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Proceduralist-Directed Rapid On-Site Pathologic Evaluation (ROPE) Using Dynamic Cell Imaging

A Pilot Study of Peripheral Pulmonary Lesion Sampling Through Robotic Bronchoscopy

Ismael Matus, MD, DAABIP, Sameer Akhtar, DO,† Haroon Raja, MD, DAABIP,* and Phoebe Holmes, MD‡*

Background: Diagnostic evaluation of peripheral pulmonary lesions (PPLs) relies on complementary technologies. Rapid onsite evaluation (ROSE) of cytopathology's role is established for mediastinal lymph nodes, though less studied for PPLs. VanGogh, a novel Dynamic Cell Imaging (DCI) technology for on-site histopathologic analysis (formerly CelTivity, used in the study) may offer intraprocedural confirmation of diagnostic tissue during the evaluation of PPLs. This study looks to quantify the concordance between rapid onsite pathologic evaluation (ROPE) under DCI and a pathologist's diagnosis in evaluating PPLs.

Methods: Single-center study of robotic bronchoscopic PPL samples using 21G fine needle aspiration, cryobiopsy, and forceps biopsy. Samples interpreted at the point of care by the proceduralist using DCI as either lesional or nonlesional, then evaluated by a pathologist as either diagnostic or nondiagnostic. The concordance between the proceduralist's and the pathologist's assessments was calculated at the patient-level and biopsy-level and stratified by tissue acquisition technique.

Results: Fifty patients with 146 samples were included. Overall concordance between the proceduralist's DCI interpretation and the pathologist's diagnosis was 88% (95% CI: not defined) at the patient level. Overall concordance was 84% (95% CI: 77%-90%). Stratified by tissue acquisition technique, concordance was highest for cryobiopsy (88% [95% CI: not defined]) and forceps biopsy (86% [95% CI: 74-94]).

Conclusion: ROPE, using cryoprobe and forceps biopsy of PPLs, demonstrated high concordance with final pathology, improving intraprocedural confidence in procuring representative diagnostic specimens. Further studies may determine DCI's impact on diagnostic yields and confirmatory role in target localization for future bronchoscopy ablative treatments.

Key Words: bronchoscopy, cryobiopsy, dynamic cell imaging (DCI), fine needle aspiration, forceps biopsy, lymph node, peripheral pulmonary lesions (PPLs), rapid onsite evaluation (ROSE), rapid onsite pathologic evaluation (ROPE)

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The diagnostic evaluation of peripheral pulmonary lesions (PPLs) has rapidly evolved in the past decade. Unfortunately, the synergism between differing advanced bronchoscopic platforms, real-time procedural imaging to confirm "tool-in lesion," complimentary tissue acquisition tools, and rapid on-site evaluation (ROSE) of cytopathology has yet to fully close the gap between real-time localization of PPLs and obtaining representative diagnostic specimens. Intraprocedural confirmation remains paramount.

In the diagnosis of lung cancer, ROSE has provided intraprocedural confirmation of accurate and sufficient sampling; however, it has most commonly been limited to the examination of cytology specimens obtained from either endobronchial ultrasound-transbronchial needle aspirations or a brush. For mediastinal staging through endobronchial ultrasound-transbronchial needle aspirations, ROSE does not improve diagnostic yield, nor does it reduce procedural time during transbronchial needle aspirations. However, it has high concordance while reducing samples needed and requirements for additional bronchoscopies to define a final diagnosis.[1,2]

One study performing multimodal tissue sampling in cone beam computed tomography-guided navigation bronchoscopy suggested that ROSE, in the case of PPLs, predicted procedural malignant pathology outcome in only 47.5% of cases, while not reducing the number of biopsies taken nor the procedure time.[3] The performance of ROSE from histopathologic samples obtained through "touch preparation" has limited supporting literature[1] and has not been widely adopted. Low-quality evidence supported Roy-

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From the *Thoracic Surgery and Interventional Pulmonology Service, Helen F. Graham Cancer Center and Research Institute; †Department of Medicine; and ‡Division of Pathology, Christiana Care Health System, Newark, DE.

