Diagnostic accuracy of transbronchial lung cryobiopsy for interstitial lung disease diagnosis (COLDICE): a prospective, comparative study

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Summary

Background Transbronchial lung cryobiopsy (TBLC) is a novel technique for sampling lung tissue for interstitial lung disease diagnosis. The aim of this study was to establish the diagnostic accuracy of TBLC compared with surgical lung biopsy (SLB), in the context of increasing use of TBLC in clinical practice as a less invasive biopsy technique.

Methods COLDICE was a prospective, multicentre, diagnostic accuracy study investigating diagnostic agreement between TBLC and SLB, across nine Australian tertiary hospitals. Patients with interstitial lung disease aged between 18 and 80 years were eligible for inclusion if they required histopathological evaluation to aid diagnosis, after detailed baseline evaluation. After screening at a centralised multidisciplinary discussion (MDD), patients with interstitial lung disease referred for lung biopsy underwent sequential TBLC and SLB under one anaesthetic. Each tissue sample was assigned a number between 1 and 130, allocated in a computer-generated random sequence. Encoded biopsy samples were then analysed by masked pathologists. At subsequent MDD, de-identified cases were discussed twice with either TBLC or SLB along with clinical and radiological data, in random non-consecutive order. Co-primary endpoints were agreement of histopathological features in TBLC and SLB for patterns of definite or probable usual interstitial pneumonia, indeterminate for usual interstitial pneumonia, and alternative diagnosis; and for agreement of consensus clinical diagnosis using TBLC and SLB at MDD. Concordance and κ values were calculated for each primary endpoint. This study is registered with the Australian New Zealand Clinical Trials Registry, ACTRN12615000718549.

Findings Between March 15, 2016, and April 15, 2019, we enrolled 65 patients (31 [48%] men, 34 [52%] women; mean age 66·1 years [SD 9·3]; forced vital capacity 83·7% [SD 14·2]; diffusing capacity for carbon monoxide 63·4% [SD 12·8]). TBLC (7·1 mm, SD 1·9) and SLB (46·5 mm, 14·9) samples were each taken from two separate ipsilateral lobes. Histopathological agreement between TBLC and SLB was 70·8% (weighted κ 0·70, 95% CI 0·55–0·86); diagnostic agreement at MDD was 76·9% (κ 0·62, 0·47–0·78). For TBLC with high or definite diagnostic confidence at MDD (39 [60%] of 65 cases), 37 (95%) were concordant with SLB diagnoses. In the 26 (40%) of 65 cases with low-confidence or unclassifiable TBLC diagnoses, SLB reclassified six (23%) to alternative high-confidence or definite MDD diagnoses. Mild-moderate airway bleeding occurred in 14 (22%) patients due to TBLC. The 90-day mortality was 2% (one of 65 patients), following acute exacerbation of idiopathic pulmonary fibrosis.

Interpretation High levels of agreement between TBLC and SLB for both histopathological interpretation and MDD diagnoses were shown. The TBLC MDD diagnoses made with high confidence were particularly reliable, showing excellent concordance with SLB MDD diagnoses. These data support the clinical utility of TBLC in interstitial lung disease diagnostic algorithms. Further studies investigating the safety profile of TBLC are needed.

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Introduction

Interstitial lung diseases comprise a heterogeneous group of conditions with variable natural history and treatment response. Through the integration of comprehensive clinical, serological, and radiological data within a multidisciplinary discussion (MDD), an underlying interstitial lung disease subtype can usually be identified.[1–3] In particular, the high-resolution CT scan can provide detailed information on the probable disease pattern, with specific clinical context allowing for refinement of potential differential diagnoses. In up to 30% of cases, however, the high-resolution CT and clinical findings are not sufficient to allow for confident clinical diagnosis, requiring a surgical lung biopsy (SLB) for histopathological evaluation.[4] The current accepted standard practice for obtaining SLB is through video-assisted thoracoscopic

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See Online for appendix

Research in context

Evidence before this study

Transbronchial lung cryobiopsy (TBLC) is an emerging technique to obtain lung tissue for diagnosis of interstitial lung disease. As a minimally invasive technique, TBLC has been adopted into clinical practice in many centres; however, it requires validation as an accurate diagnostic test. Due to an absence of high-quality evidence, diagnostic guidelines for idiopathic pulmonary fibrosis do not recommend for or against TBLC in its diagnostic algorithms. The safety of TBLC has been raised as a potential concern, with varying amounts of reported cases of pneumothorax and airway bleeding. Furthermore, the diagnostic accuracy of TBLC has not been verified in adequately powered prospective studies comparing TBLC against the accepted histopathological standard of surgical lung biopsy (SLB). We searched PubMed using the terms "cryobiopsy" or "cryoprobe" and "interstitial lung disease" or "diffuse parenchymal lung disease" or "pulmonary fibrosis", for all clinical trials published from database inception up until July 8, 2019, with no language restrictions. Most of the publications were retrospective single-centre case series. The four systematic reviews of TBLC reported data for diagnostic yield and safety, but none assessed diagnostic accuracy. We identified only one small study that directly compared TBLC and SLB sampled sequentially from the same patients. The study retrospectively used a single pathologist to analyse the histopathological patterns after initial unmasked assessment and multidisciplinary discussion for clinical diagnosis, showing low concordance between the findings of the two forms of biopsy. Because of the very small sample size and other methodological limitations of this study, the issue of diagnostic accuracy of TBLC in interstitial lung disease diagnosis remains unresolved.

Added value of this study

The COLDICE study was a prospective, multicentre, investigator-initiated study designed to evaluate diagnostic accuracy of TBLC in interstitial lung disease diagnosis. The study was adequately powered to compare diagnostic agreement between TBLC and SLB obtained from the same patients at the same time from the same lobes, for both masked histopathological analysis, and for clinical diagnosis at multidisciplinary discussion. The study showed high concordance between the paired biopsy specimens for both histopathological pattern and multidisciplinary discussion diagnosis. The data from TBLC specimens were informative and reliable, particularly when high-confidence patterns were reported by the pathologist. Although our study was not designed to address the true safety aspects of TBLC independent of SLB, we did not find any new safety signals.

