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Review Article

Molecular Diagnosis of Lung Cancer (LC) In the ERA of Cryobiopsy (CB). A Literature Review

Lojo-Rodríguez I[1], Botana-Rial M[2*], Ramos-Hernández C[1], Ruano-Raviña A[3], González-Montaos A[1], Rodríguez-Otero I[1], Fernández-Villar A[2]

[1]Pulmonology Department, Hospital Álvaro Cunqueiro, EOXI Vigo. Pneumovigo I+I Research Group. Health Research Institute Galicia Sur (IIS Galicia Sur). Vigo, Spain.

[2]Pulmonology Department, Hospital Álvaro Cunqueiro, EOXI Vigo. Pneumovigo I+I Research Group. Health Research Institute Galicia Sur (IIS Galicia Sur). CIBERES-ISCIII. Vigo, Spain.

[3]Preventive Medicine and Public Health, School of Medicine, University of Santiago de Compostela, La Coruña, Spain; CIBER de Epidemiología y Salud Pública (CIBERESP), Spain. Health Research Institute of Santiago de Compostela (Instituto de Investigación Sanitaria de Santiago de Compostela - IDIS), Santiago de Compostela, Spain.

***Corresponding author:** Botana-Rial M, Pulmonary Department, Hospital Álvaro Cunqueiro, Vigo. Street Clara Campoamor, 341. 36312. Vigo. Spain.

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Abstract

Background: Cryobiopsy (CB) is a recent technique that proved its utility diagnosing lung cancer (LC). It is unknown whether the alterations caused by freezing may influence molecular diagnosis of LC. The main objective of this narrative review was to evaluate the diagnostic yield of CB detecting biomarkers, including the expression of PD-L1 protein. **Summary:** We performed a bibliographic search in PubMed (Medline) and Embase, electronic academic databases to identify relevant studies. 14 were included. 615 patients received a diagnosis of LC through CB. One study analysed the utility of CB in endobronchial lesions, 7 studies of PPLs, 2 studies of pleural effusion and 4 studies analysed the usefulness through CB of mediastinal adenopathies. CB of endobronchial lesions increased the detection of EGFR mutations compared to conventional biopsy at 19% vs 6.5%. The efficacy of CB in molecular study of PPLs ranged 90%-100%. Pleural CB was sufficient to allow the analysis of EGFR mutations in all samples. Referring to studies that performed molecular testing, the diagnostic yield of CB versus endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) always exceeded 90%. CB can improve the molecular diagnosis of LC when used to obtain tissue from endobronchial lesions, peripheral pulmonary lesions (PPLs), mediastinal adenopathies, or pleural tissue. **Key Messages:** CB can improve molecular diagnosis of LC. In the current era where cancer treatment is based on precision medicine, the use of CB could improve prognostic of patients. Research is needed to analyse performance of CB when applied to different tissues to determine all molecular alterations in patients with LC.

Keywords: Cryobiopsy; Biomarker; Lung Cancer; Molecular Diagnosis;

Introduction

The diagnosis of lung cancer (LC) has evolved from a simple histological diagnosis to the need to characterise different molecular subtypes, with different prognostic and therapeutic implications. Thus, optimising sampling for diagnosis can represent a challenge in some cases [1, 2]. The gold standard for the confirmation is the biopsy. Thus, the tissue samples obtained from biopsies must contain adequate material to be able to identify the LC subtype by immunohistochemical and histopathological procedures and to allow complete molecular characterisation [1-3]. Molecular testing has become a mandatory component of the non-small cell LC (NSCLC) management.

The detection of EGFR, BRAF, and MET gene mutations, as well as analysis of ALK, ROS1, RET, and NTRK translocations have already been incorporated into non-small cell LC NSCLC diagnostic standards, and inhibitors of these kinases are now in routine clinical practice. In addition, there are emerging biomarkers such as KRASG12C substitutions and HERB2 activating alterations wich are likely to enter NSCLC guidelines up on the approval of the corresponding drugs. In addition to genetic examination, the expression of the PD-L1 protein must also be examined in order to direct the use of immunotherapy in these patients [4].

Hence, the number of molecular markers that need to be determined to fully understand each case of LC has significantly increased, with this trend expected to continue as different molecular alterations are discovered that may help improve patient survival. All this reflects the need to determine which sample types are sufficient to allow the molecular characterisation of diseased individuals. In this context, cryobiopsy (CB) is a tool traditionally indicated for the extraction and recanalisation of endobronchial tumours or the removal of clots and foreign bodies from airways. In recent years we have known an increase in its use as a novel diagnostic tool [5, 6].

