Ultrasound in Med. & Biol., Vol. 44, No. 7, pp. 1556–1562, 2018

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2018.03.003

● *Clinical Note*

ONE-LUNG FLOODING ENABLES ULTRASOUND-GUIDED TRANSTHORACIC NEEDLE BIOPSY OF PULMONARY NODULES WITH HIGH SENSITIVITY

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(*Received* 23 *November* 2017; *revised* 27 *February* 2018; *in final form* 1 *March* 2018)

Abstract—Ultrasound-guided transthoracic needle biopsy (USgTTNB) can only be used for peripheral tumours that contact the pleura. Sonographic accessibility of the entire lung can be achieved using one-lung flooding. In this study, feasibility, sensitivity and complication rate of USgTTNB of lung nodules after one-lung flooding in an *ex vivo* and *in vivo* lung tumour model were assessed. USgTTNB was performed *ex vivo* after one-lung flooding in 10 resected human lung lobes containing carcinoma or metastasis. USgTTNB after one-lung flooding and simulation of a lung nodule was conducted *in vivo* in 5 animals. Transthoracic sonography and chest X-ray were obtained 30 min after reventilation. The lungs were examined macroscopically and histopathologically. The pathologic diagnosis was confirmed in 85.7% and 100% of tumours after first and second puncture attempts, respectively. The successful puncture rate *in vivo* was 90%. Neither pneumothorax nor bleeding was observed. One-lung flooding enables USgTTNB of lung nodules with a high sensitivity and minimal risk of complications in a pre-clinical model. (E-mail: Thomas.Lesser@srh.de)

Key Words: **One-lung flooding, Ultrasound, Transthoracic needle biopsy, Pulmonary nodules.**

INTRODUCTION

Since publication of the National Lung Screening Trial (NLST) results, lung cancer screening has gained considerable interest not only in the United States but also elsewhere in the world. Low-dose computed tomography (LD-CT) lung cancer screening leads to a high detection rate of pulmonary nodules. In the NLST, 51.8% of cancer patients with a positive LD-CT result had Stage IA tumours. However, 96.4% of positive screening results in the LD-CT group were false-positive results (National Lung Screening Trial Research Team 2011). Most small nodules are not malignant. The American College of Chest Physicians Guidelines for Diagnosis and Management of Lung Cancer recommend pathologic confirmation of pulmonary nodules, except when the clinical probability of malignancy is very low (<5%) or the clinical probability is low (<30%–40%) and the results of a functional imaging test are negative (Gould et al. 2013).

Current non-surgical biopsy methods, such as computed tomography (CT)-guided transthoracic needle biopsy (CTgTTNB) and transbronchial biopsy (TBB), have significant risks of complications and are frequently non-diagnostic. For nodules measuring up to 15 mm in diameter, a false-negative biopsy occurs in 10%–49% of CTgTTNB and 30%–70% of TTB (Fontaine-Delaruelle et al. 2015; Gould et al. 2013). Pneumothorax and haemorrhage are serious potential complications of CTgTTNB or TBB. Rzyman et al. (2013) reported that approximately 60% of patients with pulmonary nodules underwent surgery without earlier pathologic examination; 35% of these operations were unnecessary, as the lesions were benign. These findings emphasize the need for a safe, minimally invasive non-surgical biopsy method with sufficient sensitivity, accuracy and negative predictive value.

Traditional ultrasound-guided TTNB (USgTTNB) can only be used for peripheral tumours that contact the pleura. In ventilated lung, nodules localised to more central areas are not visible by ultrasound. Animal experiments have shown that sonographic accessibility of the entire lung can

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be achieved using one-lung flooding (OLF) (Lesser et al. 1998). OLF enables complete sonographic imaging of the whole lung, including parenchyma, vessels and bronchi, as well as lung tumours (Lesser et al. 2013). A good sonographic imaging of the lesion is indispensable for a successful biopsy. However, there are various aspects that influence the biopsy outcomes, such as lesion size, depth, localization, needle size, the approach path to the lesion, strategies for clear visualization of the needle. Although solid pancreatic masses were visible by ultrasound, percutaneous ultrasound-guided core needle biopsy resulted in a negative predictive value of 75% (Kahriman et al. 2016).

