A Pilot Study of the Ultrathin Cryoprobe in the Diagnosis of Peripheral Pulmonary Ground-Glass Opacity Lesions

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Research

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Abstract

Background: It is very difficult to obtain samples of peripheral pulmonary ground-glass opacity lesions (GGOs) by traditional transbronchial biopsy. This study was conducted to evaluate the diagnostic efficacy and safety of transbronchial cryobiopsy (TBCB) for GGOs by a newly developed ultrathin cryoprobe whose outer diameter was 1.1 mm.

Methods: Twenty patients with 23 GGOs received TBCB using the ultrathin cryoprobe were retrospectively analyzed from October 2018 to November 2019 in Shanghai Chest Hospital. TBCB was performed under the guidance of virtual bronchoscopic navigation (VBN), electromagnetic navigation bronchoscopy (ENB), endobronchial ultrasound and fluoroscopy. We collected baseline information of subjects, reported diagnostic yield and complications, analyzed factors may affect the diagnostic yield.

Results: Twenty-three GGOs (12 pure GGOs, 11 mixed GGOs), with an average diameter of 21.58 ± 11.88mm, received TBCB and the diagnostic yield was 82.61% (19/23). Of the 19 GGOs diagnosed by TBCB, 12 were adenocarcinoma, 5 were inflammation, one was occupational interstitial lung disease, one was pulmonary meningothelial-like nodule. The undiagnosed 4 lesions confirmed to be adenocarcinomas by further analysis. None of the factors, including size (GGOs ≥ 20mm, GGOs < 20mm), navigation (VBN, ENB), fluoroscopic visibility (visible, invisible), GGO-component (pure GGOs, mixed GGOs) and guide sheath (K-201, K203), changed the diagnostic yield. No pneumothorax or severe hemorrhage presented.

Conclusions: The ultrathin cryoprobe is feasible and safe for the diagnosis of pulmonary GGOs with high diagnostic yield, which provided a huge potential tool for the diagnosis of GGOs, especially for those suspicious of early-stage lung cancer.

Trial registration: Clinical Trials.gov. No: NCT03716284. Registered: 20 October, 2018. URL: Clinical Trials.gov.

Introduction

Ground-glass opacity lesions (GGOs) were characterized as an elevation of lung opacity on high-resolution computer tomography (HRCT) without obscuring the existence of bronchi and vessels [1]. Any changes induce partial airspace filling and thickening of the interlobular septa will lead to GGOs, such as interstitial pneumonia, inflammation, preinvasive and invasive carcinoma [2, 3]. They can be divided into pure and mixed type of GGOs according to whether solid component involved [4]. Pulmonary GGOs have growing as a unique consideration from solid lesions, because they are more probably to be malignant but paradoxically predict a better prognosis [5]. and their unique structures also challenged the effectiveness of traditional non-surgical diagnostic tools that have been widely used for solid lesions.

Transthoracic needle aspiration (TTNA), with a diagnostic sensitivity of 90% for lung cancer [6], may not be very efficient in the diagnosis of GGOs. Apart from the multiple complications induced by TTNA [7]. troubles also derive from the fact that GGOs are small and changing their position apparently with

respiratory motion, and their loose structure is easy to be obscured by bleeding [8]. all of which makes a second biopsy difficult. Advanced bronchoscopy, including endobronchial ultrasound (EBUS), virtual bronchoscopic navigation (VBN) and guide sheath (GS), has been conducted to improve the diagnostic yield of GGOs [9–12]. Cryobiopsy, which was commonly used to identify interstitial pulmonary diseases and endobronchial tumors, presented unique value in providing large and well-preserved specimens, thus expanding its diagnostic scope to the peripheral pulmonary lesions (PPLs) [13–16]. But until now, there has not been any study conducted transbronchial cryobiopsy (TBCB) for peripheral pulmonary GGOs.

We innovatively used an ultrathin cryoprobe, with an outer diameter of 1.1 mm, incorporated with VBN or electromagnetic navigation bronchoscopy (ENB), as well as EBUS-GS and X-ray fluoroscopy for peripheral pulmonary GGOs diagnosis. As far as we know, this is the first time it has been clinically applied for peripheral pulmonary GGOs.

Subjects And Methods

Twenty consecutive patients with 23 GGOs that received TBCB by the ultrathin cryoprobe were prospectively recorded and retrospectively analyzed from October 2018 to November 2019 in Shanghai Chest Hospital. All patients provided written informed consent and patients with contraindications to bronchoscopy were excluded from the study (eg, bleeding diathesis, anticoagulation treatment, severe cardiopulmonary dysfunction, pregnancy). The protocol was approved by local ethics committee of Shanghai Chest Hospital (KS1829).

Each patient adopted HRCT before bronchoscopy to evaluate lesion size, lobar location, bronchus sign, nodule numbers and the proportion of GGO component. Lesion size is defined as the longest diameter on the largest section. The ratio of GGO component refers to the diameter of GGO element attenuated at mediastinal window compared to the diameter of lesion at the lung window. Bronchus sign describes the positional relationship of the lesion and nearby bronchus on HRCT [17]. A lesion with bronchus directly leading into is defined as bronchus sign positive.