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Correspondence: Ismael Matus, MD, DAABIP, Thoracic Surgery and Interventional Pulmonology Service, Helen F. Graham Cancer Center and Research Institute, Christiana Care Health System, 4701 Ogletown-Stanton Road, Suite 2100, Newark 19713, DE (e-mail: ismael.matus@christianacare.org).

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Chowdhuri et al[5] joint guideline recommending touch preparations from histologic forceps samples for ROSE.[4,5] Recently, investigators studied the clinical utility of touch imprint cytology in PPL evaluation using cryobiopsy specimens.[1,6] Kops et al[7] reported that in PPLs, higher concordance with procedural outcomes was observed for ROSE of touch imprint cytology (72.8%) and ROSE of touch imprint cytology with traditional ROSE (77.3%) compared with traditional ROSE alone (66.7%). Unfortunately, ROSE has limitations beyond its diagnostic value for PPLs, including the need for access to an experienced cytopathologist or a dedicated cytotechnician, as well as challenges with cost reimbursement and time requirements of up to 56 minutes.[8–10]

The Van Gogh system (Aquyre Biosciences, Weston, MA) utilizes Dynamic Cell Imaging (DCI), a novel technology that enables on-site procedural suite analysis of histopathologic specimens without special tissue preparation. This technology has the potential to increase immediate intraprocedural confidence in obtaining representative diagnostic tissue for PPL evaluation. In this study, we introduced a process we termed "rapid onsite pathologic evaluation" (ROPE) using the DCI system. This method focuses on evaluating tissue specimens (eg, cryobiopsies and forceps biopsies) as opposed to cytologic samples (eg, ROSE of cytopathology). Our objective was to quantify the concordance between the proceduralist's DCI interpretation of tissue specimens and the pathologist's diagnosis in evaluating PPLs.

MATERIALS AND METHODS

Study Design and Patients

We conducted a single-center study of consecutive patients undergoing routine robotic-assisted bronchoscopy with radial probe endobronchial ultrasound-guided bronchoscopic sampling of PPLs from December 2022 to March 2023. Cone beam computed tomography imaging was not integrated. Samples were collected in a standard clinical fashion in the following sequence: 21G fine needle aspiration, cryobiopsy, forceps biopsy, brushes, and bronchoalveolar lavage. The proceduralist determined which sampling strategies to use based on clinical need. All samples were collected for clinical purposes and no additional samples were collected for research. When more than one biopsy was collected using the same sampling strategy, we included the first biopsy in the analytic data set. In the case where there was more than one nodule from a single patient, the first nodule biopsied was retained for this study. This study was approved by our institutional review board. Because all procedures were performed in accordance with standard clinical protocols and posed minimal risk to participants, the requirement for informed consent was waived.

Specimen Handling and Preparation

For each sample collected through fine needle aspiration, cryobiopsy, and forceps biopsy, we used DCI to assess whether the sample was lesional. This assessment was compared against the gold standard, which was whether a pathologist deemed the sample diagnostic. The primary objective was to quantify the concordance between the proceduralist's DCI-based interpretation and the pathologist's final assessment at both the patient and biopsy levels.

All bronchoscopies were performed by 1 of 2 interventional pulmonologists. Lesion confirmation was performed under general anesthesia through an endotracheal tube (Monarch; Johnson & Johnson, IN), robotic-assisted bronchoscopy with radial probe-endobronchial ultrasound (Olympus; Medical, Japan). Tissue was obtained for all lesions in the following sequence: 21G ARC Point (Medtronic, MN), 1.1 mm ultrathin probe cryobiopsy (Erbecryo 2; Erbe, Tuebingen, Germany), forceps biopsy (Radial jaw; Boston Scientific, MA), brushes (Cellebrity; Boston Scientific, MA), and bronchoalveolar lavage.