The objective of this study is to investigate the feasibility, sensitivity and safety of USgTTNB of pulmonary nodules, using OLF in pre-clinical *ex vivo* and *in vivo* lung tumour models.

MATERIALS AND METHODS

Ex vivo *examinations*

An *ex vivo* human lung model was used to obtain biopsy specimens of central pulmonary lung nodules. Lung lobes containing central tumours were obtained from 10 patients who underwent curative lobar resection. The experimental protocol was approved by a local institutional review board, and informed consent for the study was obtained from all patients. The lobes were flooded *ex vivo* with saline (Lesser et al. 2013). Biopsy was performed by placing the sonographic probe on the visceral pleura of the flooded lobe and inserting an automated cutting core biopsy needle (BARD MAX-CORE Disposable Core Biopsy Instrument, 14 gauge; BARD GmbH Karlsruhe, Germany) (Fig. 1). Each tumour was punctured up to three times. A sonographic hit was defined as the needle being visible within the nodule during B-mode imaging. A pathologic hit was defined as the needle biopsy results exhibiting the same histology as the final pathology results of the resected lobe. Of the 10 lung lobes (4 right lower lobe, 3 left upper lobe, 1 left lower lobe, 2 right upper lobe) examined, 5 contained non–small cell lung cancer (NSCLC; 2 adenocarcinoma, 2 neuroendocrine tumour, 1 squamous cell carcinoma) and 5 contained metastases (2 renal cell carcinoma, 1 colorectal cancer, 1 breast cancer, 1 lung carcinoma). The mean nodule diameter was 2.9 cm (1.8–4.5 cm) on CT examination.

In vivo *examinations*

In vivo experiments were performed using a large porcine model, with 5 female pigs ("Deutsches Landschwein," 30–34 kg) in the supine position. Permission for all animal experiments was granted by the Veterinary Department of the Thuringian State Authority for Food Protection and Fair Trading in compliance with the National Animal Protection Act.

After the animals were anaesthetized with propofol (10 mg kg^{-1} h^{-1}), sufentanil (0.02 µg kg^{-1} min^{-1}) and pancuronium bromide (2.5 µg kg^{-1} min^{-1}) a left-sided animal-specific Robertshaw double-lumen tube with an extra-long bronchial channel (39 Fr; special product by Mallinckrodt Medical, Dublin, Ireland) was inserted. The correct position of the tube was checked by fibre optic bronchoscopy (BF 3 C30; Olympus, Tokyo, Japan). Mechanical ventilation was performed with an intensive care unit respirator (Servo 900, Siemens AG, Munich, Germany) in volume-controlled mode (FiO_2 = 0.4; tidal volume = 10 mL kg^{-1} weight; respiratory rate adjusted to maintain an end-tidal CO_2 of 35–45 mm Hg; inspiratory:expiratory ratio = 1:1; positive end-expiratory pressure = 5 cm H_2O). After 30 min of double-lung ventilation with FiO_2 = 1.0, the right endobronchial channel was disconnected from the respirator. The infusion system was immediately connected to this channel, and the right lung was slowly filled

