Accuracy of cell typing in nonsmall cell lung cancer by EBUS/EUS–FNA cytological samples

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ABSTRACT: Endoscopic ultrasound-guided transbronchial or transoesophageal lymph node aspiration is increasingly used as a method of diagnosing nonsmall cell carcinoma. Data validating the accuracy of cell typing of nonsmall cell carcinoma using these cytological samples has not been assessed.

23 samples were identified in Edinburgh, UK and a further 25 in Cambridge, UK, with matching histological samples. The morphological cell type, as assessed on the cytological preparations and cell blocks, was recorded and immunohistochemical staining was performed, where possible, as an adjunct. The final cell type, as assessed by morphology with or without immunohistochemistry, was correlated with that reported in the paired histological samples.

Cell blocks with tumour were available in 39 out of 48 cases. The accuracy of cell typing when no cell block was available was four out of nine cases. This increased to 25 out of 39 when a cell block was available, increasing to 33 out of 39 with the addition of immunohistochemistry. The overall accuracy of classification was 37 out of 48 cases.

Accurate cell typing of nonsmall cell carcinomas can be performed using endoscopically derived fine-needle aspirates. The importance of obtaining sufficient material for the production of cell blocks is critical in allowing optimal assessment.

KEYWORDS: Classification, cytology, fine-needle aspiration, immunohistochemistry, nonsmall cell lung carcinoma

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Endobronchial ultrasound (EBUS)- or endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) has been used as a minimally invasive method of sampling mediastinal and hilar lymph nodes as part of the staging process in patients with lung carcinoma [1–4]. Studies from various centres have demonstrated such techniques to have a high degree of accuracy and diagnostic yield in identifying node involvement by metastatic carcinoma [1–4]. This has led to increasing popularity over the last few years and the role of EBUS/EUS-FNA has evolved; it is now perceived as being a method of allowing simultaneous diagnosis and staging of lung cancer in one procedure [4].

Developments in oncological therapeutics have simultaneously led to the concept of individualised therapy for nonsmall cell lung cancers (NSCLCs) and the recent introduction of new drugs licensed for tumours with nonsquamous histology [5]. This has led to expectations on pathologists to robustly identify squamous and glandular differentiation in small biopsy and cytological samples, when previously the term ''NSCLC, not otherwise specified (NSCLC-NOS)'' would have been sufficient to allow patient management [6].

Accurate cell typing of nonsmall cell carcinomas in small diagnostic biopsy specimens has been recognised for many years to present a significant problem to pathologists, given the focal nature of specific diagnostic features and their frequent absence in small diagnostic specimens [7–9]. Similar issues exist when assessing cytological specimens, with the added complication that the architectural features that indicate glandular differentiation in histology specimens may not be present, making the distinction between adenocarcinoma and poorly differentiated nonkeratinising malignant squamous cells particularly difficult [10].

Studies using a wide range of monoclonal antibodies have been described for use as an adjunct to morphological assessment in indicating probable glandular or squamous differentiation [11–17]. Robust validation of these approaches in routine practice has, however, been difficult, but a

recent study has provided evidence that the use of p63 or cytokeratin (CK)5/6 and thyroid transcription factor (TTF)1 staining of bronchial biopsies provides good prediction of cell type, as assessed by matched resection specimen histology as the gold standard [18]. We have previously demonstrated that EBUS-FNA specimens can be processed to provide cell blocks on which immunohistochemistry can be performed [3]. Validation of the accuracy of cell typing in EBUS/EUS-FNA specimens with the use of adjunct immunohistochemistry has, however, not previously been assessed in comparison with histology. If EBUS/EUS-FNA cytological specimens are to be increasingly used for primary diagnosis rather than tissue biopsy for histology, then validation of the accuracy of cell typing using these samples is crucial.

METHODS
EBUS-FNA has been carried out in Edinburgh, UK since 2004 and ~1,800 patients have undergone the procedure. All the specimens have been reported in the Pathology Dept at the Royal Infirmary of Edinburgh. The specimens identified for this study were processed using thin-layer cytological techniques coupled with any residual material being processed for a cell block, as we have previously described [3]. Using the laboratory computer system, we searched for cases where EBUS- or EUS-FNAs had been performed and reported as showing squamous carcinoma, adenocarcinoma or malignant cells not otherwise specified. This list was then searched to identify cases where separate specimens had been submitted for histology and reported as squamous carcinoma, adenocarcinoma or NSCLC-NOS.

