Transbronchial cryobiopsy in diffuse parenchymal lung diseases

Venerino Poletti[a,b], *Claudia Ravaglia*[a], *and Sara Tomassetti*[a]

Purpose of review
The diagnostic yield of conventional transbronchial lung biopsy varies among various parenchymal lung diseases: in pulmonary sarcoidosis and lymphangitis carcinomatosa, a diagnosis can be obtained in up to 80% of patients; this method is considered inadequate, however, in identifying more complex histological patterns such as usual interstitial pneumonitis or nonspecific interstitial pneumonitis, mainly because the specimens are tiny and the interpretation is confounded by crush artifacts. Recently, the use of cryoprobes has achieved a significant impact on this issue. This review is about this promising application of cryobiopsy in the diagnostic process of diffuse parenchymal lung diseases.

Recent findings
Recent studies document that with transbronchial cryobiopsies, the diagnosis of usual interstitial pneumonitis can be made confidently by pathologists with a good interobserver agreement. Pneumothorax is the main complication (reported in up to one-quarter of cases in some series); bronchial bleeding is usually controlled using Fogarty balloon.

Summary
Transbronchial cryobiopsy is a promising new technique that may become a valid alternative to surgical lung biopsy in the near feature.

Keywords
diffuse parenchymal lung diseases, interstitial lung diseases, interventional pulmonology, transbronchial lung cryobiopsy

INTRODUCTION: CONVENTIONAL TRANSBRONCHIAL LUNG BIOPSY

Transbronchial lung biopsy (TBB) was introduced into clinical practice as a diagnostic procedure in the evaluation of diffuse lung diseases in the mid-1960s [1,2]. The lung sampling was carried out through a rigid bronchoscope in patients under local anesthesia, and biopsies were obtained using semiflexible forceps guided under fluoroscopy. Among the first 450 cases reported [3], lung tissue was obtained in 84%. However, discomfort for the patients was evident, mortality and morbidity were not negligible, and the technique was available only in very specialized centers.

After the development of flexible bronchoscopy by Ikeda, TBB became widely used as a relatively noninvasive and safe method to obtain lung tissue [4]. Nowadays, indication for TBB is determined mainly by computed tomography features of lesions [5]. Nodular lesions with a positive bronchus sign [6], alveolar opacifications, nodular or reticular features with a perilymphatic distribution and 'tree in bud pattern' have been shown to predict a high diagnostic yield from the technique [7]. This correlation may in great part be understood considering that tissue captured by TBB is representative mainly of the centrilobular zone, the zone centered by small airways [7–9]. Complications because of TBB are manageable, with pneumothorax being the most frequent (2–10% of cases); hemorrhage more often is sustained by bronchial artery tearing and is observed in less than 2% of cases [10]. This

[a]Department of Diseases of the Thorax, Azienda USL Romagna, GB Morgagni Hospital, Forlì, Italy and [b]Department of Respiratory Diseases and Allergy, Aarhus University Hospital, Aarhus, Denmark

Correspondence to Venerino Poletti, MD, Department of Diseases of the Thorax, Azienda USL Romagna, GB Morgagni Hospital, via C. Forlanini 34, 47100 Forlì, Italy. Tel: +39 0543 735042;
e-mail: venerino.poletti@gmail.com

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KEY POINTS

- Conventional TBB has a limited role in evaluating patients with suspected idiopathic interstitial pneumonias.
- Transbronchial lung cryobiopsy was shown to have a meaningful impact on diagnostic confidence in the multidisciplinary diagnosis of idiopathic interstitial pneumonias.
- Information provided by cryo-TBB has the same impact of information provided by surgical lung biopsy in defining the confidence of the diagnosis of IPF in a multidisciplinary diagnostic context.
- TBLCB seems to have significant lower incidence of adverse events compared with VATS.

latter percentage increases when pulmonary hypertension is present, probably because in this setting bronchial-pulmonary shunts are more numerous. Much more rarely, air embolism, cardiac arrhythmias, pulmonary edema and deaths have been reported.