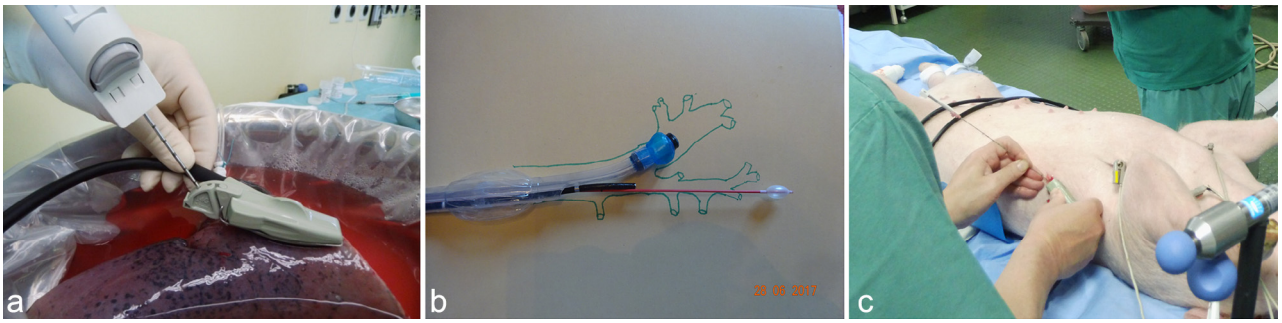

Fig. 1. Experimental setup. (a) Transpleural ultrasound-guided core needle biopsy (using a needle-guidance device) of a central localized lung malignancy after flooding of the resected human lobe *ex vivo*. (b) *In vivo* experimental setup of simulated lung lesion, showing transbronchial insertion of a Fogarty catheter in the flooded right lung *via* the right lumen of a double-lumen tube, using fibreoptic bronchoscopic control. The Fogarty balloon is filled with saline. (c) Ultrasound-guided transthoracic needle biopsy of a simulated lung lesion after one-lung flooding *in vivo*. A small-calibre core biopsy needle (18 gauge) was inserted by the "freehand" technique.

with isotonic saline. This was followed by TTNB of a simulated lung lesion, as described later in this report.

For reventilation, fluid was partly drained from the right lung in a passive manner by placing the animal in the Trendelenburg position. Double-lung ventilation was then resumed for 30 min, using the initial settings. Vital signs were monitored using pulse oximetry and electrocardiogram (Datex AS/3 Patient Monitor; Datex-Ohmeda Corp., Helsinki, Finland) throughout. A chest X-ray and sonographic pleural examination were performed 30 min after double-lung ventilation under the condition of spontaneous breathing to exclude pneumothorax. After this, the animals were euthanized with sodium pentobarbital and potassium chloride. During necropsy, multiple cross sections of the lung were obtained to search for intraparenchymal bleeding as a sign of blood vessel damage.

Transthoracic needle biopsy of simulated lesion

After achieving complete OLF, as confirmed by sonographic assessment of the entire lung plus adjacent organs (liver and heart), a Fogarty embolectomy catheter (4 Fr, Fogarty Fortis, Edwards Lifesciences, Unterschleissheim, Germany) was inserted through the right endobronchial channel into the right lower segmental bronchi without imaging (Fig. 1). The position of the balloon was completely unknown before transcutaneous sonography. The location of the balloon was varied from animal to animal by bronchoscopic guiding in different segmental bronchi. Thereafter, the balloon was filled with saline (1 mL) to represent a simulated lung nodule. The expanded balloon was localised by transcutaneous sonography, and a small-calibre core biopsy needle (18 gauge, Magnum Biopsy Needle, BARD GmbH) was inserted by the "freehand" technique under real-time ultrasound guidance (Fig. 1). A puncture attempt was considered successful if the balloon could be moved by contact with the needle. This was performed three times by two examiners (six times per animal). For sonographic examinations of the *ex vivo* and *in vivo* models, a sonographic system (Flex Focus 800, BK Medical, Arhus, Denmark) with linear (8870, 18-6 MHz) and curved (8815, 10-4 MHz) arrays were used.

RESULTS

Ex vivo *examinations*

All central pulmonary nodules were sonographically detectable and clearly demarcated in the flooded lung. Lung tumours were characterised as hypo-echoic sonographic lesions surrounded by hyper-echoic flooded-lung parenchyma. Functional structures, such as bronchi and vessels, near the tumour were also detectable. The biopsy needle appeared as a strongly hyper-echoic object. The position of the needle tip was tracked real-time in the flooded lung. After needle removal, the needle tract remained slightly visible, characterised by minor residual air and an echoless central appearance. Figure 2 illustrates the needle position during ultrasound-guided transpleural biopsy of a lung nodule.

A total of 28 core biopsies were performed in 10 lung lobes. The sonographic hit rate (sensitivity) was 100%. The pathologic hit rate (sensitivity) after 1 puncture was 85.7% (24/28); 4 biopsies were false-negative (false-negative rate: 14%). A second puncture resulted in the correct diagnosis in all cases. All *ex vivo* results and specimen characteristics are summarized in Table 1.