The reports and slides for both the FNA specimens and the matched histology specimens were reviewed. For the cytological specimens, it was noted whether a cell block containing tumour cells was available, and an assessment of cell type, based on the morphology of the tumour cells on the Papanicolaou-stained thin-layer cytology specimen, and, if available, a cell block was made. In all cases, including those where a confident cell type was identified morphologically and a cell block containing tumour cells was available, immunohistochemistry was performed if this had not been performed at the time of initial reporting. Sections were stained using monoclonal antibodies to TTF1 (clone 8G7G3/1; Dako, Ely, UK) and p63 (clone 4A4; Dako) using a BOND-MAX™ automated immuno-histochemical staining machine (Leica, Milton Keynes, UK). The results of this staining were correlated with the morphological impression of the cell type and in those cases where the assessment had been NSCLC-NOS, this was used to suggest a probable cell type, as has been described for bronchial biopsy specimens [18]. The final cytology cell type for these cases was then compared with the histological classification suggested by the biopsy specimens, where appropriate supported by immunohistochemical assessment, in those cases showing no definitive squamous or glandular differentiation and regarded as NSCLC-NOS.

In order to further validate this approach to cell typing of NSCLC in these samples, a retrospective search of the departmental archives was also carried out in the Pathology Dept of Papworth Hospital in Cambridge, UK. This was to allow assessment of the robustness of the results within the Edinburgh test cohort by comparing them with routinely reported cases from another pathology department, which

used identical processing procedures for these FNA samples. The reports were retrieved and the following information was noted, whether cell blocks were available, the morphological diagnosis made on the specimen and whether immunohistochemistry had been performed. The cell type reported was then compared with that of the matched histology specimen.

RESULTS
Edinburgh
23 EBUS-FNA samples (table 1) were identified in Edinburgh, which had corresponding histology samples (12 bronchial biopsies, three percutaneous computed tomography (CT)-guided core biopsies, five resection specimens and in three cases, material obtained at autopsy). In three of the cases, the EBUS-FNA samples were obtained to confirm recurrence rather than to establish diagnosis or stage at the time of initial diagnosis. The nodal sites sampled and found to contain malignant cells are shown in table 1. These were predominantly mediastinal nodes (L4, R4, station (Stn) 7 and precarinal) but also hilar nodes (Stns 10 and 11) and in one case, a paraoesophageal mass.

In 14 out of 23 cases, cell blocks containing malignant cells were identified and immunohistochemistry was performed on each of these. In all cases where a confident cell type had been established by morphology (eight squamous carcinomas and one adenocarcinoma), the immunohistochemical staining was in agreement and correlated with the histological classification. In the remaining five cases, three were found to stain for TTF1 but not p63, thus favouring designation as an adenocarcinoma, consistent with the histological cell type seen (fig. 1). In two cases, the tumour cells were negative for both markers, indicating that no further comment on probable cell type could be made. In one of these cases, a subsequent resection was performed and the tumour classified as a large cell undifferentiated carcinoma and in the second case, histology obtained at autopsy post-chemotherapy was too autolytic for any specific comment on cell type to be made on the basis of morphology.

Of the nine cases where no material was available in a cell block, four were morphologically regarded as showing features consistent with squamous cell carcinoma consistent with the histological classification. Five cases were regarded as showing no specific features to allow subclassification and the histology specimens revealed three of these to be adenocarcinomas and two squamous carcinomas.

Cambridge
A total of 25 cases were identified in Cambridge, 10 of which had been performed to confirm recurrence following previous resections (table 2). The sites sampled were similar to those in the Edinburgh group but also included an EUS-FNA of an adrenal metastasis and two endobronchial aspirates of tumour masses, which had been processed in the same manner. The immunohistochemical stains used in this group were more variable, reflecting preferences and practices within the department and the fact that this was a retrospective review rather than a study cohort. In particular, CK5/6 and p63 were both used to identify squamous differentiation. In all 25 cases, cell blocks containing tumour were available. In 16 cases, a confident cell type was established morphologically (five adenocarcinomas and 11 squamous carcinomas). Of the remaining nine cases, where the morphological features were