The DCI system uses a specialized phase contrast microscope that employs an advanced form of Michelson interferometry to target appropriate tissue and capture metabolic activity of individual cells (Fig. 1). DCI is a proprietary technology that overlays metabolic activity onto a traditional morphologic assessment, displaying live cells with a bright color contrast that highlights abnormalities, distinguishing them from normal alveolar-parenchymal tissue. This contrast is displayed in the form of a heatmap, allowing the sampled abnormality to be distinctive from any surrounding normal lung tissue. Finally, standard cytologic assessments support the reader in identifying cellular features representative of malignant or non-malignant, benign lesions.[11]

Samples were placed into the tissue sample holder specifically designed for the microscope of the DCI system. For fine needle aspiration, the aspirate was pushed out of the needle directly on the sample platform. For cryobiopsy, tissue samples were immersed in a balanced salt solution for 60 seconds before being transferred onto the sample platform. For a forceps biopsy, the biopsy was placed directly on the sample platform with a drop of balanced salt solution on top. In each scenario, the sample holder was then closed and inserted into the device, where image acquisition software facilitated a point-of-care assessment. After completing DCI and interpretation, specimens were removed from the DCI specimen holder and transferred into a separate formalin solution container for formal pathologic preparation and analysis. Notably, pathologists observed that DCI did not alter the integrity of the tissue specimens.

Each DCI image was classified as either "lesional" or "nonlesional." Lesional specimens displayed the primary feature of highly contrasted cell colors in the yellow-to-red spectrum. Additional findings to further stratify the sample include cells clustering together with little to no extracellular spacing, morphologic pleomorphism, enlargement of the cells into 10 to 20 μm range, or organelle (ie, nucleolus) visibility. Nonlesional specimens demonstrated normal bronchial epithelium (low contrasted, nonmetabolic connective lung tissue, demonstrated in blue tone, with little or no small, darker green healthy cells) (Fig. 1). The interventional pulmonologist followed the protocol described above for 5 procedures to learn how to interpret the DCI images before data collection.

Pathologist Assessment

Routine histology processing of biopsies was performed according to the College of American Pathology requirements and checklist, as well as the manufacturer's instructions for the tissue processor. The tissue was placed in 10% neutral buffered formalin and sent to the histology laboratory. Following tissue processing and paraffin embedding, the tissue was cut on a microtome at a thickness of 4 to 5 μm and placed on glass slides. Five slide levels of each biopsy were prepared with 2 to 3 sections placed on each slide. The microtome was advanced between levels during

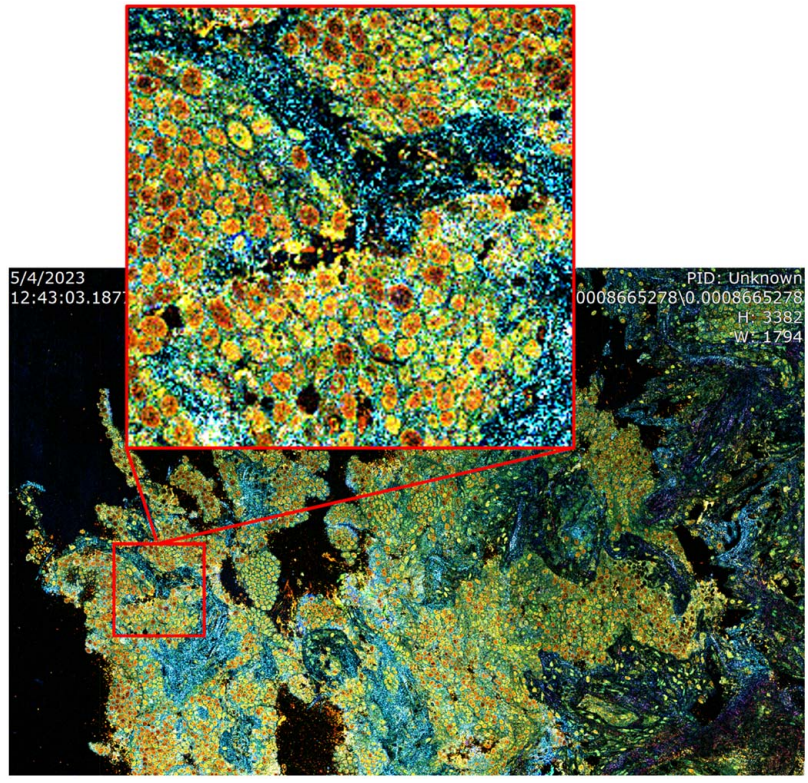

FIGURE 1. Dynamic Cell Image of a Lung Nodule Cryobiopsy. Representative DCI image of lesional lung tissue obtained through cryobiopsy. The DCI system uses phase contrast microscopy with Michelson interferometry to visualize metabolic activity.