Implications of all the available evidence

To our knowledge, the COLDICE study is the first comparative study showing a high agreement between TBLC and SLB for interstitial lung disease diagnosis. Together with the data from case series, the evidence suggests that TBLC is a valid first-line diagnostic tool for patients with interstitial lung disease deemed to require histopathological diagnosis. Although further studies of a similar design would enrich the existing data, we appreciate that larger studies will be difficult to do in the clinical setting. Studies focusing on safety and standardisation of the TBLC procedure will be important adjuncts to clinical practice.

surgery (VATS), done by a thoracic surgeon in the operating room under general anaesthesia. This procedure poses substantial risks, including a reported 1·7% mortality in patients subjected to elective SLB.[5] Although this mortality risk might be lower at centres with staff experienced in SLB, the decision to proceed with invasive biopsy should not be undertaken lightly. Poor cardiopulmonary reserve, advancing age, and comorbid disease often render patients with interstitial lung disease unsuitable candidates for SLB. Thus, diagnostic uncertainty will remain in a substantial proportion of patients with interstitial lung disease. For these patients requiring histopathological assessment, the benefit of obtaining the lung biopsy with a less invasive procedure than SLB is evident.

Transbronchial lung cryobiopsy (TBLC) has emerged over the past decade as an alternative diagnostic technique, with increasing use across many centres. The diagnostic yield, accuracy, potential safety, and health resource use advantages of TBLC over SLB are important considerations that need to be addressed in well designed studies. Before widespread implementation of TBLC can take place, it is necessary to directly compare histopathological interpretation of this small tissue sampling with larger SLB specimens obtained from the same patients. Although it is apparent that the diagnostic yield of TBLC is lower than that of SLB, TBLC might be established as a potentially safer, but reliable substitute if accuracy can be shown. To date, the literature reports a diagnostic yield for an identifiable histopathological pattern in 73–81% of TBLC specimens, compared with around 95% for SLB specimens.[6–8] Although the TBLC yield is less than that of SLB, there is evidence to suggest a similar clinical utility, at a lower risk to the patient. For example, TBLC has been shown to affect diagnostic confidence to a similar degree as SLB, within the context of MDD.[9] The diagnostic accuracy of TBLC, however, has not been addressed in a robust manner. This important issue can only be assessed through the direct verification of TBLC findings against SLB specimens, obtained from the same anatomical sites, from the same patients. Romagnoli and colleagues[10] attempted to address this issue in a small interstitial lung disease cohort, observing poor agreement for TBLC and SLB histopathological analyses with a κ-concordance coefficient of only 0·22. Because of methodological

limitations, including underpowering and the use of a single pathologist review after MDD diagnosis, these findings should be interpreted with caution. In the absence of rigorous direct comparison with the SLB, the role of TBLC in interstitial lung disease diagnostic algorithms remains unclear. The increasing dichotomy between the European and North American perceived role of TBLC (ie, generally, the technique has been used more in Europe than in North America), highlights the urgent need to settle the issue of the clinical utility of TBLC for interstitial lung disease diagnosis.[11,12] We therefore did the cryobiopsy versus open lung biopsy in the diagnosis of interstitial lung disease alliance (COLDICE) study, designed to evaluate the agreement between TBLC and SLB as a means of assessing diagnostic accuracy, at both histopathological assessment and at MDD.

Methods

Study design and participants

The COLDICE study was a comparative, multicentre, prospective, investigator-initiated, diagnostic accuracy study done across nine Australian tertiary hospitals with interventional pulmonology and interstitial lung disease expertise. Eligible patients were aged between 18 and 80 years, requiring lung biopsy to support their interstitial lung disease diagnosis, were able to give informed consent, and were without contraindications for lung biopsy. Key exclusion criteria were hypoxaemia while breathing room air (SpO_2 <90%), diffusing capacity for carbon monoxide less than 40% predicted, total lung capacity less than 50% predicted, excessive or uncorrectable bleeding risk, body-mass index more than 40 kg/m², pulmonary hypertension (with estimated right ventricular systolic pressure >40 mm Hg or signs of right ventricular dysfunction on echocardiogram), or advanced comorbid conditions (appendix p 1). Following baseline assessment by referring specialists, all potential study candidates were screened through a centralised MDD. Participants were enrolled into the study if the central MDD deemed that a lung biopsy was indicated. Detailed clinical data including physician-verified history of exposures, connective tissue disease symptoms, disease severity indices, and serology were presented in conjunction with standard high-resolution CT imaging (appendix p 1).

Participants gave fully informed written consent. All study-related activities followed the International Conference on Harmonisation Guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. The study protocol followed the STARD guidelines for assessment of diagnostic accuracy. Ethics approval of the trial protocol was provided by an independent ethics committee at each participating site. Both the steering committee and data and safety monitoring board provided trial oversight, including contemporaneous review of all safety events.

Procedures

Both TBLC and SLB were done sequentially under a single general anaesthetic. Detailed procedural protocols are included in the appendix (p 2) and have previously been published.[13] In brief, for TBLC, patients were intubated with either a rigid bronchoscope or a flexible endotracheal tube. A 1·9 mm or 2·4 mm cryoprobe (Erbe Elektromedizin, Tübingen, Germany) was inserted through the working channel of a therapeutic bronchoscope and advanced under fluoroscopic guidance to a subpleural location and activated for 3–7 s. The cryoprobe, with attached lung parenchyma, and flexible bronchoscope were then removed en bloc and the sample placed into formalin. Prophylactic endobronchial balloon blockers were placed in the targeted airways, and inflated after each TBLC until haemostasis was achieved. Between four and seven specimens were obtained from two separate, ipsilateral lobes. At TBLC completion, severity of bleeding was recorded (following the grading defined by Hetzel and colleagues[14] included in appendix p 2), and thoracic ultrasound or fluoroscopy was done to assess for pneumothorax.[14] Following TBLC, two SLB (from the same lobes corresponding to those sampled by TBLC) were done by a thoracic surgeon using VATS with ventilation via a double-lumen endotracheal tube. Patients were managed with standard post-operative practices for VATS, with ongoing assessment according to clinical need. For study purposes, vital status and postoperative complications were ascertained at 6 weeks and 3 months after the procedure, and at study completion. Lung function test measurements were done at 6 months after the procedure.

