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Journal: *Interactive CardioVascular and Thoracic Surgery*
Article Doi: 10.1093/icvts/ivx373
Article Title: The use of ultrasound in detecting and defining ground-glass opacities: results of an *ex vivo* evaluation
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Corr. Author: Alfonso Fiorelli

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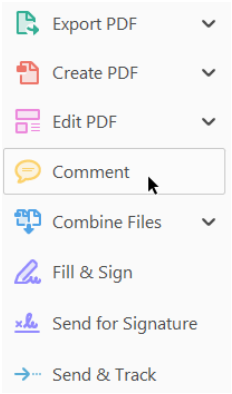

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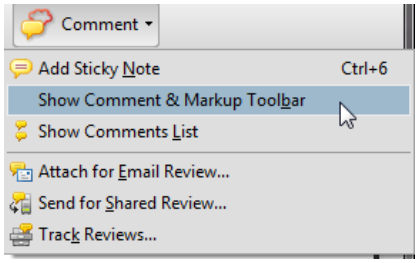

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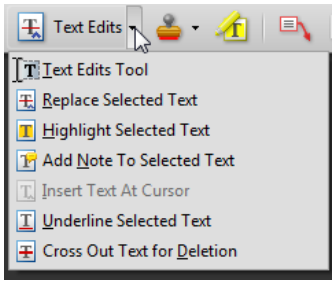

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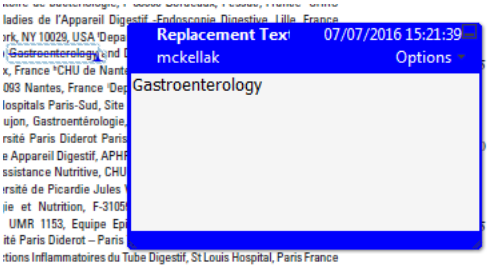

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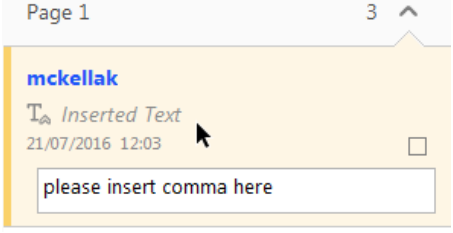

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Interactive CardioVascular and Thoracic Surgery (2017) 1–8
doi:10.1093/icvts/ivx373

Cite this article as: Fiorelli A, Messina G, Frongillo E, Accardo M, Montella M, Panarese I *et al*. The use of ultrasound in detecting and defining ground-glass opacities: results of an *ex vivo* evaluation. Interact CardioVasc Thorac Surg 2017; doi:10.1093/icvts/ivx373.

The use of ultrasound in detecting and defining ground-glass opacities: results of an *ex vivo* evaluation†

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Received 4 July 2017; received in revised form 17 September 2017; accepted 3 October 2017

Abstract

OBJECTIVE: To evaluate the role of ultrasound in detecting and defining ground-glass opacities (GGOs) in surgical specimens of patients undergoing thoracoscopic diagnostic resection.

METHODS: We performed an observational single-centre study of all consecutive patients undergoing thoracoscopic diagnostic resection of GGOs. In each patient, the specimen was scanned with ultrasound; then, a needle was inserted into the lesion to facilitate its detection by the pathologist. We evaluated the rate of detection with ultrasound, compared the size and depth measurements of the lesions as determined from ultrasound scans with those from the histological specimens and correlated the ultrasound findings with the histological subtypes of adenocarcinomas.

RESULTS: We reviewed 17 tissue samples. The final diagnoses were 1 (6%) atypical adenomatous hyperplasia, 5 (29%) adenocarcinomas *in situ*, 4 (24%) minimally invasive adenocarcinomas and 7 (41%) invasive adenocarcinomas. All tumours were successfully identified using ultrasound. The size ($P = 0.87$) and depth ($P = 0.25$) of the lesions measured with ultrasound did not significantly differ from the measurements obtained from the histological specimens. In addition, ultrasound size ($r = 0.945$; $P < 0.0001$) and depth ($r = 0.588$; $P = 0.013$) were significantly correlated with the pathological measurements. All lesions with hyperechoic findings ($n = 6$) were pure GGOs, whereas lesions with mixed echoic ($n = 11$) patterns were mixed GGOs. We were unable to differentiate the histological subtypes of adenocarcinomas using the ultrasound scans.

CONCLUSIONS: Detection of GGOs on ultrasound scans is feasible, but differentiation of the histological subtypes of adenocarcinomas is not possible. The next step is to evaluate the intraoperative reproducibility of our results.

Keywords: Ground-glass opacities • Ultrasound • Lung • Lung nodule • Video-assisted thoracoscopic surgery

INTRODUCTION

Ground-glass opacity (GGO) is a radiological term indicating an area of hazy increased lung opacity with visible vessels and bronchial structures [1]. The introduction of low-dose helical computed tomography (CT) scans for lung cancer screening has increased the detection of GGOs. Because GGOs can be observed in both benign and malignant conditions, differential diagnosis is crucial to define a prompt treat [2]. The role of bronchoscopic or percutaneous biopsy in diagnosing GGOs is controversial; diagnostic resection remains the best choice for obtaining a definitive diagnosis [3, 4]. However, GGOs are often impalpable and deep, and their detection during video-assisted thoracoscopic surgery (VATS) can be technically challenging [5, 6]. Several techniques, including ultrasound (US), which have been used successfully for the intraoperative localization of solid lung nodules [7, 8, 9], have also been adopted for detecting GGOs but with poor results so far.

