

Diagnostic patterns of non-small-cell lung cancer at Princess Margaret Cancer Centre

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ABSTRACT

Background Accurate classification of lung cancer subtypes has become critical in tailoring lung cancer treatment. Our study aimed to evaluate changes in diagnostic testing and pathologic subtyping of advanced non-small-cell lung cancer (NSCLC) over time at a major cancer centre.

Methods In a review of patients diagnosed with advanced NSCLC at Princess Margaret Cancer Centre between 2007–2009 and 2013–2015, diagnostic method, sample type and site, pathologic subtype, and use of immunohistochemistry (IHC) staining and molecular testing were abstracted.

Results The review identified 238 patients in 2007–2009 and 283 patients in 2013–2015. Over time, the proportion of patients diagnosed with adenocarcinoma increased to 73.1% from 60.9%, and diagnoses of NSCLC not otherwise specified (NOS) decreased to 6.4% from 18.9%, $p < 0.0001$. Use of diagnostic bronchoscopy decreased (26.9% vs. 18.4%), and mediastinal sampling procedures, including endobronchial ultrasonography, increased (9.2% vs. 20.5%, $p = 0.0001$). Use of IHC increased over time to 76.3% from 41.6% ($p < 0.0001$). Larger surgical or core biopsy samples and those for which IHC was performed were more likely to undergo biomarker testing (both $p < 0.01$).

Conclusions Customizing treatment based on pathologic subtype and molecular genotype has become key in treating patients with advanced lung cancer. Greater accuracy of pathology diagnosis is being achieved, including through the routine use of IHC.

Key Words Diagnostic testing, pathologic subtypes, immunohistochemistry, molecular testing, lung cancer

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INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide¹. Treatment selection and outcomes rely on accurate diagnostic subtyping of non-small-cell lung cancer (NSCLC), including molecular testing^{2–9}. For example, pemetrexed and bevacizumab are associated with superior outcomes in adenocarcinoma and inferior outcomes in squamous cell carcinoma^{2,7,10}. In patients with nonsquamous histology, the introduction of targeted therapy agents such as gefitinib, alectinib, and crizotinib for the treatment of oncogene-addicted lung cancer has made genomic testing an essential component of the diagnostic algorithm in NSCLC^{3,4,8,9}. Reflex molecular profiling of diagnostic samples has become indispensable in the Canadian system to identify candidates for those and other targeted therapies in a timely manner³. Pathologic subtype remains important in selecting relevant cases. For instance,

all patients with nonsquamous histology should routinely undergo profiling¹¹. However, those diagnosed with pure squamous, small-cell, or neuroendocrine subtypes have a lower likelihood of *EGFR*, *ALK*, or *ROS1* aberration.

Multiple sampling methods are available for lung cancer diagnosis^{12–14}. Although minimally invasive procedures are often preferred to minimize the risk to patients, their use must be balanced against successful acquisition of sufficient material for pathologic and molecular evaluation. Commonly, however, diagnostic tumour tissue remains quite limited in patients with advanced lung cancer, and cancer morphology alone is often insufficient for precise tumour characterization. Immunohistochemistry (IHC) has emerged as an important technique to better subtype lung cancer when morphology alone is nonspecific^{15–22}. Current

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guidelines recommend the routine use of IHC in biopsy samples when accurate subtyping cannot be performed based on morphologic assessment²³. A final diagnosis of NSCLC not otherwise specified (NOS) has been associated with an unfavourable prognosis in patients with stage IV NSCLC and should be refined wherever possible²⁴.

Precise histologic characterization of NSCLC has become essential for therapeutic decision-making because the results can predict response to various systemic treatments^{25,26}. With the development of novel therapies and diagnostic techniques, shifts have occurred over time in the use of various diagnostic procedures in routine practice. Our study examined the changes in diagnostic testing and pathologic subtyping of advanced NSCLC, including the use of IHC, over time at a major cancer centre in Canada.

METHODS

This study was approved by the University Health Network Research Ethics Board. All patients with stage IV NSCLC diagnosed at the Princess Margaret Cancer Centre or University Health Network during January 2007–January 2009 and January 2013–May 2015 (data cut-off) were reviewed. The initial cohort consisted of patients diagnosed during 2007–2009, when pemetrexed was being introduced into the Canadian lung cancer treatment landscape and pathologic subtype was emerging as a critical factor in treatment selection. Patients diagnosed during 2013–2015 were chosen for the comparator cohort, when targeted therapy was being integrated into standard practice. Patients were excluded if the initial diagnosis was made at another institution, if no histologic or pathology diagnosis had been confirmed, and if the initial diagnosis was not advanced NSCLC.