Morphologic interpretation of TBB specimens takes place in the context of clinical-radiologic findings [4,7–8]: lung specimens may contain very specific and informative lesions (also using immunohistochemical staining) that are diagnostic by themselves [e.g. carcinomatous lymphangitis, other neoplasms, infections when pathologic microorganisms are detected inside the lesion (i.e. tuberculosis), alveolar proteinosis and Langerhans cell histiocytosis]; lung specimens may show characteristic but not specific features that are considered diagnostic when combined with the clinical-radiologic context and hypotheses (e.g. organizing pneumonia, sarcoidosis and hypersensitivity pneumonitis); or they may not be informative when not containing features, recognizable morphologic patterns or when morphologic findings are incongruous with clinical-radiologic context. In general, TBB specimens are diagnostically less useful in immunocompetent patients compared with immunocompromised patients; TBB has a limited role in evaluating patients with suspected idiopathic interstitial pneumonias including idiopathic pulmonary fibrosis (IPF), idiopathic nonspecific interstitial pneumonia (NSIP), and desquamative interstitial pneumonia (DIP). Tomassetti *et al.* [11] showed that in this setting, TBB has a high specificity for usual interstitial pneumonitis (UIP) pattern, a very low specificity when other patterns (NSIP and DIP) are identified and a very low sensitivity in diffuse lung diseases.

TRANSBRONCHIAL CRYOBIOPSY: HOW DOES IT WORK AND TECHNICAL ASPECTS

The use of cryoprobes for bronchoscopic procedures was described as early as 1977 [12,13]. They were used for therapeutic purposes and for 'debulking' of tumors occluding the lumen of large airways [14,15]. More recently, cryoprobes have been used to obtain lung tissue [16]. The cryosurgical equipment operates by the Joule–Thompson effect, which dictates that a compressed gas released at high flow rapidly expands and creates a very low temperature [17]. The cooling agent is applied under high pressure (45 bar) through the central canal of the probe (Erbokryo CA, ERBE, Tubingen, Germany). Carbon dioxide (CO_2) is the cooling agent commonly used. The gas at the tip expands due to the sudden difference in pressure relative to the atmospheric pressure, resulting in a drop in temperature at the tip of the probe (in tissue, approximately –50 to 60 °C). Weight and diameter of cryobiopsies correlate positively with longer activation times and larger diameters of the cryoprobe. Transbronchial cryobiopsies of lung tissue (TBCLB) are carried out during flexible bronchoscopy. The majority of authors, however, intubate patients either with a rigid tube or with flexible orotracheal tubes and obviously deeply sedated them with intravenous propofol with or without remifentanil. Spontaneous breathing is maintained during the whole procedure or, if patients are paralyzed by the use of nondepolarizing blocking agents, jet ventilation is used. Oxygen saturation, blood pressure, ECG and transcutaneous carbon dioxide partial pressure are monitored continuously. A bronchial blocker (Fogarty balloon or other balloons) is usually positioned at the entrance of the preselected segmental or lobar bronchus and always insufflated after each biopsy in order to control major bleeding [18,19]. A minority of authors do not intubate patients and do not use bronchial blockers [20]. The cryoprobe is introduced into the selected area under fluoroscopic guidance via flexible bronchoscope. A distance of approximately 10–20 mm from the thoracic wall and a perpendicular relation between the thoracic wall and the probe are considered optimal. Once brought into position, the larger probe (2.4 mm) is cooled for approximately 3–6 s, whereas when a smaller probe is used the freezing time is longer (up to 7–8 s). The frozen tissue attached to the probe's tip is removed by pulling the cryoprobe together with the bronchoscope. The frozen specimen is thawed in physiological saline and fixed in formalin. The number of biopsies taken is usually 3–6. A chest radiograph or an ultrasonographic evaluation after the procedure is performed

when a pneumothorax is clinically suspected (pain and oxygen need) or routinely 3 h after the procedure.