In this study, we performed US on surgical specimens of patients undergoing thoracoscopic diagnostic resection of GGOs with the goal of assessing its effectiveness in detecting and defining the characteristics of GGOs and in providing the pathologist with a representative tissue sample for diagnosis.

MATERIALS AND METHODS

Study design

We conducted an observational single-centre study in the thoracic surgery unit of the University of 'Luigi Vanvitelli' of Naples from January 2014 to December 2016. We included all

†Presented at the 25th European Conference on General Thoracic Surgery, Innsbruck, Austria, 28–31 May 2017.

consecutive patients undergoing diagnostic resection of GGOs via VATS. Exclusion criteria included the lack of (i) US evaluation of the surgical specimen; (ii) a definite histological diagnosis and/or (iii) data regarding CT, ultrasound or pathological findings. The end-points of the study were (i) to evaluate the ability of US to detect GGOs and to define the size and depth of the lesions in comparison with the true measurements acquired from the histological specimens and (ii) to correlate the US patterns with the histological subtypes of GGOs. All data were collected prospectively and then analysed retrospectively. The US analysis of the surgical specimen did not change the standard diagnostic workup of the GGO; thus, specific consent from the institutional ethics committees was not necessary. All patients gave signed written informed consent for surgical treatment of GGOs, and they were aware that the US exploration of the surgical specimen would have not changed the standard of care for their disease and that their data could be used for scientific purposes only.

Study population

All patients underwent preoperative chest high-resolution CT scans that revealed the presence of GGOs. Based on the CT findings, the lesions were initially defined as possibly benign, indeterminate or possibly neoplastic. After reviewing the cases, a multidisciplinary team, which included a radiologist, a pneumologist and a thoracic surgeon, formulated a tentative diagnostic consensus according to the results of antibiotic therapy and short-term follow-up high-resolution CT scans. In agreement with the guidelines of the Fleischner Society [5] and of the Japanese Society [6] for management of incidental pulmonary nodules detected on CT images, patients with GGOs that had a solid component $\geq$5 mm or patients with GGOs $\geq$15 mm were suspected of having a cancer and underwent an invasive diagnostic procedure such as percutaneous biopsy or bronchoscopy. In the absence of a definitive diagnosis, a VATS diagnostic resection with an intraoperative microscopic examination of a frozen section specimen was carried out. Then, an anatomical resection completed the operation, if indicated. Patients were followed up according to standard clinical practice.

Radiological evaluation

All patients had 1-mm, thin-section chest CT scans using an 80 detector CT. Images were obtained using a lung window setting with a level of 1.500 HU, and a mediastinal window setting with a level of 30 HU and a width of 400 HU. The size, the depth and the tumour disappearance rate were also measured. A pure GGO was defined as a lesion without a solid component, whereas a mixed GGO was defined as a lesion with heterogeneous attenuation and any solid component.

Video-assisted thoracoscopic surgery resection

To facilitate its identification during VATS, the GGO was marked with methylene blue in the radiology room immediately prior to surgery. A 20-gauge needle and a solution of 0.7 ml of methylene blue and 0.3 ml of non-ionic contrast were used to tattoo the lesion. In a way similar to the way a fine-needle aspiration biopsy is guided by CT, the GGO was identified on the CT scan, and the site on the skin for the introduction of the needle was chosen, taking into consideration the relation of the lesion with the ribs and the scapula and the shorter distance between the lesion and the skin. The methylene blue was injected immediately adjacent to the GGO and along the needle tract right up to the pleural surface as the needle was retracted [10, 11]. In this way, a mark on the pleura was present that allowed the surgeon to identify the deep lesion during VATS. Then, a CT check of the labelling was performed, and the presence or the absence of a pneumothorax or of other possible complications was also noted. The patient was then transferred to the operating theatre to avoid any delay between the labelling and the VATS procedure. The diagnostic resection of the lesion was performed using the standard 3-port VATS approach, with the patient under general anaesthesia and using 1-lung ventilation. The palpation of the lung with dedicated endoscopic forceps was carried out in the region of the lung stained by methylene blue, and a standard wedge resection of the target area was performed with a stapler. As soon as we could confirm the diagnosis from the analysis of the frozen section, we performed an anatomical resection according to standard clinical practice.

Ultrasonography evaluation

The specimen was evaluated immediately after the resection without washing, incising or filling it with saline solution. A standard convex probe was connected to a dedicated US processor and used with frequencies of 3.5 MHz and 5 MHz, according to the depth of the lesions seen on the preoperative CT scan. The probe was placed directly on the surface of the specimen; performance of the US was based on our ability to identify the lesion and measure its diameter and depth. In addition, the echogenic patterns were recorded and correlated with the solid component and the histological results. Finally, a needle was inserted into the lesion under US guidance to facilitate its identification by the pathologist and to guide the analysis of the frozen section (Fig. 1). AQ9

Pathological evaluation

After US evaluation, the surgical specimen was sent to the pathologist. The entire resected lesion was fixed in formalin and embedded in paraffin. Several 3-μm-thick sections around the maximum diameter of the tumour were stained with haematoxylin and eosin and examined with a light microscope. The pathologist measured the size and the depth of the lesion (distance between the tumour and the lung surface) and classified the tumour according to the latest classification of adenocarcinomas from the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) [3]. Two senior pathologists analysed the histological preparations and a third was consulted in case of disagreement.

