Elastography during convex-probe (endobronchial ultrasound) for optimal biopsy sample and gene identification in non-small-cell lung cancer

Paul Zarogoulidis^{*1}, Christoforos Kosmidis¹, Varvara Fyntanidou², Anastasios Barmpas¹, Charilaos Koulouris¹, Zoi Aidoni¹, Haidong Huang³, Chong Bai³, Wolfgang Hohenforst-Schmidt⁴, Kosmas Tsakiridis⁵ & Konstantinos Sapalidis¹

¹3rd Department of Surgery, 'AHEPA' University Hospital, Aristotle University of Thessaloniki, Medical School, Thessaloniki, Greece

²Anesthesiology Department, 'AHEPA' University Hospital, Aristotle University of Thessaloniki, Medical School, Thessaloniki, Greece

³Department of Respiratory & Critical Care Medicine, Changhai Hospital, the Second Military Medical University, Shanghai, PR China

⁴Department of Cardiology/Pulmonology/Intensive Care/Nephrology, Sana Clinic Group Franken, 'Hof' Clinics, University of Erlangen, Hof, Germany

⁵Thoracic Surgery Department, 'Interbalkan' European Medical Center, Thessaloniki, Greece

*Author for correspondence: pzarog@hotmail.com

“The radial endobronchial ultrasound (EBUS) and convex EBUS are the excellent diagnostic tools for the investigation of a nodule and diagnosis-staging lung cancer”

First draft submitted: 21 August 2019; Accepted for publication: 23 August 2019; Published online: 27 September 2019

Keywords: EBUS • elastography • lung cancer

"We need methods to update the rapid on-site evaluation of needle biopsy. Convex-probe (endobronchial ultrasound [EBUS]) is an endoscope equipped with elastography a nonspecific method of thickness evaluation. However, it provides useful information as a rapid on-site biopsy evaluation tool. This probe can target with the help of elastography a specific site to puncture for in order to acquire the best possible tissue sample with live cancer cells. Can elastography identify regions with mutations within a tumor or lymph node?"

Lung cancer is still diagnosed at a late stage although we have novel endoscopic diagnostic equipment. The radial endobronchial ultrasound (EBUS) and convex EBUS are the excellent diagnostic tools for the investigation of a nodule and diagnosis-staging lung cancer [1,2]. In the case where a patient is inoperable we have to acquire good tissue samples in order to have as possible the 'tumor heterogeneity' [3]. Taking samples from different site of the tumor can be achieved in most of the inoperable patients since the disease is spread to different lymph nodes, but also because the main lesion is >2 cm and several punctures can be made in different positive lymph nodes and different sites of the main lesion. We need to acquire from a patient tissue with tumor heterogeneity in order to have the best chances of finding *EGF* or *ALK* mutation, *BRAF*, *ROS1* and *pPD-L1*. Based on these genes we can provide targeted therapy with tyrosine kinase inhibitors or immunotherapy [4,5].

Endoscopes & imaging techniques

Acquiring large tissue samples with live cancer cells can be difficult in some cases like squamous cell carcinoma where tumor necrosis is observed. The video bronchoscope is used to obtain tissue samples with forceps and blind biopsies with 19G, 21G and 22G needles. However, with the forceps we take biopsies only from one site of the tumor and we lose the heterogeneity. Blind biopsies are not being used any more since we have the convex-probe (EBUS); with this system the operator can acquire multiple samples from different sites of a lesion or lymph node therefore the heterogeneity of the tumor is obtained. Acquiring large tissue samples with live cancer cells can be difficult in some cases like squamous cell carcinoma where tumor necrosis is observed. In the everyday clinical practice positron emission computed tomography can be used especially in large central mass with tumor necrosis in

order to visualize the best site for biopsy within the mass (live cancer cells). Moreover, we have the first information if a mass is possibly cancer and which lymph nodes might be positive with malignancy [6]. Rapid on-site evaluation is a method to identify after puncture of a mass or lymph node if the site of the puncture contains adequate material for sufficient evaluation of malignancy or benignancy. It is a method of rapid evaluation performed by cytologists or pulmonary physicians specialized to this method.

