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Transbronchial cryobiopsy in the era of precision thoracic diagnostics: Histopathology, omics, radiomics, and AI converge

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ABSTRACT

In the era of precision medicine, transbronchial cryobiopsy (TBCB) has emerged as a significant technological advancement supporting the diagnosis of diffuse lung diseases and thoracic malignancies. The advent of targeted therapies, along with the increasing need to identify new diagnostic and prognostic biomarkers, has made it essential to adopt innovative surgical techniques capable of obtaining large, well-preserved tissue samples. This approach not only enables accurate histopathological assessment but also allows for comprehensive molecular analyses, while meeting the requirement for minimal invasiveness in particularly fragile patients or those with severe comorbidities. Tissue samples obtained via TBCB are immediately frozen, offering a precise snapshot of the disease at the time of collection. The sample size is generally sufficient for further molecular testing, thereby reducing the need for repeat biopsies and enabling prompt and accurate diagnosis—an essential element in the treatment pathway for patients with complex pulmonary conditions, both oncologic and interstitial. Although surgical lung biopsy remains the gold standard in terms of diagnostic accuracy, TBCB has demonstrated superiority over conventional forceps biopsy techniques, both in terms of sample quality and safety profile, with a significantly lower risk of serious complications. This review aims to critically evaluate the available evidence supporting TBCB compared to traditional techniques, with a particular focus on its role in the molecular characterization of thoracic tumors and interstitial lung diseases. Furthermore, we explore its integration into a forward-looking framework where TBCB contributes to radiomics and artificial intelligence training, fostering the development of predictive tools for diagnosis, prognosis, and therapeutic response.

"Ice can preserve all kinds of things that way, cleanly, clearly. That's the essence of ice, the role it plays."

—Haruki Murakami

1. Introduction

The origin of Transbronchial Lung Cryobiopsy (TBCB) is rooted in the fundamental concept of the role that freezing has progressively

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played in medicine. From the outset, cryopreservation has represented a breakthrough for the conservancy of the human species, making it possible to store oocytes and spermatozoa, thereby ensuring reproductive continuity. This principle was later extended to experimental research through cellular biobanks, enabling the availability of stocks of both healthy and pathological cells, essential for scientific investigation. Freezing was subsequently applied in therapeutic contexts, such as lesion cryoablation, and eventually in surgery, where it allowed for better preservation of biopsy specimens, thus facilitating more accurate diagnoses and molecular biomarker analysis, while maintaining a good safety profile for patients [1]. It is important to clarify that both forceps and cryobiopsy specimens are immediately fixed in formalin, a process that is responsible for tissue preservation. The primary advantage of cryobiopsy lies in the reduced mechanical compression applied to the sample, resulting in superior preservation of tissue architecture and an enhanced diagnostic yield for ancillary molecular analyses.

In this review, we aimed to thoroughly analyse TBCB by exploring its procedural aspects, efficacy and safety profile, and comparing it with other techniques, including established gold standards. We also contextualized its use in specific clinical settings such as oncology and interstitial lung diseases. Finally, we projected this technique into the future by integrating it with emerging technologies such as radiomics and artificial intelligence-based predictive tools. TBCB is indeed a method capable of generating essential data to train these technologies, enabling the discovery of correlations between imaging, tissue, and diagnosis, and paving the way for a future in which image-based automated analysis could significantly contribute to early and accurate diagnosis.

2. Transbronchial cryobiopsy: mechanism, procedure, and complications

2.1. Technical mechanism and basic procedure

TBCB represents a significant advancement in interventional pulmonology, built upon the fundamental principle of rapid freezing for tissue acquisition. The procedure utilizes a flexible cryoprobe, typically 1.9 mm or 2.4 mm in diameter, inserted through the working channel of a flexible bronchoscope. The cryoprobe operates based on the Joule-Thomson effect, wherein rapid expansion of compressed gas (usually nitrous oxide or carbon dioxide) produces an extreme temperature drop at the probe tip, reaching approximately −89.5 °C [2]. This rapid freezing creates strong adhesion between the probe and surrounding tissue through crystallization of water molecules. After activation for several seconds, the entire bronchoscope and probe are withdrawn together, retrieving an intact tissue sample significantly larger than that obtained through conventional forceps biopsy.

TBCB procedures are generally performed under fluoroscopic or endobronchial ultrasound (EBUS) guidance to optimize probe positioning and minimize complications. The procedural approach may utilize either flexible bronchoscopy under moderate-to-deep sedation or rigid bronchoscopy under general anesthesia, with the choice dependent on patient factors, anticipated risks, and institutional expertise [3].

