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DOI: 10.1164/rccm.201309-1703ED

Endobronchial Ultrasound–guided Transbronchial Needle Aspiration for Lymphoma: The Final Frontier

In 2002, endobronchial ultrasound (EBUS) was developed by integrating a convex transducer with a frequency of 7.5 MHz into the tip of a flexible fiber-optic bronchoscope (XBF-UC40P, Olympus, Tokyo, Japan). For the first time, the linear curved array transducer allowed real-time sampling of lymph nodes adjacent to the airway by scanning in parallel to the insertion direction of the bronchoscope. The immediate application for the new technology in lung cancer staging was clear, and EBUS-guided transbronchial needle aspiration (EBUS-TBNA) is now performed by trained interventional pulmonologists and thoracic surgeons in selected centers around the world for a range of indications.

However, the technique has faced many hurdles along the way, and uptake of the procedure was surprisingly slow initially. This was due not just to capital costs but also to problems with appropriate reimbursement and the fact that many pulmonologists preferred conventional TBNA. However, we believe that most pulmonologists across the world now agree that EBUS-TBNA is superior to blind or conventional TBNA. In 2008, Wallace and colleagues showed in a prospective study where patients underwent conventional TBNA and EBUS-TBNA (as well as endoscopic ultrasound–guided fine needle aspiration) that EBUS-TBNA had a significantly better sensitivity than conventional TBNA for diagnosing lymph node metastases in patients with lung cancer (1). EBUS-TBNA allows sampling of smaller lymph nodes and in lymph node locations that are challenging for conventional TBNA. It has long been the argument that EBUS-TBNA is not required when lymph nodes are bulky. However, in patients with sarcoidosis, a condition often characterized by bulky intrathoracic lymphadenopathy, a randomized controlled trial of EBUS-guided versus conventional TBNA demonstrated a statistically higher diagnostic yield for the EBUS group (83% vs. 54%, $P < 0.05$) (2).

Thoracic surgeons were understandably anxious and skeptical about the rise of EBUS-TBNA and the potential fall of mediastinoscopy. In a crossover trial in patients with lung cancer, mediastinoscopy had a sensitivity of 78% for detecting lymph node metastases, whereas EBUS-TBNA had a sensitivity of 90%, primarily due to the ability of EBUS-TBNA to sample posterior subcarinal lymph nodes (3). However, many thoracic surgeons have embraced the new technology, taken it into the operating theater, and also helped to advance the field. A recent trial led by pioneering thoracic surgeons demonstrated a similar sensitivity for mediastinoscopy and EBUS-TBNA and led them to conclude that EBUS-TBNA could replace mediastinoscopy in patients with lung cancer (4). Although EBUS-TBNA may be a barrier to training in mediastinoscopy, many thoracic surgeons value the presence of EBUS-TBNA as it frees operating room time for more therapeutic rather than diagnostic surgical procedures.

While pulmonologists and surgeons were being persuaded that EBUS-TBNA was superior to conventional TBNA and a suitable alternative to mediastinoscopy in patients with lung cancer, the landscape of lung cancer management was changing. Oncologists became increasingly interested in tissue acquisition to allow suitable samples for the personalized management of patients with advanced lung cancer. EBUS-TBNA has risen to this challenge as well. In a study of 774 patients undergoing EBUS-TBNA, 90% of samples submitted were suitable for epidermal growth factor receptor mutation status testing (5). Samples obtained in routine practice were also suitable for immunohistochemistry to aid subtyping of lung cancer. Many oncologists understand the merits of EBUS-TBNA, as it offers a technique that allows sampling of multiple tumor metastatic sites, which may be important as we increasingly recognize tumor heterogeneity (6). Because EBUS-TBNA is easily repeated, it can also be performed after disease progression to recharacterize tumor phenotype and genotype. This may be of particular significance if the patient is on a first-line TKI, given the occasional transformation of epidermal growth factor receptor–positive adenocarcinomas to small cell carcinoma (7). EBUS-TBNA has a role in the restaging of the mediastinum after chemotherapy (8) and can also biopsy parenchymal lesions adjacent to the airway (9). Radiation oncologists may use EBUS-TBNA for mapping disease in the mediastinum to guide radiotherapy fields in conjunction with integrated positron emission tomography–computed tomography and also for minimally invasive mediastinal staging prior to stereotactic radiotherapy. In patients with extrathoracic malignancy and enlarged mediastinal lymphadenopathy, oncologists often turn to EBUS-TBNA to confirm metastases and exclude other processes in the mediastinum (10).