Each set of slides for the TBLC and SLB tissue samples were labelled with randomly generated, de-identifying code numbers of 1–130, unrelated to the other specimen slides from the same patient. Three masked expert pathologists reviewed all 130 sets of slides, recording individual interpretation and then consensus agreement for: (1) international guideline-directed histopathological categories (definite usual interstitial pneumonia, probable usual interstitial pneumonia, indeterminate for usual interstitial pneumonia, and alternative diagnosis);[1] and (2) specific interstitial lung disease histopathological patterns, including usual interstitial pneumonia-idiopathic pulmonary fibrosis, non-specific interstitial pneumonia, organising pneumonia, usual interstitial pneumonia associated with connective tissue disease-interstitial lung disease, lymphocytic interstitial pneumonia, hypersensitivity pneumonitis, respiratory bronchiolitis-interstitial lung disease and desquamative interstitial pneumonia, sarcoidosis, lymphangioleiomyomatosis, miscellaneous, and unclassifiable disease. Pathologists' confidence in their findings (high, intermediate, or low) were also recorded.

Following completion of recruitment, a centralised MDD team comprised of expert pathologists, radiologists, and clinicians convened to consider each tissue sample in combination with clinical details and radiology

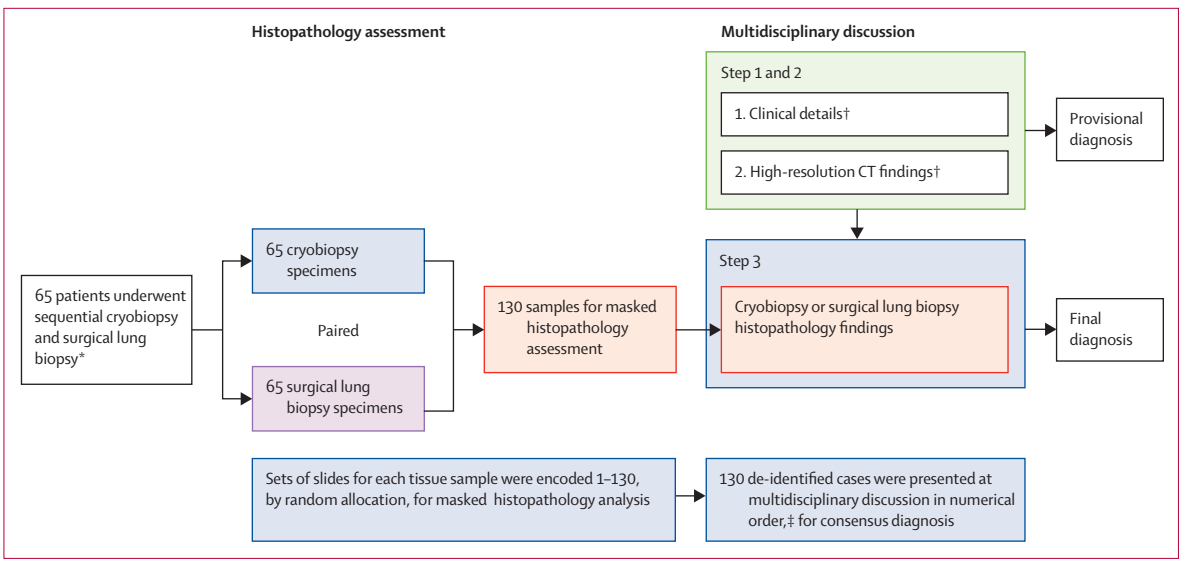

Figure 1: **Study profile**
*Surgical lung biopsies done immediately after the transbronchial lung cryobiopsies; paired samples were taken from two corresponding lobes of the ipsilateral lung. †All clinical details and high-resolution CT images were duplicated and presented twice under separate code numbers, along with either transbronchial lung cryobiopsies or surgical lung biopsy histopathology. ‡Cases were presented in numerical order, except in a minority in which paired cases fell within ten presentations of one another.

(figure 1). Each de-identified case (numbered 1–130) was presented in a standardised fashion in three steps (step one was clinical data; step two was high-resolution CT findings; and step three was consensus histopathological interpretation of either TBLC or SLB). After step two, MDD participants recorded their individual diagnosis, then convened to reach a consensus provisional diagnosis and diagnostic confidence level (definite [90–100% confident], high [70–89% confident], or low [51–69% confident]).[15] Unclassifiable cases were not assigned diagnostic confidence levels. Following step three, participants again recorded diagnoses and confidence levels individually, and then reached a final consensus diagnosis and diagnostic confidence level. For step three, participants were masked to the nature of the biopsy, through careful concealment of the dimensions of the specimen by the pathologist. Paired cases were not linked in any way during the process.

Outcomes

Co-primary endpoints were: (1) agreement of histopathological interpretation between TBLC and SLB for the 2018 guideline-refined categories of definite or probable usual interstitial pneumonia, indeterminate for usual interstitial pneumonia, or alternative diagnosis; and (2) agreement between final consensus clinical-radiological-pathological diagnoses for matched TBLC and SLB specimens at MDD. Prespecified key secondary endpoints comprised agreement between the specific histopathological patterns, (eg, usual interstitial pneumonia, non-specific interstitial pneumonia, hypersensitivity pneumonitis) identified by pathologists for TBLC and SLB; interobserver agreement between the three pathologists for each biopsy technique for both guideline-refined interpretation and specific histopathological patterns; proportions of TBLC and SLB cases where biopsy led to a change in diagnostic confidence from low to high or definite; or an unanticipated diagnosis, (eg, from unclassifiable to respiratory bronchiolitis-interstitial lung disease or idiopathic pulmonary fibrosis); and procedural features predictive of diagnostic agreement.

Statistical analysis

A calculated sample size of 65 would enable estimation of a true κ of at least 0·8 with a lower 95% confidence limit of at least 0·6, for histopathological agreement between TBLC and SLB for the guideline-refined categories of definite or probable for usual interstitial pneumonia pattern, indeterminate for usual interstitial pneumonia pattern, or alternative diagnosis. This calculation followed the assumption that of cases in this population, 55% would be classified as definite or probable usual interstitial pneumonia, 15% as indeterminate for usual interstitial pneumonia, and 30% as an alternative diagnosis. These estimates were based on prevalence data within a specialist interstitial lung disease clinic from one of the participating sites.