2.2. Early retrospective studies and case series

The initial clinical applications of TBCB were documented in several early retrospective studies that established its feasibility and potential advantages. Schumann et al. (2010) conducted one of the pioneering investigations, demonstrating that cryobiopsy yielded significantly larger and more intact tissue specimens compared to conventional forceps biopsy in the diagnosis of endobronchial lesions [4]. This initial report showed a diagnostic yield increase from 65 % with conventional forceps to 95 % with cryobiopsy, establishing proof-of-concept for the technique.

Subsequent early studies expanded the application of TBCB to diffuse parenchymal lung diseases. Babiak et al. (2009) reported a case series of 41 patients, obtaining specimens averaging 5.2 mm in diameter with a 95 % diagnostic yield, though reporting moderate bleeding in 56 % of cases [5]. Pajares et al. (2014) documented significantly larger biopsy specimens (14.7 ± 11 mm^2 vs. 3.3 ± 4.1 mm^2) in their retrospective analysis [6].

These early investigations, while promising, had notable limitations including small sample sizes, single-center designs, variable procedural techniques, and potential selection bias. The learning curve effect was particularly evident in early safety profiles, with complication rates varying considerably between centers. Additionally, most early studies lacked standardized protocols for procedural approach, freezing time, and complication management, limiting generalizability.

2.3. Systematic reviews and meta-analyses

As clinical experience with TBCB expanded, systematic reviews and meta-analyses provided more robust assessments of its efficacy and safety. Ganganah et al. (2016) published a comprehensive meta-analysis comparing cryobiopsy to forceps biopsy, analyzing 11 studies including 731 patients. This analysis demonstrated significantly superior diagnostic yield with TBCB (relative risk 1.36; $p = 0.0002$), but also highlighted increased bleeding risk (pooled severe bleeding risk of 6.8 % versus 0.3 % with conventional biopsy) [7].

A subsequent meta-analysis by Iftikhar et al. (2017) compared TBCB to surgical lung biopsy (SLB) across 15 studies, finding comparable diagnostic yields (83.7 % vs. 92.7 %) but significantly lower complication rates and mortality with TBCB. Pooled analysis revealed pneumothorax in 9.4 % of TBCB cases, moderate-to-severe bleeding in 14.2 %, and mortality of 0.1 %, compared to surgical mortality of 2.3 % [8]. Notably, this analysis identified significant heterogeneity in reported complication rates, reflecting inconsistent procedural approaches and variable definitions of adverse events.

Giri et al. (2022) performed an updated meta-analysis of 27 studies encompassing 2823 patients, confirming TBCB's superior diagnostic yield over conventional biopsy (RR = 1.32, 95 % CI 1.05–1.66; $p = 0.02$) with a nonsignificant trend toward increased bleeding complications [9]. This comprehensive analysis established that TBCB represented a favorable risk-benefit profile when performed in appropriately selected patients and settings.

2.4. Prospective controlled studies and randomized trials

The strongest evidence supporting TBCB comes from prospective controlled studies, particularly the landmark COLDICE trial. This multicenter Australian study, published in 2019, prospectively enrolled 65 patients with suspected interstitial lung disease (ILD) who underwent both TBCB and surgical lung biopsy during the same procedure. Pathological specimens were independently assessed by blinded pathologists, with diagnostic concordance as the primary endpoint. The study demonstrated 70.8 % concordance between TBCB and surgical biopsy ($\kappa = 0.70$), with similar diagnostic confidence in multidisciplinary discussions [10]. Notably, TBCB showed a more favorable safety profile, supporting its clinical adoption as a less invasive alternative.

Pajares et al. conducted a randomized trial comparing TBCB to conventional forceps biopsy in 77 patients with ILD, demonstrating significantly higher diagnostic yield with TBCB (51.3 % vs. 29.1 %; $p = 0.038$) [6]. This prospective evidence validated the findings of earlier retrospective studies in a controlled experimental setting.

The MULTICRIO study, a multicenter prospective investigation across 10 Spanish hospitals, compared 124 patients undergoing TBCB with 97 patients undergoing conventional biopsy, confirming superior diagnostic yield for TBCB (47.6 % vs. 19.4 %; $p < 0.0001$), particularly in fibrotic patterns [11]. This study highlighted the importance of standardized protocols, including prophylactic Fogarty balloon placement and intubation, in minimizing complications.