Statistical analysis

The Kolmogorov–Smirnov test and graphic histograms were used to check the normality/skewness of the continuous variables, and appropriate statistical tests were applied. Data were summarized as the mean and standard deviation for continuous variables or the absolute number and percentage for categorical variables. The Mann–Whitney test was used to compare the continuous variables of tumour size and depth. The Pearson correlation test was used to evaluate the correlation between the US and the

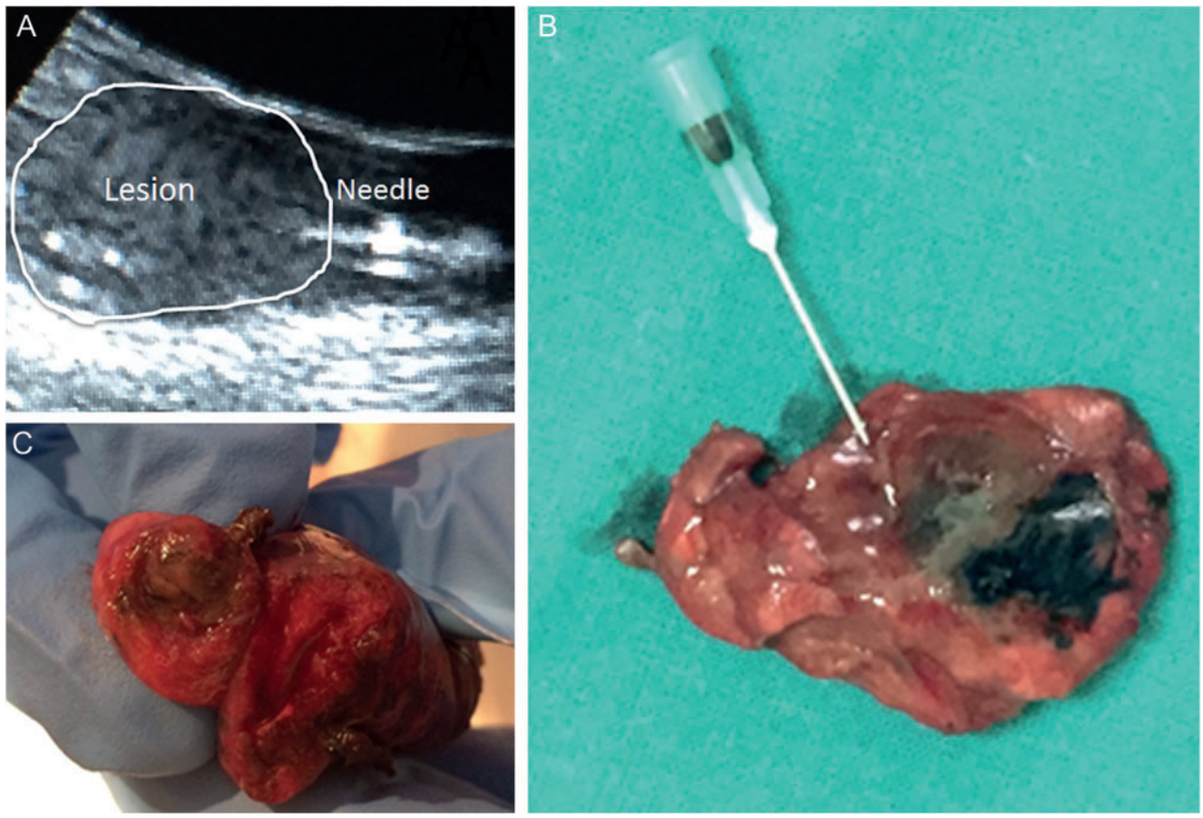


Figure 1: (**A**) Ultrasound scan showing the needle inserted into the lesion, (**B**) specimen with needle and (**C**) detection of lesion for frozen section analysis.

histological measurements, and the Bland–Altman plots provided a graphical presentation of the agreement between the different measurements. A *P*-value <0.05 was considered statistically significant. MedCalc statistical software (version 12.3; Broekstraat 52, 9030 Mariakerke, Belgium) was used for the analysis.

RESULTS

During the study period, 33 patients were referred to our department because GGOs were detected on high-resolution CT scans performed for other clinical reasons. Of these, 19 GGOs were suspected to be malignant because of an increase during the follow-up period in the solid component ≥5 mm ($n = 11$) or in tumour size ≥15 mm ($n = 8$). In 2 of 19 (10%) cases, a diagnosis of poorly differentiated adenocarcinoma was made with a transcutaneous biopsy, and a lobectomy was performed immediately. The remaining 17 (90%) patients underwent a diagnostic resection of the GGOs via VATS. The specimens ($n = 17$) were analysed with US and represented the objects of this analysis. A flow chart is shown in Fig. 2. The mean age of the study population was 57 years; 11 were women and 6 were men. The results of respiratory and cardiac tests were within normal ranges, and no other preoperative comorbidities contraindicated an operation. In addition, the chest CT scans showed no pleural adhesions that made VATS technically unfeasible. Based on the CT consolidation, 6 lesions were classified as pure GGOs and 11 as mixed GGOs. Based on the results of the frozen section analysis, diagnostic resection was followed by wedge resection in 3 cases, by segmentectomy in 7 and by lobectomy in 5. The characteristics of study population are shown in Table 1.