the cutting process to fully sample through the tissue. After cutting, levels 1, 3, and 5 were stained with Hematoxylin and eosin based on the manufacturer's instructions of the tissue stain. Levels 2 and 4 were retained for possible future immunohistochemical or H&E staining. As a result, at least 5 levels through the tissue were obtained, each 4 to 5 μm in thickness, spanning ~1 to 2 mm through the tissue. The pathologist then determined whether each sample was diagnostic. Diagnostic samples were categorized as malignant or specific benign, and nondiagnostic samples were deemed inconclusive.

Statistical Analyses

We calculated the concordance between the proceduralist's assessment using DCI immediately after sampling and the pathologist's later assessment of the target tissue by final histopathology and cytopathology. At the patient level, concordance was defined as the proceduralist identifying at least one sample as lesional on DCI and the pathologist identifying at least one as diagnostic, or the proceduralist identifying all samples as nondiagnostic on DCI and the pathologist identifying all samples as nonlesional (nondiagnostic). At the biopsy level, concordance was defined as the proceduralist identifying a given sample as lesional on DCI and the pathologist identifying that sample as lesional (diagnostic), or the proceduralist identifying the sample as nonlesional on DCI and the pathologist identifying it as nonlesional (nondiagnostic).

To further evaluate DCI, we calculated its sensitivity, specificity, positive predictive value, and negative predictive value. Corresponding 95% CIs were obtained through the bootstrap method to account for repeated biopsies within individuals.

All measures were calculated for all specimens collected and then stratified by tissue acquisition technique (ie, fine needle aspiration, cryobiopsy, and forceps biopsy) and by final diagnosis from pathology (malignant disease, specific benign diagnosis, and inconclusive). Given that fine needle aspiration was primarily used as a method to gain transbronchial access into targeted PPLs with limited passes and not used to obtain optimal diagnostic specimens, we further evaluated DCI's performance within each diagnostic subgroup excluding specimens collected through fine needle aspiration. Given that the study was not designed to evaluate DCI influence on diagnostic yield, this measure was not calculated. As a pilot study, the sample size was not based on formal power calculations.

RESULTS

We collected 146 biopsies from 50 patients, including a fine needle aspiration sample from 46 patients, a cryobiopsy sample from 46 patients, and a forceps biopsy sample from 50 patients. The majority of our patients were male (60%) with a median [interquartile range (IQR)] age of 71 (65 to 78) years. In this study, 74% of participants received a conclusive diagnosis of either malignant disease or a specific benign diagnosis.

The PPLs' median (IQR) longest diameter of 12 (1 to 30) mm was obtained from measuring nodules on axial, coronal, and sagittal computed tomography planes. The PPLs' closest edge to pleural distance was a median (IQR) of 7 (0.0 to 30.0) mm. A majority were solid (92%), localized

to the upper lobes (64%), and with a negative bronchus sign (76%) (Table 1).

Median procedure time was 35 minutes and 8 seconds. No deviations from standard workflow or block time constraints were observed following DCI integration.

Concordance

Compared with final pathology, which defined a specimen to be diagnostic or nondiagnostic, the proceduralist's interpretation of DCI as lesional or nonlesional, demonstrated an overall concordance of 88% (95% CI: not defined) at the patient level. When considering each of the 146 biopsies, overall concordance was 84% (95% CI: 77%-90%). When assessed at the patient level, DCI had 100% (95% CI: not defined) sensitivity, though low specificity with high negative and positive predictive values. In addition, DCI showed a sensitivity, specificity and predictive values of at least 80% when evaluating each of the 146 biopsies individually. A flow of patients and samples through the study is shown in Figure 2.

Stratified by tissue acquisition technique, concordance was highest for cryobiopsy (88% [95% CI: not defined]) and lowest for fine needle aspiration (79% [95% CI: 67%-90%]), seen in Table 2. Specimens obtained through cryobiopsy had the highest sensitivity and lowest specificity, while fine needle aspiration samples had the lowest sensitivity and highest specificity. Positive predictive value was highest for cryobiopsy; negative predictive value was highest for forceps biopsy, which is shown in supplementary E-Table 1, Supplemental Digital Content 1, http://links.lww.com/LBR/A377.