2.5. Current procedural standards and safety protocols

Based on accumulated high-quality evidence, standardized protocols have emerged to optimize TBCB safety and efficacy. The 2018 expert statement from the Cryobiopsy Working Group and subsequent guidelines from major respiratory societies (ATS/ERS/JRS/ALAT) have established evidence-based recommendations for procedural technique [12,13].

Current evidence supports using the 1.9 mm cryoprobe over the 2.4 mm probe, as it provides comparable diagnostic yield with lower pneumothorax risk and better access to peripheral lung regions. Optimal freezing time is established at 3–6 s, balancing sample adequacy with safety. Fluoroscopic guidance is strongly recommended to maintain a safe distance from the pleura (typically >1–2 cm) and avoid large vessels.

Prophylactic measures to prevent complications now include routine placement of a bronchial blocker or Fogarty balloon, which is inflated immediately after biopsy to control bleeding. Evidence supports taking multiple samples [3–5] from different segments of the same lobe to improve diagnostic yield, rather than sampling from different lobes, which increases complication risk without diagnostic benefit [14].

The 2022 ATS/ERS/JRS/ALAT guidelines for diagnosis of idiopathic pulmonary fibrosis conditionally recommend TBCB in centers with appropriate expertise, multidisciplinary teams, and established safety protocols [13]. This recommendation recognizes both the diagnostic value of TBCB and the importance of procedural expertise in minimizing complications.

3. Transbronchial cryobiopsy vs conventional and surgical techniques: comparative efficacy, safety, and molecular diagnostic yield

In a multidisciplinary setting where thoracic disease management requires integrated specialist discussion, combining histological analysis with genomic and molecular assessments has become essential for accurate diagnosis and personalized therapy [15,16]. TBCB has emerged as an indispensable tool providing high-quality tissue samples, prompting thorough evaluation of its risk-benefit profile. Compared to surgical lung biopsy (SLB), TBCB demonstrated an 85 % diagnostic yield with larger samples (mean diameter 5.7±2 mm, area 40±2 mm^2), though with a 60 % bleeding risk via flexible bronchoscopy [17]. When combined with conventional transbronchial biopsy (TBLB), TBCB significantly improved diagnostic yield in diffuse parenchymal lung diseases [18], with randomized studies showing superior yield (51.3 % vs. 29.1 %) and larger samples (14.7 ± 11 mm^2 vs. 3.3 ± 4.1 mm^2) compared to forceps biopsy [3,6]. Strategic sampling from different segments of the same lobe further increased yield to 96 % [14]. TBCB's superior tissue quality enables advanced molecular analyses including NGS, RNA-seq, PD-L1 expression, and TMB assessment [19,20], while supporting sophisticated RNA sequencing for investigating oncogenic pathways and IPF mechanisms [21,22]. In oncology, TBCB combined with EBUS-TBNA achieved excellent lymph node sampling (90.6 %), with tumor content >30 % and superior DNA/RNA yields [23–26]. Major societies now recommend TBCB in expert centers for various patient populations [27–31]. Despite advantages over conventional biopsies, TBCB's superiority to VATS remains controversial, with the COLDICE study showing 70.8 % diagnostic concordance ($\kappa = 0.70$) but a more favorable safety profile [16,32,33]. TBCB carries risks including bleeding (10 % vs. 3 % with forceps), pneumothorax (11–19.2 %), and rare mortality (0.3–0.4 %), but demonstrates shorter hospital stays than SLB [32,34–38]. Standardization efforts continue to optimize parameters and training protocols, while growing interest in TBCB is driven by its ability to provide high-quality samples for multi-omics analyses, AI applications, and radiomics in a single procedure.

3.1. Compression and freezing artifacts in cryobiopsy versus forceps biopsy

It is well recognized that during the pre-fixation phase, biopsy forceps or other sharp instruments can penetrate or compress the tissue, producing areas of crushing, tearing, fragmentation, or voids within the specimen. These alterations may distort the tissue architecture, alter cellular morphology and stromal organization, and ultimately compromise diagnostic interpretation, particularly in small biopsy samples [39].

One of the principal histopathological differences between transbronchial cryobiopsy (TBCB) and conventional forceps biopsy lies precisely in the nature and extent of these artifacts. As demonstrated by Alexander Babiak et al. [40], cryobiopsy specimens are significantly larger and exhibit minimal crush or compression artifacts compared with samples obtained by forceps. The freezing mechanism allows tissue adherence to the probe surface without the mechanical pressure exerted by the forceps jaws, thereby maintaining the alveolar and interstitial architecture.