TRANSBRONCHIAL CRYOBIOPSY IN DIFFUSE PARENCHYMAL LUNG DISEASE

In 2009, Babiak *et al.* [16] reported 41 patients with diffuse lung disease evaluated with transbronchial lung cryobiopsy (TBLCB). In this study, the size of the specimens retrieved by cryoprobes was significantly larger than that observed in specimens obtained by flexible forceps (11.11 mm^2 compared with 5.82 mm^2). Pneumothorax was observed in two patients (4.87%) that resolved after tube drainage, and biopsy-associated bleeding did not require any intervention. Pajares *et al.* [21] described their experience in 10 patients with interstitial lung disease (ILD) who were suitable for TBB. No major complications were reported. After these first experiences focusing on safety and feasibility of the procedure, studies investigating the clinical role of this approach appeared. Kropski *et al.* [22] published a retrospective study of patients who had undergone bronchoscopic cryobiopsy for evaluation of diffuse parenchymal lung disease at an academic tertiary care center. Twenty-five eligible patients were identified. With a mean area of 64.2 mm^2, cryobiopsies were larger than that typically encountered with traditional transbronchial forceps biopsy. In 19 of the 25 patients, a specific diagnosis was obtained. The overall diagnostic yield was 80%. The most frequent diagnosis was UIP ($n=7$). Poletti's group [23] prospectively studied 69 cases of fibrotic diffuse parenchymal lung disease with nondiagnostic high-resolution computed tomography (HRCT) features using TBCLB. Patients were at least 18 years old with a forced vital capacity (FVC) higher than 50% of predicted value, a diffusing capacity for carbon monoxide higher than 35% of predicted value and a pulmonary systolic arterial pressure estimated by echocardiography less than 40 mmHg. Exclusion criteria included coagulopathy (platelets $<70\,000 \times 10^9$/l, prothrombin time international normalized ratio>1.5 was considered an exclusion criteria), forced expiratory volume in the first second less than 0.8 l, diffuse bullous disease, hemodynamic instability and severe hypoxemia (PaO_2 <55 mmHg on room air). A flexible cryoprobe of 2.4 mm in diameter was used and the probe was cooled with carbon dioxide (CO_2) for 5–6 s. Biopsies were always obtained in the more affected areas and in the lower lobes and always in the same segment in the vast majority of cases. Adequate cryobiopsies were obtained in 68 cases (99%). The median size of cryobiopsies was 43.11 mm^2 (range, 11.94–76.25) (Fig. 1). Among 68 cases with adequate TBCLB samples, the pathologists were confident ('high confidence') that histopathologic criteria defined a specific pattern in 52 patients (76%), including 36 of 47 with UIP (77%). Agreement between the pathologists in the detection of UIP was very good with a kappa coefficient of 0.83 [95% confidence interval (CI), 0.69–0.97]. Fruchter *et al.* [24] described the diagnostic role of TBCLB performed in 75 patients (mean age 56.2 years) with clinical and radiological features suggestive of ILD. Patients were only sedated using midazolam and not intubated. The mean cross-sectional area of the biopsy specimen obtained was 9 mm^2 with an average of 70% alveolated tissue. The most common pathological diagnoses were idiopathic nonspecific interstitial pneumonitis ($n=22$), cryptogenic-organizing pneumonia ($n=11$) and usual interstitial pneumonitis ($n=7$). There were three patients with pulmonary Langerhans cell histiocytosis and one patient with pulmonary lymphangioleiomyomatosis. A definite and probable clinical-pathological consensus diagnosis was possible in 70 and 28% of patients, respectively. In only 2% of patients, diagnosis could not be established. Fruchter *et al.* [25] reported their experience in 40 lung transplant patients (mean age, 58.3 years) who underwent TBLCB, either routine post-lung transplantation surveillance bronchoscopy ($n=27$), or clinically indicated bronchoscopy ($n=13$). During the procedure, two to three biopsy samples were taken. The mean diameter of the specimen taken by TBLCB was 10 mm^2 compared with only 2 mm^2 using regular forceps ($P<0.05$). The increased size and quality of biopsy samples in the study group translated to a significant increase in the percentage of alveolated tissue (65 vs. 34%, respectively, $P<0.05$) that enabled a clear histological detection of acute rejection ($n=4$), pneumonitis ($n=3$), diffuse alveolar damage ($n=1$) along with

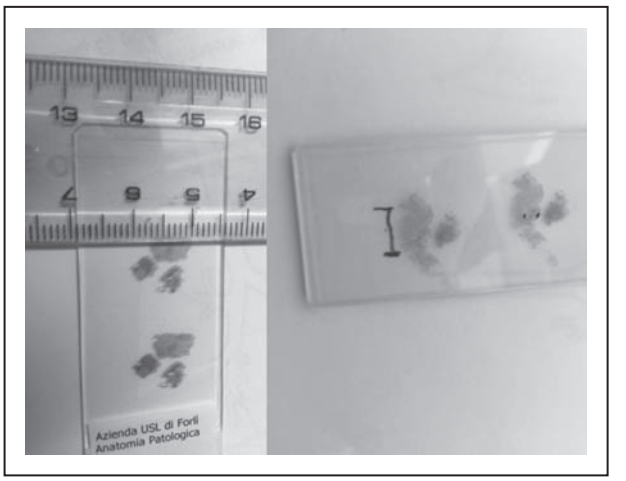

FIGURE 1. Transbronchial cryobiopsy samples. The largest diameter is almost 1 cm.