Agreement between TBLC and SLB for MDD clinical diagnoses was assessed using simple κ statistics, presented with 95% CIs. For agreement between TBLC and SLB guideline-directed histopathological patterns, weighted κ values using Fleiss-Cohen weights were calculated. Fleiss κ statistics were used for the agreement among all three raters for each biopsy type. McNemar's test was used to assess change in proportions of unclassifiable cases before

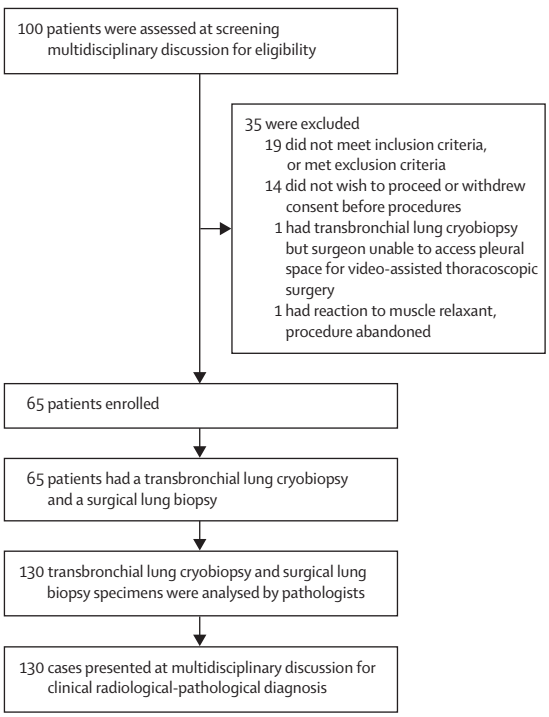

Figure 2: **Enrolment and study protocol**

	Value
Sex	
Women	34 (52%)
Men	31 (48%)
Age, years	
Mean	66·1 (9·3)
Range	32–79
Body-mass index, kg/m²	28·8 (4·2)
Smoking status	
Never	26 (40%)
Former	37 (57%)
Current	2 (3%)
Lung function measurements	
Forced vital capacity, % of predicted value	83·7 (SD 14·2)
DLCO, % of predicted value	63·4 (SD 12·8)
6-min walk test (n=30)	
Distance, m	458·1 (119·1)
Nadir SpO_2, %	91·9 (SD 5·2)
Exposure history*	
Domestic	22 (34%)
Occupational	18 (28%)
Iatrogenic	2 (3%)
Ancillary clinical features	
Family history of ILD	9 (14%)
Positive serum autoantibodies†	12 (19%)
Connective tissue disease features‡	8 (12%)
Comorbid conditions	
Gastro-oesophageal reflux disease	23 (35%)
Hypertension	17 (26%)
Cardiac disease	14 (22%)
Airways disease	11 (17%)
Previous malignancy	7 (11%)
Obstructive sleep apnoea	6 (9%)

Data are n (%) or mean (SD), unless otherwise stated. DLCO=diffusion capacity of the lungs for carbon monoxide. SpO_2=oxygen saturation of peripheral blood. ILD=interstitial lung disease. *Exposures included birds, mould, hay, rural organic dusts, chemicals of warfare, welding, and occupational dusts (eg, asbestos, silica, coal). †Autoantibodies included anti-nuclear antibody titres of 320 or more of any pattern or any titre with nucleolar pattern, extractable nuclear antibodies, rheumatoid factor 2 or more times the upper limit normal, anti-cyclic citrullinated protein antibodies, myositis antibodies, and anti-double stranded DNA antibodies.[16] ‡Connective tissue disease features included sicca symptoms, Raynaud phenomenon, morning joint stiffness, and myalgias, in the absence of a definable connective tissue disease syndrome.

Table 1: **Baseline characteristics of patient population**

and after the addition of histopathological assessment at MDD (with separate tests done for the two procedure types). χ^2 tests and *t* tests were used to investigate clinical, procedural, and histopathological characteristics that were associated with agreement between diagnostic methods.

A p value of less than 0·05 was considered statistically significant. A κ value equal to or less than 0·20 indicated poor agreement; 0·21–0·40 indicated fair agreement; 0·41–0·60 indicated moderate agreement; 0·61–0·80 indicated good agreement; and 0·81–1·00 indicated excellent agreement. Statistical analyses were done using SAS version 9.4.

This study is registered with the Australian New Zealand Clinical Trials Registry, ACTRN12615000718549.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Data collection, analysis, and interpretation were done solely by the authors. All listed authors contributed to the writing of the manuscript and take responsibility for the integrity of the data presented herein. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 15, 2016, and April 15, 2019, of 100 patients assessed for eligibility, we recruited 65 patients (31 [48%] men, 34 [52%] women; mean age 66·1 years [SD 9·3]; figure 2). Baseline characteristics are shown in table 1. All patients had a low-confidence diagnosis or unclassifiable interstitial lung disease at initial screening. Patients generally had mild to moderate impairment of lung function tests. No patients had a pre-existing diagnosis of connective tissue disease that would typically be associated with interstitial lung disease. During the sequential procedures, the median total number of TBLC samples was five (range 3–7), taken from two separate lobes.

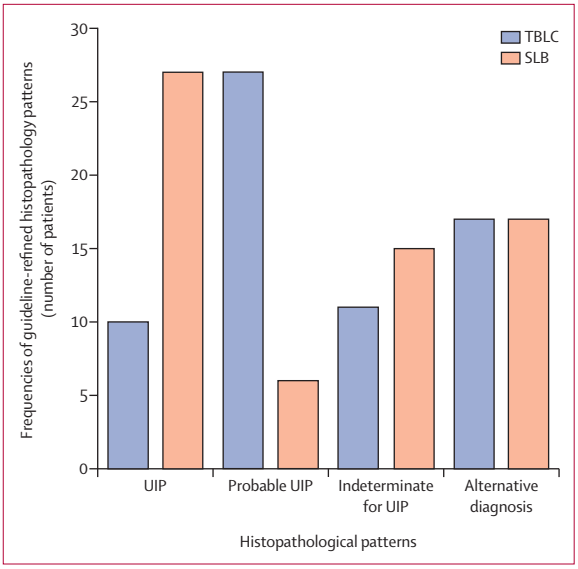

Figure 3: **Guideline-refined histopathological patterns**
UIP=usual interstitial pneumonia. TBLC=transbronchial lung cryobiopsy. SLB=surgical lung biopsy.