Data abstracted were the initial diagnostic procedure (bronchoscopy, image-guided, surgical resection, mediastinal sampling, or other), sample type [non-fine-needle aspiration (FNA) or exfoliative cytology, FNA cytology, or biopsy or surgical specimen], site of diagnostic sample (primary lung, mediastinal lymph nodes, metastasis, or effusion), final pathology diagnosis, and use of IHC. Testing for *EGFR* mutations and *ALK* rearrangements did not become routine until after 2010. Thus, data about *EGFR* or *ALK* testing were collected only for the 2013–2015 cohort.

Statistical Analysis

Patient demographics and patterns of diagnosis are summarized using descriptive statistics. Chi-square, *t*-tests, and Fisher exact tests were used, as appropriate, to compare differences in diagnostic patterns for 2007–2009 and 2013–2015. Associations between molecular testing and diagnostic sampling method or type (or both) were explored in the 2013–2015 cohort of patients with adenocarcinoma. All analyses were performed using the SAS software application (version 9.4: SAS Institute, Cary, NC, U.S.A.).

RESULTS

Patient and Sample Characteristics

The review identified 238 patients in the 2007–2009 cohort and 283 patients in the 2013–2015 cohort. Baseline characteristics in the two cohorts were similar, with the exception

that patients in the 2013–2015 cohort were slightly older ($p = 0.009$, Table 1). Over time, the proportion of patients diagnosed with adenocarcinoma increased to 73.1% from 60.9%, and cases of NSCLC NOS decreased to 6.4% from 18.9%, $p < 0.0001$. The proportion of squamous cell and large-cell subtypes remained similar over time. In the 2007–2009 cohort, 21.8% of the patients ($n = 52$) underwent more than one type of sampling on initial diagnostic evaluation (cytology and biopsy), compared with 29.3% ($n = 83$) in the 2013–2015 cohort.

Diagnostic Sampling Method and Sampling Type

Over time, a significant change in the pattern of diagnostic sampling methods was observed ($p = 0.001$, Table 1). A reduction in the use of bronchoscopy as the initial diagnostic method for advanced lung cancer was seen over time (26.9% vs. 18.4%). The use of mediastinal sampling procedures, including endobronchial ultrasound-guided (EBUS) sampling increased (9.2% vs. 20.5%). Use of other imaging-guided sampling procedures (including those guided by computed tomography and ultrasound) and surgical resection remained similar over time. A substantial reduction in cases reported as NSCLC NOS was observed for bronchoscopy, imaging-guided, and mediastinal sampling procedures (Table 1). That reduction was seen predominantly in cytology samples, with NOS diagnoses falling to 4.0% from 22.0% ($p < 0.0001$). Cytology cell block preparations were routinely made from FNA samples, but not from exfoliative samples, as long as a sufficient sample was obtained.

IHC Use in Diagnosis

The rate of IHC use increased significantly during the study period to 76.3% from 41.6% of all cases diagnosed ($p < 0.0001$, Table 1). For the group diagnosed with NSCLC NOS, the use of IHC increased to 94% (17 of 18) from 64% (29 of 45), but the difference only trended toward statistical significance ($p = 0.07$). With the exception of bronchoscopy samples ($p = 0.35$), use of IHC increased significantly for all methods of diagnosis [Figure 1(A)] and all sample types [Figure 1(B)].

Molecular Testing

Molecular testing for *EGFR* mutations and *ALK* fusions was not performed as part of routine diagnosis in 2007–2009, because a national program for testing had not yet been implemented. Of the 207 patients diagnosed with adenocarcinoma in 2013–2015, 89.9% ($n = 186$) had *EGFR* or *ALK* testing, or both, performed. In most cases, the initial diagnostic sample was used for that testing (83%, 155 of the 186). On initial diagnostic evaluation, testing involved cytology samples in 44.5% of the cases, a surgical specimen in 23.9%, and both surgical and cytology samples in 31.6%.