Patients undergo bronchoscopy with general anesthesia. Scanning data of the HRCT were transferred into a LungCare navigation system (LungCare Medical Technologies Ltd., Inc, Suzhou, China) to construct virtual image and confirm biopsy route before insertion of the bronchoscope. VBN was applied routinely and ENB was conducted according to a previous reported instruction [18]. We adopted three kinds of modalities (Fig. 1). The standard bronchoscope (BF-1T260 or BF-1TQ290, Olympus, Tokyo, Japan) was incorporated with a K-203 GS (Olympus) and an UM-S20-20R probe (Olympus). The thin bronchoscope (BF-P290 or BF-P260F, Olympus), was incorporated with a K-201 GS (Olympus) and an UM-S20-17S probe (Olympus). The ultrathin bronchoscopy (UTB, BF-XP290, Olympus) was performed without EBUS or GS. Navigation (VBN or ENB) and bronchoscopes were applied according to individual judgment of the operators. Once the bronchoscope arrived at targeted bronchus, the combined ultrasound probe and GS would be inserted, adjusting their position and orientation under the fluoroscopy, until EBUS images of the GGOs being obtained.

The ultrasound signal was recorded as “Blizzard sign” and “Mixed blizzard sign”. The "Blizzard sign" usually appeared at pure GGOs, as an increase in intensity and radius of the whitish acoustic shadow of the normal lung tissue (> 1 cm) [19, 20]. "Mixed blizzard sign" showed in mixed GGOs as a combination of “Blizzard sign” and a diffuse heterogeneity scattered with hyperechoic dots, linear arcs and vessels [19]. We divided the lesions into three groups, “within”, “adjacent to”, and “invisible”, by the relationship between the probe position and the localization of the lesions [21]. If the GGO was invisible on EBUS image, biopsy would be applied under navigation bronchoscopy and fluoroscopic guidance.

The ultrasound probe would be withdrawn after localizing the targeted lesions, and then a 1.1 mm cryoprobe (ERBE, Tuebingen, German) was inserted in, and after freezing for 3–5 s, it was removed with a specimen. The cryoprobe was small enough to remove from the working channel of the thick GS (K-203), keeping the standard bronchoscope and GS in situ. However, for a thin bronchoscope, the cryoprobe would be retreated en bloc with the thin GS (K-201) and a specimen. For UTB, the cryoprobe worked alone without EBUS or GS due to its very small working channel, the cryoprobe, and the UTB were together removed out of the airway (Fig. 2–4). The procedure was repeated several times from remote to distal ends of the targeted bronchus based on former localization under fluoroscopic guidance without rechecked by EBUS. The frozen specimens from each lesion were thawed first in saline at room temperature and afterwards transferred to formalin for fixation and then collected as one sample. The sample size was recorded. Forceps, bronchial brush and sheath wash were introduced after TBCB for histological and cytological examination if the operators considered it was necessary.

The final diagnosis was confirmed by comprehensive evidences, including histology (surgery, TBCB, forceps), microbiology and clinical follow-up. The diagnostic yield of TBCB was defined as the number of GGOs correctly diagnosed by cryobiopsy out of total number of GGOs that received cryobiopsy, which means either a definitive malignancy, or a specific benign result (eg, granuloma, fibrosis, inflammation or definitive microbiological evidence), both of whom should be in consistent with the clinic outcomes of a more than 6-month follow-up. If the sample was non-specific (eg, bronchial mucosa and normal lung tissue), it was considered as unqualified.

Statistical analysis

Descriptive statistics were recorded as frequency, percentage, median (range), mean ± standard deviation. Factors affecting the diagnosis yield were evaluated by Fisher Exact test. Univariate analysis was adopted to identify the parameters variated the diagnostic yield of TBCB. All *P* values were bilateral. A *P* value < 0.05 was considered statistically significant. The statistical analysis was conducted within SPSS version 20.0 statistical software (IBM, NewYork, United States).

Results

Twenty patients with 23 GGOs (12 pure GGOs and 11 mixed GGOs) received TBCB, of which 3 patients underwent biopsy at 2 sites respectively (Table 1). The average diameter of the GGOs was 21.58 ±

11.88 mm. All GGOs were found to be bronchus sign positive, of which 21 with ground-glass composition over 50%, 14 (10 pure GGOs and 4 mixed GGOs) could not be visualized by fluoroscopy.

Table 1
Baseline characteristics of patients with peripheral pulmonary ground-glass opacity lesions (GGOs).