Nevertheless the cryogenic process itself may introduce distinctive freezing-related artifacts, including ice-crystal formation and subtle cytoplasmic vacuolization, which represent novel alterations that pathologists should recognize during histological assessment. [41], Overall, while TBCB effectively minimizes mechanical distortion, it may introduce different but generally minor cryogenic artifacts, resulting in an overall improvement in tissue integrity and diagnostic reliability compared with conventional forceps biopsy.

4. From biopsy to therapy: the impact of sampling technique in precision oncology

In contemporary thoracic oncology, the paradigm has shifted dramatically from a histology-based treatment approach to a precision medicine model that demands comprehensive molecular characterization of tumor tissue. This evolution necessitates not only accurate histopathological classification but also high-quality tissue samples suitable for multiple molecular analyses. Current treatment algorithms for non-small cell lung cancer (NSCLC) require assessment of numerous predictive biomarkers, including driver mutations (EGFR, ALK, ROS1, BRAF, KRAS, NTRK), immunotherapy biomarkers (PD-L1 expression, tumor mutational burden), and emerging targets such as HER2 and MET alterations [42]. The technical challenge for pulmonologists and thoracic surgeons lies in obtaining sufficient high-quality tissue through minimally invasive procedures that preserve both cellular architecture and nucleic acid integrity for these increasingly complex analyses.

4.1. Early retrospective comparisons

Initial retrospective evaluations comparing TBCB to conventional forceps biopsy demonstrated promising advantages in diagnostic yield and sample quality. Schumann et al. (2010) first reported a diagnostic yield of 95 % for cryobiopsy versus 65 % for forceps biopsy in endobronchial tumors [43]. For peripheral lesions, Hetzel et al. (2012) documented increased diagnostic yield (74.2 % vs. 59.2 %) with significantly larger specimens (11.17 mm^2 vs. 4.69 mm^2) using cryobiopsy compared to forceps techniques [44].

Early retrospective analyses of molecular testing suitability showed encouraging results. Yarmus et al. (2016) compared 125 NSCLC samples obtained by cryobiopsy with 168 conventional samples, finding significantly higher rates of successful molecular testing in the cryobiopsy group (96.4 % vs. 82.9 %, $p < 0.05$) [45]. Similarly, Ussavarungsi et al. (2016) reported that TBCB provided sufficient material for EGFR and ALK testing in 92 % of cases compared to 77 % with conventional biopsy [46].

These early studies had substantial limitations, including retrospective design, potential selection bias (with cryobiopsy often performed in

cases anticipated to be challenging), inconsistent molecular testing platforms, and non-standardized biopsy protocols. Many studies also lacked blinded pathological assessment and detailed reporting of technical parameters such as freezing time and probe size. Additionally, the rapid evolution of molecular testing requirements during this period makes cross-study comparisons challenging.

4.2. Meta-analyses and systematic reviews of molecular adequacy

The first systematic review focused specifically on molecular adequacy was conducted by Cho et al. (2019), analyzing 8 studies with 581 samples. This review reported pooled rates of molecular testing adequacy of 89.5 % (95 % CI 84.2–93.1 %) for cryobiopsy compared to 73.1 % (95 % CI 65.8–79.4 %) for conventional biopsy, with a relative risk of 1.21 ($p = 0.001$) favoring cryobiopsy [47].

A meta-analysis by Liu et al. (2021) examined technical factors affecting molecular yield, finding that freezing time significantly impacted DNA quantity (optimal range 4–6 s) and that the 1.9 mm probe provided better-preserved nucleic acids than the 2.4 mm probe, likely due to less crushing artifact [48]. This analysis also reported that immediate tissue processing in RNAlater or similar preservatives improved RNA quality for gene expression profiling compared to standard formalin fixation.

Chen et al. (2020) conducted a systematic review focused on next-generation sequencing (NGS) success rates, analyzing 14 studies with 1215 samples. They reported significantly higher NGS success rates with cryobiopsy (91.8 %, 95 % CI 87.6–94.9 %) compared to conventional biopsy (78.5 %, 95 % CI 72.1–83.9 %), with particular advantages for specimens from central lesions and in cases requiring comprehensive genomic profiling panels [49].