Compared to samples obtained by conventional biopsy, when used for endobronchial lesions or peripheral pulmonary lesions (PPLs) CB has a greater diagnostic yield [6]. Indeed, two meta-analyses from 2020 (one of them by our group) on pleural CB during medical thoracoscopy demonstrated that this technique is safe and has a high diagnostic yield for the diagnosis of malignant pleural effusion, including pleural effusion caused by LC [7, 8]. More recently, and as a novel technique, it has been shown that the cryoprobe can be introduced through the echobronchoscope channel to obtain samples of adenopathies in real time, thereby improving the effectiveness of this procedure [9, 10].

CB is a recent technique that proved its utility in the diagnosis of endobronchial lesions, PPLs, pleural effusion, or mediastinal adenopathies [6-10]. CB samples are usually of a sufficient size to routinely allow the morphological and immunohistochemical study of LC, which is not always achieved with other conventional sampling techniques. Moreover, CB also provides better preserved tissue without artefacts. However, it is unknown whether the physical alterations caused by the freezing and thawing involved in obtaining CBs may influence the value of these samples for the molecular diagnosis of LC.

The results of retrospective analyses have described better detection of the EGFR oncogene mutation in patients with LC when CB had been used [11]. Thus, CB is a promising technique in the diagnosis of LC and there is increasing interest in its use as a tool to sample different tissues. The number of publications that support the effectiveness and safety of CB has increased in recent years. The randomised cohort studies are currently underway in relation to the use of CB and some reviews have described aspects of the diagnostic efficacy of CB for LC, and the publication of some evidence on the role of CB in the molecular diagnosis of LC [5, 7, 11].

However, to date, the scientific literature published that address the advantages and limitations of using CB as a technique for obtaining adequate tissue for the molecular characterisation of patients with LC, it is a low [12, 13]. Therefore, the main objective of this current review was to evaluate the diagnostic yield of CB for the detection of all molecular alterations, including the expression of PD-L1 protein in patients with histologically-confirmed LC with involvement of different anatomical regions.

Methods

We performed a bibliographic search in the PubMed (Medline) and Embase electronic academic databases to identify relevant studies. We used a predefined search strategy that used keywords to find pertinent studies published up to and including 31 May 2023 without initial publication date. We also manually searched the reference lists of all the included studies as well as relevant reviews for additional studies not detected by the electronic database searches. The terms used in the literature search were “cryobiopsy lung cancer” OR “molecular diagnostic lung cancer”, OR “genetic tests and cryobiopsy”, OR/and “cryobiopsy and molecular tests”.

Meta-analyses, systematic and narrative reviews, cohort studies, case-control studies, and case series were included in the search. The inclusion criteria were studies that analysed the diagnostic yield of molecular tests performed on CB samples from patients with LC. Studies not conducted in humans or that had not been

peer-reviewed were excluded. The search was limited to articles written in English or Spanish were included.

The predefined criteria for inclusion were any study that reported the diagnostic yield of molecular tests performed on CB samples from patients with LC. The studies that had not reported the diagnostic yield of CB narrative review articles, editorials, or letters to the editor that had not described any cases were excluded.

The studies initially obtained according to the title and abstract were reviewed and in the case of any doubt, the full text was reviewed. The citations of the included studies were also checked and any duplicates were discarded.

The main variables discribed were study year, country, design, hospital (single centre or multicentre), sample size, type of tissue/lesion biopsied, and the histological type of tumour. The molecular diagnostic yield of the CB was also collected as the main variable and the type of molecular alteration that was analysed in the sample.

The relevant information from each of the studies was collected. The results were grouped according to the type of tissue biopsied. However, because of the limited number of studies and their heterogeneity, this was not possible and so only a narrative review of the results was performed.

Results

14 were included [10, 11, 14-23]. Most of the studies were excluded because they had not provided information on the diagnostic yield of the CB samples for the detection of any molecular alterations and/or PD-L1 expression. Of the 14 studies that met the study inclusion criteria, 3 were retrospective analyses carried out in a single centre [11, 16, 25], 3 were randomised multicentre studies [10, 17, 21], and the rest were prospective single-centre studies. Most of the studies had been performed in Asian populations and only 4 had included a European population [10, 11, 21, 22] (shown in Fig.1).

