. Nevertheless, while the optimal number of cryobiopsy passes and freezing time for the ultrathin cryoprobe in PPLs remains unknown, our findings suggest that a mean activation time of 6 s and three passes may yield a diagnostic yield comparable to that of the 1.9-mm cryoprobe. Interestingly, in cases where only one activation was performed, we were able to reach a conclusive diagnosis in 75% of cases.

rEBUS orientation is crucial to the success of rEBUS procedures [10]. Consistent with the existing literature, our findings demonstrate that cryobiopsy performs exceptionally well in cases involving eccentrically and adjacently orientated lesions, yielding a diagnostic rate of 73.7%, surpassing the traditionally lower diagnostic yield observed with forceps biopsy [3, 11]. This result shown that despite the smaller diameter of the 1.1-mm cryoprobe, it retains the ability to obtain biopsies effectively in a 360° manner with adequate depth, allowing for the effective sampling of eccentrically and adjacently orientated targets. Furthermore, our study highlights that the 1.1-mm probe's smaller size and enhanced flexibility contribute to significantly shorter procedural times during rEBUS procedures. Our study also affirmed the safety of PPL-TBCB, revealing a low pneumothorax risk that aligns with that of rEBUS forceps biopsy [2, 10, 11]. In contrast, the pneumothorax risk in ILD-TBCB is notably higher, ranging from 13% to 20% in literature, likely attributed to the pronounced peripheral cryoactivation, as the majority of pathology is located in the subpleural region in ILD [12, 13].

In our study, the overall bleeding rate in our cohort is consistent with that of German and Japanese PPL-TBCB cohorts ranging from 16.2% to 79.4% [9, 11]. Despite a higher Grade 3 bleeding rate (9.3%) in our cohorts compared to reported rates of 1.2–3.5%, the majority of Grade 3 bleeding was in the

**TABLE 1** Factors influencing diagnostic yield and bleeding rate of ultrathin cryoprobe (N=69)

| | Diagnostic yield | | |
|---|---|---|---|
| | **Conclusive** | **Inconclusive** | **p-value** |
| **Mean target size** | | | |
| <2 cm | 21/27 (77.8) | 6/27 (22.2) | 0.767 |
| ⩾2 cm | 34/42 (81.0) | 8/42 (19.0) | |
| **rEBUS orientation** | | | |
| Concentric | 27/31 (87.1) | 4/31 (12.9) | 0.232 |
| Nonconcentric | 28/38 (73.7) | 10/38 (26.3) | |
| **Cryobiopsy pass** | | | |
| 2-passes cut-off | | | |
| <2 passes | 6/8 (75.0) | 2/8 (25.0) | 0.660 |
| ⩾2 passes | 49/61 (80.3) | 12/61 (19.7) | |
| 3-passes cut-off | | | |
| <3 passes | 15/23 (65.2) | 8/23 (34.8) | **0.034** |
| ⩾3 passes | 40/46 (87.0) | 6/46 (13.0) | |
| **Freeze time** | | | |
| 4-s cut-off | | | |
| <4 s | 10/11 (90.9) | 1/11 (9.1) | 0.314 |
| ⩾4 s | 45/58 (77.6) | 13/58 (22.4) | |
| 6-s cut-off | | | |
| <6 s | 29/36 (80.6) | 7/36 (19.4) | 0.855 |
| ⩾6 s | 26/33 (78.8) | 7/33 (21.2) | |
| 8-s cut-off | | | |
| <8 s | 42/51 (82.4) | 9/51 (17.6) | 0.358 |
| ⩾8 s | 13/18 (72.2) | 5/18 (27.8) | |
| 10-s cut-off | | | |
| <10 s | 51/64 (79.7) | 13/64 (20.3) | 0.987 |
| ⩾10 s | 4/5 (80.0) | 1/5 (20.0) | |
| **Total specimen size** | | | |
| <8 mm | 25/34 (73.5) | 9/34 (26.5) | 0.208 |
| ⩾8 mm | 30/35 (85.7) | 5/35 (14.3) | |
| | **Bleeding complication** | | |
| | **Bleeding** | **No bleeding** | **p-value** |
| **Cryobiopsy pass** | | | |
| 2-passes cut-off | | | |
| <2 passes | 6/8 (75.0) | 2/8 (25.0) | 0.144 |
| ⩾2 passes | 29/61 (47.5) | 32/61 (52.5) | |
| 3-passes cut-off | | | |
| <3 passes | 15/23 (65.2) | 8/23 (34.8) | 0.089 |
| ⩾3 passes | 20/46 (43.5) | 26/46 (56.5) | |
| **Freeze time** | | | |
| 4-s cut-off | | | |
| <4 s | 5/11 (45.5) | 6/11 (54.5) | 0.703 |
| ⩾4 s | 30/58 (51.7) | 28/58 (48.3) | |
| 6-s cut-off | | | |
| <6 s | 21/36 (58.3) | 15/36 (41.7) | 0.187 |
| ⩾6 s | 14/33 (42.4) | 19/33 (57.6) | |
| 8-s cut-off | | | |
| <8 s | 29/51 (56.9) | 22/51 (43.1) | 0.086 |
| ⩾8 s | 6/18 (33.3) | 12/18 (66.7) | |
| 10-s cut-off | | | |
| <10 s | 31/64 (48.4) | 33/64 (51.6) | 0.174 |
| ⩾10 s | 4/5 (80.0) | 1/5 (20.0) | |