In vivo *examinations*

OLF was performed successfully in all five animals. The entire lung with its adjacent organs was examined by transthoracic sonography, without residual air. All animals survived the procedure until the planned euthanasia, without evidence of hypoxemia or hyper-capnia. Hemodynamic parameters remained within the physiologic range throughout

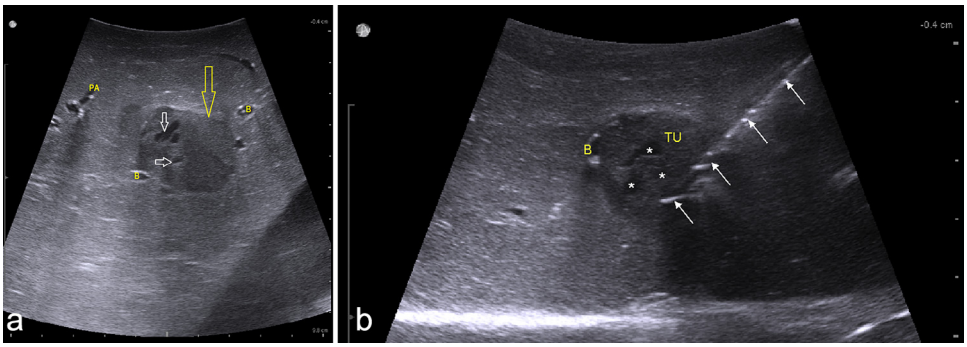

Fig. 2. (a) Ultrasound image of a human lung nodule within the flooded lung lobe before transpleural needle biopsy *ex vivo*. The *yellow arrow* marks the planned needle path to avoid injuring functional structures or puncturing liquefaction areas of the tumour (*white arrows*). B = bronchi; PA = pulmonary artery. (b) Transpleural ultrasound-guided core needle biopsy of a lung nodule after lung flooding *ex vivo*. The 14-gauge needle and biopsy channel after shutter release (*arrows*) are visualised. The location of the biopsy is outside the areas of tumour liquefaction (*asterisks*). B = bronchus; TU = tumour.

Table 1. Sensitivity of ultrasound-guided transpleural needle biopsy of malignant lung tumours in an *ex vivo* model

Number	Histology	Lobe	Diameter on CT (cm)	Puncture attempts	Sonographic hits	Pathologic hits
1	ADC	LUL	2.5	3	3	3
2	NET	RLL	3.2	3	3	3
3	NET	LLL	2.8	2	2	2
4	MTS RCC	RLL	3.7	3	3	2
5	MTS CRC	RLL	4.5	3	3	3
6	MTS BC	RLL	2.5	3	3	2
7	SQC	LUL	2.5	3	3	3
8	ADC	RUL	1.8	3	3	2
9	MTS ADC	RUL	2.5	3	3	3
10	MTS RCC	LUL	2.9	2	2	1
			Mean: 2.9	Sum: 28	Sensitivity: 100% (28/28)	Sensitivity: 85.7% (24/28)

ADC = adenocarcinoma; BC = breast carcinoma; CRC = colorectal cancer; CT = computed tomography; LLL = left lower lobe; LUL = left upper lobe; MTS = metastasis; NET = neuroendocrine tumour; RCC = renal cell carcinoma; RLL = right lower lobe; RUL = right upper lobe; SQC = squamous cell carcinoma.

the procedure. Histologic examination revealed no intrapulmonary bleeding in any animal. Chest X-ray and transpleural sonography revealed no pneumothorax at 30 min after reventilation in any animal.

The simulated lung nodules appeared hypo-echoic, with a bright hyper-echoic outer rim. They were located at a mean distance from the pleura of 45 mm (38–52 mm), with a mean length of 10.8 mm (10.4–11.2) and mean height of 9.4 mm (9.05–10.0), respectively (Fig. 3). The overall sonographic hit rate (successful puncture rate) was 90% (27/30), combining results from the two examiners. Table 2 shows the sensitivity of USgTTNB of a small simulated pulmonary nodule in our *in vivo* model.