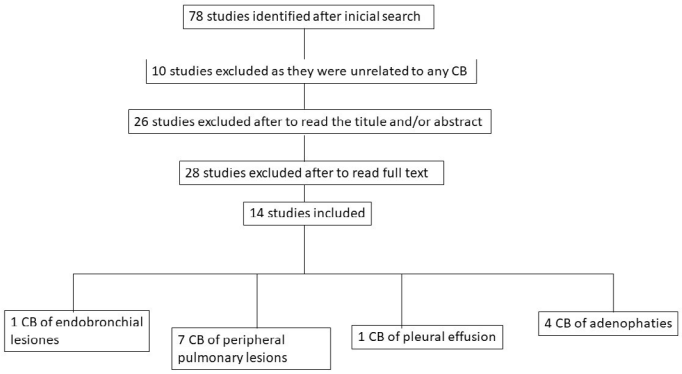

Figure 1: Figure showing the process of review and selection of studies.

A review that included 9 studies has recently been published describing the main characteristics of CB when using radial endobronchial ultrasound (EBUS) for the diagnosis of PPLs [34]. The studies that had reported the diagnostic yield of the CB samples for the detection of molecular alterations were included in the manual search [14, 17, 19, 20]

In total, 615 patients had received a diagnosis of LC through CB. In the group of patients in which the CB had been performed on endobronchial lesions, 90% (113/125) were adenocarcinomas. In the groups of PPL and adenophaties, adenocarcinoma was also the most frequent histological type. They represented 45% (96/209) and 52% (127/240) of the cases, respectively (p=0.8). In the case of patients with pleural effusion caused by LC, the histological type had been described in only 4 of the 31 cases. The 4 patients were adenocarcinomas [24].

About the quality assessment of the studies include in this review no study had as its main objective to analyze the diagnostic yield of molecular tests performed on CB samples from patients with LC.

Cryobiopsy of Endobronchial Lesions

Regarding the value of CB of endobronchial lesions, only one study had analysed how the biopsy technique had affected the detection of EGFR gene mutations. This was a retrospective single-centre study from Germany which had evaluated the percentage of detection of the EGFR gene mutation in tissue obtained by CB in 125 patients with LC and had compared it with conventional endobronchial sampling techniques. 113 (90%) cases were adenocarcinomas and 77 (61%) of these patients were men with a median age of 65 years. In this case, CB had increased the detection of EGFR gene mutations in endobronchial tumours compared to conventional biopsy at 19% versus 6.5%, respectively ($p<0.05$) [11]. About the type of study, the retrospective nature of this analysis and single center study, a prospectiove tiral will be mandatory for final assessment.

Cryobiopsy of Peripheral Pulmonary Lesions

Our search revealed that at least 7 studies had analysed the utility of the CB of PPLs for the molecular analysis of LC in a total of 286 patients [14-20]. In a study on 23 patients, Arimura K et al. evaluated the diagnostic accuracy of CB using radial EBUS to determine all the molecular alterations present next-generation sequencing (NGS) techniques [14]. These authors found that all the CB samples had provided sufficient sample quantity and quality for the complete molecular study. Similar results were also described by Tone M et al. in a study with 37 patients where CB had more effectively obtained suitable samples for genetic study by NGS in patients with LC compared to samples obtained by conventional techniques [16].

The main characteristics and results of the studies that analysed the utility of CB for the molecular characterisation of LC in PPLs shown in Table 1. Comparison of results was not possible because of the heterogeneity of the studies and because some had not specified what type of molecular study or biomarkers they had studied. In this review, only one randomized control trial was included (17) and others studies were with small number patients and differents uncontrolled variables.