TABLE 1 Edinburgh, UK cases

Case	Specimen type	Nodes involved	Cell block with tumour	Morphological cell type	TTF1 IHC	p63 IHC	Final cytology cell type	Histology cell type	TTF1 IHC	p63 IHC	Specimen type	Comments
1	EBUS	R4, Stn 7	Yes	Adeno.	+	-	Adeno.	NSCLC favouring adeno.	+	-	Bronchial biopsy	
2	EBUS	R4, Stn 7	Yes	Squamous	-	+	Squamous	NSCLC favouring squamous	-	+	Bronchial biopsy	
3	EBUS	R11	Yes	Squamous	-	+	Squamous	Squamous			Percutaneous CT-guided lung biopsy	
4	EBUS	R4	Yes	Squamous	-	+	Squamous	Squamous			Bronchial biopsy	
5	EBUS	R10, R11	Yes	Squamous	-	+	Squamous	Squamous			Bronchial biopsy	EBUS for recurrence
6	EBUS	L4	Yes	Squamous	-	+	Squamous	Squamous			Bronchial biopsy	
7	EBUS	R4	Yes	Squamous	-	+	Squamous	Squamous			Bronchial biopsy	
8	EBUS	Lung mass	Yes	Squamous	-	+	Squamous	Squamous			Resection	
9	EBUS	Stn 7	Yes	Squamous	-	+	Squamous	Squamous			Resection	EBUS for recurrence
10	EBUS	L10	Yes	NSCLC-NOS	+	-	NSCLC favouring adeno.	NSCLC favouring adeno.	+	-	Bronchial biopsy	
11	EBUS	R10, R11	Yes	NSCLC-NOS	+	-	NSCLC favouring adeno.	Adeno.			Resection	
12	EBUS	L4, Stn 7	Yes	NSCLC-NOS	+	-	NSCLC favouring adeno.	Adeno.	+		Post mortem	
13	EBUS	R11	Yes	NSCLC-NOS	-	-	NSCLC-NOS	Large cell undifferentiated	-		Resection	
14	EBUS	R4, L4, Stn 7	Yes	NSCLC-NOS	-	-	NSCLC-NOS	NSCLC-NOS			Post mortem	Morphologically very poorly preserved, post-chemotherapy
15	EBUS	Stn 7	No	Squamous			Squamous	Squamous			Percutaneous CT-guided lung biopsy	
16	EBUS	L4	No	Squamous			Squamous	Squamous			Bronchial biopsy	
17	EBUS	R11	No	Squamous			Squamous	Squamous			Bronchial biopsy	
18	EBUS	Stn 7	No	Squamous			Squamous	Squamous			Resection	
19	EBUS	R2, Stn 7, precarinal	No	NSCLC-NOS			NSCLC-NOS	Adeno.			Percutaneous CT-guided lung biopsy	EBUS for recurrence
20	EBUS	R4, Stn 7	No	NSCLC-NOS			NSCLC-NOS	Squamous			Bronchial biopsy	
21	EBUS	R2, R4, R10	No	NSCLC-NOS			NSCLC-NOS	Squamous			Bronchial biopsy	
22	EBUS	R4	No	NSCLC-NOS			NSCLC-NOS	Adeno.			Bronchial biopsy	
23	EBUS	Stn 7	No	NSCLC-NOS			NSCLC-NOS	Adeno.	+		Post mortem	