EBUS-TBNA has further been found to be of importance in patients with isolated mediastinal lymphadenopathy (IML), where the differential diagnosis often lies between sarcoidosis, tuberculosis, and lymphoma (11). In patients with sarcoidosis, EBUS-TBNA has recently been confirmed to be superior to conventional

This work was undertaken at UCLH/UCL, which received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme (N.N., S.M.J.). S.M.J. holds a Wellcome Trust senior fellowship.

bronchoscopic techniques when lymph nodes are present (12). In populations with high tuberculosis incidence, EBUS-TBNA is also a useful investigation for the diagnosis of mycobacterial disease and welcomed by microbiologists and infectious disease clinicians, as it improves the culture-positive rate (13).

Although EBUS-TBNA has been embraced by most pulmonologists, thoracic surgeons, and oncologists, its role in diagnosing lymphoma has always been controversial, and many hematooncologists remain unconvinced that the smaller samples from EBUS-TBNA are sufficient for diagnosis. This is a view shared in recent British Thoracic Society guidelines, which suggested that EBUS-TBNA was not the preferred diagnostic procedure when lymphoma was suspected (14). In this issue of the *Journal*, Moonim and colleagues (pp. 1216–1223) demonstrate the utility of EBUS-TBNA in patients with lymphoma and continue the work toward reaching this final frontier (15).

The treatment of lymphoma relies on knowing the specific subtype and histological grade, and although this may be apparently achievable with EBUS-TBNA samples, concern exists that there is a high rate of discordance between cytologic and histologic specimens. The authors conducted an analysis of EBUS-TBNA procedures performed at their tertiary institution and identified 100 patients with a diagnosis of lymphoma. Cell blocks obtained at EBUS-TBNA were subjected to immunocytochemistry, flow cytometry, and fluorescent *in situ* hybridization. Using this protocol, they demonstrated that EBUS-TBNA provided adequate information for clinical management in 84 (84%) cases. All cases of relapsed lymphoma were correctly diagnosed, as were 88% of *de novo* cases, with one false-positive result.

The study highlights several important points. The pathologist's handling, processing, and interpretation of the specimen has a critical role in the EBUS-TBNA procedure. For centers wishing to replicate the high-quality results demonstrated by Moonim and colleagues, it is important to appreciate the learning curve for our pathology colleagues as well as the operators (16). EBUS-TBNA allows sampling from all bilateral mediastinal and hilar lymph node stations other than the aortopulmonary and paraaortic areas, which is a larger area than any single surgical procedure. A further key advantage of EBUS-TBNA over mediastinoscopy is that EBUS-TBNA can be performed easily after mediastinal radiotherapy—an important scenario when considering diagnosis of lymphoma relapse.

Some nettlesome questions remain. Is the role of the on-site pathologist (routinely available in this study) relevant to obtaining sufficient tissue to allow multimodality analysis? Although favored by Moonim and colleagues, it could be argued that staining specimens on slides in the endoscopy suite may reduce the total volume of analyzable material in the cell block for the laboratory. The diagnosis of granulomatous lymphadenopathy by EBUS-TBNA in patients with lymphoma is described, but its significance and negative predictive value is unclear and requires further study in the context of lymphomas and other malignancies. Unfortunately, the small numbers of patients in each category of lymphoma preclude definitive conclusions about the utility of EBUS-TBNA in lymphoma subtypes.

When data from this study is combined with information from other studies of EBUS-TBNA in lymphoma (17–21), we can begin to conclude that EBUS-TBNA now has a key role in the diagnosis of relapsed lymphoma. The results demonstrated in this study show that it is also possible to diagnose the majority of *de novo* lymphoma. When we also consider that the incidence of lymphoma is low in patients with IML (11), the study adds great weight to the utility of EBUS-TBNA as an initial procedure in patients with IML, even when lymphoma is the most likely diagnosis on clinical and radiological grounds. More data on EBUS-TBNA in patients with known and suspected lymphoma will continue to emerge and may help to persuade our hematooncology colleagues of its clinical utility. The challenge for interventional pulmonologists is to replicate the high standards of diagnostic yield achieved by Moonim and colleagues. In the meantime, EBUS-TBNA can be proud of its progress over the last 10 years from conception to a central diagnostic tool for a wide range of disorders in pulmonology.