Study, publication year (ref Nº)	Desing, country, number of patients	Size lesion (mm)	Procedure	Malignant etiology	Yield diagnostic for the detection of molecular alterations	Time of frozen biopsy tissue	Number biopsies
Arimura 2019 (15)	Prospective, single centre, Tokio, 17	39 (10-81)	Radial EBUS	16 patients -10 adenocarcinoma -4 squamous carcinoma -2 others	PDL1>50%: 18,8% cryobiopsy vs 12,5% conventional biopsy PDL1>1%: 56,3% vs 37,5%	3-5 seconds	1-2
Udagawa 2020 (19)	Prospective, single centre, Japón, 121	38	Fluoroscopy (47% in outer third)	113 patients -49 adenocarcinoma -24 squamous carcinoma -17 small cell lung -23 others	90% sufficient simples for the detection molecular alterations. PDL1>1% 51% cryobiopsy vs 42% conventional biopsy (p=0.06)	3-5 seconds	----
Herath 2017 (20)	Prospective, single centre, Nueva Zelanda, 6	41 (19-66)	Radial EBUS	5 patients -3 adenocarcinomas - 1 squamous carcinoma -1 small cell lung	100% sufficient simples for the detection molecular alterations.	4 seconds	----
Arimura 2019 (14)	Prospective, single centre, Tokio, 23	36 (10-81)	Radial EBUS	20 patients -11 adenocarcinomas - 6 squamous carcinoma - 3 others	100% sufficient samples for the detection molecular alterations. (13 (76.5%) positive mutations, 4 (23.5%) negative mutations))	3-5 seconds	1-2
Tone 2021 (16)	Retrospective, single centre, Japón, 37 (only 18 cryobiopsies)	45 (7-92)	Radial EBUS and fluoroscopy	18 patients -----	NGS: 12 (66.7%) positive mutacions and 6 (33,3%) insufficient samples (p=0.11)	5-7 seconds	----
Herath 2022 (17)	Randomised multicentre, 3 centres of Australia y Nueva Zelanda, prospectivo, 48	33.6 (21.6)	Radial EBUS	10 patients -7 adenocarcinomas -1 squamous carcinoma -2 carcinoides	100% sufficient samples for the detection EGFR	------	2
Imabayashi 2019 (18)	Retrospective, single centre, Kioto, 36	-----	Radial EBUS	27 pacients -16 adenocarcinomas -3 squamous carcinoma -3 small cell lung -5 others	100% sufficient samples for the detection molecular alterations	3.3±0.7 seconds	1.5

Table 1: Baseline characteristics of studies describing the yield diagnostic of cryobiopsy peripheral pulmonary lesions for the detection of molecular alterations.

Cryobiopsy of Pleural Effusion

Only two studies had analysed the ability to perform a molecular characterisation of LC in CBs from pleural tissue [24, 25]. They were prospective and single centre studies. In the first case, it had been completed in a hospital in Slovenia and had included only 4 adenocarcinomas. The authors had described that the pleural biopsies with a cryoprobe and the samples had been sufficient to allow the analysis of EGFR gene mutations in all the samples [24]. In the second study, which had included 29 cases of malignant pleural effusion caused by NSCLC, all the samples had also been valid for the analysis of the EGFR gene mutation [25]. However, the authors had not described the histological type or what type of molecular determinations they had performed. None of the studies had analysed the value of CB to detect molecular alteration types other than the EGFR gene mutation and none of these studies analyzed the yield of conventional biopsy for DNA analysis in tumor samples. An adequate control group no have a gold standard diagnostic test recognized as the optimal intervention.

Cryobiopsy of Mediastinal and Hiliar Adenopathies

Only 4 studies had analysed the usefulness of the molecular diagnosis of LC through CB of mediastinal adenopathies. Zhang et al., had carried out one of the first randomised studies in 2 centres, one in Germany and the other in China, with a total of 197 patients; 93% of the CB had been suitable for the study of molecular alterations, compared to 73% of the samples obtained by fine-needle lymph node aspiration ($p=0.001$) [10]. In another randomised multicentre study in 3 hospitals in Europe and Asia with 136 patients undergoing CB and fine needle puncture aspiration, molecular testing and immunohistochemical determination of PD-L1 had been possible in almost all the samples obtained by CB (97%), while this had only been possible in 79% of those obtained by fine needle puncture ($p<0.05$) [21].

The only prospective Spanish study had included 6 cases of lung adenocarcinoma and in 4 cases of squamous LC, the previous EBUS guided transbronchial needle aspiration (EBUS-TBNA) had produced an insufficient sample. In all the cases in which a definitive diagnosis of LC had been obtained, CB of the lymph node had obtained sufficient sample to carry out an optimal and complete molecular study [22]. However, as in other studies, the molecular tests then performed had been not specified by the authors. In a similar series, Arimura K et al. Enrolled in a prospective study in Tokyo hospital which had included 16 patients. The main objective of assessing the tumour cell numbers and PD-L1 expression for CB with EBUS- guide sheath for PPLs and transbronchial biopsy in patients with LC. Detection of PD-L1 exceeding 1% had been more frequent in CB samples 56% versus 37%, respectively [23].

Nonetheless, despite excellent results, the small number of studies included and their heterogeneity, it was impossible to apply the appropriate statistical techniques for analysis of the results. Therefore, we conducted a description of the evidence and without statistical analysis.

Discussion

The data presented in this review demonstrate that samples from different tissues obtained by CB can be a useful and adequate tool to complete molecular diagnoses, including the analysis of PD-L1 expression in cases of LC. Of note, immunotherapy is now the first line therapeutic option and has been shown to be beneficial in patients with PD-L1 expression exceeding 5%. However, the number of studies analysing the value of CB in this field remains minimal.