confident exclusion of acute rejection, infection or pneumonitis ($n = 32$). Yarmus *et al.* [26] presented the safety profile and biopsy results from 21 procedures in a pilot study comparing TBLCB with standard forceps TBB in patients after lung transplantation. Mean specimen size was significantly larger with the TBLCB technique compared with the standard forceps TBB: aggregate biopsy specimen size in the TBLCB group was 50 mm^2, whereas in the standard forceps TBB group the same measure was 12.5 mm^2. There was no clinical evidence of crush artifact on any TBLCB samples, whereas all standard forceps TBB samples had significant amounts of crush artifact evident on pathologic review. Roden *et al.* [27] recently showed that in patients investigated after lung transplant, cryobiopsies were larger and contained more alveoli ($P < 0.001$, both) and small airways ($P = 0.04$) compared with conventional TBB specimens. There was no significant difference between the types of biopsies with respect to the pathologists agreement on grades of rejection. Fruchter *et al.* [28] evaluated the efficacy and safety of TBLCB in immunocompromised patients with lung infiltrates. Most patients (11) were immunocompromised due to hematological malignancies. The mean surface area of the specimen taken by TBLCB was 9 mm^2. Alveolated tissue was observed in 70% of the samples. Diagnostic information obtained by TBLCB led to change in the management of 12 patients (80%). A systematic literature review showed that overall diagnostic yield, derived from 15 investigations including 793 patients, was 0.81 (0.75–0.87) [29▪▪].

TBLCB AND THE MULTIDISCIPLINARY SCENARIO

The role of transbronchial lung cryobiopsy in the context of the multidisciplinary discussion scenario has been addressed by two studies. Pajares *et al.* [30] showed that cryobiopsy provides more relevant information compared with conventional transbronchial lung biopsy (diagnostic yield of TBLCB 51.3 vs. 29.1% of conventional TBB with a slight not statistically significant increase in moderate/severe bleeding in the first group). To address the impact of TBLCB on diagnostic confidence in the multidisciplinary diagnosis of IPF, Tomassetti *et al.* [31▪▪] collected 117 patients with fibrotic ILDs without a typical usual interstitial pneumonia pattern on HRCT. All cases underwent lung biopsies: 58 TBLCB and 59 video-assisted thoracoscopy (VATS). Two clinicians, two radiologists and two pathologists sequentially reviewed clinical-radiological findings and biopsy results, recording at each step in the process their diagnostic impressions and confidence levels. A major increase in diagnostic confidence after the addition of TBLCB, similar to VATS (from 29 to 63%, $P = 0.0003$ and from 30 to 65%, $P = 0.0016$ of high confidence IPF diagnosis, in the TBLCB group and surgical lung biopsy group, respectively) was observed. The overall interobserver agreement in IPF diagnosis was similar for both approaches (TBLCB overall kappa 0.96; VATS overall kappa 0.93). IPF was the most frequent diagnosis (50 and 39% in the TBLCB and VATS group, respectively; $P = 0.23$). After the addition of histopathologic information, 17% of cases in the TBLCB group and 19% of cases in the VATS group, mostly idiopathic nonspecific interstitial pneumonia and hypersensitivity pneumonitis, were reclassified as IPF. In conclusion, TBLCB was shown to have a meaningful impact on diagnostic confidence in the multidisciplinary diagnosis of ILDs, and might be a valid surrogate of VATS in the diagnosis of IPF. This study provides a robust rationale for future studies investigating the diagnostic accuracy of TBLCB compared with surgical lung biopsy. This study showed that information provided by cryo-TBB has the same impact of information provided by surgical lung biopsy in defining the confidence of the diagnosis of IPF in a multidisciplinary diagnostic context.