4.3. Prospective controlled studies (2019-present)

The first prospective single-arm study specifically designed to assess molecular adequacy was conducted by Udagawa et al. (2020), evaluating 121 consecutive patients who underwent TBCB for suspected lung cancer. This study demonstrated that cryobiopsy yielded significantly higher quantities of extracted DNA (1.60 μg vs. 0.58 μg; $p = 0.02$) and RNA (0.62 μg vs. 0.17 μg; $p < 0.01$) compared to conventional techniques, with higher rates of definitive histomorphological diagnosis (86 % vs. 74 %; $p < 0.01$) [50]. The study also documented higher PD-L1 expression detection (51 % vs. 42 % for expression >1 %) and superior NGS success rates (94 % vs. 81 %).

In a randomized controlled trial of 96 patients with suspected lung malignancy, Ito et al. (2022) allocated subjects to either TBCB or conventional forceps biopsy, with blinded assessment of molecular testing success as the primary endpoint. They reported significantly higher rates of successful comprehensive genomic profiling in the TBCB group (89.6 % vs. 68.8 %, $p = 0.006$) and higher mean tumor cell percentages (45.3 % vs. 28.7 %, $p < 0.001$), with a notable difference in the yield of driver mutation detection (35.4 % vs. 18.8 %, $p = 0.047$) [51].

Arimura et al. (2019) conducted a formal pathological correlation study examining PD-L1 expression between cryobiopsy samples and matched surgical specimens in 32 patients. For the 50 % expression threshold (key for first-line pembrolizumab eligibility), they reported sensitivity of 66.7 %, specificity 100 %, positive predictive value 100 %, and concordance 93.8 % ($\kappa = 0.76$), significantly better than conventional biopsy concordance ($\kappa = 0.43$) [52]. Similarly high concordance was demonstrated by Miyazu et al. (2021) for ALK and ROS1 FISH testing, with 94.3 % and 97.1 % agreement, respectively, between cryobiopsy and surgical specimens [53].

4.4. Clinical impact studies and outcome measures

The practical impact of improved molecular sampling was demonstrated in a multicenter observational study by Kang et al. (2021), which found that adoption of TBCB was associated with a 24 % reduction in inconclusive molecular diagnoses requiring repeat biopsy ($p < 0.001$) and a 35 % increase in first-line targeted therapy administration ($p = 0.003$) compared to historical controls using conventional biopsy approaches [54].

Ota et al. (2023) conducted a retrospective cohort study of 312 advanced NSCLC patients, finding that those diagnosed using TBCB ($n = 147$) had significantly higher rates of receiving molecularly-guided first-line therapy compared to those diagnosed by conventional techniques ($n = 165$) (68.7 % vs. 49.7 %, $p < 0.001$). After propensity score matching, this translated to a significant improvement in progression-free survival (median 9.8 vs. 6.3 months, HR 0.68, $p = 0.021$) [55].

In a prospective study of procedural efficiency, Saji et al. (2021) reported that TBCB reduced median time from initial evaluation to treatment initiation by 9 days (24 vs. 33 days, $p = 0.008$) compared to conventional approaches, primarily by reducing the need for sequential biopsies to obtain adequate molecular information [56]. This finding has particular relevance in aggressive malignancies where treatment delays may impact outcomes.

4.5. Current guidelines and future directions

The recognition of TBCB's molecular advantages has been increasingly reflected in guideline recommendations. The 2018 American College of Chest Physicians (CHEST) guidelines made no specific mention of cryobiopsy for molecular sampling, focusing exclusively on sample quantity regardless of technique [57]. By contrast, the 2022 European Respiratory Society (ERS) statement on lung cancer diagnostics included a conditional recommendation for cryobiopsy when extensive molecular analysis is anticipated [58]. Most recently, the 2023 College of American Pathologists (CAP)/International Association for the Study of Lung Cancer (IASLC) molecular testing guidelines specifically acknowledge the superior sample quality obtained through cryobiopsy and recommend its consideration for comprehensive biomarker assessment [59].

Several ongoing trials are addressing remaining evidence gaps. The PROMISE trial (NCT04182100) is a multicenter randomized study comparing cryobiopsy to conventional techniques with comprehensive genomic profiling success as the primary endpoint and time-to-treatment decision as a key secondary endpoint. The CRYSTAL study (NCT03651986) is evaluating whether cryobiopsy-based sampling improves detection of tumor heterogeneity and resistant subclones compared to conventional biopsies in EGFR-mutated NSCLC with acquired resistance [60].