Larger histological samples will increasingly be needed to adequately perform the molecular characterisation of tumours and this has promoted the development of new biopsy techniques such as CB [6].

In this narrative review we concluded that the use of the CB technique could continue to expand and also improves the ability of precision medicine to diagnose and optimise the treatment of patients with LC. Moreover, CB has an increasing number of applications in the field of thoracic diseases [6]. Technical advances, particularly the availability of mini probes, have further expanded such applications to other pathologies, including the diagnosis of interstitial lung diseases and the diagnostic evaluation of rejection in lung transplant patients [6, 26].

Importantly, all molecular studies must currently be performed at the time of LC diagnosis and thereby require sufficient good quality biopsy material to be able to do so. Furthermore, in recent years, there has been an increase in the approval of new targeted molecular therapies for subgroups of patients with LC with defined alterations. This means that, especially in the era of targeted cancer therapies, larger and better quality tumour samples will be of greater value for future molecular analyses. Thus, it is important that advances in pathological anatomy and molecular tests (and their procedures) not be limited by biopsy sample sizes.

Different studies have evaluated the diagnostic yield of endobronchial samples obtained by CB for histological, morphological, and immunohistochemical profile examination in patients with LC and have demonstrated an increase in the diagnostic yield of CB by more than 10% [27-29]. Indeed, in the only study whose primary objective had been to evaluate the detectability of EGFR in CB samples, CB increased detection by more than 12% [11]. However, because of the retrospective nature of study, a prospective trial will still be necessary in the future.

The detection of PPLs has increased because of the use of computed tomography (CT) imaging. However, although CT-guided biopsy may be the standard diagnostic method for PPLs, the field of interventional pulmonology has also evolved in recent years. Thus, radial EBUS and ultrathin bronchoscopy are newer technologies designed to improve access to and the diagnosis of PPLs compared to conventional bronchoscopy [30]. Despite the fact that these new tools have emerged as safe methods with acceptable diagnostic yields for the study of tumour PPLs, their main limitation continues to be the small size of the biopsies obtained through these means (30). In this context, preliminary studies comparing the diagnostic yield of CB by radial EBUS to that of conventional biopsy have shown that CB improves the diagnostic yield and obtains larger samples in patients with PPL resulting from LC [31-33].

A review that included 9 studies has recently been published describing the main characteristics of CB when using radial EBUS for the diagnosis of PPLs [34]. The main objective had been to analyse the usefulness and safety of the technique compared to conventional forceps biopsies. This study showed that the diagnostic yield of CB had been 77%, which exceeded that of conventional biopsies [20]. In the specific case of the efficacy of CB in the molecular study of PPLs, the reported diagnostic yield had ranged from 90% up to 100% in most studies [14, 17, 19, 20].

It is important to mention that some authors have criticised the rigidity of cryoprobes as a limitation to their use in bronchoscopy. Hence, to improve their results, several groups have performed CB using a guide sheath or other navigation system, all producing favourable diagnostic yields [26, 30]. Thus, CB used in this way can provide additional tissue for molecular or immunotherapy studies, especially when compared to the yield obtained by other common methods. Furthermore, studies on CB with a 1.1-mm cryoprobe for the diagnosis of PPL (NCT05046093, NCT04885595, and NCT04727190) are currently being undertaken by different groups. The use of ultrathin bronchoscopes is another novel technique in the diagnosis of PPL. Their smaller size allows for improved manoeuvrability and the ability to access smaller segmental bronchi and reach PPLs [30].

However, both the working channel and the tissue samples obtained through ultrathin bronchoscopes are small. Nonetheless, 1.1-mm cryoprobes can pass through their working channel, thereby avoiding the limitation of obtaining small samples. In relation to this, in a prospective study by Oki et al. that had included 50 patients with PPLs measuring less than 30 mm, the combination of samples obtained both by CB and conventional forceps provided a diagnosis in 74% of patients, as compared to 54% and 62% respectively for CB or forceps biopsy alone [35]. Thus, CB through an ultrathin bronchoscope is feasible, effective, and sufficiently safe for the diagnosis of PPL. However, despite these promising results, this study had been carried out in a single centre and it is unknown whether the procedure had permitted molecular studies in patient samples.

Malignant pleural effusion is associated with a very broad spectrum of mutations. EGFR oncogene mutations are more common in cases of pleural metastases or those with pleural fluid compared to primary tumours (26% vs. 15%, respectively). The frequency of KRAS mutations in cases of malignant pleural effusion were lower than in patients with LC at 19% versus 33%, respectively [36]. Therefore, the molecular characterisation of tumours is of great importance. In this sense, CB has been shown to provide a high diagnostic yield in the aetiological study of pleural effusion, allowing larger and better preserved samples to be obtained for morphological and immunohistochemical studies of cases of pleural effusion caused by LC [7, 8].