TBLCB AND ADVERSE EVENTS

Ravaglia *et al.* [29▪▪] compared the adverse events observed in 150 patients submitted to VATS with 297 patients submitted to TBCLB for diffuse parenchymal lung disease. Median time of hospitalization was 6.1 days after VATS and 2.6 days after TBLCB ($P < 0.0001$); 16 patients were discharged home the same day of the procedure. Mortality due to adverse event after VATS was observed in four patients (2.7% of total); death occurred within 60 days and was caused by acute exacerbation of IPF in all cases. Prolonged air leak was treated with prolonged chest tube drainage in three of five patients; the remaining two patients were treated with blood patch and in one case also with surgical revision. In the TBLCB group, only one patient died after 7 days (0.3% of total) with acute exacerbation of IPF. Pneumothorax was the most common complication after cryobiopsy, occurring in 60 patients (20.2%), 46 cases (15.5% of total) requiring drainage. No patients needed intervention to control bleeding and there were no cases with persistent fever or pneumonia/empyema. Other complications were transient respiratory failure (two patients, 0.7%) and neurologic manifestations (seizures in two patients, 0.7%). In the VATS group, lung function was more compromised in patients who

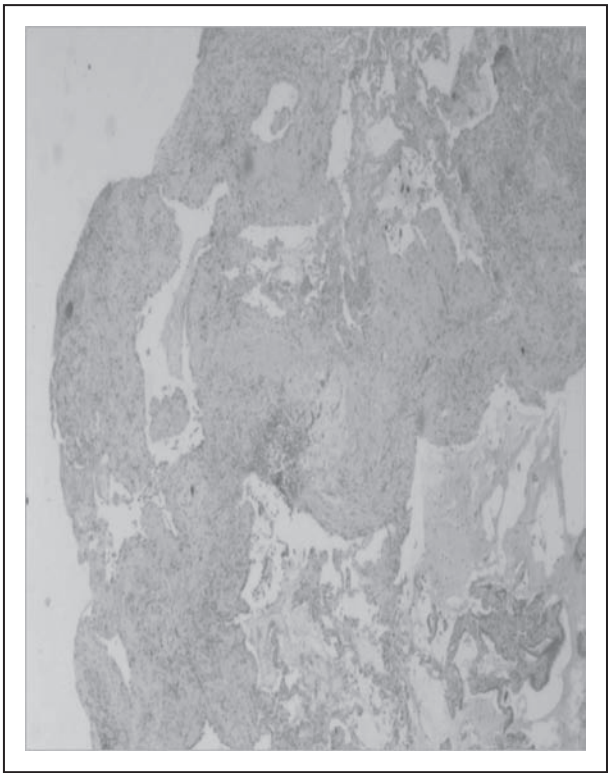

FIGURE 2. Typical aspects of usual interstitial pneumonia pattern: patchy fibrosis, fibroblastic foci and microhoneycombing, in the right lower corner (H&E, low power).

developed subsequent complications (FVC 70.2% ± 22.0 vs. 82.0% ± 21.2%, $P = 0.029$), while in the cryo group, lung function was found to be not related to the occurrence of these complications. The same authors made a systematic review of data published on adverse events observed after TBLCB for diffuse parenchymal lung disease. Data were retrieved from 15 studies including 994 patients. Overall, 100 pneumothoraces (10%), four transient respiratory failures (0.4%), two episodes of convulsions (0.2%), one death (0.1%), one acute exacerbation (0.1%) and one prolonged air leak (0.1%) were reported. Out of 100 pneumothoraces, 70 required chest tube drainage. The overall pooled probability of developing a pneumothorax was 0.06 (CI 0.02–0.11), whereas the probability of developing a pneumothorax requiring chest tube drainage was 0.03 (CI 0.01–0.08). The pooled proportions of pneumothorax according to procedural aspects, in terms of type of sedation and airways control, were 0.07 (CI 0.02–0.14) from studies with patients undergoing the procedure intubated under deep sedation (11 studies including 613 patients) and 0.01 (CI 0.00–0.06) from studies with patients not intubated under conscious sedation (three studies including 367 patients). No episodes of severe bleeding were reported. The overall pooled probability of developing a moderate bleeding from 12 studies including 383 patients was 0.12 (CI 0.02–0.25).

In conclusion, TBLCB seems to be a procedure with significant lower incidence of adverse events compared with VATS; it may also be performed in elderly and in patients with lung function impairment worsen than that accepted for VATS. Pneumothorax is the most frequent event observed. Factors associated with PNX occurrence are not yet clearly documented even if UIP pattern on histology, computed tomography fibrotic score, biopsies performed nearer to the pleura and skill level seem to be important predicting factors.