Ultrasound findings

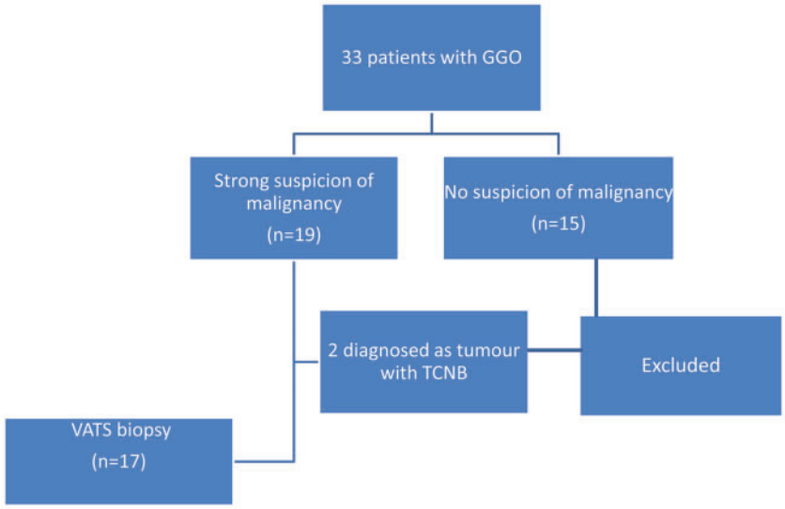
All tumours were successfully identified by US. The mean tumour size and depth were 14.1 ± 0.9 mm and 4.8 ± 0.2 mm, respectively. Six (35%) lesions had hyperechoic findings, whereas 11 (65%) lesions had mixed echogenicity where the hyperechoic patterns were irregularly mixed with hypoechoic images.

Pathological findings

In all cases, the pathologist, guided by the needle, found the lesion easily. A diagnosis was obtained at the time of the first analysis; no additional sections or surgical biopsies were needed. The final diagnoses included 1 (6%) atypical adenomatous hyperplasia, 5 (29%) adenocarcinomas *in situ* (3 mucinous predominant and 2 non-mucinous predominant), 4 (24%) minimally invasive adenocarcinomas (2 mucinous, 1 non-mucinous and 1 mixed predominant) and 7 (41%) invasive adenocarcinomas (4 lepidic, 2 papillary and 1 acinar predominant). The mean tumour size and depth were 13.9 ± 0.5 mm and 4.4 ± 0.3 mm, respectively.

Comparison of ultrasound and pathological measurements

The size ($P = 0.87$) and the depth ($P = 0.25$) of the GGOs measured from the US scans did not differ significantly from those parameters measured from the histological specimens. In addition, the size ($r = 0.945$; 95% confidence interval 0.904–0.988; $P < 0.0001$;



AQ17 **Figure 2:** Flow chart of study population. GGO: ground-glass opacity; TCNB: ~~Tru-cut needle biopsy;~~ VATS: video-assisted ~~thoracic~~ surgery.

Table 1: Characteristics of the study population

Patient	Site	Size (mm)			Depth (mm)			Findings			Operation
		CT	US	Hist.	CT	US	Hist.	CT	US	Hist.	
1	RUL	14	12	14	4	5	4	Pure	Hyperechoic	AIS	Segmentectomy
2	RUL	15	16	14	5	5	4	Mixed	Mixed	MIA	Lobectomy
3	LLL	8	10	10	4	3	4	Pure	Hyperechoic	AAH	Wedge resection
4	LLL	11	12	11	5	4	5	Mixed	Mixed	MIA	Lobectomy
5	LUL	14	13	12	6	6	5	Mixed	Mixed	MIA	Lobectomy
6	LUL	10	10	10	4	4	5	Mixed	Mixed	IA	Lobectomy
7	RLL	7	8	8	5	5	5	Pure	Hyperechoic	AIS	Segmentectomy
8	LLL	13	12	13	3	5	3	Mixed	Mixed	IA	Lobectomy
9	LLL	14	15	14	4	4	4	Mixed	Mixed	MIA	Lobectomy
10	LUL	13	14	13	2	6	4	Mixed	Mixed	IA	Lobectomy
11	LUL	15	15	16	6	6	7	Pure	Hyperechoic	AIS	Lobectomy
12	RUL	16	17	17	7	7	7	Mixed	Mixed	IA	Lobectomy
13	RLL	17	17	18	4	5	4	Mixed	Mixed	IA	Lobectomy
14	LUL	20	21	20	3	3	3	Pure	Hyperechoic	AIS	Lobectomy
15	RUL	19	20	19	2	3	2	Mixed	Mixed	IA	Lobectomy
16	LUL	11	12	12	5	6	5	Pure	Hyperechoic	AIS	Lobectomy
17	LLL	16	17	16	4	5	4	Mixed	Mixed	IA	Lobectomy

AAH: atypical adenomatous hyperplasia; AIS: adenocarcinoma *in situ*; CT: computed tomography; Hist.: histology; IA: invasive adenocarcinoma; LLL: lower left lobe; LUL: left upper lobe; MIA: minimally invasive adenocarcinoma; RLL: right lower lobe; RUL: right upper lobe; US: ultrasound.

Fig. 3A) and depth ($r = 0.588$; 95% confidence interval 0.149–0.833; $P = 0.013$; Fig. 3B) obtained from the US scans correlated significantly with the pathological measurements. The Bland–Altman plots showed an agreement between US and pathological size (Fig. 3C) and between US and pathological depth (Fig. 3D).

Comparison between ultrasound findings and histological patterns

All pure GGOs presented hyperechoic findings ($n = 6$), whereas mixed GGOs ($n = 11$) had mixed echoic patterns with both hyperechoic and hypoechoic components. In the preinvasive lesions ($n = 6$) and in the minimally invasive adenocarcinomas ($n = 4$), the hyperechoic pattern predominated over the hypoechoic component, whereas in the invasive adenocarcinoma ($n = 7$), the hypoechoic component predominated. However, we were unable to differentiate histological subtypes of adenocarcinomas from the US patterns. Examples are shown in Fig. 4.