The diagnostic yield of the CB was 95% compared to 90% when conventional flexible forceps had been used [7]. Of note, two recent meta-analyses that considered more than 300 patients also confirmed these results [7, 8]. However, given the limited amount of data currently available (only 2 studies that had included less than 40 patients had analysed the detection of the EGFR gene mutation), more studies should be performed to better understand how the use of pleural CB could help study all tumour molecular alterations, as well as to determine PD-L1 expression. In turn, lineal EBUS-TBNA was the first widely accepted procedure for the diagnosis and staging of LC. Indeed, its diagnostic yield for LC has been reported as exceeding 90% [37]. In addition, when specifically considering the study of molecular alterations, other work showed the superiority of material obtained by lineal EBUS-TBNA over endobronchial tissue samples or CT-guided biopsies [34].

Finally, lineal EBUS can also be used to apply CB inside the mediastinum [9, 10]. Recent randomised studies have also demonstrated the overall benefits of using CB, with clinically useful samples having been obtained from adenopathies in 91% of cases compared to 79% for conventional fine needle puncture, and similar results having been obtained for LC-related metastatic lymph nodes [10]. When we pooled the results of all the studies that had performed molecular testing on samples obtained from patients with LC, the diagnostic yield of CB versus EBUS-TBNA always exceeded 90% [10, 21, 22].

The main limitation of this current review was that the principal objective of most of the articles had not been to analyse the diagnostic value of CB from the molecular alterations. Thus, future research must be designed to specifically study this factor. We must also consider that most of the studies we looked at had been performed in non-Caucasian populations, which could

have affected the outcomes and the type of molecular alterations detected. The

main advantage is the review design, which allows for a homogeneous and rigorous evaluation of the evidence obtained. To date, there are hardly any critical reviews of the scientific literature that address the advantages, limitations and what molecular characterization may be more or less appropriate with the use of CB.

Conclusions:

CB can improve the molecular diagnosis of LC when used to obtain tissue from endobronchial lesions, PPLs, mediastinal adenopathies, or pleural tissue.

Although the results summarised here may be promising, further research is still needed to provide a meta-analysis or systematic review to analyse the true performance of CB more accurately when applied to different tissues to determine every kind of molecular alteration in patients with LC, including changed PD-L1 protein expression.

We believe that it would be interesting as areas for future research, studies comparing cryobiopsy with emerging diagnostic technologies as NGS, or exploring its use in different geographic region, and ethnicity.

As for every procedure, these reported findings must be balanced against the technical requirements of the biopsy types, need for training to perform these procedures, and the substantially higher cost of CB compared to that of competing strategies such as the design of new adenopathy biopsy needles or forceps. There is a huge need for advanced broncho-pleural techniques training for diagnosis of patients with suspected lung cancer among pulmonologists. A structured and well-established training program should be designed and implemented. This could include a three-step approach starting with a theoretical course for basic knowledge, followed by simulation-based training and supervised clinical learning. Objective assessment of all these steps should be mandatory for certification. Although there are no cost effectiveness studies comparing the yield of cryobiopsy with other other types of conventional biopsy, further research should be conducted. Prospective, comparative, randomized, multicenter studies need to be carried out with a large series of well characterized patients and a common, duly standardized protocol and methodology. This would help define the role of this novel modality in the diagnosis of LC.

Hence, better defining the role of CB in the diagnostic algorithm of LC remains a task for future work. The field of interventional pulmonology is rapidly evolving and, in this sense, multiple new tools are being investigated for this specialty. The increase in the diagnostic performance of bronchopleural techniques applied to study LC represents an important step in improving the management of this pathology and its survival rates. In this sense, as an innovative technique, CB allows better morphological and molecular diagnostic performance. Based on the data considered in this review, CB seems to be an extremely useful tool in the molecular diagnosis of LC. Nevertheless, given the complexity of these new techniques and technologies, it is important for interventional pulmonologists and other specialists to continue working to understand their strengths and limitations.

Statements

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Authors Contribution

All authors should have made sustancial contributions to all of the following (the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article and revising it critically for important intellectual concept).