Characteristics	Value, mean ± SD
Age (years)	62 ± 9.86
Gender, No.	
Male	7 (7 / 20)
Female	13(13 / 20)
Lesion size (mm)	21.58 ± 11.88
Lobar location	
RUL / LUL	18 (18 / 23)
RML	1 (1 / 23)
RLL / LLL	4 (4 / 23)
GGO component	
Pure GGO	12 (12 / 23)
Mixed GGO	11 (11 / 23)
GGO ≥ 50%	9 (9 / 11)
GGO < 50%	2 (2 / 11)
GS	
K-201	13 (13 / 22)
K-203	9 (9 / 22)
Bronchoscope	
Standard bronchoscope	9 (9 / 22)
Thin bronchoscope	13 (13 / 22)
Ultrathin bronchoscope	1 (1/22)
EBUS image	
Blizzard sign	13 (13 / 22)
Mixed Blizzard sign	9 (9 / 22)
X-ray fluoroscopy	

ENB = electromagnetic navigation bronchoscopy, EBUS = endobronchial ultrasound, GGO = ground-glass opacity, GS = guide sheath, LUL = left upper lobe, LLL = left lower lobe, RUL = right upper lobe, RML = right middle lobe, RLL = right lower lobe, VBN = virtual bronchoscopic navigation.

Characteristics	Value, mean ± SD
visible	9 (9 / 23)
Invisible	14 (14 / 23)
Navigation bronchoscopy	
ENB	9 (9 / 23)
VBN	14 (14 /23)
Final diagnosis	
Adenocarcinoma	16 (16 / 23)
Chronic inflammation	5 (5 / 23)
Occupational interstitial lung disease	1 (1 / 23)
pulmonary meningothelial-like nodule	1 (1 / 23)

ENB = electromagnetic navigation bronchoscopy, EBUS = endobronchial ultrasound, GGO = ground-glass opacity, GS = guide sheath, LUL = left upper lobe, LLL = left lower lobe, RUL = right upper lobe, RML = right middle lobe, RLL = right lower lobe, VBN = virtual bronchoscopic navigation.

We adopted ENB in 9 lesions and VBN in 14 lesions. Except one underwent TBCB without EBUS, all the 22 lesions (11 pure GGO and 11 mixed GGO) were identified by EBUS and all the ultrasound probe was located "within" the GGOs. Of the 11 pure GGOs revealed on EBUS, 10 showed "Blizzard sign", one showed "Mixed blizzard sign", maybe because the bronchial lavage with 20 ml normal saline was conducted before TBCB in this case, and the residual water in the lesion changed the ultrasound image. Of the 11 mixed GGOs, 8 showed "Mixed blizzard sign", and 3 showed "Blizzard sign", which probably due to the probe position was in the vicinity of the GGO component.

Each lesion underwent 3 (1–7) times of cryobiopsy, and acquired 3 (1–5) pieces of specimens. The average sample size was 3.65 ± 1.27 mm. Two samples showed non-specific, and the qualified rate was 91.30% (21/23). Nineteen GGOs was diagnosed by TBCB, with a diagnostic yield of 82.61% (19/23), of which 12 were adenocarcinoma, 5 were chronic inflammation, one was occupational interstitial lung disease, one was pulmonary meningothelial-like nodule. Of the 4 cases undiagnosed by TBCB, 2 were characterized as chronic inflammation by TBCB but turned out to be adenocarcinoma (one indicated adenocarcinoma by cytology and one was diagnosed by clinical and radiological manifestation), 2 provided unqualified samples, and one was diagnosed as adenocarcinoma by clinical and radiological manifestation, the other presented nuclear atypia by a second biopsy (cytology and pathology), and was diagnosed as adenocarcinoma based on clinic and radiological evidence (Table 1). Of the 16 cases finally recognized as adenocarcinoma, 12 performed TBCB as well as cytology (brush and/or sheath wash), of which 5 were diagnosed by both of them, 3 were undiagnosed by either TBCB or cytology, 3 were diagnosed by TBCB but not cytology, and one was identified by cytology but not TBCB. The diagnostic sensitivity of cytology was 50.00% (6/12), while it was 75.00% (12/16) for TBCB. Three cases,

whose final diagnosis were adenocarcinomas, adopted forceps as well as TBCB simultaneously, of which 2 were identified by both of them, one was diagnosed by TBCB but not forceps biopsy.

Univariate analysis was adopted to test multiple factors that may affect the diagnostic yield. Although there is a tendency that large GGOs (GGOs ≥ 20 mm) and fluoroscopic visible GGOs have a higher diagnostic yield, But the diagnostic yield did not be altered significantly by any of the variables, including lesion size (GGOs ≥ 20 mm and GGOs < 20 mm), navigation (VBN and ENB), fluoroscopic visibility (visible and invisible), GGO component (pure GGOs and mixed GGOs) and type of GS (K-201 and K203) (Table 2).

Table 2
Diagnostic yield of transbronchial cryobiopsy and univariant analysis by different variables.

Variables	Univariant analysis	
	Diagnostic yield (%)	P Value
Lesion size (mm)		
≥ 20	100.00% (9 / 9)	0.240
< 20	71.43% (10 / 14)	
X-ray fluoroscopy		
Visible	100.00% (9 / 9)	0.127
Invisible	71.43 (10 / 14)	
GGO component		
Pure GGO	83.33% (10 / 12)	1.000
Mixed GGO	81.82% (9 / 11)	
GS		
K-201	84.62% (11 / 13)	0.822
K-203	77.78% (7 / 9)	
Navigation bronchoscopy		
ENB	77.78% (7 / 9)	1.000
VBN	85.71% (12 / 14)	
Overall	82.61% (19 / 23)	

Complications

No pneumothorax or severe hemorrhage were presented in this study.