The most promising frontier for TBCB involves integration with emerging technologies requiring high-quality tissue. These include spatial transcriptomics to map gene expression within the tumor microenvironment, single-cell sequencing to resolve intratumoral heterogeneity, and multi-omic approaches integrating genomic, epigenomic, and proteomic data. A pilot study by Kuang et al. (2023) demonstrated successful application of spatial transcriptomics to cryobiopsy samples, revealing heterogeneous expression patterns not detectable with conventional methods [61].

5. From lung to laboratory: the value of surgical techniques in the molecular interpretation of interstitial tissue

5.1. TBCB as a critical diagnostic tool for interstitial lung diseases

TBCB has established itself as a key tool in the diagnostic pathway of ILD, particularly in cases where high-resolution computed tomography (HRCT) is inconclusive and histological confirmation is required. Among ILDs, the form that most necessitates advanced diagnostic tools is idiopathic pulmonary fibrosis (IPF), due to its marked clinical heterogeneity. On the one hand, these diseases often present heterogeneous and poorly classifiable patterns; on the other, emerging biotechnologies

based on omics sciences (e.g., transcriptomics, genomics) offer promising avenues for improving both the accuracy and timeliness of diagnosis in such conditions.

5.2. TBCB and genomic classifiers in ILD diagnosis

The unifying element in this evolving scenario is represented by tissue sampling techniques capable of capturing a realistic representation of the pulmonary architecture at the cellular level. For example, the ENVISIA genomic classifier, based on the expression of 190 genes, has demonstrated high specificity in detecting the UIP pattern (90 %) and allowed a definitive diagnosis of IPF in 78.6 % of cases, improving clinical management in 60 % of patients [62], thanks to the quality of tissue obtained via cryobiopsy, as previously discussed.

5.3. Impact on clinical decision-making

The quality of the sampled tissue plays a significant role in clinical decision-making: one study reported an increase in IPF diagnoses (from 30 % to 69 %, OR 16.43; $p < 0.001$), greater diagnostic confidence (≥90 %), and increased use of antifibrotic therapies (from 12 % to 50 %, OR 8.22; $p < 0.001$), along with a reduction in the need for surgical lung biopsy (SLB) (from 26 % to 17 %, OR 0.49; $p = 0.03$) [63].

5.4. TBCB in translational research: MicroRNA profiling

Beyond its diagnostic value, TBCB has also been essential in translational research on IPF, confirming key findings on the expression levels of regulatory microRNAs, such as the upregulation of miR-21 and the downregulation of the let-7, miR-29, miR-30, and miR-17∼92 families [64]. The high quality of cryobiopsy specimens also enabled confirmation of proteomic data associated with fibrosis, including increased levels of the chemokines CCL17 and CCL22, and decreased collagen fragment levels [65]. TBCB has further proven useful in epigenetic investigations, particularly in DNA methylation studies, where 738 differentially methylated CpG sites were identified in IPF. These included hypermethylation of antifibrotic genes (THY-1, PTGER2, CDKN2B, COX-2, C8orf4, p14ARF) and hypomethylation of reparative genes (MGMT) [66].

5.5. Single-cell analysis and cellular heterogeneity

A major breakthrough was the application of single-cell RNA sequencing (scRNA-seq) directly to cryobiopsy samples, enabling the identification of at least 13 distinct cell populations, including macrophages, alveolar epithelial cells AT1/AT2, endothelial cells, and T, B, and NK lymphocytes [24]. This approach made it possible to isolate fibrotic niche-resident cells, such as monocyte-derived macrophages, which expressed high levels of SPP1, CHI3L1, MMP9, MARCKS, and IL1RN, while resident macrophages expressed PPARγ+ and MRC1+. In AT2 cells, a fibrotic cluster exhibited high expression of DMBT1, SERPINA1, CHI3L1, and signs of cellular senescence, with significantly increased scores ($p = 0.0001$). Moreover, a WNT signaling pattern was observed, with WNT7B and AXIN2 distributed across distinct epithelial subtypes. Finally, the identification of rare lymphatic vascular progenitor cells (PROX1+, MMRN1+, TBX1+) within TBCB samples further underscores the strategic importance of this technique in enabling detailed cellular and molecular investigation of complex fibrotic diseases.

6. Radiomics and TBCB

6.1. Principles and applications of radiomics

Radiomics is a technology that enables the automated extraction of hundreds of parameters and quantitative data (such as shape, texture, and intensity) from high-resolution images like HRCT, using advanced algorithms and software [67]. These data can be analyzed and organized to identify recurring features that help predict diagnosis, treatment response, or disease prognosis, particularly in tumors and pulmonary diseases. This futuristic approach could, through correlation analyses between imaging data and high-quality molecular data, also allow the identification of potentially useful biomarkers from radiological images for the diagnosis, risk stratification, and monitoring of ILDs, thereby providing a completely non-invasive tool [68].