DISCUSSION

With advances in CT imaging and the growing interest of lung cancer screening, the management of lung nodules is becoming increasingly challenging. There are various ways to sample such nodules, including flexible bronchoscopy, radial endobronchial ultrasonography, surgery (thoracotomy or thoracoscopy) and CTgTTNB. The reported diagnostic yields with TBB were 65%–84% for electromagnetic navigation bronchoscopy and 46%–77% for radial endobronchial ultrasound (Herth and Eberhardt 2008). However, the yields decrease for lesions less than 2 cm in diameter. The diagnostic accuracy of

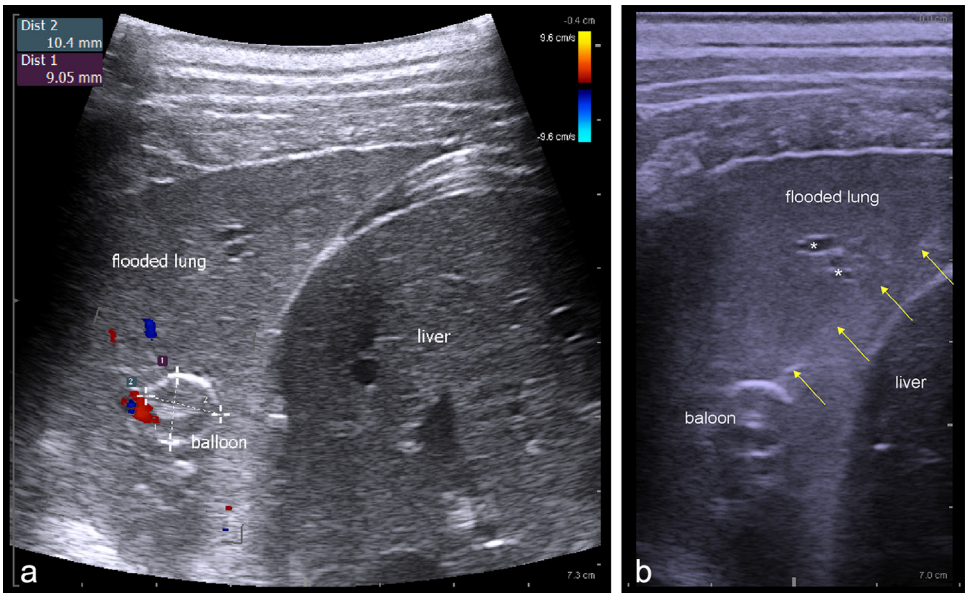

Fig. 3. (a) Transthoracic sonography of the animal's right lung during one-lung flooding before needle biopsy *in vivo*. The simulated lesion (Fogarty catheter balloon) is clearly visualised 4 cm below the costal pleura. *Coloured spots* mark the pulmonary vessels. (b) Ultrasound-guided transthoracic needle biopsy of a simulated lung lesion (Fogarty catheter balloon), using the "freehand technique," without injuring the bronchi (*asterisks*) located proximate to the needle path (*arrows*). A puncture attempt was considered successful if the balloon could be moved by contact with the needle.

Table 2. Sensitivity of ultrasound-guided transthoracic needle biopsy of a small simulated lung nodule* in an *in vivo* model

		Successful puncture†					
		Examiner A (attempts)			Examiner B (attempts)		
Animals	Lesion deep (mm)	1	2	3	1	2	3
1	38	Yes	Yes	Yes	No	Yes	Yes
2	43	Yes	Yes	Yes	Yes	Yes	Yes
3	48	Yes	Yes	Yes	Yes	Yes	Yes
4	52	Yes	No	Yes	Yes	Yes	No
5	45	Yes	Yes	Yes	Yes	Yes	Yes
	Mean: 45.2 SD: 5.3						
Hit rate		93.3% (14/15)			86.7% (13/15)		

SD = standard deviation.
* All simulated nodules were 11 mm in diameter.
† A puncture was considered successful (*yes*) if the balloon could be moved by contact with the needle.