Discussion

It is very difficult to diagnose GGOs by transbronchial lung biopsy (TBLB) when compared with solid nodules. Previous study reported the diagnostic yield of TBLB for GGOs ranges from 57–69% [9, 10, 12, 22]. While we innovatively adopted TBCB using a 1.1 mm ultrathin cryoprobe, engaged with navigation bronchoscopy (VBN/ENB), EBUS-GS and fluoroscopy, achieved a diagnostic yield of 82.61%.

Yarmus et al, conducted an animal study to evaluate the efficacy of the 1.1 mm ultrathin cryoprobe and found it provided comparable specimens with conventional TBCB in a comparable freezing period, which were much larger than what obtained by TBLB [23]. In this study, the average samples size we collected from each lesion was 3.65 ± 1.27 mm, with well-preserved cellular architecture, almost deprived of crush artifacts. The large samples provide by TBCB are very helpful to make histologic diagnosis, especially for benign lesions and highly differentiated malignancy whose histologic heterogeneity are minimal, since they are capable of presenting a mix types of pathology (Fig. 2, E), as well as normal tissues as a contrast to the pathological area (Fig. 3, E). Previous study indicated that malignancy predicted a higher diagnostic yield [24]. In this study, the diagnostic yield of benign GGOs was 100% (7/7), and the diagnostic yield of malignant GGOs was 75% (12/16). No statistical difference existed. TBCB may be more competent for GGOs diagnosis than TBLB, since persistent GGOs usually suggests an early stage of malignancy [2, 4]. For example, there is one case only presented little atypia by TBLB which was insufficient for a definitive diagnosis, but was diagnosed as highly differentiated adenocarcinoma by TBCB.

Conventional cryoprobe was thick and straight and limited to large bronchus. Hemorrhage is also a considerable problem and it induced more bleeding than forceps [15, 25–27]. Usually, the conventional cryoprobe need to be withdrawn with bronchoscope after biopsy, leaving the airway out of surveillance, so an additional insertion of a bronchoscope is required immediately after the former bronchoscope retreated [28]. On the contrary, the ultrathin cryoprobe can easily bend and extend to distal bronchus, facilitating peripheral pulmonary biopsy. Remarkably, we adopted three kinds of modalities according to different situations. For a standard bronchoscope, the cryoprobe was small enough to remove from the working channel of the K-203 GS, keeping the bronchoscope and GS in situ, so the GS acts as a locator to provide a simple and precise way for repetitive cryobiopsy without constantly checked by EBUS. It also blocked the airway and prevented potential bleeding. No additional insertion of bronchoscope and balloon catheter is needed. The thin bronchoscope was used to dealing with remote targets, although it needs an en bloc removal of the ultrathin cryoprobe and the K-201 GS, the remained bronchoscope on guard still enables a quick handling of potential complications. Besides, there was one case we engaged the ultrathin cryoprobe with UTB under the guidance of VBN and fluoroscopy. The ultrathin cryoprobe is the first cryoprobe which can incorporate with UTB. Both the UTB and the cryoprobe were very thin and flexible enough to overcome the complicated and sharp angles of far distal left superior bronchus where the GGO located that we almost conducted a direct vision biopsy.

Pulmonary GGOs being not visible by fluoroscopy may contribute to a lower diagnostic yield [22, 29]. Meanwhile, GGOs beyond EBUS detection often frustrated in biopsies [9, 30]. Navigation bronchoscopy, including VBN and ENB, facilitating confirmation of targets by visualize GGOs with image reconstruction [10]. thus improving the diagnostic yield. In this study, 60.87% (14/23) GGOs were fluoroscopic invisible, but all the GGOs (22/22), excepted one that did not adopt EBUS, were localized by EBUS. Fluoroscopic invisibility did not result a poorer diagnostic yield. In this study, ENB was selectively adopted for GGO-predominant lesions (GGO ≥ 50%), and lesions that were hard to operate. Nine GGOs adopted ENB, of which 5 were fluoroscopic invisible, 8 were GGO-predominant. The diagnostic yield was 77.78%. A comprehensive use of navigation, EBUS and fluoroscopy made up for the deficiency of one single technology.

Cytology examination, with diagnostic sensitivity of 50%, was not very effective for malignancy in this study, because GGOs that seldom infiltrate bronchus makes it difficult for malignant cells to be retrieved by brushing and sheath wash. We usually adopt cytology after TBCB to improve its sensitivity, since TBCB would penetrate the bronchus and destroy the integrity of GGOs, makes cytological samples easier to be retrieved. Cytology is an essential complement for histology. For example, there was one case undiagnosed by TBCB as a cavitary lesion, however, presented malignancy in brushing and cytopathology of sheath wash. Cavitary lesions with very little cellular structure was not easy to be localized, while brushing expanded the sampling area and washing conducted after TBCB let the lesions more probably to be identified.