	Transbronchial lung cryobiopsy	Surgical lung biopsy
Histopathological patterns		
Usual interstitial pneumonia pattern consistent with idiopathic pulmonary fibrosis	41 (63%)	39 (60%)
Hypersensitivity pneumonitis	10 (15%)	15 (23%)
Sarcoidosis	3 (5%)	2 (3%)
Respiratory bronchiolitis-ILD or desquamative interstitial pneumonia	2 (3%)	2 (3%)
Non-specific interstitial pneumonia overlapping with organising pneumonia pattern	2 (3%)	2 (3%)
Usual interstitial pneumonia pattern consistent with connective tissue disease-ILD	0	2 (3%)
Unclassifiable	3 (5%)	1 (2%)
Non-diagnostic tissue	3 (5%)	1 (2%)
Non-ILD diagnosis*	1 (2%)	1 (2%)
Multidisciplinary discussion diagnoses		
Idiopathic pulmonary fibrosis	38 (58%)	35 (54%)
Hypersensitivity pneumonitis	15 (23%)	18 (28%)
Sarcoidosis	2 (3%)	2 (3%)
Smoking-related ILD†	1 (2%)	2 (3%)
Connective tissue disease-ILD‡	1 (2%)	2 (3%)
Lymphangioleiomyomatosis	1 (2%)	1 (2%)
Unclassifiable ILD	6 (9%)	3 (5%)
Non-ILD diagnosis*	1 (2%)	1 (2%)

Data are n (%). ILD=interstitial lung disease. *One patient had lepidic mucinous adenocarcinoma on both transbronchial lung cryobiopsy and surgical lung biopsy. †Smoking-related ILD comprised respiratory bronchiolitis-ILD or desquamative interstitial pneumonia pattern with an associated smoking history. ‡Connective tissue disease-ILD comprised Usual interstitial pneumonia pattern consistent with connective tissue disease-ILD or Non-specific interstitial pneumonia overlapping with organising pneumonia patterns with associated clinical or serological features of connective tissue disease. Notably, no patients had a definable connective tissue disease syndrome.

Table 2: **Histopathological patterns and multidisciplinary discussion diagnoses for specimens**

38 (59%) of 65 were done via rigid bronchoscopy. In most cases, the 1·9 mm cryoprobe was used (62 [95%] of 65). A mean freeze time of 4·6 s (SD 0·7) was applied, yielding mean tissue samples of 7·1 mm (SD 1·9), by longest axis. Pleura was observed in seven (11%) of 65 TBLC samples. At VATS, two SLB specimens were obtained from each patient, with mean long axis of 46·5 mm (14·9). Median length of hospital stay was 4 days (IQR 3–4).

At consensus histopathological assessment, raw agreement between TBLC and SLB for guideline-refined patterns was 70·8%, with a weighted κ of 0·70 (95% CI 0·55–0·86). Frequencies of each category are shown in figure 3. Agreement for the specific histopathological pattern identified by pathologists for paired TBLC and SLB was 69·2%, with κ 0·47 (0·30–0·64).

For the MDD final diagnoses, raw agreement between TBLC and SLB was 76·9% with a κ of 0·62 (0·47–0·78). The spectrum of final MDD diagnoses and histopathological patterns for each biopsy type is shown in table 2 and figure 4. High confidence or definite final MDD diagnoses were reached in 39 (60%) of 65 TBLC cases and in 48 (74%) of 65 SLB cases (p=0·090). In those with high confidence or definite TBLC MDD diagnoses, there was concordance with the SLB MDD diagnosis in 37 (95%) of 39 cases, as shown in figure 5. In the 26 unclassifiable or low-confidence TBLC MDD diagnoses, six (23%) were reclassified into alternative high confidence or definite diagnoses by SLB. Within these six cases, TBLC MDD favoured idiopathic pulmonary fibrosis in three cases but SLB MDD favoured hypersensitivity pneumonitis with high confidence as the diagnosis (for the same three cases); hypersensitivity pneumonitis was favoured with TBLC, but SLB MDD favoured idiopathic pulmonary fibrosis in one case; and the other two cases were unclassifiable at TBLC, but yielded high confidence idiopathic non-specific interstitial pneumonia and respiratory bronchiolitis interstitial lung disease diagnoses with SLB. The histopathology and MDD findings of every participant, and clinical details of discordant cases are included in the appendix (pp 4–6).

Between step two (provisional diagnosis) and step three (final diagnosis) at MDD, the addition of biopsy information was deemed helpful if it changed the diagnosis from low to high confidence or definite, or provided an unanticipated diagnosis. This was the case in 48 (74%) of 65 TBLC samples and in 50 (77%) of 65 SLB samples (p=0·55). The majority of these changes were due to a change in diagnosis (usually from unclassifiable to a specific diagnosis), rather than a change in diagnostic confidence level. Of note, there were eight individuals (12%) in whom neither the TBLC or SLB provided any further diagnostic certainty.

No specific TBLC procedural variables (including freeze time, number or size of samples, or site of biopsy) were associated with agreement between TBLC and SLB (appendix p 7).

For guideline-refined histopathological patterns, interobserver agreement between the three pathologists was κ 0·53 (95% CI 0·43–0·63) for TBLC, compared with κ 0·64 (0·54–0·75) for SLB. For specific histopathological pattern interpretation, the interobserver agreement for each technique was similar (TBLC κ 0·52, 95% CI 0·44–0·60 *vs* SLB κ 0·50, 0·42–0·58; table 3).