CTgTTNB for identifying malignancy in patients with nodules up to 20 mm in diameter is 69.6% (Kothary et al. 2009). More important, Fontaine-Delaruelle et al. (2015) found in a large multi-centric study of CTgTTNBs that 49% of TTNB specimens with negative or non-malignant results missed a cancer diagnosis. That means the negative predictive value for a malignant disease diagnosis is only 51%. Thus, negative CTgTTNB samples are a diagnostic challenge. Currently there is no clear consensus as to what diagnostic workup should be initiated. One way is surgery with the diagnosis by frozen section, which is associated with a number of unnecessary procedures attributable to a benign diagnosis (Grogan et al. 2011; Sihoe et al. 2013). The USgTTNB using one-lung flooding could close the diagnostic gap.

Our results show the feasibility and high sensitivity of USgTTNB for pulmonary nodules, using OLF during both *ex vivo* human and *in vivo* animal examinations. The transbronchial insertion of a Fogarty catheter *in vivo* allows us to simulate small lesions centrally in the lung lobe near the functional structures. Contrary to the transpleural injection technique, the locations of the lesions are completely unknown. Of note, the 90% sonographic hit rate for very small simulated pulmonary lesions (mean length of 10.8 mm and mean height of 9.4 mm) implies that USgTTNB using OLF could improve diagnostic yields in future applications on humans.

Our results of the *ex vivo* needle biopsies show a lower false-negative rate (14%) in comparison with the latest clinical study in this field (49%) (Fontaine-Delaruelle et al. 2015). The most important requirements for successful USgTTNB are fulfilled during OLF. Except for lepidic adenocarcinoma, all other NSCLCs and metastases are well visualised sonographically because of clear demarcation from the lung parenchyma. Furthermore, superior imaging within the tumours, including areas of liquefaction, helps avoid non-diagnostic biopsies. OLF allows the sonographic visualization and the real-time guidance of any transthoracic needle insertion. Our personal experience with intraoperative needle biopsy of pulmonary lesions during thoracotomy or thoracoscopy showed that small consistent nodules in the air-filled lung cannot be sufficiently punctured without manual fixation. We observed that a possible local shift of the tumour out of the pathway after needle contact does not appear in flooded lung.

The CTgTTNB complication rate appears noteworthy. The incidences of any pneumothorax and pneumothorax requiring chest tube during CTgTTNB were 9%–54% (mean: ~ 20%) and 5%–10%, respectively (Boskovic et al. 2014; Tam et al. 2013; Tuna et al. 2013). Pulmonary haemorrhage and haemoptysis after CTgTTNB occur in approximately 10% of cases (Ashraf et al. 2017).

As in most clinical studies, we used a small-calibre core biopsy needle (18 gauge) for USgTTNB in our animal model to make the results of potential complications comparable. With OLF, complications such as pneumothorax and haemorrhage can be avoided. By six needle passes per animal, no complications occurred. Despite the obstruction by the rib cage, sonographic guidance allows us to find the optimum needle path to avoid injuries of vessels or bronchi. It is to be assumed, if very small vessels not visualised by ultrasound are injured, intra-parenchymal bleeding generally should not occur because pressure exerted by fluid within the lung compresses these tiny vessels. During OLF, fluid in the lung also eliminates the possibility of pneumothorax. After 30 min of reventilation of the flooded lung, absence of pneumothorax was confirmed by X-ray and ultrasound in all animals. OLF reduces movement of the ipsilateral diaphragm and thus lung motion (Lesser et al. 2016), which can be considered ideal for avoiding intra-parenchymal or pleural laceration after needle insertion.

The study was performed using simple portable and state-of-art B-mode imaging systems. A higher resolution

could be achieved using Harmonic (THI) imaging, which would be beneficial for imaging in a flooded lung because of its low attenuation (Wolfram et al. 2014). Shear wave elastography would be a useful sonographic feature that could enable detection of dense tumour areas and therefore vital tissue. It can also be hypothesized that shear wave elastography would enhance visibility of bronchoalveolar carcinomas, which were difficult to detect in previous studies (Lesser et al. 2013). Unfortunately, such advanced imaging systems were not available to us. We showed that with the most common baseline, a simple B-mode that supports all ultrasound systems, puncture of central pulmonary nodules is feasible, any more advanced imaging modalities will increase hit rate.

