363. EBUS-TBNA: a never ending story

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Rapid on-site cytologic evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for diagnosing lung cancer: A randomized study

Masahide Oki[1], Hideo Saka[1], Chiyoe Kitagawa[1], Yoshihito Kogure[1], Naohiko Murata[1], Misaki Ryuge[1], Takashi Adachi[1], Saori Oka[1], Rie Tsuboi[1], Masahiko Ando[2]. *[1]Department of Respiratory Medicine, Nagoya Medical Center, Nagoya, Japan; [2]Department of Kyoto University Health Service, Kyoto University, Kyoto, Japan*

Introduction: Although rapid on-site cytologic evaluation (ROSE) is widely used during endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), its utility remains unknown.
Aims and objectives: The purpose of the present study was to evaluate the efficacy of ROSE during EBUS-TBNA in the diagnosis of lung cancer.
Methods: One hundred twenty patients with highly suspected lung cancer who had hilar/mediastinal lymphadenopathy or a tumor adjacent to the central airway were enrolled in this study and randomized to undergo EBUS-TBNA with and without ROSE.
Results: Twelve patients with visible endobronchial lesions were excluded in the analysis. Thus, a total of 108 patients (55 in ROSE group; 53 in non-ROSE group) were included in the analysis. Additional procedures including EBUS-TBNA for other lesions and/or transbronchial biopsy were performed in 11% of patients in the ROSE group and 57% in the non-ROSE group ($P < 0.001$). Mean puncture number was significantly fewer in the ROSE group (2.2 vs. 3.1 punctures, $P < 0.001$). Mean bronchoscopy time was similar in both groups (22.3 vs. 22.1 min, $p = 0.95$). The sensitivity and accuracy for diagnosing lung cancer were 88% and 89% in the ROSE group, and 86% and 89% in the non-ROSE group, respectively. No complications were associated with the procedures.
Conclusion: Although ROSE during EBUS-TBNA can reduce the puncture number or eliminate the need for additional bronchoscopic procedures, it is not associated with the total bronchoscopy time or diagnostic accuracy in patients with suspected lung cancer.

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A way to optimize efficiency of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA): Evaluation of the aspiration on site by a non-pathologist – Results on a monocentric study about 98 procedures

Isabelle Rault[1], Florence Le Meunier[1], Coraline Hybiak[1], Houcine Bentayeb[1], Marie Boutemy[1], Emmanuelle Lecuyer[1], Remi Suguenot[1], Patrick Dumon[1], Faouzi Amirats[1], Claire Andrejak[2], Vincent Jounieaux[2], Sophie Carton[3], Youcef Douadi[1], Charles Dayen[1]. *[1]Pneumology Unit, Saint Quentin Hospital, Saint Quentin, France; [2]Pneumology Unit, Amiens Hospital, Amiens, France; [3]Pathology Unit, Saint Quentin Hospital, Saint Quentin, France*

Background: EBUS-TBNA is now an usual technique in the diagnosis of mediastinal adenopathy. To have the best degree of performance in the diagnosis, the number of needle-aspirations must be not less than three. The length of this technique and the number of sites to evaluate are some difficulties of EBUS-TBNA, particularly with loco-regional anesthesia. To improve the diagnosis and reduce the number of procedures, some authors describe the use of a pathologist on site.
Objectives: Optimize the efficiency of EBUS-TBNA by a non-pathologist.
Method: We realize a study about patients who get an EBUS-TBNA in 2011. On each of them, our nurses were asked to evaluate the efficiency of the EBUS aspiration through the macroscopic aspect of the sample. The nurses' responses (RON) were: needle-aspiration negative or needle-aspiration positive. Nurses' on site evaluations were compared with the final diagnosis obtained by the cytologist (ROC). Our nurses have an experience of EBUS-TBNA since 3 years in routine.
Results: 98 patients (72 men and 26 women) were included in this study. We realize 536 needle aspirations. The most often punctured areas were 7 (66/156, 42%) and 4R (34/156, 21,8%). Lung cancer was diagnosed in 52 of the 98 patients (53%) with most often adenocarcinoma (32/52).
We found 85,8% of correlation between RON and ROC.
Conclusion: On site evaluation by a non pathologist may optimize efficiency of EBUS-TBNA and is cost-effectiveness.