Over the course of the study, 25 adverse and serious adverse events were recorded (table 4). Because of the study design, comparison of safety for each procedure was not possible. There were, however, some adverse events that could be attributed to TBLC, with 14 (22%) episodes of mild to moderate airway bleeding, and one pneumothorax immediately evident before VATS procedure. There were no cases of severe airway bleeding. Other procedure-related adverse events included chest wall wound infection, desaturation less than 90% during anaesthesia, intra-operative hypotension, and acute intraoperative bronchospasm. None of these events were associated with any long-term consequences for the patients. Included in the serious adverse events were two acute exacerbations of idiopathic pulmonary fibrosis (onset of each within 2 weeks of surgery). The 90-day mortality was 2% (one of 65 patients), including one of the patients with acute exacerbation, who died from respiratory failure 50 days after surgery. In 44 patients with 6-month follow-up lung function testing, mean forced vital capacity was 76·5% (SD 15·1) and diffusing capacity for carbon monoxide 61·0% (SD 11·6) of the predicted value. At study completion, 56 patients were alive and nine were deceased. Aside from the aforementioned death following acute exacerbation of idiopathic pulmonary fibrosis, only one other death occurred within 6 months of surgery. This death was due to cerebrovascular accident 4 months after the procedures. The remaining seven deaths were related to malignancy, progression of interstitial lung disease, and cardiovascular disease.

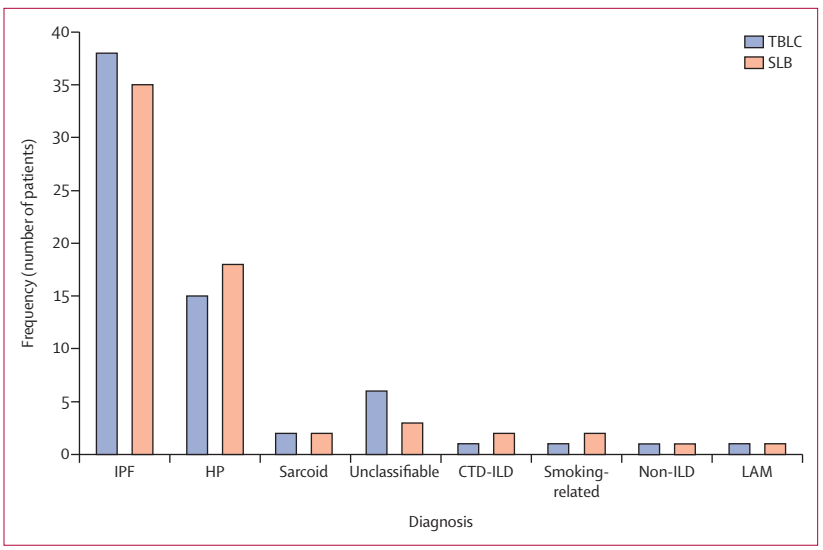

Figure 4: **Diagnoses from multidisciplinary discussion**
The Non-ILD diagnosis was adenocarcinoma of the lung, identified by both TBLC and SLB. TBLC=transbronchial lung cryobiopsy. SLB=surgical lung biopsy. IPF=idiopathic pulmonary fibrosis. HP=hypersensitivity pneumonitis. CTD-ILD=connective tissue disease-associated interstitial lung disease. LAM=lymphangioleiomyomatosis.

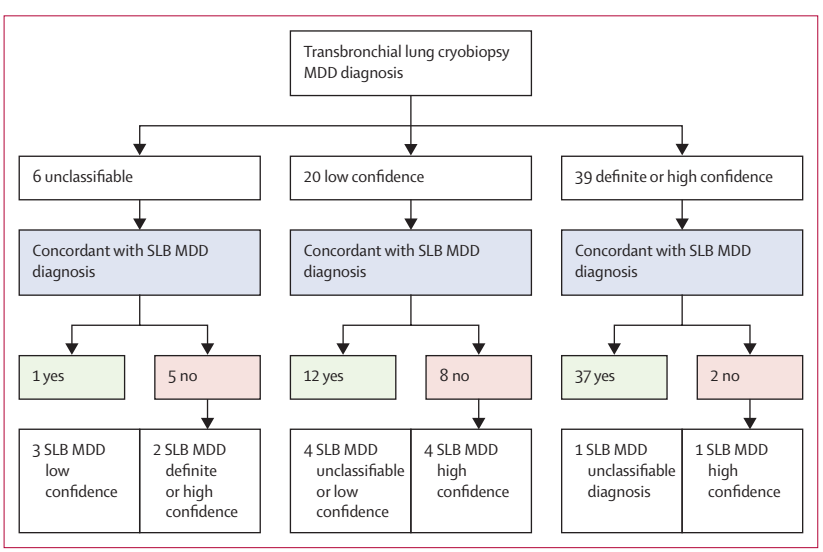

Figure 5: **MDD results for transbronchial lung cryobiopsy compared with surgical lung biopsy**
MDD=multidisciplinary discussion. SLB=surgical lung biopsy.

Discussion

Our prospective, multicentre study showed good agreement between TBLC and SLB obtained sequentially from the same patients, supporting the clinical utility of TBLC as an alternative to SLB for patients requiring lung tissue for interstitial lung disease diagnosis. This study is the largest to date, comparing the two techniques for the evaluation of diagnostic accuracy of TBLC. To our knowledge, this is the very first prospective study that shows good concordance for both the interpretation of histopathological pattern and consensus MDD diagnosis. Importantly, when high-confidence or definite TBLC MDD diagnoses were obtained, SLB added minimal diagnostic value. For unclassifiable or low confidence TBLC MDD diagnoses, although the agreement with SLB-MDD was lower, the SLB provided an alternative definite or high confidence diagnosis in only a minority. These results suggest that if a definite or high-confidence TBLC MDD diagnosis is made, SLB will provide limited additional information for the physician. The same holds true to a lesser extent in the setting of unclassifiable or low confidence TBLC MDD diagnoses.

Our study aimed to replicate clinical practice. TBLC specimens were considered independently of the associated SLB. Not only was a masked assessment of histopathology done upfront by three expert pathologists before MDD, but the TBLC findings were also subsequently presented with de-identified data at MDD, for specific clinical diagnoses and ratings of diagnostic confidence. These assessments were made without the influence of SLB, thus minimising potential bias.