DISCUSSION

Thoracoscopic resection is the strategy of choice to obtain an adequate specimen for diagnosing GGOs. However, intraoperative detection is technically challenging, especially with deep and pure GGOs. US has yielded good results in localizing solid lung nodules [7, 8, 9] that were occult at first intraoperative inspection, but it remains underused for the detection of GGOs. Several explanations are possible. The complete collapse of the lung

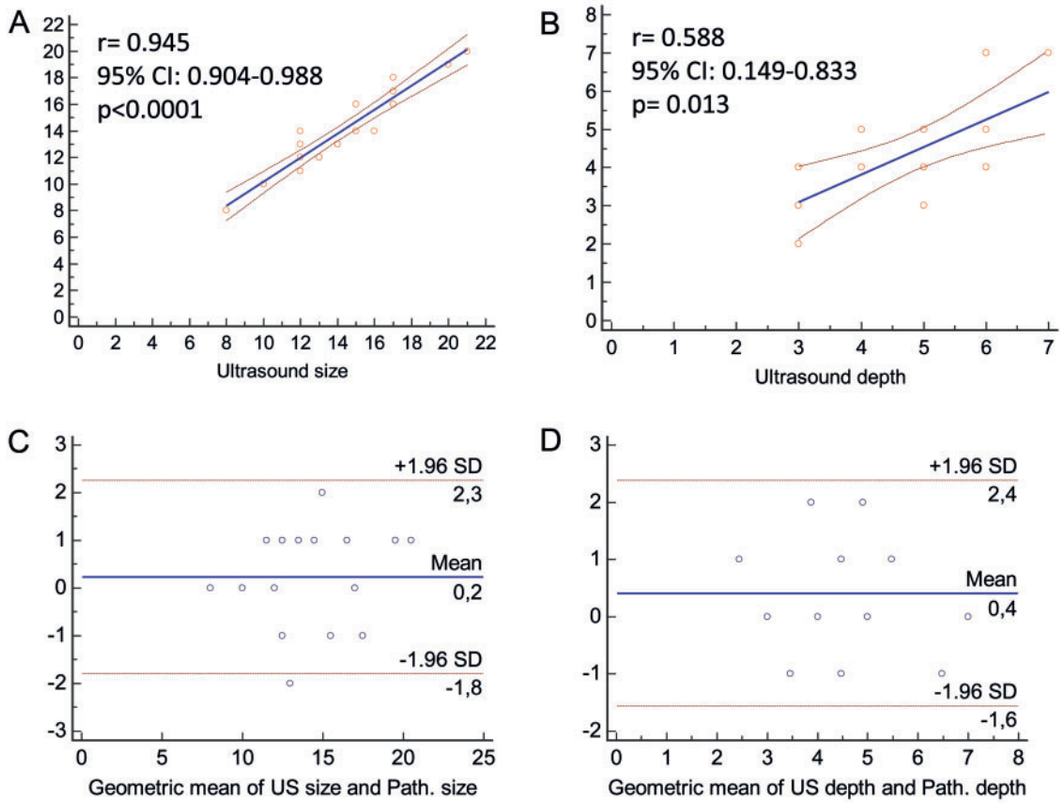


Figure 3: A significant correlation was found between the results determined from the US scan and the histological analysis regarding the size (**A**) and the depth measurements (**B**); good agreement was found between the results determined from the US scan and the histological specimen regarding the size (**C**) and the depth measurements (**D**). CI: confidence interval; SD: standard deviation; US: ultrasound.

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parenchyma is mandatory to detect GGOs by US, but it is difficult to obtain in emphysematous patients or in patients who did not tolerate prolonged single-lung ventilation. Kondo *et al.* [12] used US during VATS to guide the diagnostic resection of GGOs and obtained high-quality echo images in 56% (30/53) of patients in whom the complete collapse of the lung was achieved. Furthermore, GGOs are lesions with a density similar to that of adjacent normal parenchyma and thus identifying them with US is difficult, even for thoracic surgeons with extensive experience. Several experimental studies have evaluated the possibility of detecting small lung nodules including GGOs with US to define the US settings and to improve the skills of thoracic surgeons in this strategy. Daddi *et al.* [13], in an *ex vivo* porcine lung perfusion model, simulated 2 different types of lung lesions: They simulated solid nodules using a sample of porcine cardiac muscle and a GGO, using a sample of mediastinal fat tissue and then evaluated 3 different microconvex probes (an EBUS probe, a laparoscopic probe and a fingertip probe) for their detection. The EBUS probe used at 5 MHz frequency had the best performance in terms of image quality and detection rate of lesions. However, the main limit of this article is the lack of a realistic model as GGOs are heterogeneous lesions that cannot be accurately mimicked by a single sample of fat tissue. Ujiie *et al.* [14] evaluated the feasibility of using a new US thoracoscope to localize nodules ($n = 25$) in resected *ex vivo* human lungs. Solid nodules ($n = 16$) were more easily visualized than GGO lesions ($n = 9$). A strong positive correlation was found between the US and the true pathological measurements, but the US findings of GGOs did not correlate with their solid component and the histological subtypes of adenocarcinomas.

We evaluated the ability of US to detect and define the characteristics of GGOs. In addition, we inserted a needle into the lesions under US guidance to facilitate their detection by the pathologist and to reduce the number of frozen sections to be analysed. To facilitate the intraoperative localization, the lesion was marked with methylene blue that was injected near the lesion and, after labelling, the patient was quickly transferred to the operating theatre to prevent the dye from diffusing into the lesion and destroying the histological specimens. Filling the specimen with saline would result in a better resolution by improving the surface contact with the US transducer, but we decided against it to avoid any potential histological alteration of the lesion. In agreement with previous studies [12, 13], a convex rather than a linear probe was used at 2 different frequencies (e.g. 3.5 MHz and 5 MHz), which were chosen according to the distance of the lesion from the pleural surface and considering that the depth of penetration of the US beam is inversely proportional to the US frequency.