ILR: Conceptualization, Methodology, Writing

MBR: Conceptualization, Methodology, Writing, Formal Analysis

CRH: Methodology, Writing

AGM: Validation, Formal Analysis

ARR: Validation, Supervision

IRO: Methodology, Formal Analysis

AFV: Methodology, Supervision

References

1. Roy-Chowdhuri S (2021) Molecular Pathology of Lung Cancer. Surg Pathol Clin 14:369-377.
2. Rodriguez-Canales J, Parra-Cuentas E, Wistuba II (2016) Diagnosis and Molecular Classification of Lung Cancer. Cancer Treat Res 170:25-46.
3. Belanger AR, Akulian JA (2017) An update on the role of advanced diagnostic bronchoscopy in the evaluation and staging of lung cancer. Ther Adv Respir Dis 11:211-221.
4. Imyanitov EN, Iyevleva AG, Levchenko EV (2021) Molecular testing and targeted therapy for non-small cell lung cancer: Current status and perspectives. Crit Rev Oncol Hematol 157:103194.
5. Simon M, Simon I, Tent PA, Todea DA, Haranguș A (2021) Cryobipsy in lung cancer diagnosis- a literatura review. Medicina (Kaunas) 57:393.

6. DeMaio A, Thiboutot J, Yarmus L (2022) Sryma. Applications of cryobiopsy in airway, pleural, and parenchymal disease. Expert Rev Respir Med 16:875-886.

7. Rial MB, Rodríguez IL, Roibás CM, Fernández VL, Delgado MN, et al. (2020) Diagnostic Yield and Safety of Pleural Cryobiopsy during Medical Thoracoscopy to Diagnose Pleural Effusion. A Systematic Review and Meta-Analysis. Arch Bronconeumol 56:784-791.

8. Shafiq M, Sethi J, Ali MS, Ghori UK, Saghaie T, et al. (2020) Pleural Cryobiopsy: A Systematic Review and Meta-Analysis. Chest 157:223-230.

9. Mondoni M, Sotgiu G (2022) Optimizing the endoscopic diagnosis of mediastinal lymphadenopathy: a glimpse on cryobiopsy. BMC Pulm Med 22:355.

10. Zhang J, Guo JR, Huang ZS, Fu WL, Wu XL, et al. (2021) Transbronchial mediatinal cryobiopsy in the diagnosis of mediastinal lesions:randomised trial. Eur Respir J 58:2100055.

11. Haentschel M, Boeckeler M, Ehab A, Wagner R, Spengler W, et al. (2020) Cryobiopsy increases the EGFR detection rate in non-small cell lung cancer. Lung Cancer 141:56-63.

12. Botana-Rial M, Lojo-Rodríguez I, Leiro-Fernández V, Ramos-Hernández C, González-Montaos A, et al. (2023) Is the diagnostic yield of mediastinal lymph node cryobiopsy (cryoEBUS) better for diagnosing mediastinal node involvement compared to endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA)? A systematic review. Respir Med 218:107389.

13. Maturu VN, Prasad VP, Vaddepally CR, Dommata RR, Sethi S (2024) Endobronchial Ultrasound-guided Mediastinal Lymph Nodal Cryobiopsy in Patients With Nondiagnostic/Inadequate Rapid On-site Evaluation: A New Step in the Diagnostic Algorithm. J Bronchology Interv Pulmonol 31:2-12.

14. Arimura K, Tagaya E, Akagawa H, Nagashima Y, Shimizu S, et al. (2019) Cryobiopsy with endobronchial ultrasonography using a guide sheath for peripheral pulmonary lesions and DNA analysis by next generation sequencing and rapi don-site evaluation. Respir Investig 57:150-156.

15. Arimura K, Kondo M, Nagashima Y, Kanzaki M, Kobayashi F, et al. (2019) Comparison of tumo cell numbers and 22C3 PD-L1 expresiion between cryobiopsy and transbronchial biopsy with endobronchial ultrasonography-guide sheath for lung cancer. Respir Res 20:18.

16. Tone M, Inomata M, Awano N, Kuse N, Takada K, et al. (2021) Comparison of adequacy between transbronchial lung cryobiopsy simples and endobronchial ultrasound-guided transbronchial needle aspiration simples for next-generation seguencing analysis. Thorac Cancer 12:251-258.

17. Herath S, Wong C, Dawkins P, Veale A, Yap E, et al. (2023) Cryobiopsy with radial.endobronchial ultrasound (cryo-radial) has comparable diagnostic yield wuth higher safety in comparison with computed tomography-guided transthoracic biopsy for peripheral pulmonary lesions: an exploratory randomised study. Intern Med J 53:1390-1399.

18. Imabayashi T, Uchino J, Yoshimura A, Chihara Y, Tamiya N, et al. (2019) Safety and usefulness of cryobiopsy and stamp cytology for the diagnosis of peripherl pulmonary lesions. Cancers (Basel) 11:410.