There are some limitations. Firstly, it is a retrospective study with limited subjects. A large-scale research is needed to explore the diagnostic value and safety profile of the ultrathin cryoprobe. We also need randomized tests to confirm whether it is more efficient for GGOs than forceps. Secondly, bronchoscopes and sampling devices (e.g. cryoprobe, biopsy forceps, brush and sheath) were not fully randomized but applied based on the operator's individual experience, which made it difficult to confirm the diagnostic efficacy of each devices and their complementarity. Lastly, the comprehensive use of guided instruments, including navigation, X-ray fluoroscopy and EBUS, was managed to achieve a maximum diagnostic yield in the real word, but also has introduced considerable bias when analyzing factors that affecting the diagnostic yield.

Conclusion

We confirm that TBCB using the novel ultrathin cryoprobe can be engaged with a variety of bronchoscopes and guided instruments, to carry out peripheral pulmonary GGOs diagnosis. It was a feasible and safe approach, with a high diagnostic yield and little complications, especially for patients who suspicion of an early stage of pulmonary cancer.

List Of Abbreviations

ENB = electromagnetic navigation bronchoscopy, GGO = ground-glass opacity, GS = guide sheath, LUL = left upper lobe, LLL = left lower lobe, PPLs = peripheral pulmonary lesions, RUL = right upper lobe, RML = right middle lobe, RLL = right lower lobe, TTNA = transthoracic needle aspiration, TBLB = transbronchial lung biopsy, TBCB = transbronchial lung cryobiopsy, UTB = ultrathin bronchoscopy, VBN = virtual bronchoscopic navigation.

Declarations

Ethics approval and consent to participate:

The protocol was approved by local ethics committee of Shanghai Chest Hospital (KS1829). All patients provided written informed consent before the procedure.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors have no conflicts of interest to declare.

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Authors' Contributors:

Conception and design: J Sun. Collection, analysis and interpretation of data: X Liu, J chen, S Jiang, H Ma, F Xie, J Sun. Manuscript writing: S Jiang, X Liu. All authors read and approved the final manuscript.