Complications

Complications included pneumothorax and bleeds. Four patients (8%) experienced pneumothorax, which all required intervention, per our protocol for the management of iatrogenic pneumothorax.[12] One was discharged on the same day with removal of the small-bore chest tube through which manual aspiration was successfully performed. One was discharged on the same day with a small-bore chest tube for ambulatory management after failing manual aspiration. Two were hospitalized with a small-bore chest after manual aspiration attempts failed and were then discharged within 72 hours with chest tube removal. In addition, 4 patients (8% experienced bleeding), which were categorized as grade 2 per Nashville Bleeding Scale.

TABLE 1. Patient and Biopsy Characteristics

	N = 50*
Male	29 (58)
Age, y	71 [65-78]
Peripheral pulmonary lesion	
Longest diameter (mm)	12 [1-30]
Closest distance to pleural edge (mm)	7 [0-30]
Solid morphology	46 (92)
Biopsy lobe	
Lingula	1 (2)
Left lower lobe	4 (8)
Left upper lobe	13 (26)
Right lower lobe	8 (16)
Right middle lobe	5 (10)
Right upper lobe	19 (38)
Positive bronchus sign	12 (24)
Radial probe-endobronchial ultrasound orientation	
Concentric	34 (68)
Eccentric	11 (22)
Adjacent	2 (4)
Not visualized	2 (4)
Complications	
Bleeds (Grade 2)	4 (8)
Pneumothorax	4 (8)

Median [25th percentile to 75th percentile] for continuous variables; N (%) for categorical variables.

*One patient had 2 lobar samples obtained; one lobar sample was obtained from all remaining patients.

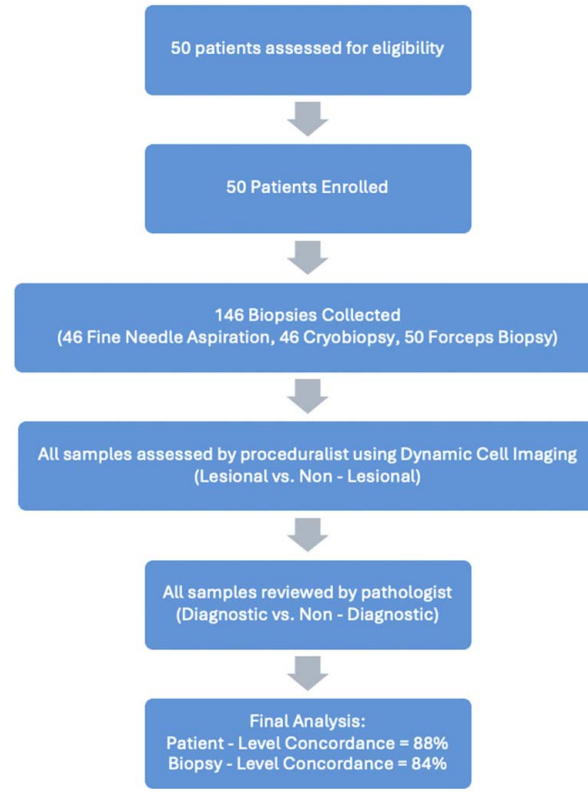

FIGURE 2. STARD flow diagram of participant and sample inclusion. Flow of enrolled patients and biopsy samples through the study, including DCI interpretation by proceduralists and diagnostic assessment by pathologists. All patients underwent robotic bronchoscopy with sample acquisition using fine needle aspiration, cryobiopsy, and/or forceps biopsy.

Pathology Diagnosis and Clinical Follow-Up

On the basis of index bronchoscopy results, 44% of patients were diagnosed with malignancy by the pathologist, 32% had a specific benign diagnosis, and 21% were inconclusive (Table 3). The 14 patients deemed inconclusive by the pathologist were followed clinically; 4 were lost to follow-up. Of the remaining 10 patients, 4 were diagnosed with malignancy on follow-up; all 4 had at least one sample that was identified as lesional by DCI.