Summary table of 23 Edinburgh cases with results of cell typing studies on both endobronchial ultrasound (EBUS)-guided fine-needle aspirations and matched histology samples. TTF: thyroid transcription factor; IHC: immunohistochemistry; Stn: station; adeno.: adenocarcinoma; NSCLC: nonsmall cell lung cancer; NOS: not otherwise specified; +: positive staining; -: negative staining; CT: computed tomography.
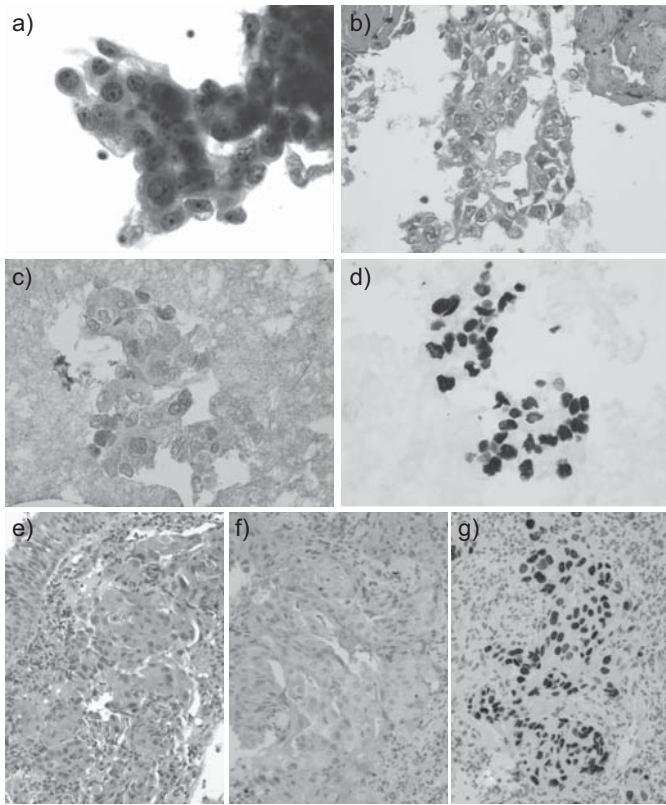
FIGURE 1. Photomicrographs of an endobronchial ultrasound-guided fine-needle aspiration sample processed using the thin-layer technique with cell block preparation. Groups of malignant cells are present in a) the Papanicolaou-stained cytological preparation, consistent with metastatic nonsmall cell carcinoma, no specific features of either squamous or glandular differentiation are evident in this slide or b) the subsequent cell block stained with haematoxylin and eosin. Immunohistochemistry for c) p63 and d) thyroid transcription factor (TTF)1 showed nuclear expression of TTF1 but not p63, favouring adenocarcinoma. (All ×400 original magnification). e) A bronchial biopsy obtained from the same patient demonstrated submucosal infiltration by nonsmall cell carcinoma. Histologically, no specific features to confirm squamous or glandular differentiation were observed and mucin stains were negative. Immunohistochemistry showed the tumour cells to be f) negative for p63 and g) express TTF1, thus favouring adenocarcinoma. (All ×200 original magnification).

nonspecific, the immunohistochemical profile correctly favoured three as adenocarcinomas and two as squamous carcinomas. In the remaining four cases, the immunohisto-chemistry showed expression of both TTF1 and CK5/6 in two cases, and in a third, the tumour cells were negative for TTF1, p63 and CK5/6, precluding any specific comment on probable cell type. In the fourth case, the immunohistochemistry favoured squamous differentiation (TTF1 negative and CK5/6 positive) but the matched CT lung biopsy showed features of adenocarcinoma.

The overall accuracy of cell typing in the samples from both centres is summarised in table 3. For cases where no cell blocks were available, accurate classification was obtained in four out of nine cases. The accuracy of morphological classification using the cytological preparation and cell block together increased to nine out of 14 and 16 out of 25 in the Edinburgh

and Cambridge cohorts, respectively. With the addition of immunohistochemical staining, this further increased to 12 out of 14 and 21 out of 25, respectively. The overall diagnostic accuracy rate for the entire series from both centres was 37 out of 48 cases.

DISCUSSION

Ideally, the introduction of new methodologies for tissue sampling should be subject to robust validation to ensure that they are able to reliably provide the information required for patient management. EBUS- and EUS-guided lymph node FNA were originally introduced as staging tools in lung cancer and studies demonstrated that it was a reliable method of identify-ing the presence of malignant cells in lymph nodes [1–4]. The subsequent change to using this approach for primary diag-nosis, at the same time as oncological practice, has required more robust cell typing of nonsmall cell carcinomas and led to concerns as to whether these samples are adequate to address this clinical issue. In particular, while immunohistochemistry has been validated for use in small bronchial biopsies [18] and its use has been described in EBUS-FNA samples [3, 19], no data have been available defining the accuracy of classification of nonsmall cell carcinomas using this approach.

The majority of patients who have EBUS- or EUS-FNA samples showing evidence of NSCLC will have had their samples obtained from mediastinal nodes and will, therefore, not be subjected to mediastinoscopy or be suitable for surgical resection. This means that although large numbers of these samples are being obtained and reported, most patients will not have any matched histology. This has been reflected in our searches, where only 23 patients in Edinburgh and 25 in Cambridge could be found where histology was also available, despite the fact that both centres are performing large numbers of these procedures.

