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

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 testing2–5. For example, pemetrexed and bevacizumab are associated with superior outcomes in adenocarcinoma and inferior outcomes in squamous cell carcinoma2,7,10. 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 NSCLC3,4,8,9. 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 manner. 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 diagnosis12–14. 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 nonspecific15–22. Current

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guidelines recommend the routine use of IHC in biopsy samples when accurate subtyping cannot be performed based on morphologic assessment. 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 possible.

Precise histologic characterization of NSCLC has become essential for therapeutic decision-making because the results can predict response to various systemic treatments. 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 subtyping 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 2). 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
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TABLE I   Patient demographics and diagnostic sampling characteristics

| Variable                  | 2007–2009 | 2013–2015 | p Value |
|---------------------------|-----------|-----------|---------|
| 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)| 0.10    |
| Cytology                  | 36 (15.1)| 21 (7.4) |         |
| 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      | 22 (9.2) | 58 (20.5)|         |
| Cytology                  | 9 (3.8)  | 58 (20.5)| <0.0001 |
| Biopsy                    | 13 (5.4) | 0        |         |
| Other                     | 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                  | 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 thoracotomy.
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 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.
provide more diagnostic material for analysis, advantages for cytology specimens for mutation testing have been identified. In addition, molecular testing of cytology cell blocks yields success rates similar to those achieved with histology samples, with several cytology methods and preparations being available. 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. Advances in bronchoscopic technique, including ultrasound guidance and rapid onsite evaluation of cytology have led to greater precision in diagnosis. 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. Compared with conventional diagnostic procedures, EBUS has also been shown to reduce time to treatment decisions. 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, 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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