The novel 19G endobronchial USS (EBUS) needle samples processed as tissue “core biopsies” facilitate PD-L1 and other biomarker testing in lung cancer specimens: case report and the viewpoint from the Respiratory Physician and the Pathologist

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Abstract
The immunohistochemical expression of Programmed Death Ligand (PD-L1) predicts responses to PD-1/PD-L1 inhibitors in non-small cell lung cancer (NSCLC). PD-L1 testing is currently only recommended on tissue specimens; however, in many patients, cytology samples are the only specimens available. The introduction of the novel 19G “core-biopsy” needle has revolutionized the utility of endobronchial USS-guided biopsy (EBUS) by providing solid tissue “microbiopsies” rather than traditional liquid cytology samples. We report a case of metastatic adenocarcinoma with the only accessible site of biopsy being a hilar lymph node. Using the 19G core-biopsy needle and processing the microbiopsy samples in formalin provided more material for predictive biomarker testing, including PD-L1 immunohistochemistry, when traditional processing was inadequate. This case highlights the need for close multidisciplinary discussions between the pathologist and the respiratory physician regarding emerging biomarkers and novel biopsy techniques to obtain maximum utility of the tools and avoid repeated procedures for the patient.

Introduction
Positivity for Programmed Death Ligand (PD-L1) expression as detected by immunohistochemistry (IHC) helps predict patient response to anti-PD-1/PD-L1 immunotherapeutic agents in advanced non-small cell lung cancer (NSCLC), and multiple drugs have recently been approved for use in the first- and second-line setting [1].

Historically, PD-L1 IHC was undertaken on solid tissue samples, such as those obtained via CT-guided lung biopsy. Cytology samples are currently not recommended for PD-L1 testing as these samples were excluded from the clinical trials evaluating immunotherapeutic agents, and there is concern about the technical performance of PD-L1 IHC assays when different fixatives are used, such as alcohol-based fixatives that are often used for cytological cell block preparations [1, 2]. However, for some patients with advanced-stage NSCLC, cytology samples are the only specimens available [3]. In some patients, the only accessible site for biopsy may be mediastinal or hilar lymph nodes (LN) using endobronchial USS (EBUS)-guided needle biopsy. This is an accepted and widely used practice with a good safety profile; however, the traditional biopsy tool used in Linear EBUS—a 21, 22 or 25 G needle—obtains a traditional liquid cytology sample that is processed as smears for morphological assessment and a cell block preparation for immunohistochemical markers. While these samples may be adequate for other biomarker...
testing, such as \textit{EGFR} and \textit{ALK} assessment \cite{4}, they are currently suboptimal for PD-L1 IHC as solid tissue samples are required.

The recent introduction of a larger EBUS biopsy needle with a 19G that can obtain solid tissue microbiopsies has revolutionized the way biopsies are taken from mediastinal/hilar LNs \cite{5}. However, the optimal processing method to best utilize this new technique has not been well established.

This is the first paper to our knowledge that has demonstrated the superiority of processing the 19G EBUS samples as “solid tissue microbiopsies” for optimal biomarker testing, including PD-L1 IHC, where solid tissue samples are required.

**Case Report**

We report the case of a 65-year-old male patient who presented to hospital with worsening back pain of a few weeks duration. He was a current smoker with a 20-pack year smoking history, and the only comorbidities were hypertension and reflux disease, for which he was on treatment. He was full-time employed and had no previous respiratory symptoms. On physical examination, the patient had oxygen saturation of 96% on room air, a blood pressure of 130/90 mm Hg and a pulse rate of 86 min. The respiratory and cardiovascular examinations were unremarkable. There was no cervical or axillary lymphadenopathy. Apart from a weakness in the left leg, his exam was unremarkable.

A CT scan of the lumbar spine demonstrated malignant deposits, and a CT head confirmed small cerebral metastasis. A staging CT chest-abdomen-pelvic demonstrated the only abnormality, a marked left hilar lymphadenopathy of 30mm. A PET/CT confirmed that this lesion, as well as the bony metastases, was FDG avid in keeping with a malignant process. At this point, the only easily accessible site for biopsy to determine the primary site of disease and undertake any necessary biomarker testing was the left hilar LN using EBUS.

Therefore, with the patient’s consent, an EBUS procedure was performed, and nine biopsies were obtained from the 11L LN using three passes of the 19G core biopsy needle and six passes from the 21G needle. There were no side effects noted, and bleeding was less than 1 mL. At the time of the procedure, processing of the sample was performed as per routine practice by an onsite cytologist, and once malignancy was confirmed on smears, the residual sample was deposited in Hanks solution for cell block preparation. Abundant tissue fragments were present in the cell block, which showed metastatic adenocarcinoma with >100 cells hpf and tumour cells comprising >80% of the sample. The tumour was wild type for \textit{EGFR}, and ALK IHC was negative. A PD-L1 test was requested by the oncologist, and despite the abundance of the cells, the sample was deemed unsatisfactory for PD-L1 testing as no solid tissue specimen was available (Fig. 1).

Following this, in discussion with the pathologist, the procedure was repeated with a similar number of passes, and the liquid sample was placed in Hanks solution, and the solid core tissue fragments were extracted using a pipette and placed directly in formalin to process as a solid tissue biopsy. There were approximately 30 tissue fragments ranging in size from <1 mm up to 10 mm. This method enabled optimal PD-L1 IHC to be undertaken, which showed more than 100 viable tumour cells present and 0% of tumour cells with membranous staining (Fig. 2).

**Discussion**

The 19G EBUS needle can provide large samples, including solid tissue microbiopsies. To obtain the maximal potential from this new advancement in sampling technique, we found that processing the solid tissue microbiopsy samples in formalin, like a true tissue biopsy, rather than the traditional cytological approach of suspending in solution for cytocentrification and cell block preparation provided the best results. This enables the EBUS sample to be used for multiple ancillary biomarker tests, including PD-L1 IHC testing, which is increasingly required for lung cancer treatment decisions. In addition, participation in clinical trials generally requires a tissue biopsy sample from the patient’s tumour, and this technique would provide a suitable sample.
While the biopsy specimen was more fragmented than a traditional transthoracic core biopsy sample, there was a greater number of tissue fragments that were embedded across multiple blocks, similar to how a curettage specimen would be embedded and sectioned. As there were multiple smaller biopsy fragments compared to a traditional core biopsy, specimen orientation was less important during embedding.

We feel that the dissemination of this information would enable other institutions that perform EBUS procedures incorporating the novel 19G needle to adopt this processing as a true solid tissue biopsy in formalin, potentially avoiding repeated procedures as occurred in our patient, with the ability to not only perform EGFR and ALK testing but PD-L1 IHC as well.

In this era, with rapid introduction of novel biopsy techniques and requirement of emerging molecular marker testing, this case report highlights the need for ongoing close communication between the pathologist, proceduralist and the oncologist to acquire the maximal advantage of novel techniques and enable the optimum outcome for the patient whilst avoiding repeated procedures.

Disclosure Statements
No conflict of interest declared.
Appropriate written informed consent was obtained for publication of this case report and accompanying images.

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