Elastography option

All companies in the market that produce these systems have elastography as an option. Rapid tissue evaluation is performed with structural deformation, which is caused by compression or vibration and produces color images representing the relative elasticity or stiffness of the tissue compressed/viewed. The produced images can be classified as malignant or benign with a division in three different types. Each type has a different grading of color. This information comes from our previous knowledge with esophageal ultrasonography-elastography [7]. Elastography is actually an imaging modality that maps the elastic properties and stiffness of soft tissue [8]. Either this is a lymph node or a mass or an unknown lesion/finding gray-scale ultrasonography assesses the distribution, size, and internal architecture of masses and lymph nodes [9]. Doppler ultrasonography can evaluate the intranodal vascular pattern and resistance of lymph nodes and masses [10]. The contrast-enhanced ultrasonography provides information on lung masses and lymph nodes parenchymal perfusion [11]. With elastography we can assess qualitatively and quantitatively the stiffness of lymph nodes [12]. Moreover; elastography is also known as E-mode. The morphologic characteristics of the lymph nodes have been recorded as follows based on convex-probe (CP)-EBUS images: shape (oval or round); margin (indistinct or distinct); presence or absence of central hilar structure; short-axis size $</>1$ cm; echogenicity (homogeneous or heterogeneous); and presence or absence of coagulation necrosis sign. It was observed that when biopsy was performed these results were compared between convex-probe-EBUS image classification and final pathology, and the following observations were made: indistinct margins; lymph nodes with small sizes; round shapes; presence of central hilar structure; and homogeneous echogenicities, tended to be benign. In the case where central hilar structure was observed, they tended to be malignant. Moreover; a size of ≥ 10 mm, heterogeneous echogenicity, round shape, absence of central echogenic hilum, distinct margin and coagulation necrosis were considered to be signs of malignant infiltration of the lymph node. It has been concluded based on these findings that sonographic features of lymph nodes based on the new EBUS imaging classification can predict metastatic lymph nodes during EBUS- transbronchial needle aspiration procedure [13]. Furthermore; B-mode morphological ultrasound criteria are a useful tool for the detection of malignant lymph nodes during EBUS [13,14]. Elastography, to date, even when we use only the combination of the color distribution and strain ration greater than or equal to eight, for some studies greater than or equal to four, it is sufficient to identify the best region to puncture and obtain tissue sample, but also to have the first evaluation for malignancy or benignancy [15].

Future perspective

Elastography is actually an imaging modality that characterizes the elastic properties and stiffness of tissue [8]. This tissue can be a lymph node, a mass or surrounding. Based on the elasticity we will have diagnostic information about the presence or status of a disease. It has been observed that in the case of cancer tumors, the surrounding tissue will be harder. Elastography is currently used in many organs for the investigation of several disease conditions. It provides additional diagnostic information compared with a mere anatomical image, and moreover, it is used to guide biopsies. Maybe one day it will replace them entirely. Biopsies are invasive and in several situations present a risk of hemorrhage, infection or even malignancy transplantation in surrounding tissue. Elastography on the other hand is completely noninvasive. Major drawback is that we have only one information whether it is benignancy or malignancy, but in the case of malignancy, we do not know the histological type. Elastography has been previously used for many years to investigate liver disease. Elastography can evaluate in the liver stiffness, which is usually an indication of fibrosis or steatosis (fatty liver disease). This method has the advantage because when fibrosis is diffused, a biopsy can easily miss sampling of the diseased tissue, as it might happen with non-small-cell lung cancer (NSCLC) large masses. The results then are false negative. Elastography has been also applied as an external procedure (use of transdermal convex probe) for the evaluation of breast, pancreatic, thyroid and prostate tumors. Furthermore, it can evaluate lymph node involvement of esophageal and rectal cancers [7,16–22]. Based on the color of the lesion and, in specific, the percentage of the color in the whole lesion, we have the first evaluation of malignancy or benignancy. Based on previous studies it is reported that blue areas inside a lymph node represent stiff areas, which are usually correlated with malignancy. Blue color, which again represents the