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Figures

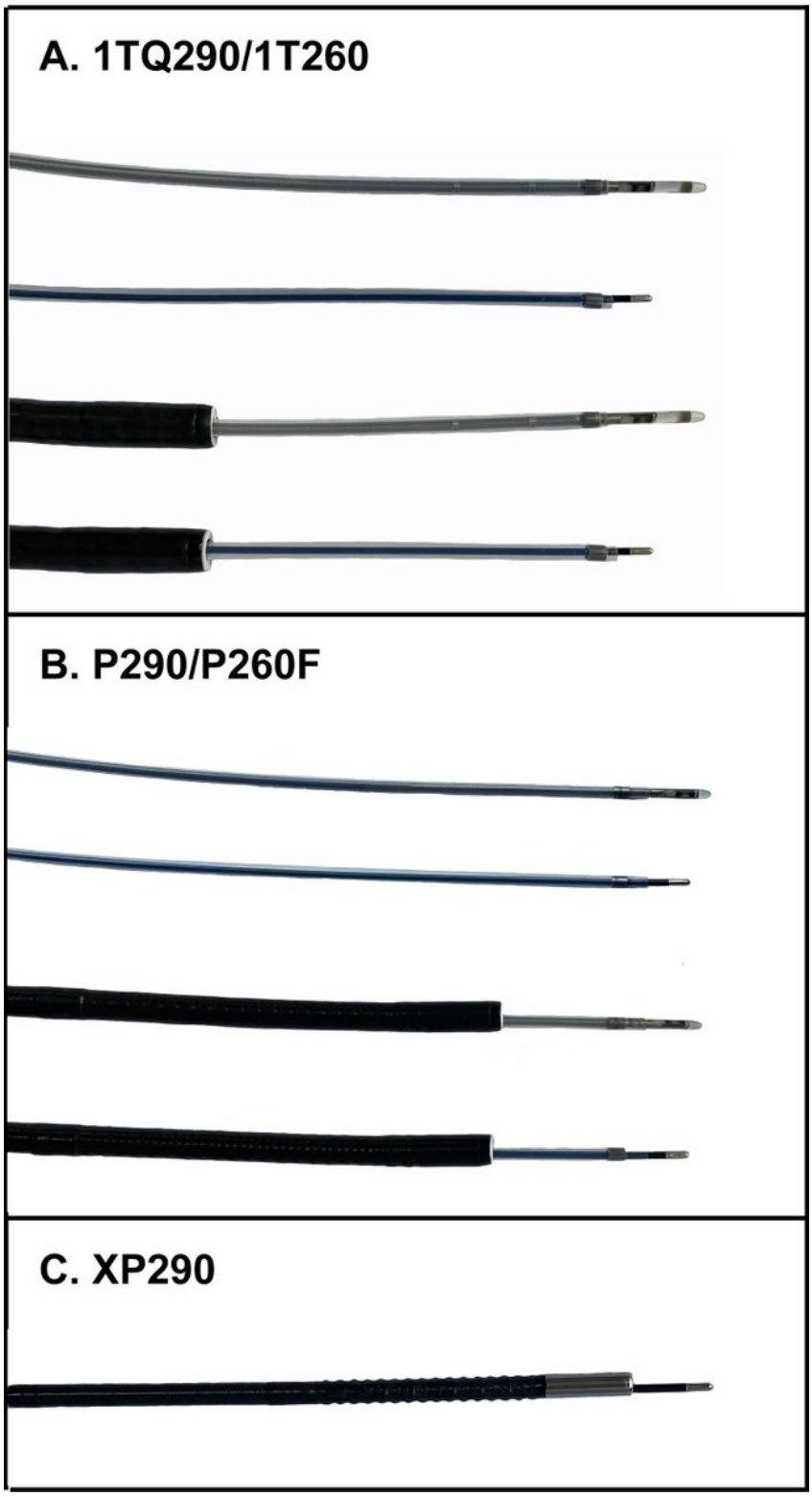

Figure 1

Different assembly of bronchoscope, endobronchial ultrasound (EBUS), guide sheath (GS) and ultrathin cryoprobe. [A] The standard bronchoscope (BF-1TQ290/BF-1T260, distal end outer diameter 5.9mm, working channel 3.0mm), EBUS (UM-S20-20R, outer diameter 1.7mm), GS (K-203, outer diameter 2.55mm), and the ultrathin cryoprobe (outer diameter 1.1mm) were used together. [B] The thin bronchoscope (BF-P290/BF-P260, distal end outer diameter 4.2mm, working channel 2.0mm) was engaged with EBUS (UM-S20-17S, outer diameter 1.4mm), GS (K-201, outer diameter 1.95mm), and the ultrathin cryoprobe. [C] The ultrathin bronchoscopy (UTB, BF-XP290, distal end outer diameter 3.1mm, working channel 1.2mm) was directly combined with the ultrathin cryoprobe.