Biomarker testing was more commonly performed in surgical samples, mediastinal samples, and samples obtained from image-guided procedures, $p = 0.01$ [Figure 2(A)]. Biomarker testing was also more likely to be performed using larger surgical or biopsy samples than cytology FNA or exfoliative samples, $p = 0.003$ [Figure 2(B)]. In addition, IHC use was found to be associated with biomarker testing, because 93% of samples undergoing IHC evaluation (167 of 180), compared with 70% not undergoing

TABLE I Patient demographics and diagnostic sampling characteristics

Variable	Patient cohort		p Value
	2007–2009	2013–2015	
Patients (n)	238	283	
Age at diagnosis (years)			0.009
Median	66	68	
Range	30–87	36–94	
Sex [n (%)]			0.28
Men	145 (60.9)	166 (58.7)	
Women	93 (39.1)	117 (41.3)	
Pathologic subtype [n (%)]			<0.0001
Adenocarcinoma	145 (60.9)	207 (73.1)	
Squamous cell carcinoma	32 (13.5)	38 (13.4)	
Large-cell carcinoma	14 (5.9)	9 (3.2)	
NSCLC NOS	45 (18.9)	18 (6.4)	
Other	2 (0.8)	11 (3.9)	
Initial sampling method [n (%)]			0.001
Bronchoscopy	64 (26.9)	52 (18.4)	
Cytology	36 (15.1)	21 (7.4)	0.10
Biopsy	28 (11.8)	31 (11.0)	
Image-guided	73 (30.7)	88 (31.1)	
Cytology	65 (27.3)	31 (11.0)	<0.0001
Biopsy	8 (3.4)	57 (20.1)	
Surgical resection	31 (13.0)	45 (15.9)	0.0001
Mediastinal sampling ^a	22 (9.2)	58 (20.5)	
Cytology	9 (3.8)	58 (20.5)	<0.0001
Biopsy	13 (5.4)	0	
Other ^b	48 (20.2)	40 (14.1)	
Cytology	43 (18.1)	38 (13.4)	0.45
Biopsy	5 (2.1)	2 (0.7)	
Initial sampling site [n (%)]			0.001
Lung	139 (58.4)	133 (47.0)	
Mediastinal lymph node	24 (10.1)	65 (23.0)	
Metastasis	49 (20.6)	59 (20.8)	
Effusion ^c	26 (10.9)	26 (9.2)	
Initial sampling type [n (%)]			0.0001
Cytology, non-FNA	58 (24.4)	38 (13.4)	
Cytology, FNA	106 (44.5)	111 (39.2)	
Core biopsy or surgical	74 (31.1)	134 (47.4)	
IHC performed [n (%)]			<0.0001
Yes	99 (41.6)	216 (76.3)	
No	139 (58.4)	67 (23.7)	

^a Including mediastinoscopy and endobronchial ultrasonography.^b Including thoracentesis.^c Including pleural and pericardial effusions.

NSCLC NOS = non-small-cell lung cancer not otherwise specified; IHC = immunohistochemistry; FNA = fine-needle aspiration.

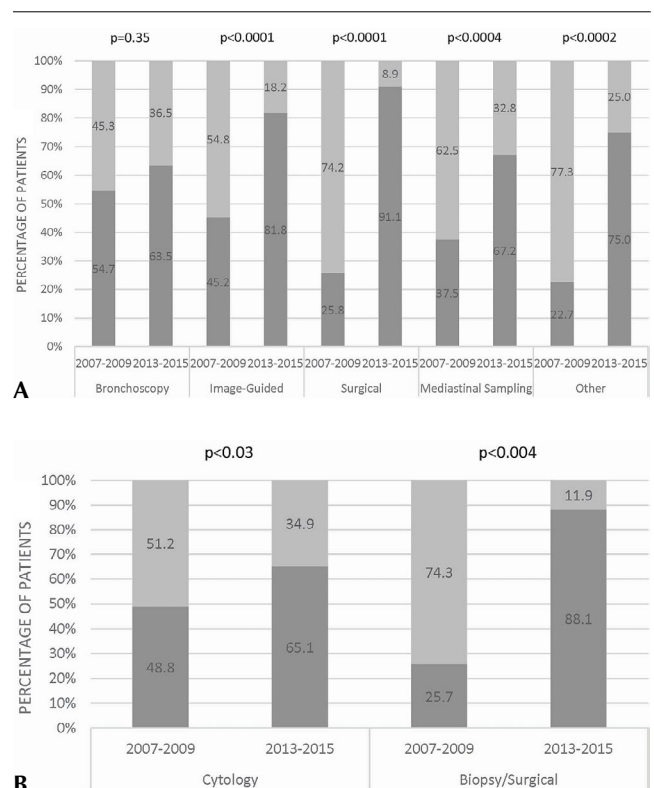
IHC evaluation (19 of 27), also subsequently underwent molecular analysis ($p = 0.002$).