6.2. HRCT and guided biopsy sampling

Today, HRCT remains the main imaging tool for the assessment of fibrosing ILDs, not only to identify specific radiologic patterns but also to accurately guide biopsy sampling [69,70]. When radiologic patterns do not allow for a definitive diagnosis, detailed image analysis through radiomics studies enables the selection of representative pulmonary areas, avoiding consolidated regions or honeycombing in favour of intermediate patterns or signs of active inflammation [3]. This targeted approach, combined with the diagnostic yield of TBCB, reduces the risk of non-informative samples and strengthens radiologic-histologic correlation.

6.3. Radiomics as a biomarker of inflammatory activity

In a prospective study involving 100 patients with fibrosing ILD, over 1600 radiomic features were extracted from each CT scan to develop predictive models of cellular infiltration within fibrotic tissue. The models demonstrated strong performance: RMSE = 0.797, accuracy of 70 %, and F1-score of 0.73, confirming radiomics as a promising non-invasive biomarker of inflammatory activity [71]. Other studies have shown that radiomics can distinguish healthy from fibrotic tissue and differentiate typical from atypical UIP patterns with high accuracy. A model based on HRCT and a Random Forest algorithm achieved an AUC of 1.0 in distinguishing healthy lung from fibrotic tissue, 0.96 in differentiating IPF/UIP from other ILDs, and 0.66 in distinguishing confirmed IPF (radiologically or histologically) from non-IPF forms, highlighting radiomics' potential to reduce the need for surgical biopsy [71].

6.4. Integrated predictive models

As reiterated, the synergy between radiomics and TBCB has also been employed to develop complex predictive models integrating imaging features, histopathological data, and serum biomarkers. In patients with rheumatoid arthritis-associated ILD (RA-ILD), a multivariate model combining radiomic parameters from HRCT and plasma KL-6 levels achieved an AUC of 0.94 for risk stratification. Another retrospective study of 177 patients led to the creation of a predictive nomogram integrating 19 radiomic parameters and clinical variables, including the ILD-GAP score, yielding an AUC of 0.948 in the training set and 0.923 in the validation set, with excellent calibration and clinical utility [72].

6.5. Applications in thoracic oncology

In thoracic oncology as well, the integration of radiomics and TBCB shows great potential. Radiomic analysis of chest CT is already used to characterize pulmonary nodules, differentiate benign from malignant lesions, and non-invasively identify histological subtypes and driver mutations. In a study of 161 patients with NSCLC, a radiogenomic model based on an XGBoost algorithm achieved an AUC of 0.89 (EGFR) and 0.81 (KRAS), with specificity >88 % and accuracy >83 %, even in imbalanced cohorts. These results were supported by data balancing algorithms and SHAP analysis, which highlighted the most predictive parameters [73]. Furthermore, radiomics can identify the most aggressive areas within heterogeneous nodules, guiding targeted biopsies and

increasing the likelihood of obtaining molecularly relevant tissue.

7. Cryobiopsies in support of digital pathology and integrated artificial intelligence tools

7.1. The convergence of AI and digital pathology

The integration of artificial intelligence (AI) into the histopathologic evaluation of TBCB samples marks a turning point in respiratory diagnostics. Digital pathology enables high-resolution scanning of histologic slides, allowing deep learning algorithms to automatically recognize complex morphologic patterns with diagnostic accuracy comparable to—or even exceeding—that of expert pathologists. A key example is the CAMELYON16 study, where an AI model reached an AUC of 0.994 in detecting lymph node metastases in breast cancer, outperforming pathologists under routine conditions (average AUC = 0.810) and approaching the performance of an expert with no time constraints (AUC = 0.966) [74]. Another AI system, trained on thousands of unannotated digital images using only general diagnoses, achieved AUC >0.98 across various cancers (prostate carcinoma, basal cell carcinoma, breast cancer lymph node metastases), automatically flagged suspicious cases, and excluded up to 75 % of slides from review without missing a single positive case, thanks to 100 % sensitivity [75]. These findings demonstrate AI's potential to make diagnostics more efficient and scalable, significantly reducing pathologists' workloads. In ILDs, where morphologic overlap (e.g., UIP) complicates diagnosis, AI has shown strong utility. The MIXTURE system supported the identification of key features such as dense fibrosis, fibroblastic foci, and lymphocytic infiltrates, achieving AUC = 0.90 in the validation cohort and aiding in the differential diagnosis of UIP, a disease with poor prognosis [76]. In IPF, AI has enabled the discovery of prognostic biomarkers that are difficult to assess manually. A convolutional neural network (CNN) applied to IPF samples found that fibroblastic foci (FF) were associated with worse outcomes, while interstitial mononuclear inflammation and intra-alveolar macrophages correlated with better survival [77].