Data are presented as n/N (%) unless otherwise stated. rEBUS: radial endobronchial ultrasound. Bold type represents statistical significance.

1.9-mm group (7.3%), while the bleeding rate in the 1.1-mm group (2%) was more consistent with the literature [9, 11]. We also demonstrated that the overall bleeding rates between both cryoprobes were similar, with less severe bleeding in the 1.1-mm cryoprobe group. This observation may be attributed to the significantly higher number of peripheral lesions in the 1.1mm cryoprobe group, which allowed for the avoidance of biopsies in the middle zone of the hemithorax where bleeding is more likely to occur [4]. However, as our data are retrospective, the lower bleeding rate with the 1.1-mm cryoprobe may also reflect a temporal, maturing learning curve, as the 1.1mm probe was introduced more recently.

Furthermore, PPL-TBCB procedures exhibit significant heterogeneity, varying across centres globally, underscoring the imperative for a standardisd protocol. The German cohort both used a different bleeding classification and did not deploy a prophylactic balloon blocker with rigid bronchoscopy, while the Japanese cohort deployed a dual-scope system, without a prophylactic balloon blocker. [9, 11] In our cohort, TBCB was performed *via* natural airway in 38.7% with conscious sedation, a practice previously reported in an ILD-TBCB cohort [14]. Anecdotally, we have observed that ILD-TBCB cases require rigorous control of bleeding and spillage of bleed due to underlying poor lung reserve leading to a lack of compensatory mechanisms. In contrast, PPL-TBCB patients are generally fit and likely able to tolerate bleeding better. These findings necessitate further investigation to facilitate the development of a standardised PPL-TBCB protocol in the future. Meanwhile, irrespective of the methods employed, it remains crucial to have bleeding mitigation measures in place for all TBCB procedures.

The main limitation of our study was its retrospective, nonrandomised, single-centre design with small sample size; thus, causality cannot be demonstrated. Additionally, the lack of individual analysis for each cryobiopsy specimen and the absence of a standardised protocol for total passes and activation time make it challenging to determine the true technical superiority of the 1.1-mm cryoprobe. Decreased bleeding rates and procedural times in the 1.1-mm group may reflect maturing proceduralist experience rather than intrinsic advantages of the probe. Of note, the recent discontinuation of the 1.9-mm probe necessitates further comparative studies between the 1.7- and 1.1-mm probes, while taking into consideration that the 1.7-mm probe cannot be deployed through an ultrathin bronchoscope. Nevertheless, we believe that our findings raise important questions, and emphasise the need for further research and standardisation of this procedure in the future.

In conclusion, PPL-TBCB may not require larger specimen sizes, as quality of tissue outweighs the quantity of tissue for accurate diagnosis. Comparable diagnostic yields in PPL-TBCB can be achieved by ~6 s of freezing time and three or more passes using the 1.1-mm cryoprobe with rEBUS guidance. The use of the 1.1-mm cryoprobe is associated with improved accessibility, shorter procedural time and potentially reduced incidence of severe bleeding. In the effort to enhance PPL diagnostic accuracy, further prospective studies are needed to establish standardised protocols for the optimal use of the ultrathin cryoprobe in PPLs.

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