DISCUSSION

Optimal management of lung cancer relies on the pathologic and molecular characterization of the tumour to select

TABLE II Non-small-cell lung cancer not otherwise specified diagnosed during 2007–2009 and 2013–2015

Variable	Patient cohort		p Value
	2007–2009	2013–2015	
Initial sampling method [n (%)]			
Bronchoscopy	12/64 (18.8)	0/52 (0)	0.0005
Image-guided	15/73 (20.5)	4/88 (4.6)	0.003
Surgical	8/31 (25.8)	9/45 (20.0)	0.59
Mediastinal	4/22 (18.2)	2/58 (3.4)	0.046
Other	6/48 (12.5)	3/40 (7.5)	0.50
Initial sampling type [n (%)]			
Cytology ^a	36/164 (22.0)	6/149 (4.0)	<0.0001
Biopsy or surgical	9/74 (12.2)	12/134 (9.0)	0.48

^a Combined fine-needle aspiration and non-fine-needle aspiration cytology.**FIGURE 1** Immunohistochemistry (IHC) use during 2007–2009 and 2013–2015 (light grey, IHC not performed; dark grey, IHC performed). (A) By sampling method. (B) By sampling type.

the best treatment approach. We observed a clear shift in diagnostic testing patterns over time, with greater use of mediastinal sampling procedures and significant uptake of IHC and biomarker testing as part of routine pathology assessment. In addition, the pathologic subtyping of lung cancers has substantially improved, with a decrease in the proportion of NSCLC NOS diagnoses to 6.4% from 18.9% over the period of the study.

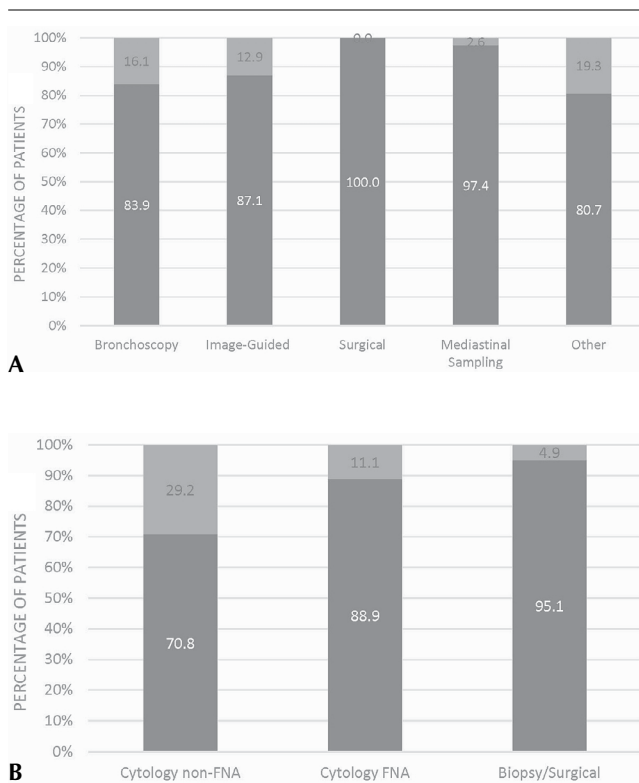


FIGURE 2 Molecular testing in adenocarcinoma (light grey, molecular testing not completed; dark grey, molecular testing completed; 2013–2015 cohort). (A) By sampling method. (B) By sampling type.

The rate at which IHC is being used in lung cancer diagnosis continues to rise, particularly in cases of NSCLC NOS. Studies have shown that appropriate use of IHC could result in an additional classification rate of 72%–86% for samples originally classified as NSCLC NOS using morphology analysis alone^{27,28}. When histology cannot be confidently established because of scarce material or lack of definite morphology (or both), IHC should be used to further characterize the tumour after the initial morphology examination and to provide guidance about appropriate treatment selection²⁹. Even in cases of clear-cut morphology, IHC could still be used to exclude metastatic extrapulmonary carcinoma³⁰. Greater use of IHC has led to greater accuracy of pathologic subtyping and allowed clinicians to strategize both testing and treatment. Notably, rather than an exhaustive set of stains being used, which could lead to insufficient samples being available for molecular testing, a selected IHC panel should be used. For example, a diagnostic panel for lung cancer could include a limited number of stains, such as TTF1, cytokeratin 7, napsin A, and p40 or another squamous carcinoma marker.