For practical guiding during OLF, the use of needle guidance systems such as magnetic methods (Gadsden et al. 2015) will have an impact on accuracy and hit rate, which will be implemented in future human trials.

OLF involves complete filling of one lung with saline, generating a gas-free saline lung field that permits acoustic access to the whole lung and adjoining organs. The safety of OLF was intensively investigated in large animal models in acute and chronic experiments; no haemodynamic and oxygenation compromise or surfactant washout was observed (Klinzing et al. 1999, 2000). The authors found that in comparison with one lung ventilation, fluid flooding of the non-ventilated lung reduced the pulmonary right-left shunt considerably and increased the arterial oxygen partial pressure. Histologic and immunologic investigations demonstrated that OLF is not associated with destruction of alveolar texture, atelectasis-promoting surfactant loss or any irreversible pulmonary parenchymal damage. In chronic animal experimentations up to 10 wk after OLF, we observed a slightly increased infiltration of the lung interstitium with inflammatory cells 24 h after OLF. A total of 48 h after OLF the inflammatory cell infiltrates was decreased and completely disappeared after 6 d (Lesser et al. 2008). In comparison, the whole-lung lavage (a repeated lung flooding) is a recognized therapeutic method to clean the lung of patients suffering from pulmonary alveolar proteinosis. The international survey found that whole-lung lavage is safe and effective as therapy for pulmonary alveolar proteinosis and pneumoconiosis (Awab et al. 2017; Campo et al. 2016; Zhang et al. 2016).

OLF requires general anaesthesia and double-lumen tube insertion. However, these are routine procedures and may be done every day in lung cancer centres with highly experienced anaesthesiologists in thoracic surgery. On the other hand, OLF offers the possibility to diagnose and treat the patients in one session. If the lesion represents a malignancy, the procedure can be changed to a therapeutic surgical resection without further steps. In contrast with exploratory thoracoscopy or thoracotomy with parenchyma resection attributable to undetermined pulmonary lesions, OLF seems to be less invasive. Another important advantage may be the ability to offer local ablative methods, such as radiofrequency, microwave or high-intensity focused ultrasound ablation, to treat lesions in the same session, immediately after the diagnostic procedure (Wolfram et al. 2014).

This study has some limitations. First, we could not fully assess the accuracy of our technique because the availability of resected human lobes containing benign tumour is very limited. Furthermore, because of the lack of a lung tumour model in swine, we were unable to determine a pathologic hit rate for the *in vivo* experiments. Second, the presence of a pneumothorax was excluded by evaluation 30 min after reventilation. The occurrence of later pneumothorax was not studied; however, the incidence of late pneumothorax in the literature is below 10%. Additionally, the animals had no pulmonary diseases, such as chronic obstructive pulmonary disease or emphysema, which are risk factors for TTNB-related complications.

The present work represents a preclinical study on a lung tumour model to evaluate the feasibility, sensitivity and complication rates of USgTTNB, using one-lung flooding. Based on this, we are planning ongoing work on human patients. In the future, study patients with non-diagnostic results of CTgTTNB or TBB of pulmonary nodules will be included. The aim is to show that USgTTNB using one-lung flooding can improve the negative predictive value of the biopsy. This is important because increased number of benign diagnosis leads to a reduced surgical biopsy rate (thoracoscopy, thoracotomy) and thus to avoid futile operations.

CONCLUSION

OLF enables USgTTNB of lung nodules with a high sensitivity and minimal risk of complications in a preclinical lung tumour model. Requirements for successful and safe ultrasound-guided needle biopsy, such as high-quality imaging of the tumour, lung parenchyma, proximate functional structures and biopsy needle, are fulfilled during OLF.

Acknowledgments—The authors thank Dr. Leder and Mr. Peuke for organizational support, as well as Mr. U. Ziemann. This research was supported by the German Society of Ultrasound in Medicine (DEGUM) and the SRH Waldklinikum Gera, Germany.

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