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Role of molecular and cellular techniques applied to EBUS-TBNA of mediastinal lymph nodes for lung cancer staging

Antonio Bugalho[1,2,3], Gloria Nunes[1], Manuela Correia[1], Catarina Martins[1], Sara S. Dias[4], Paula A. Videira[1], Maria João Marques Gomes[1]. *[1]CEDOC, Faculdade de Ciências Médicas, UNL, Lisbon, Portugal; [2]Interventional Pulmonology Unit, Hospital Pulido Valente, Lisbon, Portugal; [3]Interventional Pulmonology Unit, Hospital Beatriz Angelo, Loures, Portugal; [4]Departamento Universitário de Saúde Pública, Faculdade de Ciências Médicas, UNL, Lisbon, Portugal*

Introduction: Accurate NSCLC staging is of major importance since it dictates the choice of treatment and prognosis. EBUS-TBNA is a minimally invasive method to sample mediastinal lymph nodes. The concomitant identification of cancer biomarkers is important to improve EBUS-TBNA staging.
Aim: Assess the feasibility and role of EBUS-TBNA combined to the identification of tumour-associated antigens and tumour-associated immune responses to diagnose lymph node metastases and pathological characteristics in lymph node aspirates.
Methods: In a prospective study, EBUS-TBNA samples from 57 patients with confirmed or suspected lung cancer were analysed by cytopathology, flow cytometry (FACS) and reverse-transcriptase polymerase chain reaction (RT-PCR).
Results: All samples were adequately processed by the 3 different techniques. Among the 47 samples diagnosed with tumour cell by cytopathology, 70% showed the presence of cytokeratin-19 (CK-19) cells by FACS and 83% of the SCLC were CK-19 negative. CK-19 phenotype and gene expression were significantly correlated ($r= 0.901$) and cells with this phenotype also expressed CEA, sialyl Lewis X and CD44+ in 22.2%, 25.0% and 18.7% of cases. The expression of the *EPCAM* gene was significantly higher in the cytopathologically diagnosed cases ($p=0.03$). The analysis of immune cells profile in the aspirates of these patients revealed a decrease in total leukocytes ($p=0.022$) and a increase in monocytes ($p= 0.039$).
Conclusions: The combination of molecular and cellular biology techniques with EBUS-TBNA might be a feasible option to improve NSCLC staging and offer an individualized diagnostic and therapeutic approach to lung cancer patients.

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Endobronchial ultrasound guided biopsy followed by real-time PCR is an applicable method to analyse SHOX2 methylation level in mediastinal lymph nodes of lung cancer patients

Katrin Bähner, Lutz Freitag, Claudia Ivascu, Dirk Theegarten, Jeremias Wohlschläger, Roze Imsak, Kaid Darwiche. *NRW, Ruhrlandklinik Essen, Germany*

Previous studies have shown that SHOX2 DNA methylation can potentially serve as a tumour marker in lung cancer patients. DNA methylation of SHOX2 could have been assessed in serum and bronchial aspirates.
This study aimed to find out, if there is an opportunity to analyse SHOX2 DNA methylation level in lymph node tissue obtained by endobronchial ultrasound guided biopsy in context of diagnosis and lymph node staging.
Tissue from mediastinal lymph nodes was taken from ten patients with proven lung

cancer. In five cases the tissue was obtained by transbronchial forceps biopsy, in the other five by transbronchial needle aspiration (TBNA). Samples were treated with bisulphite, excluding the unmethylated SHOX2 DNA from amplification. A real-time PCR duplex assay was then used to quantify the amount of total DNA on one hand and the number of methylated SHOX2 gene copies on the other hand. Finally the DNA methylation level of SHOX2was determined relating the number of methylated SHOX2 gene copies to the total amount of DNA.
DNA was successfully extracted and analysed by real-time PCR duplex assay in all samples. Valid measurements of SHOX2 DNA methylation level could be achieved in all samples, as well.
Endobronchial ultrasound guided biopsy as a minimal invasive method followed by real-time PCR is an applicable procedure to analyse SHOX2 DNA methylation level in mediastinal lymph nodes of lung cancer patients.
These outcomes allow comparing SHOX2 DNA methylation level in patients suspected to have lung cancer in further studies. DNA methylation level of SHOX2 has the potential to be a useful biomarker in lung cancer.