The histological classification of 27 out of 48 cases as squamous carcinomas and 17 out of 48 as adenocarcinomas on histology may suggest a slight bias towards histology being available more frequently in squamous carcinoma. This may have contributed to the overall accuracy of cell typing by morphol-ogy, as evidence of keratinisation is a reliable indicator of squamous differentiation [10]. In contrast, the identification of adenocarcinoma often requires architectural clues that are less often present in cytology samples compared with histology samples and, thus, confident distinction between adenocarci-noma and a less well differentiated squamous carcinoma can pose more problems in cytological samples. This difficulty was illustrated in the five Edinburgh cases where morphologically, the tumour cells were regarded as showing no specific features of either squamous or glandular differentiation (NSCLC-NOS) and no cell block was available. The histology from these cases showed three to be adenocarcinomas and two to be squamous carcinomas.

In five cases classified as NSCLC-NOS on morphological criteria, immunohistochemical staining results were inconclu-sive. Three showed no staining with the antibodies used and two stained with both markers, given an equivocal phenotype. Three of these cases were regarded as adenocarcinomas on biopsies, one resection was classified as large cell undiffer-entiated carcinoma and two remained classified as NSCLC-NOS

TABLE 2 Cambridge, UK cases

Case	Specimen type	Site involved	Cell block with tumour	Morphological cell type	TTF1 IHC	p63 IHC	CK5/6 IHC	Final cytology cell type	Histology cell type	TTF1 IHC	p63 IHC	CK5/6 IHC	Specimen type	Comment
1	EBUS	Stn 7, Stn 11	Yes	Adeno.	+			Adeno.	Adeno.	+			Lymph node biopsy	
2	EBUS	Stn 7	Yes	Adeno.	+	-		Adeno.	Adeno.	+			Percutaneous CT-guided lung biopsy	
3	EBUS	RLL mass	Yes	Adeno.	-	-		Adeno.	Adeno.	-			Resection	EBUS for recurrence
4	EBUS	Stn 7	Yes	Adeno.	+	-		Adeno.	Adeno.	+			Percutaneous CT-guided lung biopsy	
5	EBUS	Stn 7	Yes	Adeno.	+			Adeno.	Adeno.	+			Lymph node biopsy	
6	EUS and EBUS	Stn 7	Yes	Squamous			+	Squamous	Squamous				Bronchial biopsy	
7	EUS	R 8	Yes	Squamous				Squamous	Squamous				Bronchial biopsy	
8	EUS and EBUS	Stn 7	Yes	Squamous				Squamous	Squamous	-			Resection	EBUS/EUS for recurrence
9	EUS	Retrotracheal mass	Yes	Squamous				Squamous	Squamous				Resection	EUS for recurrence
10	EBUS	Stn 7	Yes	Squamous				Squamous	Squamous				Resection	EBUS for recurrence
11	EBUS	R 4	Yes	Squamous			+	Squamous	Squamaous	-		+	Resection	EBUS for recurrence
12	Endobron. FNA		Yes	Squamous				Squamous	Squamous				Percutaneous CT-guided lung biopsy	
13	EBUS	RLL mass	Yes	Squamous				Squamous	Squamous				Resection	EBUS for recurrence
14	EBUS	L 10	Yes	Squamous				Squamous	Squamous				Resection	EBUS for recurrence
15	EBUS	Stn 7	Yes	Squamous				Squamous	Squamous	-		+	Bronchial biopsy	
16	Endobron. FNA		Yes	Squamous				Squamous	Squamous				Resection	EBUS for recurrence
17	EUS	Left adrenal	Yes	NSCLC-NOS	+	-		NSCLC favouring adeno.	Large cell/adeno.	+			Resection	EBUS for recurrence
18	EUS	Pericardial node	Yes	NSCLC-NOS	+			NSCLC favouring adeno.	NSCLC favouring adeno.	+			Lung and pleural biopsies	
19	EBUS	Stn 7	Yes	NSCLC-NOS	+	-		NSCLC favouring adeno.	Adeno.	+		+	Bronchial biopsy	
20	EBUS	RLL mass	Yes	NSCLC-NOS	-	+	+	NSCLC favouring squamous	NSCLC favouring squamous	-	+	+	Percutaneous CT-guided lung biopsy	
21	EBUS	R 4	Yes	NSCLC-NOS	-		+	NSCLC favouring squamous	NSCLC favouring squamous	-		+	Percutaneous CT-guided lung biopsy	
22	EBUS	L11	Yes	NSCLC-NOS	+		+	NSCLC-NOS	Adeno	+		+	Resection	EBUS for recurrence
23	EBUS	Azygos mass	Yes	NSCLC-NOS	+		+	NSCLC-NOS	NSCLC-NOS	-			Percutaneous CT-guided lung biopsy	
24	EBUS	Stn 3	Yes	NSCLC-NOS	-		+	NSCLC favouring squamous	NSCLC favouring adeno.				Percutaneous CT-guided lung biopsy	
25	EBUS	R4 and 7	Yes	NSCLC-NOS		-	-	NSCLC-NOS	NSCLC favouring adeno.	+			Percutaneous CT-guided lung biopsy	