The decrease in NSCLC NOS over time was driven largely by improved subtyping of cytology samples. However, in our study, we also saw significant uptake in IHC use in core biopsy and resection specimens. Recent studies have concluded that cytologic subtyping of NSCLC is feasible and accurate for diagnosis³¹ and that, compared with small biopsy samples, it yields comparable rates of definitive NSCLC subtyping³². Although biopsy samples might sometimes

provide more diagnostic material for analysis, advantages for cytology specimens for mutation testing have been identified^{33–36}. In addition, molecular testing of cytology cell blocks yields success rates similar to those achieved with histology samples³⁷, with several cytology methods and preparations being available³⁸. Thus, cytology samples represent an effective and minimally invasive diagnostic option for the histologic and molecular classification of NSCLC.

Although the use of image-guided procedures remained unchanged, mediastinal sampling procedures (that is, by EBUS), increased substantially over time, and diagnostic bronchoscopy decreased. Although bronchoscopy remains widely used (in particular, to rule out other diagnoses), it is less likely to yield a definitive diagnosis if limited specimen amounts are obtained^{39–41}. However, advances in bronchoscopic technique, including ultrasound guidance and rapid onsite evaluation of cytology have led to greater precision in diagnosis⁴². Furthermore, cytology specimens obtained from EBUS procedures have been found to be suitable for diagnostic use in routine practice and, combined with IHC, to reduce the rate of NSCLC NOS^{43,44}. Compared with conventional diagnostic procedures, EBUS has also been shown to reduce time to treatment decisions⁴⁵. The increasing use of EBUS sampling can effectively guide individualized patient therapy with samples suitable for pathologic subtyping and molecular analysis, recognizing the importance of operator performance and rapid onsite evaluation to enhance diagnostic success.

In our study, we were unable to capture the proportion of pathologic diagnoses that were made on the basis of IHC results when morphology alone was nonspecific. Another limitation is that, although patients might have had multiple diagnostic samples collected on initial lung cancer assessment, we used the most definitive sample report. Further staining and classification were often deferred to specimens with the most cell content when multiple sample types were available. Surgical resection was used as the initial method of diagnosis in a small number of cases, in which patients diagnosed with clinical early-stage lung cancer were found, on resection, to have advanced-stage disease—for example, pleural involvement. Lastly, it must be recognized sample quality can be affected by operator expertise—for example, samples obtained by EBUS. However, the latter limitation did not appear to negatively affect the results of our study, which showed high rates of pathologic subtyping regardless of EBUS use.

CONCLUSIONS

Customizing treatment based on pathologic subtype and molecular genotype has become essential in the diagnosis and treatment of patients with advanced-stage NSCLC. A clear shift in diagnostic testing practices has occurred over time at our centre, with greater uptake of routine IHC use and biomarker testing. As novel therapies emerge and diagnostic techniques evolve for patients with lung cancer, assuring the greater accuracy and consistency of pathologic and molecular diagnosis will help to optimize treatment and therapeutic outcomes for patients with lung cancer.

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CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology's* policy on disclosing conflicts of interest, and we declare the following interests: DMH reports personal fees from Pfizer, grants and personal fees from Merck, grants from AstraZeneca, grants and personal fees from Novartis, personal fees from Takeda, and personal fees from Roche, outside the submitted work. MST reports personal fees from AstraZeneca, Pfizer, Bristol Myers Squibb, Hoffmann–La Roche, Bayer, Takeda, and Merck, outside the submitted work. NBL reports institutional research funding from Roche, AstraZeneca, Array, Guardant, and Merck Sharp and Dohme outside submitted work; honoraria or travel expenses (or both) for independent continuing medical education lectures from AstraZeneca, Bristol Myers Squibb, Merck Sharp and Dohme, Roche, and Pfizer outside the submitted work; and advisor fees from Xcovery. The remaining authors have no conflicts of interest to declare.

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