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Initial experience in endobronchial and endoscopic ultrasound-guided fine needle aspiration in the same procedure
Salvador de la Torre Carazo[1], Ricardo García-Luján[1], Rodrigo Alonso Moralejo[1], Antolina Gómez López[1], Beatriz Arias Arcos[1], Nuria Alberti[2], Eduardo de Miguel Poch[1]. *[1]Pneumonology, Hospital 12 de Octubre, Madrid, Spain; [2]Pathology, Hospital 12 de Octubre, Madrid, Spain*

Introduction: Current papers show usefulness of a combined endobronchial and endoscopic ultrasound-guided fine needle aspiration (EBUS-FNA and EUS-FNA) of mediastinic adenopathies (1,2).
Aim: To analize the initial results of EUS-FNA of mediastinic adenopathies in a tertiary hospitals bronchoscopic unit.
Methods: Descriptive, prospective study of all the ecobronchoscopic procedures done May 2011-January 2012. EUS-FNA was performed when no endobronchial accessible adenopathies or its aspiration considered non representative by rapid onsite evaluation. Mediastinoscopy was performed when no diagnosis was yield with the endobronchoscope.
Results: 54 patients underwent a bronchoscopic procedure in that period. In 9 cases EUS-FNA was performed (16,7%). Region 7 in 4 cases and 4L in 5 cases were sampled. In every case we started with an EBUS exploration: 5 of them were non representative with EBUS-FNA and in 4 cases no accessible adenopahies were found with EBUS
EUS-FNA yield the following diagnosis: 3 non Hodgkin lymphomas, 2 lymphoid hyperplasia with anthracosis (with posterior mediastinoscopies in which no evidence of malignancy was proven), 1 epidermoid lung cancer, 1 colorectal carcinoma's metastasis, 1 sarcoidosis and 1 secondary amyloidosis. Every EUS-FNA was representative and no technique complications were described.
Conclusion: Every case in which EUS-FNA was performed, was diagnostic, no false negatives were observed. This suggests that EUS-FNA done in the same procedure is beneficial in those procedures in which EBUS-FNA does not yield a conclusive diagnosis.
1 Herth FJ et al. Chest. 2010;138:790-4
2Hwangbo B et al. Chest. 2010;138:795-802.

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Endobronchial ultrasound-guided sampling can be used for successful molecular sampling in routine clinical practice
Lianne Castle[1], Takahiro Nakajima[1], Marcelo Cypel[1], Marc de Perrot[1], Andrew Pierre[1], Gail Darling[1], Thomas Waddell[1], Shaf Keshavjee[1], Gilda Santos[2], Scott Boerner[2], William Geddes[2], Kazuhiro Yasufuku[1]. *[1]Thoracic Surgery, Toronto General Hospital, Toronto, ON, Canada; [2]Cytology, Toronto General Hospital, Toronto, ON, Canada*

Treatment in oncology is becoming increasingly driven by tumour genetics and biomarker status. In non-small cell lung cancer, mutations within the gene encoding for the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) are associated with improved response to tyrosine kinase inhibitors. This has led to need for genetic analysis for this mutation in lung cancer patients.
Initial studies assessed EGFR status in surgical biopsies. Many patients have advanced disease at presentation and there is a requirement to be able to perform this analysis on fine needle aspirates.
Whilst studies have shown cytological samples specifically prepared are suitable for this analysis, few studies have assessed the ability to perform molecular analysis on routine samples.
We hypothesised that molecular analysis would be possible from routine samples collected at our institution.
All procedures performed between 2008 and 2011 were reviewed. Using patient and pathology records, data was collected on patient demographics, adequacy of samples, cytological diagnosis and molecular analyses performed.
Cancer was diagnosed in 352 out of 741 cases (48%). Molecular analysis was performed in 70 cases (20% cancer cases). Molecular analysis included PCR, flow cytometry and FISH and occurred in varying cancer diagnoses including: lymphoma (B cell and Hodgkins), melanoma, sarcoma and lung and breast cancer. In a subgroup, EGFR analysis was possible in 87% (47/54) of cases with mutations present in 34% (16/47).
Samples from EBUS-TBNA can be used for molecular analysis in routine clinical practice. Their use extends not only to lung but other metastatic cancers of the thorax.