Summary table of 25 Cambridge cases with results of cell typing studies on all fine-needle aspiration (FNA) samples and matched histology samples. TTF: thyroid transcription factor; IHC: immunohistochemistry; CK: cytokeratin; EBUS: endobronchial ultrasound; EUS: endoscopic ultrasound; endobron.: endobronchial; Stn: station; RLL: right lower lobe; adeno.: adenocarcinoma; NSCLC: nonsmall cell lung cancer; NOS: not otherwise specified; +: positive staining; -: negative staining; CT: computed tomography.

TABLE 3	Summary of combined Edinburgh, UK and Cambridge, UK data		
	Edinburgh	Cambridge	Total
Total cases	23	25	48
Total cases with cell blocks containing tumour cells	14	25	39
Cases correctly classified when no cell block with tumour cells available	4/9	NA	4/9
Cases correctly classified on morphology alone (cytological + cell block)	9/14	16/25	25/39
Cases correctly classified on morphology (cytology + cell block) with additional immunohistochemical staining	12/14	21/25	33/39

Data are presented as n or n/N. Summary of accuracy of classification of nonsmall cell carcinoma in fine-needle aspirate samples in comparison with histology, stratified for the availability of cell blocks with tumour cells and immunohistochemistry. NA: not applicable.

even on histology. The fact that some cases that were regarded as adenocarcinomas histologically were not identified as such by TTF1 staining on the cell blocks probably reflects the fact that around 25–30% of primary lung adenocarcinomas may be negative for this antigen [12]. Studies on bronchial biopsies have indicated that most tumours with this ''null'' phenotype are likely to be adenocarcinomas or large cell carcinomas [18], consistent with our findings.

Only one case out of 48 was identified where the immunohistochemistry gave a false indication of cell classification, with the results suggesting squamous differentiation and the biopsy demonstrating adenocarcinoma. The reason for this is unclear, but it is quite likely to reflect the fact that lung carcinomas are heterogeneous and frequently show variable differentiation [20].

Our study has reiterated the importance of cell blocks in the assessment of EBUS- or EUS-FNA samples in patients with lung carcinoma. In the absence of these, accuracy of cell typing for NSCLC is only 44%, but when cell blocks are prepared and immunohistochemical staining is performed, this increases to >80%, which is comparable with that reported previously for bronchial biopsies [18]. Other groups have described methodologies for immunohistochemical staining of previously stained Papanicolaou slides [21] and while this might allow some further information to be obtained in those cases where no cell block is available, the ability to use a panel of antibodies to optimise diagnosis is not possible with this approach. The importance of having cell blocks containing tumour cells for immunohistochemistry has, in our personal experience, also been useful in identifying or confirming the presence of metastatic carcinoma from sites other than lung. We have successfully identified and confirmed the presence of metastatic colorectal carcinomas, breast carcinomas, renal carcinoma and melanoma.

In conclusion, this study indicates that EBUS/EUS samples can provide cytological samples that will allow accurate cell typing of nonsmall cell carcinomas, but that the proportion of cases in which this is possible relies heavily on the availability of sufficient material being obtained to produce cell blocks, rising from 44% in cases with no cell block to >80% when cell blocks suitable for immunohistochemistry are available. This compares favourably with the results of studies on bronchial biopsies [13] and indicates that EBUS-FNA specimens with cell blocks are sufficient to allow accurate cell typing of nonsmall cell carcinoma.

STATEMENT OF INTEREST

A statement of interest for W.A.H. Wallace can be found at www.erj. ersjournals.com/site/misc/statements.xhtml

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