	κ (95% CI)
Transbronchial lung cryobiopsy guideline-refined pattern*	0·53 (0·43–0·63)
Surgical lung biopsy guideline-refined pattern	0·64 (0·54–0·75)
Transbronchial lung cryobiopsy specific histopathological pattern	0·52 (0·44–0·60)
Surgical lung biopsy specific histopathological pattern	0·50 (0·42–0·58)

Data are Fleiss κ coefficients with 95% CIs. *Guideline-refined patterns comprised definite or probable usual interstitial pneumonia, indeterminate for usual interstitial pneumonia, or alternative diagnosis, following the recommendations of Raghu and colleagues.[1]

Table 3: **Interobserver agreement of three expert pathologists for histopathological patterns**

	Value (%)	Attributable cause
Adverse events		
Airway bleeding, mild-moderate*	14 (22%)	TBLC
Airway bleeding, severe	0	NA
Pneumothorax, evident before VATS	1 (2%)	TBLC
Hypotension from anaesthetic	1 (2%)	Undetermined
Desaturation during procedure	1 (2%)	Undetermined
Bronchospasm	1 (2%)	Undetermined
Chest wall wound infection	1 (2%)	SLB
Serious adverse events		
Acute exacerbation of idiopathic pulmonary fibrosis	2 (3%)	Undetermined
Death within 90 days†	1 (2%)	Undetermined
Rehospitalisation, chest wall pain management	1 (2%)	SLB
Rehospitalisation, mild hypoxia	1 (2%)	Undetermined
Bleed at VATS port site requiring intervention‡	1 (2%)	SLB

Data are n (%). VATS=video-assisted thoracoscopic surgery. TBLC=transbronchial lung cryobiopsy. SLB=surgical lung biopsy. NA=not applicable. *Mild to moderate bleeding defined as bleeding controllable with endobronchial balloon or topicalised treatment (or both) without the need for surgical intervention or specific haemodynamic support. †Death at day 50 after the procedure, following acute exacerbation of idiopathic pulmonary fibrosis. ‡Patient required surgical control of chest wall bleeding and transfusion of 2 units packed red blood cells.

Table 4: **Adverse procedure-related events**

Our findings contradict the results of Romagnoli and colleagues,[10] which showed poor agreement between TBLC and SLB in a smaller cohort of 21 patients. In their study, both TBLC and SLB were presented together at MDD to inform the discussion and final diagnosis. Due to their study design, the final MDD diagnosis was affected by the SLB data, introducing substantial bias into the process. The subsequent masked assessment of TBLC specimens by a single pathologist had limited agreement with MDD diagnosis. It is unlikely however, that this aspect of the study design would have affected the masked biopsy interpretation, with poor histopathological agreement potentially relating to additional factors.[10] Given the smaller sample size and the limitations discussed, no firm conclusions regarding the diagnostic utility of TBLC could be made from the study by Romagnoli and colleagues.[10]

To translate the findings of the COLDICE study into clinical practice, it is important to understand the role of TBLC currently in the clinical setting. Early retrospective and observational data for TBLC suggested high diagnostic yield and an acceptable safety profile.[17–19] The promise of a minimally invasive technique has led to its uptake in many centres, arguably ahead of rigorous evidence. An example of this is shown in European Registry data in which TBLC has largely supplanted the traditional VATS-SLB over the past 4 years for new diagnoses of idiopathic pulmonary fibrosis.[4] Perhaps reflecting the widespread usage of TBLC, subsequent studies have shown lower diagnostic yield than initially reported.[8,12,20,21] The safety profile of TBLC has also been brought into question, with substantial risks of airway bleeding and pneumothorax.[8,12] Accordingly, the updated American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Society idiopathic pulmonary fibrosis guidelines do not recommend for or against TBLC in suspected idiopathic pulmonary fibrosis cases, because of these ongoing safety concerns and absence of procedural standardisation.[1]

The technical specifications for TBLC within this study protocol have been shown as reliable for obtaining an accurate diagnosis. By taking a minimum of four specimens from two separate, involved lobes, the TBLC agreed with the SLB more than 70% of the time. In most cases, a 1·9 mm cryoprobe was applied for a mean freeze time of 4·6 s, yielding mean tissue samples of more than 7 mm. In a 2018 expert statement on the practice of TBLC, a freeze time of 7 s with a 1·9 mm probe was recommended, and a minimum diameter of 5 mm was suggested as adequate.[14] Our study shows that shorter freeze times can achieve biopsies of acceptable size and accuracy. It is possible, however, that sampling fewer than four specimens might yield less accurate results.

Our study provides important information for the pathologist. We incorporated the updated 2018 idiopathic pulmonary fibrosis guideline histopathological patterns as a primary endpoint. Although guideline criteria were predicated from studies using SLB, the use of these broad yet prescriptive categories allowed for a standardised approach to interpretation of TBLC specimens. Notably, the proportion of probable usual interstitial pneumonia relative to usual interstitial pneumonia was higher in TBLC, and the converse was seen with SLB. Reflecting what is observed in clinical practice, the smaller TBLC samples that were obtained from the centrilobular position did not always display the attributes of the larger SLB specimens, with peripheral structures such as pleura and septae absent or not easily identifiable.[22] This result made the confirmation of predominant subpleural and paraseptal distribution of fibrosis difficult in some cases, and thus a probable usual interstitial pneumonia rather than a definite usual interstitial pneumonia pattern was more likely to be observed in TBLC. It follows that the

application of guideline criteria, derived from SLB specimens, contributed to the slightly higher interobserver agreement between three pathologists for SLB, compared with TBLC interpretation. The interobserver agreement for the varying degrees of diagnostic confidence at histopathology assessment is the subject of secondary analysis in a planned sub-study.

At MDD, both guideline-refined and specific histopathological patterns were presented, along with the degree of confidence in the consensus findings and any other differential diagnoses. Integrating all data into the MDD, the agreement between TBLC and SLB increased to 76·9%. Overall, both TBLC and SLB provided useful information to aid the MDD diagnosis to a similar degree (ie, 48 (74%) of 65 TBLC samples *vs* 50 (77%) of 65 SLB samples), highlighting the important contribution of histopathology in many cases. It is interesting to observe that diagnostic uncertainty (ie, unclassifiable or low confidence) remained in a proportion of patients, both with representative TBLC and SLB data at MDD. This finding brings to light the limited role of biopsy in some cases. It is clear that specific labelling of a patient's interstitial lung disease is not always constrained by the adequacy of the tissue sample, suggesting inherent limitations in our current understanding of the pathobiology of many interstitial lung diseases.