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Combined ultrasound-guided needle aspiration in restaging of the non-small-cell lung cancer – A three years experience
Artur Szlubowski[1], Stanislaw Orzechowski[1], Jerzy Soja[2], Juliusz Pankowski[4], Anna Obrochta[4], Magdalena Jakubiak[4], Wojciech Czajkowski[3], Marcin Zielinski[3]. *[1]Endoscopy Unit, Pulmonary Hospital, Zakopane, Poland; [2]Dept. of Medicine, Jagiellonian University, Krakow, Poland; [3]Dept. of Thoracic Surgery, Pulmonary Hospital, Zakopane, Poland; [4]Dept. of Pathology, Pulmonary Hospital, Zakopane, Poland*

Objectives: The aim of the prospective study was to assess the diagnostic yield of the combined approach – endobronchial (EBUS) and endoscopic (EUS) ultrasound-guided needle aspiration (CUS-NA) in restaging of the non-small-cell lung cancer (NSCLC) patients after neo-adjuvant therapy.
Methods: In a consecutive group of NSCLC patients with pathologically confirmed N2 disease, who underwent neo-adjuvant chemotherapy, CUS-NA was performed. All negative patients underwent subsequently the transcervical extended bilateral mediastinal lymphadenectomy (TEMLA) as a confirmatory test.
Results: 73 patients underwent restaging CUS-NA from Jan. 2009 to Dec. 2011. There were 123 mediastinal lymph nodes biopsied (stations: 2R – 4, 4R – 24, 2L – 3, 4L – 27, 7 – 61, 8 – 4). CUS-NA revealed metastatic lymph node involvement in 21/73 patients (28.8%). In 52 (71.2%) patients with negative or uncertain CUS-NA, who underwent subsequent TEMLA metastatic nodes were found in 13 patients (17.8%) and there was "minimal N2" in 8 out of them. In 7 patients CUS-NA occurred to be false-negative in the right paratracheal stations 2R and 4R, only accessible for EBUS. A diagnostic sensitivity, specificity, accuracy, PPV and NPV of the restaging CUS-NA was 62% (95% CI – 60–90), 95% (95% CI – 85–97), 80%, 91% (95% CI – 80–100) and 74% (95% CI – 71–91), respectively. No complications of CUS-NA were observed.
Conclusions: CUS-NA is a reasonable and safe technique for mediastinal restaging in NSCLC patients, and after our data, in patients with negative results of the combined endoscopic technique, a surgical restaging of the mediastinum might not be mandatory.

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Combined endosonographic staging followed by cervical mediastinoscopy in the real world, do we still need both?
Erik (H.F.M.) van der Heijden[1], Ad F.T.M. Verhagen[2], Olga C.J. Schuurbiers[1]. *[1]Pulmonary Diseases, Radboud University Medical Center, Nijmegen, Netherlands; [2]Cardiothoracic Surgery, Radboud University Medical Center, Nijmegen, Netherlands*

Introduction: The combination of EUS- and EBUS-FNA is an accurate preoperative staging method in NSCLC, challenging the status of cervical mediastinoscopy (CM) as the gold standard. In a recent RCT combined endoscopic staging (CES) had greater sensitivity compared to CM. After negative CES, a CM had to be performed in 11 patients to find one positive result [Annema JAMA 2010]. Defranchi [ATS 2010] found a NNT of 3.6 and Tournoy [ERS 2011] presented a NNT near infinite in imaging negative and lymphocyte positive CES samples. Our goal was to assess the need to perform a CM after negative CES in our center.
Methods: Records of 100 consecutive patients with negative CES referred for CM or surgery between January 2009 and June 2011 were analysed. All patients were treated in strict accordance with national guidelines: preoperative CES mediastinal staging is performed in all patients with potentially resectable NSCLC and mediastinal nodes with short axis $\geq$10 mm on CTscan, PET-positive mediastinal or hilar nodes and/or centrally located tumors. If found negative CES is followed by CM.
Results: Of 100 CES negative patients 82 underwent CM and 18 a thoracotomy as next step procedure in which N2+ nodes were found in 11/82 and 3/18 patients. After negative CM surgery was performed in 63 of 71 patients. N2+ nodes were found in 7/63 patients. A total of 21 patients were found N2 positive after negative CES (NPV 79%). The NPV of CM after negative CES is 89%.
Conclusion: Endosonographic mediastinal staging using strict guidelines is reliable and accurate. In our center one patient with tumor positive mediastinal nodes can be found by CM after 7 negative CES procedures in (suspected) NSCLC.