Using US, we identified all GGOs with a success rate of 100%. The labelling of GGOs and the characteristics of the lesions could explain these results. Methylene blue not only guided the intraoperative localization of the lesions but also restricted the region that should be scanned by US, thereby facilitating their detection. In addition, the size and the depth of a GGO could also have influenced the successful detection. Suzuki *et al.* [15] reported that target nodules ≤10 mm in size or >5 mm beneath the pleural

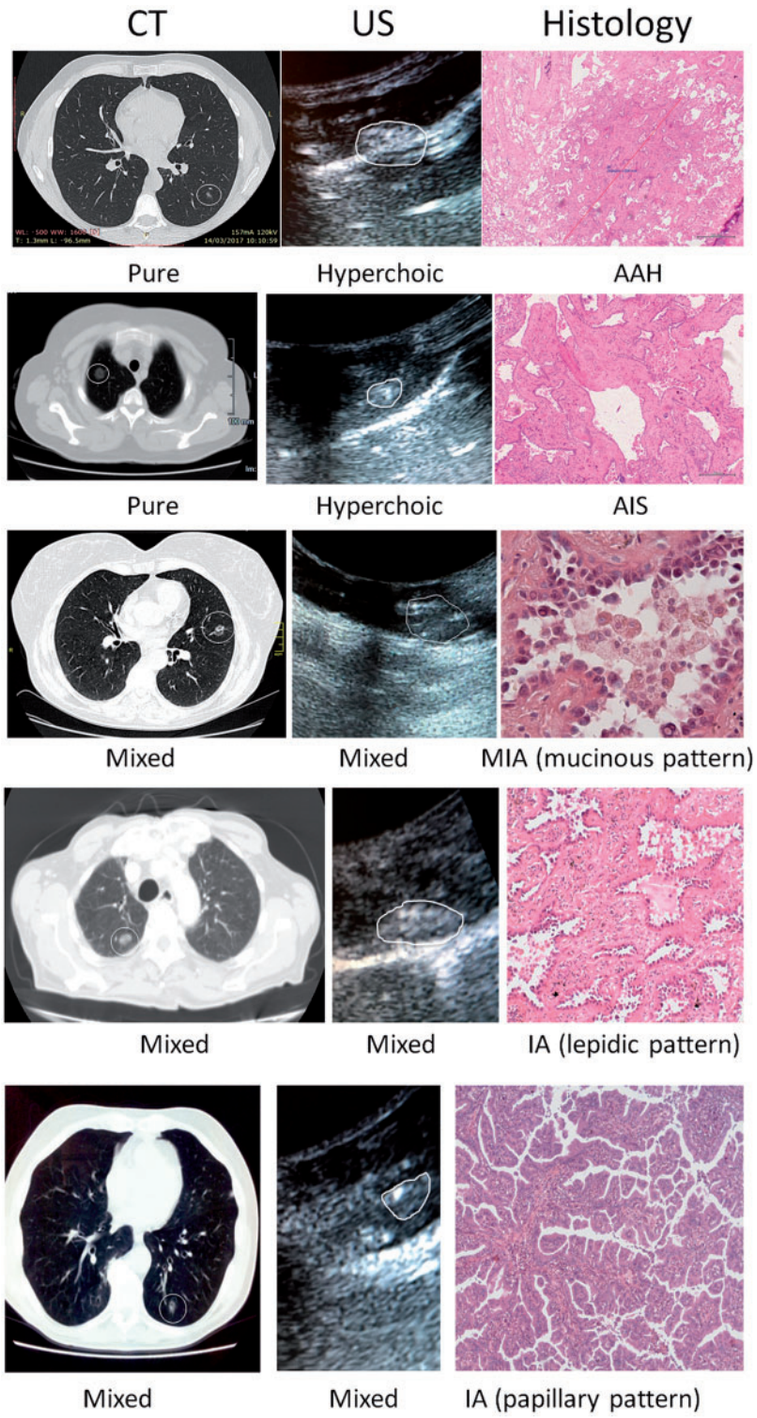

Figure 4: Examples of comparisons between US, CT and histological patterns. Haematoxylin and eosin staining: 20× magnification. AAH: atypical adenomatous hyperplasia; AIS: adenocarcinoma *in situ*; CT: computed tomography; IA: invasive adenocarcinoma; MIA: minimally invasive adenocarcinoma; US: ultrasound.

surface exhibited a 63% detection failure rate during the surgical procedure. Tamura *et al.* [16] reported that it was difficult to identify lung nodules <15 mm in size when the distance from the pleural surface was >10 mm. In our series, the pathological mean size of the nodule was 13.9 ± 0.5 mm, and in 11 of 17 (65%) cases, it was less than 15 mm; the pathological mean depth was 4.4 ± 0.3. Thus, a larger sample size including smaller and deeper tumours is needed to confirm our results. From a technical point of view, high frequency (5 MHz) allowed us to visualize shallower tumours and low frequency (3.5 MHz) to deeper lesions (> 4 mm). In line with previous studies [12, 13, 14], we found a strong correlation between the US and the histological measurements,

though it was less significant for the depth measurement. Pressing the probe on the surface of the specimen allowed us to push away the peritumoural residual air and to improve visualization of GGOs [8, 12, 14], but in theory, it affected the exact measurement of the distance between the lesion and the pleural surface. However, from a surgical point of view, removing as little lung tissue as possible containing GGOs at the first instance is of great importance, because some of them are benign. On the other hand, all lesions that turned out to be malignant at frozen section analysis required anatomical resections, at least a segmentectomy. Thus, the measurement of their margins with US could be irrelevant.