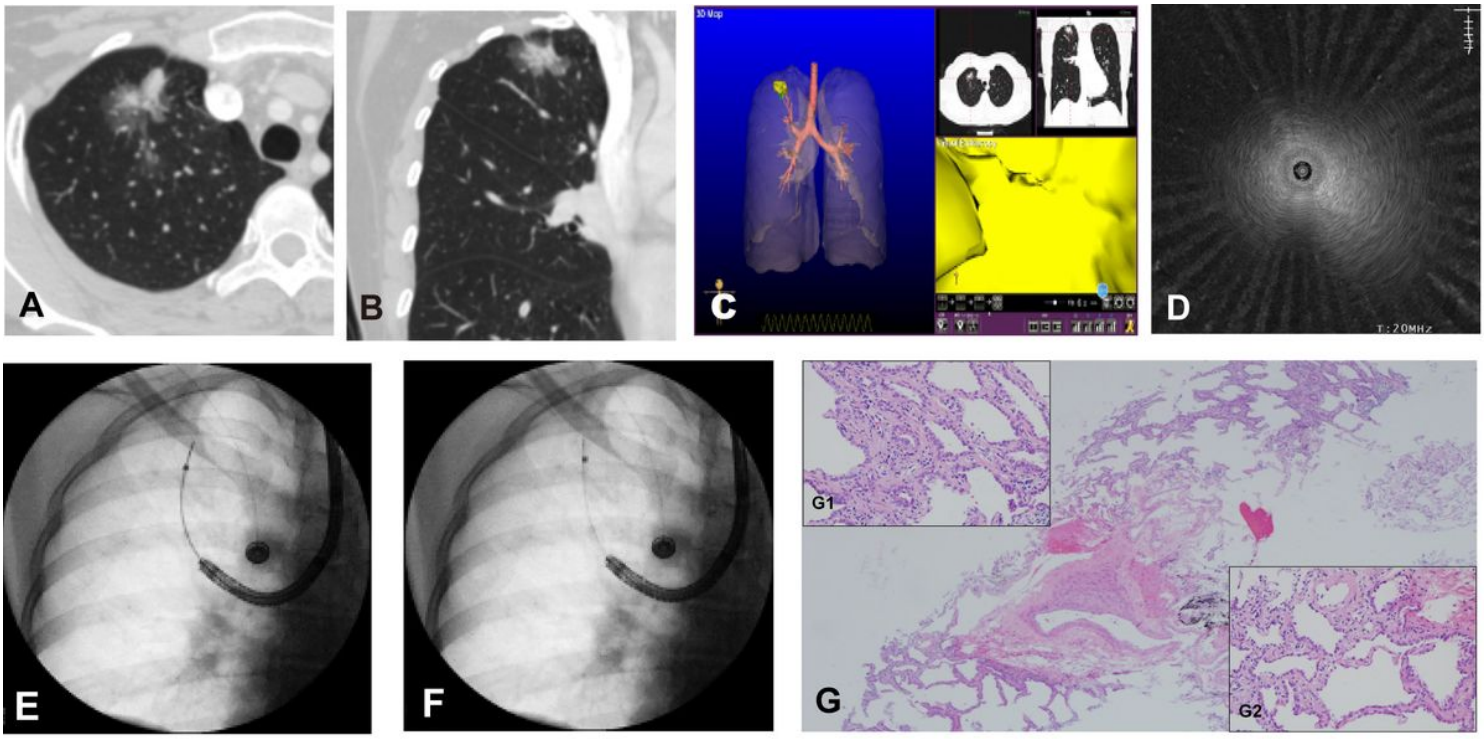

Figure 2

Representative case 1 of transbronchial cryobiopsy (TBCB) using the standard bronchoscope (BF-1TQ290) with electromagnetic navigation bronchoscopy (ENB). [A] The axial section of high-resolution computer tomography (HRCT) showed that the GGO was located in the apical segment of the right upper lobe (RUL). [B] The coronal plane of HRCT showed where the GGO located. [C] ENB presented the real-time interface where the tracking wire reaches the target. [D] EBUS image showed “Blizzard sign”. [E] The location of EBUS probe (UM-S20-20R) was visualized by X-ray fluoroscopy to confirm its arrival. [F] After confirming the arrival of bronchoscope, EBUS probe was retreated and cryobiopsy was performed via the K-203 GS. [G] H&E staining of the specimen with the magnification of 4×. [G1] Magnification of the right upper corner of the specimen showed acinar adenocarcinoma (magnification, 20×). [G2] Magnification of the left lower corner of the specimen displays adenocarcinoma with lepidic pattern (magnification, 20×).

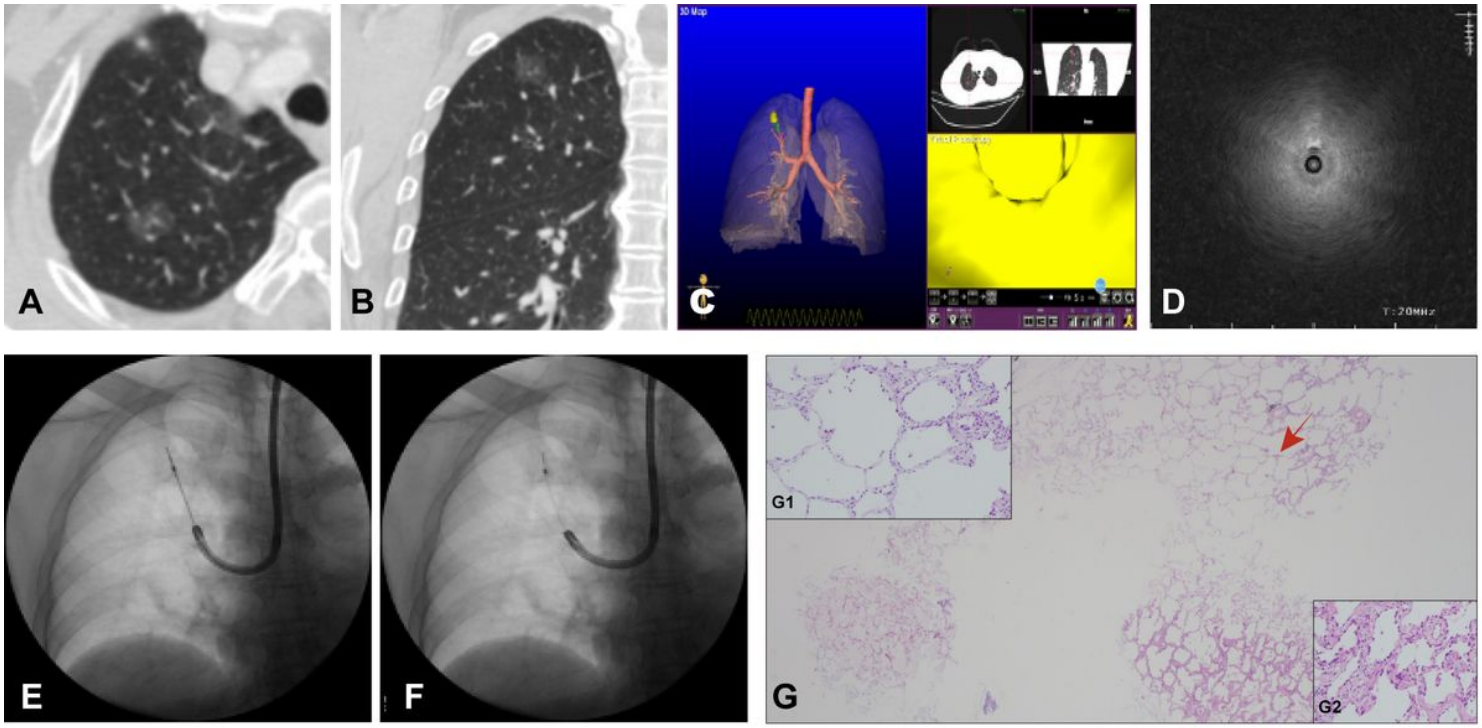

Figure 3

Representative case 2 of TBCB using the thin bronchoscope (BF-P290) with ENB. [A] The HRCT axial section showed that the GGO was located in the apical segment of the RUL. [B] The HRCT coronal plane showed where the GGO located. [C] ENB showed the real-time interface where the positioning wire reaches the target. [D] EBUS displayed “Blizzard sign”. [E] The location of ultrasound probe (UM-S20-17S) was visualized by X-ray to confirm its arrival. [F] After confirming the arrival of bronchoscope, ultrasound probe was retreated and cryobiopsy was performed via the K-201 GS. [G] H&E staining of the specimen with the magnification of 2×. [G1] magnification of the pointed area displays the transition from normal pulmonary alveoli to atypical hyperplastic area (magnification, 10×). [G2] Magnification of the left lower parts of the specimen showed adenocarcinoma with lepidic pattern (magnification, 10×).