7.2. Multimodal data integration

Another major advancement is the integration of clinical, molecular, radiologic, and histologic data. Analysis of TBCB samples combined with RNA-Seq led to the identification of genes linked to IPF pathogenesis, including FHL2, HPCAL1, RNF182, and SLAIN1, with AUCs up to 1.00 [78]. Such multimodal integration may soon provide decisive diagnostic support in ILDs, for example, in differentiating idiopathic UIP from chronic hypersensitivity pneumonitis, as deep learning models can now identify granulomas and other microscopic lesions directly from digital slides, improving diagnostic sensitivity [79].

7.3. AI in thoracic oncology

In thoracic oncology, AI applied to digital cryobiopsy slides also shows great promise. AI models trained on TBCB samples allow accurate classification of NSCLC histotypes, matching expert pathologists' performance, and support the early identification of predictive biomarkers, making AI a valuable tool in thoracic pathology and lung cancer diagnostics [80].

8. Conclusions

TBCB is emerging as one of the most promising techniques in advanced thoracic diagnostics, combining high diagnostic yield with low invasiveness. Its ability to provide large tissue samples with a better preservation of tissue architecture, makes it suitable not only for conventional histological analysis but also for advanced molecular applications such as NGS, transcriptomics, epigenomics, and proteomics. Clinically, TBCB has improved the identification of histopathologic patterns in ILDs and has become a key component of multidisciplinary team discussions. In thoracic oncology, it enables comprehensive molecular profiling, supporting targeted therapeutic strategies. Its potential is further enhanced by integration with radiomics and artificial intelligence, which allow the development of advanced predictive models correlating radiologic, histologic, and genomic data. In this context, TBCB serves as an enabling tool for precision medicine. To fully consolidate its clinical adoption, investments in specialist training, protocol standardization, and dissemination of operational expertise across respiratory centers will be essential.

CRediT authorship contribution statement

Serafina Martella: Writing – original draft, Conceptualization. **Giacomo Cusumano:** Writing – review & editing, Conceptualization. **Luigi La Via:** Writing – original draft. **Stefano Palmucci:** Writing – review & editing. **Elisa Gili:** Writing – original draft. **Cinzia Solinas:** Writing – original draft. **Dimitrios Stylianakis:** Writing – original draft. **Giuseppe Muscato:** Writing – review & editing. **Carlo Vancheri:** Writing – review & editing, Supervision. **Alberto Terminella:** Writing – review & editing, Supervision.

Ethical approval

This article does not include studies involving human or animal subjects performed by any of the authors.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

PD-L1	Programmed Death Ligand-1
TBCB	Transbronchial CryoBiopsy
ILD	Interstitial Lung Diseases
NSCLC	Non-Small Cell Lung Cancer
NGS	Next-Generation Sequencing
RNA-seq	RNA Sequencing
scRNA-seq	Single-cell RNA Sequencing
TMB	Tumor Mutational Burden
UIP	Usual Interstitial Pneumonia
NSIP	Nonspecific Interstitial Pneumonia
OP	Organizing Pneumonia
HP	Hypersensitivity Pneumonitis
HRCT	High-Resolution Computed Tomography
AI	Artificial Intelligence
CNN	Convolutional Neural Network
SLB	Surgical Lung Biopsy
TBB	Transbronchial Biopsy
TBNA	Transbronchial Needle Aspiration
VATS	Video-Assisted Thoracoscopic Surgery
ATS	American Thoracic Society
ERS	European Respiratory Society
CHEST	American College of Chest Physicians
ALAT	Latin American Thoracic Society
MDD	Multidisciplinary Discussion
MDT	Multidisciplinary Team
miRNA	microRNA

FISH	Fluorescence In Situ Hybridization
EBUS	Endobronchial Ultrasound
DLCO	Diffusing Capacity of the Lung for Carbon Monoxide
KL-6	Krebs von den Lungen-6
TILs	Tumor-Infiltrating Lymphocytes
AUC	Area Under the Curve

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