Author disclosures are available with the text of this article at www.atsjournals.org.

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DOI: 10.1164/rccm.201309-1701ED

The Lung Microbiome and Viral-induced Exacerbations of Chronic Obstructive Pulmonary Disease: New Observations, Novel Approaches

Chronic obstructive pulmonary disease (COPD) is responsible for an enormous global burden of morbidity, mortality, and healthcare expense, and is the only leading cause of death with a rising prevalence (1). The course of COPD is complicated by acute exacerbations (AEs), which are associated with high mortality, accelerated loss of lung function, and a large fraction of the disease's direct cost (2). The majority of AEs are associated with isolation of bacterial or viral pathogens from respiratory specimens (3), and the concurrent isolation of both is associated with increased inflammation and exacerbation severity (4). The mechanisms of interaction between viruses, bacteria, and the host in the pathogenesis of AEs have not been elucidated, as prior investigations have been hampered by inadequate animal models (5) and by dependence on limited culture-based techniques of microbial identification (6).

In this issue of the *Journal*, Molyneaux and coworkers (pp. 1224–1231) demonstrate how novel investigative approaches may be integrated to bypass these difficulties and elucidate the contributions of microbes to the pathogenesis of AEs (7). They employed a previously described human model of AEs in which rhinovirus is instilled into the nares of subjects with mild COPD, provoking the clinical, physiologic, and inflammatory features associated with AEs (8). The authors collected induced sputum from subjects with and without COPD at multiple time points before and after rhinovirus instillation and characterized the bacterial microbiota of the respiratory tract using a modern culture-independent technique (pyrosequencing of bacterial 16S ribosomal RNA gene libraries). They observed no significant difference in the microbiota of COPD subjects and control subjects at baseline, but significant changes detectable at 15 days after instillation. Unlike that of control subjects, the postexposure sputum of COPD subjects exhibited an increase in the amount of bacterial DNA and a significant change in bacterial community composition, driven by a phylum shift toward Proteobacteria and away from Firmicutes and Bacteroidetes.

Several studies in recent years have applied similar molecular techniques to respiratory specimens from patients with COPD (9–12), though only one to date has included patients experiencing an AE (13). Most reports have found a distinct microbiome in COPD lungs when compared with those of controls, often also reporting a relative increase in Proteobacteria over other prominent phyla (6). In the current study, the apparent lack of difference observed between the preintervention sputum microbiota of COPD subjects and control subjects likely reflects the relatively mild severity of disease in this study's participants, an unavoidable limitation of this model and the necessities of safe and ethical study of human subjects. Yet the significant differences detected in sputum microbiota 15 days after rhinovirus exposure in the same subjects illustrate the importance of treating the lung microbiome in COPD as a dynamic and context-dependent phenomenon. Importantly, the authors observe that the specific members of Proteobacteria that exhibited the most dramatic postexposure increase in abundance (*Haemophilus* sp. and *Neisseria* sp.) were present in lower numbers among the microbiota of the same COPD subjects before rhinovirus exposure. This key observation illustrates the plausibility of the hypothesis that bacteria in AEs are not newly introduced pathogens, but instead reflect selective outgrowth of newly favored species from a previously homeostatic microbial ecosystem.

Important as this observation may be, the most significant impact of this study should be what it illustrates about the future and potential of lung microbiome studies. With few exceptions, studies of lung microbiota to date have been cross-sectional, observational, and descriptive (6). With their experimental design, Molyneaux and colleagues have demonstrated a means of testing mechanistic hypotheses in human subjects to a degree previously unapproached in the field. Rather than relying on comparisons of pooled averages of populations, the serial analysis of subject specimens at predefined time points after a controlled exposure permits comparison of subjects with themselves, a more rigorous and informative analysis. The use of changes in lung microbiota as the primary endpoint of an experimental intervention is novel, and will be a necessary approach as future studies attempt to