These findings emphasise the importance of dynamic multidisciplinary evaluation and discussion for interstitial lung disease diagnosis. Through a process of information sharing between the pathologist, radiologist, and clinician at every step, including the degree of confidence in findings, a rational approach can be taken for the diagnostic tests of patients with interstitial lung disease.

One of the key questions that the COLDICE study could not reconcile because of its design was the safety comparison between SLB and TBLC. This highly selected patient population included only individuals deemed suitable for SLB in terms of safety. The amount of serious adverse events was in-keeping with the published data for SLB, however the specific cause for these events cannot be confirmed.[23] For TBLC-specific complications, there were 14 episodes of mild to moderate airway bleeding. The routine use of an endobronchial balloon to occlude the segmental or subsegmental bronchus after each biopsy allowed control of bleeding, with avoidance of hypoxaemia or haemodynamic instability. In published meta-analyses of TBLC,[6–8] the reported range of substantial bleeding is wide, at 0·3–26·6% of cases. The true incidence is uncertain because of publication bias (ie, adverse outcomes are underreported), the fact that standardised definitions for bleeding have only been proposed in 2018, and that prophylactic endobronchial balloons have not been used routinely in all studies. We were unable to accurately assess the pneumothorax prevalence, given that TBLC and SLB were done sequentially.

We acknowledge several limitations. Although small, COLDICE represents the largest study to date comparing TBLC and SLB samples obtained from the same patient. The complexity of COLDICE constrained the number of participants, but our study was appropriately powered for the primary endpoint. The prespecified strategy to combine the two categories of usual interstitial pneumonia and probable usual interstitial pneumonia for our analysis was considered clinically appropriate in the context of idiopathic pulmonary fibrosis guidelines, and the anticipated implications of these patterns at MDD. However, it is possible that in clinical practice there will be some scenarios in which these histopathological categories do not result in the same MDD diagnosis. Thus, it might be appropriate to gather further histopathological evaluation with SLB when diagnostic uncertainty remains after TBLC.

Notably, the patient cohort included a high proportion of idiopathic pulmonary fibrosis and hypersensitivity pneumonitis diagnoses. This finding was probably a consequence of the higher relative frequencies of these diseases in the general interstitial lung disease population, and the deliberate exclusion of patients with definable disease without biopsy, such as connective tissue disease-interstitial lung disease. Notably, in individuals with discordant diagnoses, a substantial proportion were idiopathic pulmonary fibrosis versus chronic hypersensitivity pneumonitis, highlighting a common challenge in clinical practice. The histopathological differences for these two disease entities can be subtle. In the discordant cases in which the SLB MDD diagnosis was made with high confidence, the larger volume of tissue was clearly advantageous, allowing for identification of disease-specific features missed in the smaller cryobiopsy specimen.

A further potential issue was the recall of specific clinical details for paired cases at MDD, introducing bias into the process of consensus diagnosis. Many measures were taken to reduce this risk, including standardised presentation of clinical data, de-identified high-resolution CT discussion, and the separation of matched cases in time. The generalisability of study findings could be limited by the involvement of expert pathologists and MDD panellists in this study. It is uncertain if TBLC accuracy will be similar in the wider clinical setting. As previously mentioned, the study design did not allow for assessment of the safety of TBLC, apart from immediate bleeding after biopsy. The COLDICE population excluded patients with high risk for adverse outcomes from SLB, and thus the study findings do not support the application of TBLC in patients considered marginal or unsuitable for SLB. Procedural and technical practices that are endorsed in the expert consensus TBLC statement by Hetzel and colleagues[14] including the routine use of an endobronchial balloon blocker and insertion of a rigid or flexible airway under anaesthesia, were used by the experienced interventional bronchoscopists in the COLDICE study. Moreover, procedures took place in centres with adequate resources, familiarity, and expertise

in handling potential complications. Thus, the outcomes reported in our study might not be the same in centres in which these criteria are not routinely followed in clinical practice. Aside from a standardised, guideline-driven approach to TBLC, there is a clear need to scientifically evaluate its safety in a rigorous manner beyond what was possible in our study. Unanswered questions include the role of TBLC in patients with advanced disease, or in individuals who are unfit for invasive lung biopsy due to comorbidities or poor lung reserve.

The COLDICE trial provides evidence supporting the clinical utility of TBLC for interstitial lung disease diagnosis within the context of MDD. Compared with the current accepted histopathological standard SLB, TBLC specimens provide data that are useful and reliable, particularly when high-confidence patterns are reported. These data suggest that TBLC, when done by an experienced proceduralist, is a valid first-line minimally invasive diagnostic tool for patients with interstitial lung disease deemed to require lung biopsy.

Contributors

LKT, EMTL, TJC, CG, JPW, PJT, WAC, MPV, MJP, MS, JER, and GR are members of the COLDICE steering committee and have all made contributions to the design and execution of the study. Further major contributions to study design were made by JLM, AM, EM, SL and HEJ. LKT, EMTL, CG, TJC, JPW, MPV, HEJ, MS, JPWr, BH, GD, PJCW, and BJN contributed to participant recruitment. JLM, WAC, and AM did masked pathology analyses. SL and EM did radiological assessment. LKT, EMTL, CG, TJC, JPW, MPV, WAC, AM, HEJ, SEW, QTL, JPWr, BH, GD, and PJCW participated in masked multidisciplinary discussion. LKT, QTL, JER, and SEW were responsible for data entry. LKT, EMTL, HEJ, and CO did data analysis. LKT wrote the manuscript, with input from all authors. All authors reviewed the manuscript and are in agreement with regard to the contents.

Declaration of interests

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Data sharing

Because of restrictions in patient consent and institutional review board, raw data collected for this study will regrettably not be made available for the purposes of data sharing.

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