19. Udagawa H, Kirita K, Naito T, Nomura S, Ishibashi M, et al. (2020) Feasibility and utility of transbronchial cryobiopsy in precisión medicine for lung cancer: prospective single-arm study. Cancer Sci 111:2488-2498.

20. Herath S, Yap E (2017) Novel hybrid cryo-radial method: and emerging alternative to CT_guided biopsy in suspected lung cancer. A prospective case series and description of technique. Respirol Case Repor 6:e00287.

21. Fan Y, Zhang AM, Wu XL, Huang ZS, Kontagiianni K, et al. (2023) Transbronchial needle aspiration combined with cryobiopsy in the diagnosis of mediastinal diseases: a multicentre, open.label, randomised trial. Lancet Respir Med 11:256-264.

22. Ariza-Prota M, Pérez-Pallarés J, Fernández-Fernández A, García-Alonso L, Cascón JA, et al. (2023) EBUS-guided transbronchial medistinal cryobiopsy in the diagnosis of mediastinal lesions: safety, feasibility adn diagnosti yield-experience in 50 cases. ERJ Open Res 9:00448-2022.

23. Arimura K, Kondo M, Nagashima Y, Kanzaki M, Kobayashi F, et al. (2019) Comparison of tumor cell numbers and 22C3 PD-L1 expresion between cryobiopsy and transbronchial biopsy with endobronchial ultrasonography-guide sheath for lung cancer. Respir Res 20:185.

24. Rozman A, Camlek L, Malovrh MM, Kern I, Schonfeld N (2016) Feasibility and safety of parietal pleural cryobiopsy during semi-rigid thoracoscopy. Clin Respir J 10:574-8.

25. Tousheed SZ, Manjunath PH, Chandrasekar S, Murali Mohan BV, Kumar H, et al. (2018) Cryobiopsy of the pleural. An improved diagnostic tool. J Bronchology Interv Pulmonol 25:37-41.

26. Criner GJ, Eberhardt R, Fernandez-Bussy S, Gompelmann D, Maldonado F, et al. (2020) Interventional bronchoscopy. Am J Respir Crit Care Med 202:29-50.

27. Hetzel J, Hetzel M, Hasel C, Moeller P, Babiak A (2008) Old meets modern: the use of traditional cryoprobes in the age of molecular biology. Respiration 76:193-7.

28. Schumann C, Hetzel J, Babiak AJ, Merk T, Wibmer T, et al. (2010) Cryoprobe biopsy increases the diagnostic yield in endobronchial tumor lesions. J Thorac Cardiovasc Surg 140:417-21.

29. Hetzel J, Eberhardt R, Herth F J F, Petermann C, Reichle G, et al. (2012) Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. Eur Respir J 39:685-90.

30. Shepherd RW (2016) Bronchoscopic pursuit of the peripheral pulmonary lesion: navigational bronchoscopy, radial endobronchial ultrasound, and ultrathin bronchoscopy. Curr Opin Pulm Med 22:257-64.

31. Gupta A, Youness H, Dhillon SS, Harris K (2019) The value of using radial endobronchial ultrasound to guide transbronchial lung cryobiopsy. J Thorac Dis 11:329-334.

32. Schuhmann M, Bostanci K, Bugalho A, Warth A, Schnabel PA, et al. (2014) Endobronchial ultrasound-guided cryobiopsies in peripheral pulmonary lesions: a feasibility study. Eur Respir J 43:233-9.

33. García FA, Torky M, Clemente CC, Mitja PS, Gratacós AR, et al. (2021) Transbronchial Cryobiopsy of Peripheral Pulmonary Lesions Guided With Real-Time Transthoracic Ultrasonography. Arch Bronconeumol 57:772-774.

34. Sryma PB, Mittal S, Madan NK, Tiwari P, Hadda V, et al. (2023) Efficacy of Radial Endobronchial Ultrasound (R-EBUS) guided transbronchial cryobiopsy for peripheral pulmonary lesions (PPLs): A systematic review and meta-analysis. Pulmonology 29:50-64.

35. Oki M, Saka H, Kogure Y, Niwa H, Yamada A, et al. (2023) Ultrathin bronchoscopic cryobiopsy of peripheral pulmonary lesions. Respirology 28:143-151.

36. Agalioti T, Giannou AD, Stathopoulos GT (2015) Pleural involvement in lung cancer. J Thorac Dis 7:1021-30.

37. Fielding D, Kurimoto N (2018) Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Diagnosis and Staging of Lung Cancer. Clin Chest Med 39:111-123.