stiffness, is the same for metastatic lymph nodes from lung cancer or other extra thoracic malignancies. During the elastographic evaluation we should avoid large vessels because structures with very low elasticity might induce artifacts in the evaluation of stiffness distribution [22]. However, with elastography we can evaluate whether the wall of a large thoracic vessel (pulmonary artery/aorta) has been infiltrated by malignancy. Moreover, we can distinguish a thrombus within a large thoracic vessel from tumor tissue when using the EBUS. In the study by Gu *et al.* [23], the diagnostic yield of elastography was investigated for differentiating malignant and benign mediastinal lymph nodes. Conventional EBUS B-mode features, shape, including size, echogenicity, border distinction, coagulation necrosis and central hilar structure with central blood vessel were also evaluated. The combined model investigated was superior to elastography alone. The most important criteria were the following: elastography, heterogeneity, size and shape, and when these four were positive when the odds ratio for malignancy was 9.44 with a 95% CI of 3.99–22.32; $p < 0.0001$. In a recent publication positron emission computed tomography technique presented moderate effectiveness in predicting EGFR-positive NSCLC cancers [24]. The same was observed for *ALK* mutation expression [25]. Regarding elastography and possible identification of NSCLC mutations we do not have any published data. The only association between elastography and gene expression association comes from published studies from other cancer types such as breast cancer. Indeed a thorough investigation of the somnographic findings can provide the first rapid diagnosis of tumor gene mutations before of course the tissue confirmation [26–28]. In the near future we do not expect a new ultrasound processor to be used by the three companies producing the convex probes. We do expect to have a new software that adds information to the already existing (color and strain ratio) like size of lymph node calcification, lymph node barrier condition and angiogenesis. A new algorithm with more information included simultaneously will make the technique more efficient since the evaluation will be done automatically and faster for the operator. Furthermore, elastography has been used and can be used to evaluate whether the bronchial wall/esophageal wall or surrounding tissue has been infiltrated in the case of malignancy. In any case we cannot evaluate the gene expression of a tumor with elastography, maybe in the future with the addition of a contrast enhancer.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1. Zaric B, Stojisic V, Carapic V *et al.* Radial endobronchial ultrasound (EBUS) guided suction catheter-biopsy in histological diagnosis of peripheral pulmonary lesions. *J. Cancer* 7(1), 7–13 (2016).
2. Oezkan F, Khan A, Zarogoulidis P *et al.* Efficient utilization of EBUS-TBNA samples for both diagnosis and molecular analyses. *OncoTargets Ther.* 7, 2061–2065 (2014).
3. Domvri K, Darwiche K, Zarogoulidis P, Zarogoulidis K. Following the crumbs: from tissue samples, to pharmacogenomics, to NSCLC therapy. *Translational Lung Cancer Res.* 2(4), 256–258 (2013).
4. Domvri K, Zarogoulidis P, Darwiche K *et al.* Molecular targeted drugs and biomarkers in NSCLC, the evolving role of individualized therapy. *J. Cancer* 4(9), 736–754 (2013).
5. Zarogoulidis K, Zarogoulidis P, Darwiche K *et al.* Treatment of non-small-cell lung cancer (NSCLC). *J. Thorac. Dis.* 5(Suppl. 4), S389–396 (2013).
6. Groeux D, Quere G, Blanc E *et al.* FDG PET-CT for solitary pulmonary nodule and lung cancer: literature review. *Diagn. Interv. Imaging* 97(10), 1003–1017 (2016).
7. Janssen J, Dietrich CF, Will U, Greiner L. Endosonographic elastography in the diagnosis of mediastinal lymph nodes. *Endoscopy* 39(11), 952–957 (2007).
8. Wells PN, Liang HD. Medical ultrasound: imaging of soft tissue strain and elasticity. *J. R. Soc. Interface* 8(64), 1521–1549 (2011).
9. Ahuja A, Ying M, King W, Metreweli C. A practical approach to ultrasound of cervical lymph nodes. *J Laryngol. Otol.* 111(3), 245–256 (1997).
10. Wu CH, Chang YL, Hsu WC, Ko JY, Sheen TS, Hsieh FJ. Usefulness of Doppler spectral analysis and power Doppler sonography in the differentiation of cervical lymphadenopathies. *AJR. Am. J. Roentgenol.* 171(2), 503–509 (1998).
11. Jin Y, He YS, Zhang MM *et al.* Value of contrast-enhanced ultrasonography in the differential diagnosis of enlarged lymph nodes: a meta-analysis of diagnostic accuracy studies. *Asian Pac. J. Cancer Prev.* 16(6), 2361–2368 (2015).