Second, all pure GGOs presented a hyperechoic pattern, and mixed GGOs had a mixed component with hyperechoic and hypoechoic patterns. The hyperechogenicity seen in pure GGOs could be explained by the large amount of residual air in the intact alveoli (without stromal invasion), whereas the heterogeneous pattern seen in mixed GGOs could be due to the presence of air and a solid component. These findings may also predict tumour invasion beyond the alveolar spaces, which usually happens when a GGO lesion develops a solid component. Similarly, GGO patterns found using the EBUS were classified by Izumo *et al.* [17] as (i) the blizzard sign, indicative of a pure GGO lesion and characterized by the presence of an increase in intensity and a radius of the whitish acoustic shadow noted while scanning from normal lung tissue to the ground-glass area and (ii) the mixed blizzard sign, indicative of a mixed GGO and characterized by the presence of diffuse heterogeneity with several hyperechoic dots, linear arcs that are distributed irregularly and combined with the blizzard sign. In the preinvasive lesions ($n = 6$) and in the minimally invasive adenocarcinomas ($n = 4$), the hyperechoic pattern seemed to be more predominant than that of the invasive adenocarcinoma ($n = 7$); however, we were unable to differentiate the histological subtypes of adenocarcinomas from the US patterns. The different types of echogenicities could also be the result of artefacts [18]. In fact, if the lung is not completely deflated, the structures of the interior lungs were likely to display a 'spotted hyperechoic pattern' because of the residual air echo artefacts.

Third, in clinical practice, our strategy could help thoracic surgeons become familiar with the identification of GGOs in lung parenchyma. Thus, after an initial learning curve performed in collaboration with expert radiologists, they could be ready to use US for intraoperative identification of the GGOs. In addition, the identification of a GGO in the specimen using US could also help the pathologist identify the lesion and reduce the operative time. Because a GGO is similar to normal lung parenchyma in density, its identification in the specimen can be technically challenging for pathologists, and it may require sectioning and microscopic examination of the entire portion of the sample where the lesion is thought to exist. Therefore, marking the lesion on the specimen using a US-guided needle can lead to quick localization by the pathologist, reduce the number of sections needed for examination and, thus, shorten the anaesthesia and operative time. In all our cases, the pathologists found the lesions easily, and the results from the frozen sections of the specimens were conclusive from the first analysis without the need for additional attempts. Our theory was confirmed by Li *et al.* [19] who evaluated the validity of CT-guided fine-needle localization of GGOs in reaerated lung specimens and found rapid, accurate localization of lesions by the pathologist with reduction of operative time. However, this strategy is more cumbersome than US, because it requires a radiology room with a dedicated CT scanner. In addition, moderate mechanical aeration of the specimen is needed to detect the GGOs with CT, but this requirement is unfeasible when the specimen is damaged with air leaks at reinflation.

Limitations

The experimental nature of the study and its several limitations do not permit us to draw definitive conclusions on the reproducibility of our results in clinical practice. (i) Small, deep lesions are more challenging to detect with US, but they are poorly represented in our study population. (ii) To obtain clear findings using US, it is mandatory to completely deflate the lung, a clinical requirement that is not feasible in all patients. (iii) The lack of a control group prevents the determination of whether the identification of GGOs in a surgical specimen using US could really reduce the number of frozen sections needed for analysis and the operative time.

CONCLUSIONS

The results of our study showed that we could successfully detect GGOs and define their characteristics in terms of the size, depth and solid component using US but that we were unable to differentiate the different subtypes of adenocarcinomas. Working with US in clinical practice can help thoracic surgeons become familiar with US before using it intraoperatively and can lead to the quick localization of the lesion by the pathologist. Due to the experimental nature of the study, the lack of a control group and the small sample size, the next step is to evaluate the reproducibility of our results in clinical practice and in a larger population.

Conflict of interest: none declared.

REFERENCES

[1] Nakata M, Saeki H, Takata I, Segawa Y, Mogami H, Mandai K *et al.* Focal ground-glass opacity detected by low-dose helical CT. Chest 2002;121:1464–7.

[2] Infante M, Lutman RF, Imparato S, Di Rocco M, Ceresoli GL, Torri V *et al.* Differential diagnosis and management of focal ground-glass opacities. Eur Respir J 2009;33:821–7.

[3] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y *et al.* International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244–85.

[4] Kobayashi Y, Mitsudomi T. Management of ground-glass opacities: should all pulmonary lesions with ground-glass opacity be surgically resected? Transl Lung Cancer Res 2013;2:354–63.

[5] MacMahon H, Naidich DP, Goo JM, Lee KS, Leung ANC, Mayo JR *et al.* Guidelines for management of incidental pulmonary nodules detected on CT images: from the Fleischner Society 2017. Radiology 2017;284:228–43.

[6] Takizawa H. Report on survey of chest CT examinations conducted at Japan Society of Ningen Dock member facilities. Ningen Dock 2009;24:657–64.