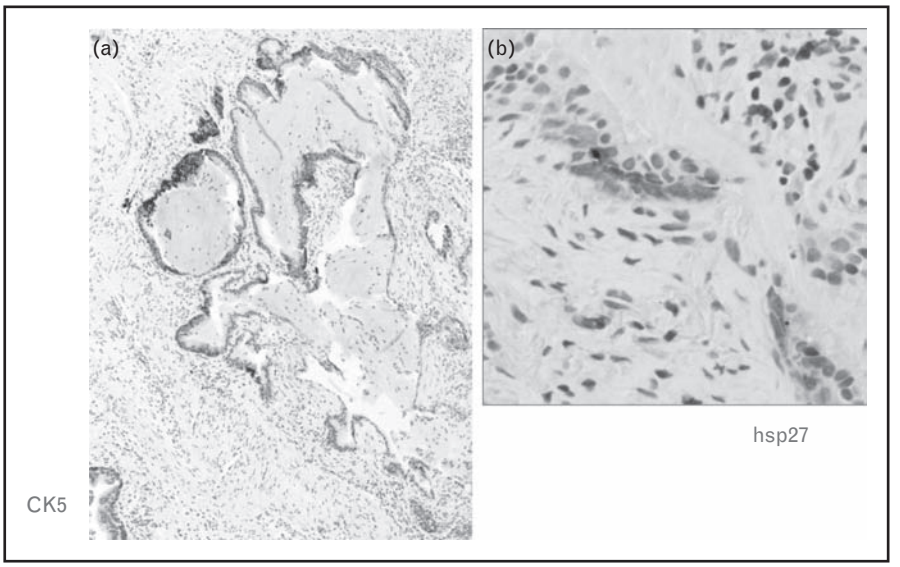

FIGURE 3. Usual interstitial pneumonia pattern. Immunohistochemical investigations showing positivity for cytokeratin 5 of the epithelial cells lining cysts of microhoneycombing (a), and heat shock proteins in the basal cells part of a fibroblastic focus (b).

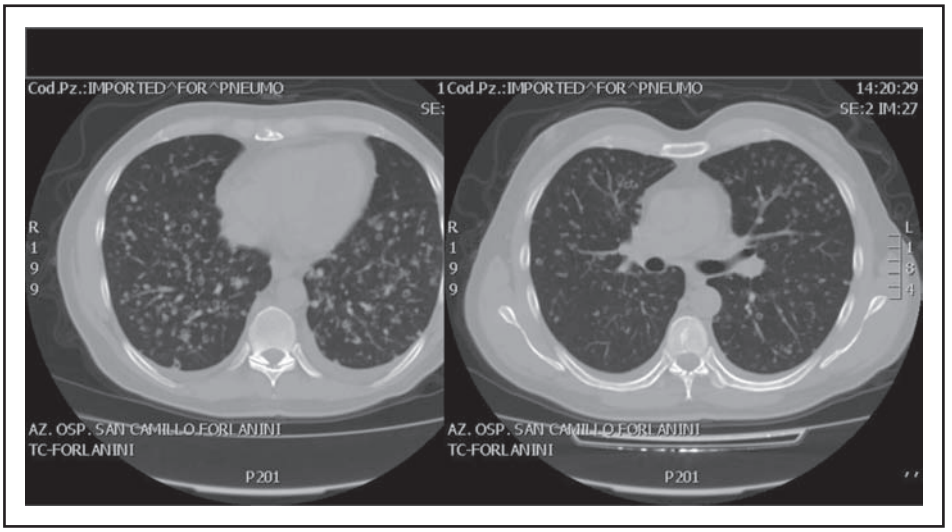

FIGURE 4. Fifty-two-year-old man: allogeneic bone marrow transplant for chronic myeloid leukemia 10 years before. HRCT: 'Cheerios in the lung'.