12. Choi YJ, Lee JH, Baek JH. Ultrasound elastography for evaluation of cervical lymph nodes. *Ultrasonography* 34(3), 157–164 (2015).
13. Fujiwara T, Yasufuku K, Nakajima T *et al.* The utility of sonographic features during endobronchial ultrasound-guided transbronchial needle aspiration for lymph node staging in patients with lung cancer: a standard endobronchial ultrasound image classification system. *Chest* 138(3), 641–647 (2010).
14. Garcia-Olive I, Monso E, Andreo F *et al.* Sensitivity of linear endobronchial ultrasonography and guided transbronchial needle aspiration for the identification of nodal metastasis in lung cancer staging. *Ultrasound Med. Biol.* 35(8), 1271–1277 (2009).
15. Huang H, Huang Z, Wang Q *et al.* Effectiveness of the benign and malignant diagnosis of mediastinal and hilar lymph nodes by endobronchial ultrasound elastography. *J. Cancer* 8(10), 1843–1848 (2017).
16. Barr RG, Destounis S, Lackey L 2nd, Svensson WE, Balleyguier C, Smith C. Evaluation of breast lesions using sonographic elasticity imaging: a multicenter trial. *J. Ultrasound Med.* 31(2), 281–287 (2012).
17. Brock M, von Bodman C, Palisaar RJ *et al.* The impact of real-time elastography guiding a systematic prostate biopsy to improve cancer detection rate: a prospective study of 353 patients. *J. Urol.* 187(6), 2039–2043 (2012).
18. Lyshchik A, Higashi T, Asato R *et al.* Thyroid gland tumor diagnosis at US elastography. *Radiology* 237(1), 202–211 (2005).
19. Giovannini M, Hookey LC, Bories E, Pesenti C, Monges G, Delperio JR. Endoscopic ultrasound elastography: the first step towards virtual biopsy? Preliminary results in 49 patients. *Endoscopy* 38(4), 344–348 (2006).
20. Saftoiu A, Vilmann P, Hassan H, Gorunescu F. Analysis of endoscopic ultrasound elastography used for characterisation and differentiation of benign and malignant lymph nodes. *Ultraschall in der Medizin* 27(6), 535–542 (2006).
21. Giovannini M, Thomas B, Erwan B *et al.* Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreatic masses: a multicenter study. *World J. Gastroenterol.* 15(13), 1587–1593 (2009).
22. Saftoiu A, Vilmann P, Ciurea T *et al.* Dynamic analysis of EUS used for the differentiation of benign and malignant lymph nodes. *Gastrointest. Endosc.* 66(2), 291–300 (2007).
23. Gu Y, Shi H, Su C *et al.* The role of endobronchial ultrasound elastography in the diagnosis of mediastinal and hilar lymph nodes. *Oncotarget* 8(51), 89194–89202 (2017).
24. Yang B, Wang QG, Lu M *et al.* Correlations study between (18)F-FDG PET/CT metabolic parameters predicting epidermal growth factor receptor mutation status and prognosis in lung adenocarcinoma. *Front. Oncology* 9, 589 (2019).
25. Lv Z, Fan J, Xu J *et al.* Value of (18)F-FDG PET/CT for predicting *EGFR* mutations and positive ALK expression in patients with non-smal-cell lung cancer: a retrospective analysis of 849 Chinese patients. *Eur. J. Nucl. Med. Mol. Imaging* 45(5), 735–750 (2018).
26. Wang D, Zhu K, Tian J *et al.* Clinicopathological and ultrasonic features of triple-negative breast cancers: a comparison with hormone receptor-positive/human epidermal growth factor receptor-2-negative breast cancers. *Ultrasound Med. Biol.* 44(5), 1124–1132 (2018).
27. Denis M, Gregory A, Bayat M *et al.* Correlating tumor stiffness with immunohistochemical subtypes of breast cancers: prognostic value of comb-push ultrasound shear elastography for differentiating luminal subtypes. *PLoS ONE* 11(10), e0165003 (2016).
28. Au FW, Ghai S, Lu FI, Moshonov H, Crystal P. Quantitative shear wave elastography: correlation with prognostic histologic features and immunohistochemical biomarkers of breast cancer. *Acad. Radiol.* 22(3), 269–277 (2015).