DISCUSSION

Dynamic cell imaging leverages the Warburg effect—a Nobel-recognized hallmark of cancer metabolism characterized by a metabolic shift favoring aerobic glycolysis—detecting this distinct cellular activity in real time.[13] The technology overlays vibrant, heat map-style color contrast

TABLE 2. Concordance of Dynamic Cell Imaging With Pathologist Assessment for Peripheral Pulmonary Lesions Collected During Robotic-Assisted Bronchoscopy

	No. samples	Concordance % (95% CI)
All patients (N = 50)*		88*
All biopsies	146	84 (77-90)
Biopsy procedure		
Fine needle aspiration	48	79 (67-90)
Cryobiopsy	48	88*
Forceps biopsy	50	86 (74-94)
Diagnosis		
Malignant disease 22 patients	All 65	89 (82-97)
	Cryobiopsy and forceps biopsy 43	93 (86-100)
Specific benign 14 patients	All 41	76 (62-88)
	Cryobiopsy and forceps biopsy 28	82 (71-93)
Inconclusive 14 patients	All 40	85 (73-95)
	Cryobiopsy and forceps biopsy 27	81 (67-93)

(N = 50).
*A meaningful 95% CI could not be estimated due to sparse table cell counts.

onto traditional morphologic assessment, highlighting and clearly distinguishing abnormalities from surrounding normal lung parenchyma. These capabilities distinguish DCI from our traditional cytopathologist-ROSE technique. Furthermore, it enables rapid, in-depth on-site tissue analysis by generating default virtual slices at 7 μm intervals and analyzing at a higher resolution 1 μm slice, compared with traditional 4 to 5 μm slice performed during pathologic assessment. At the proceduralist's discretion, further meticulous analysis can be performed on site at 1 μm intervals.

TABLE 3. Pathology Diagnosis From Bronchoscopy

Malignant (44%)	N = 22 (%)
Adenocarcinoma, lung	11
Squamous cell lung cancer	11
Non–small cell lung cancer – not otherwise specified	6
Neuroendocrine carcinoma	4
High-grade B-cell lymphoma	2
Low-grade B-cell lymphoma	2
Small cell carcinoma	2
Lymphomatoid granulomatosis	2
Poorly differentiated carcinoma	2
Uterine carcinoma	2
Benign (32%)	N = 15
Non-necrotizing granuloma	9
Organizing pneumonia with Pseudomonas	6
Pseudomonal focal acute on chronic inflammation	4
Fungal granuloma	2
Necrotizing mycobacterium tuberculosis granuloma	2
Fungal organisms with morphed interstitial fibrosis and elastosis, myo-interstitial hyperplasia (fungal infection)	2
Organizing pneumonia + mycobacterium abscessus	2
Interstitial pneumonia with multinucleated giant cells	2
Anthracotic nodule	2
Inconclusive (21%)	N = 14 (4 lost to follow-up)
Normal alveolar parenchyma	9
Nonspecific Inflammation (ie, acute or chronic inflammation)	4
Atypical cells	2
Focal squamous metaplasia	2
Fibrous tissue	4

We demonstrate that proceduralist's ROPE using DCI to discriminate lesional from nonlesional samples of the targeted PPL demonstrated an overall concordance of 84% when compared with the pathologist's determination of whether the sample was diagnostic at both the patient and biopsy levels. The novel DCI system also performed well in terms of sensitivity, specificity, positive predictive value, and negative predictive value. Our findings demonstrate the value of this technology in obtaining representative diagnostic specimens by providing imaging feedback to the proceduralist at the point of care and highlight the potential of DCI as a powerful tool for promoting intraprocedural confidence and efficiency.