[7] Piolanti M, Coppola F, Papa S, Pilotti V, Mattioli S, Gavelli G. Ultrasonographic localization of occult pulmonary nodules during video-assisted thoracic surgery. Eur Radiol 2003;13:2358–64.
[8] Mattioli S, D'Ovidio F, Daddi N, Ferruzzi L, Pilotti V, Ruffato A *et al.* Transthoracic endosonography for the intraoperative localization of lung nodules. Ann Thorac Surg 2005;79:443–9.
[9] Santambrogio R, Montorsi M, Bianchi P, Mantovani A, Ghelma F, Mezzetti M. Intraoperative ultrasound during thoracoscopic procedures for solitary pulmonary nodules. Ann Thorac Surg 1999;68:218–22.
[10] Vandoni RE, Cuttat JF, Wicky S, Suter M. CT-guided methylene-blue labelling before thoracoscopic resection of pulmonary nodules. Eur J Cardiothorac Surg 1998;14:265–70.
[11] Findik G, Demiröz SM, Apaydın SMK, Ertürk H, Biri S *et al.* Computed tomography-guided methylene blue labeling prior to thoracoscopic resection of small deeply placed pulmonary nodules. Do we really need palpation? Thorac Cardiovasc Surg 2017;65:387–91.
[12] Kondo R, Yoshida K, Hamanaka K, Hashizume M, Ushiyama T, Hyogotani A *et al.* Intraoperative ultrasonographic localization of pulmonary ground-glass opacities. J Thorac Cardiovasc Surg 2009;138:837–42.
[13] Daddi N, Sagrini E, Lugaresi M, Perrone O, Candoli P, Ragusa M *et al.* Ex vivo pulmonary nodule detection with miniaturized ultrasound convex probes. J Surg Res 2016;202:49–57.
[14] Ujiie H, Kato T, Hu HP, Hasan S, Patel P, Wada H *et al.* Evaluation of a new ultrasound thoracoscope for localization of lung nodules in *ex vivo* human lungs. Ann Thorac Surg 2017;103:926–34.
[15] Suzuki K, Nagai K, Yoshida J, Ohmatsu H, Takahashi K, Nishimura M *et al.* Video-assisted thoracoscopic surgery for small indeterminate pulmonary nodules: indications for preoperative marking. Chest 1999;115:563–8.
[16] Tamura M, Oda M, Fujimori H, Shimizu Y, Matsumoto I, Watanabe G. New indication for preoperative marking of small peripheral pulmonary nodules in thoracoscopic surgery. Interact CardioVasc Thorac Surg 2010;11:590–3.
[17] Izumo T, Sasada S, Chavez C, Matsumoto Y, Tsuchida T. Radial endobronchial ultrasound images for ground-glass opacity pulmonary lesions. Eur Respir J 2015;45:1661–8.
[18] Hasan AA, Makhlouf HA. B-lines: transthoracic chest ultrasound signs useful in assessment of interstitial lung diseases. Ann Thorac Med 2014;9:99–103.
[19] Li M, Shen G, Gao F, Zheng X, Hua Y, Xiao L. CT-guided fine-needle localization of ground-glass nodules in re-aerated lung specimens: localization of solitary small nodules or multiple nodules within the same lobe. Diagn Interv Radiol 2015;21:391–6.

APPENDIX. CONFERENCE DISCUSSION

Dr. Z. Szanto *(Pecs, Hungary)*: You probably did not get typical ultrasound pattern of each lesion. Would you rely on this only to do a lobectomy or do you need a fast frozen section to prove it is a malignancy. What would you do?

Dr. A. Fiorelli *(Naples, Italy):* Those are only preliminary results of this study. We were unable to define typical ultrasound patterns for each GGO lesion. As you can see, the final pattern of the ultrasound in the GGO lesion is very different than in solid lesion, because the characteristics of the tumour are completely different. Literature usually says to use intraoperative ultrasound during operation for solid nodule, but the intraoperative use of ultrasound for detecting GGO lesion is under evaluation. Solid tumour presents a hypoechoic pattern that makes its identification easier. Conversely, it is difficult to identify and define the ultrasound patterns of GGO, especially in case of deep and pure GGO. The air is the enemy of ultrasound, and the presence of air in the lesion does not allow the beam of ultrasound. Pure GGO presents hyperechoic findings due to the presence of the air. Mixed ground glass presents an invasion of the cancer cell into stroma of the alveoli, the solid component, which explains the hypoechoic pattern. In theory, the hypoechoic dots mixed with hyperechoic could suppose invasiveness of the tumour.

Dr. Szanto: Would you do a surgery just relying on the ultrasound?

Dr. Fiorelli: No. It is only an observational study that did not change the standard diagnostic workup for the GGO lesion. Our strategy could guide the pathologists to identify the GGO on the frozen section analysis. If the intraoperative identification of the GGO is difficult for the thoracic surgeon, similarly its identification could also be difficult for the pathologist who cannot palpate the GGO in specimen. So, our strategy could reduce the operative time, since it helps the pathologist to identify the lesion, reduces the number of frozen section analysis, gives to the thoracic surgeon a fast intraoperative diagnosis that remains the only strategy to define the malignancy of the lesion and to decide whether performing a lobectomy or other types of resection.

Dr. A. Turna *(Istanbul, Turkey)*: Did you look at the false-positive rate, false-negative rate and the accuracy of this ultrasound method? I think that ROC curve would be beneficial to understand the value of this method. The second question is what is the deepest level that you can see effectively to understand the nature of the GGO?

Dr. Fiorelli: Obviously, this is a preliminary result. The final goal of this article is to learn the ultrasound pattern of GGO on the specimen before its intraoperative use. Pure GGO and deep lesions are very difficult to be detected. The presence of air makes pure GGO lesions similar to normal lung parenchyma, while in mixed GGO lesion, the solid component facilitates its identification. Obviously, the identification of deep lesions on surgical specimen is easier than during operation, because the parenchyma of surgical specimen is fully collapsed.