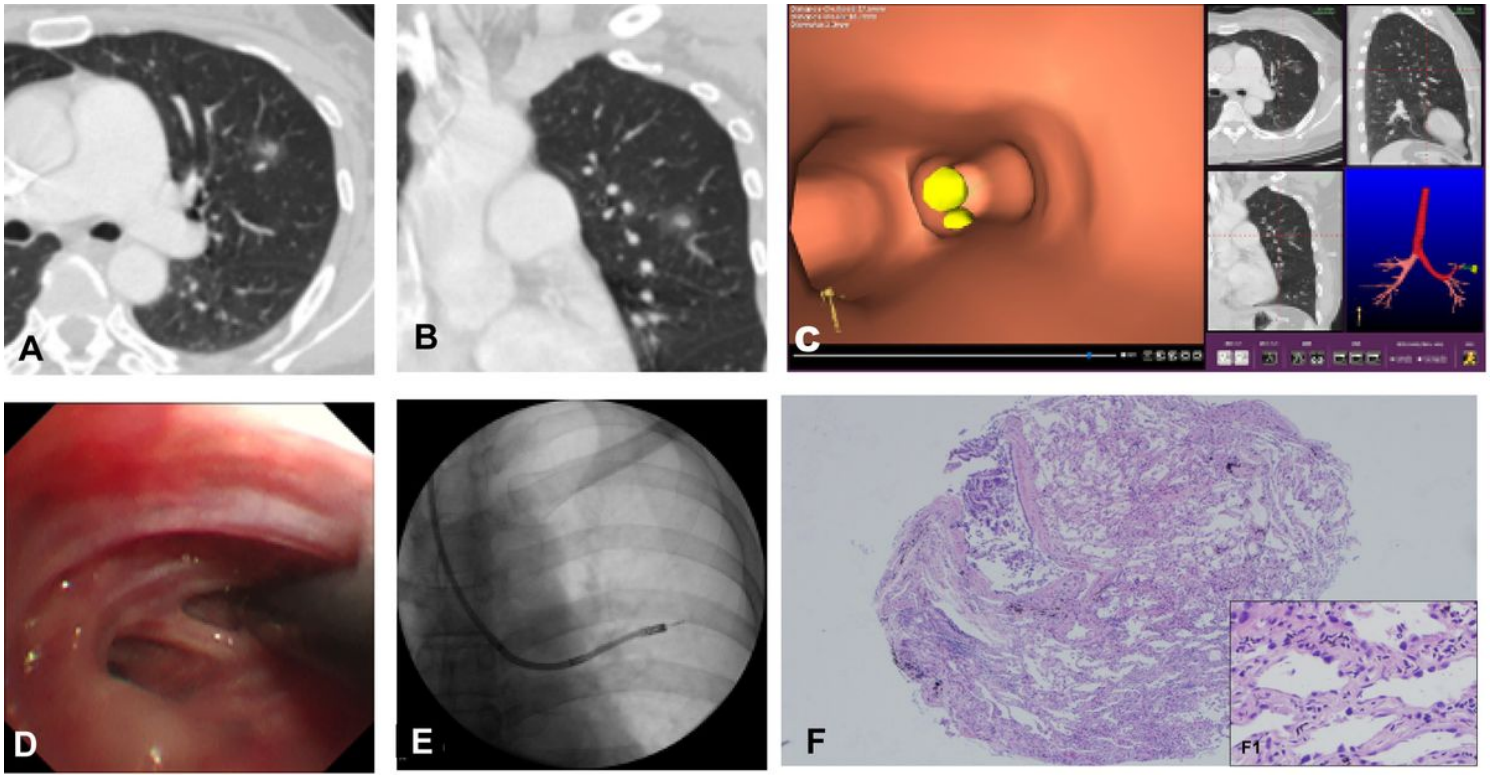

Figure 4

Representative case 3 of TBCB using the UTB (BF-XP290) guided by virtual bronchoscopic navigation (VBN). [A] The HRCT axial section showed a pure GGO located in the anterior segment of the left upper lobe, with blood vessel involved in. [B] The HRCT coronal plane showed where the GGO located. [C] Bronchial path and virtual bronchography of VBN. [D] The ultrathin cryoprobe was inserted into the target bronchus under the direct vision of the UTB. [E] Cryobiopsy was conducted under X-ray fluoroscopy. [F] H&E staining of the specimen with the magnification of 4×. [F1] Enlarged picture displays atypical hyperplasia (magnification, 20×). It was confirmed as adenocarcinoma in situ by surgery.