The value of ROSE for mediastinal staging in lung cancer is established, and this practice is widely implemented. Its role in the diagnostic evaluation for PPLs is less studied; however, it is reported to be a simple and rapid technique for the cytologic assessment of transbronchial biopsy specimens by placing and gently rolling the specimen over a microscope slide to release cells for rapid on-site examination.[14,15] Shikano et al[16] demonstrated that ROSE-touch imprint cytology when compared with final diagnosis showed a sensitivity of 75.3%, specificity of 91.6%, positive predictive value of 97.6%, NPV of 45.0%, and diagnostic accuracy values of 78.3%, respectively, with discordant results in 21.7%.[16]

Comparing across tissue acquisition techniques, cryobiopsy specimens were found to have the highest concordance, sensitivity and positive predictive value at 85%, 98%, and 87%, respectively. However, the lower specificity of 19% observed with cryobiopsy specimens may stem from the inclusion of non-neoplastic yet reactive or fibrotic tissue within larger more heterogenous cryobiopsy specimens that were classified as lesional on dynamic cell imaging. DCI's visualization of cellular metabolic activity highlights reactive tissue as metabolically "active," leading to an inflated rate of "lesional" interpretations and thus more false positives, coupled with the evolving interpretive proficiency required for proceduralist-led DCI analysis as contributors to the lower specificity observed with cryobiopsy specimens. Cryobiopsy is becoming widely adopted with an

encouraging safety profile and a suggested value of an incremental diagnostic yield.[17–20] By synergizing the cryobiopsy technique with ROPE-DCI, further investigation may determine this approach's impact on diagnostic yields for PPLs.

Of the 4 patients deemed nondiagnostic on initial pathologic assessment and later diagnosed with malignancy, all had samples identified as lesional by DCI. This raises the question as to whether the DCI system's high-resolution virtual slices can reveal malignant features that may be missed on standard 3 to 5 μm pathologic sections from the same specimen.[21] In such cases, DCI findings may prompt the pathologists to obtain additional sections from the specific DCI-lesional specimen, highlighting a potential area for future investigation.

The limitations of this study include a relatively small sample size, which limited the ability to estimate some CIs, and the study was conducted at a single site with only 2 proceduralists. While the latter may limit the generalizability of the results, the patient population included a range of both benign and malignant diagnoses. Future studies would benefit from including multiple proceduralists to confirm reproducibility.

This study presents a novel application of DCI, offering an innovative approach with the potential to enhance procedural decision-making and diagnostic accuracy. The absence of selection bias strengthens the validity of the findings, as all eligible patients during the study period were included without prescreening. Furthermore, the patient cohort reflected a diverse and representative clinical population, encompassing both benign and malignant diagnoses, which supports the relevance of the findings to routine clinical practice. Despite being conducted at a single site, the real-world setting and consistency in technique provide a valuable foundation for future multicenter investigations.

The diagnostic evaluation of PPLs depends heavily on the use of complementary technologies, which include advanced bronchoscopy platforms for precise localization, real-time intraprocedural imaging feedback, tissue acquisition tools and ideally, on-site confirmation of diagnostic sampling. Real-time imaging feedback with radial probe-endobronchial ultrasound and 3D imaging with Cone Beam Chest Tomography is leveraged to further aid in diagnostic precision of navigational and robotic-assisted bronchoscopy platforms. However, they do not eliminate variables such as tissue acquisition tools' interface with bronchial and parenchymal tissue and respiratory motion. Ultimately, achieving precise placement of a "sampling tool in a lesion" does not always equate to obtaining a representative "lesion in the sampling tool". Hence, a process such as ROPE through DCI may prove of value not only for enhancing diagnostic yield of PPLs, but for boosting confidence in target localization through onsite lesional confirmation desirable for the delivery of oncoming bronchoscopic ablative treatments of pulmonary malignancy.

CONCLUSIONS

The proceduralist-directed rapid on-site pathologic evaluation (ROPE) using dynamic cell imaging of cryobiopsy and forceps biopsy specimens of PPLs demonstrated promising concordance with final pathology. These preliminary findings suggest that DCI may enhance intraprocedural confidence in obtaining representative diagnostic tissue, thereby promoting procedural efficiency, potentially increasing diagnostic yields and serving as a complimentary technology to reinforce target localization for future bronchoscopic ablative therapies. However, given the limited sample size and single-center design, larger multicenter studies are warranted to validate the diagnostic impact and workflow integration of this technology.

Future development leveraging artificial intelligence integration into DCI may offer more "diseased-tissue-targeted" analysis of sampled specimens in addition to generating integrated machine learning onsite histopathologic interpretations.

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