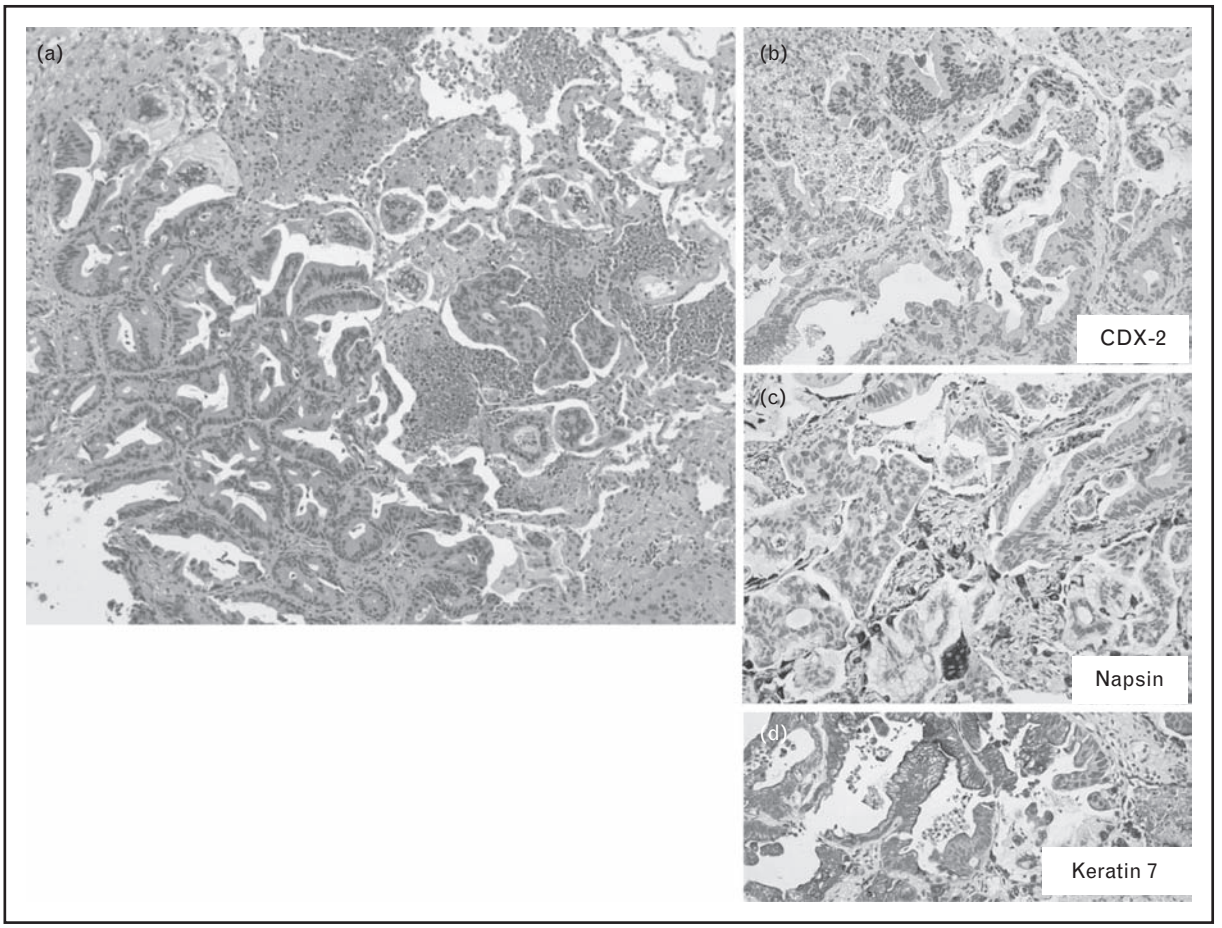

FIGURE 5. (a) Adenocarcinoma: enteric phenotype. The neoplastic cells express (b) CDX-2, (c) Napsin, and (d) keratin 7.

CONCLUSION

TBLCB is a method that allows to obtain lung parenchyma samples large enough for identification of complex morphological patterns such as UIP [18,19], nonspecific interstitial pneumonia, desquamative interstitial pneumonia [32], neuroendocrine cell hyperplasia with associated acellular bronchiolitis and lymphoproliferative disorders [33,34]. Immunohistochemical and molecular biology investigations may be successfully completed in samples obtained by this method. The most frequent complication related to the procedure is pneumothorax although life-threatening bleeding and acute exacerbation of the underlying interstitial lung disease have been reported; however, these potentially fatal complications have been reported only in a few cases and the balance between diagnostic yield/accuracy and complications is definitely better than that observed using surgical lung biopsy [29▪▪]. The modalities by which this procedure is carried out vary (as reported in the literature since 2009) [18,32] and a standardization of the procedure is deemed necessary [35]. This technique could represent a valid surrogate to surgical lung biopsy in diffuse parenchymal lung diseases in the near future, in particular if new investigative technique will be applied to samples so recruited [36▪▪,37,38] (Figs 2–5).

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Conflicts of interest

There are no conflicts of interest.

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