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Original Research: Biomarker Testing in a Canadian Centre for Patients with Non-small Cell Lung Cancer: Assessing Residual Risks

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Biomarker testing is critical for guiding treatment decisions and clinical management in patients with non-small cell lung cancer (NSCLC). Although the clinical utility of comprehensive testing for point mutations and gene rearrangements is well established, access to next-generation sequencing (NGS)-based assays in Ontario has historically been limited due to provincial funding constraints.

We conducted a retrospective chart review of 215 patients diagnosed with lung adenocarcinoma over a five-year period (2016-2021) and report the observed biomarker testing practice. Testing primarily comprised polymerase chain reaction (PCR)-based detection of common epidermal growth factor receptor (EGFR) mutations and immunohistochemistry (IHC) for anaplastic lymphoma kinase (ALK) overexpression, with or without confirmatory fluorescence in situ hybridization (FISH), and programmed death-ligand 1 (PD-L1) IHC. IHC for ROS1 overexpression, as a surrogate for ROS1 fusion, was observed in the first quarter of 2020. Routine panel-based NGS testing was implemented in the first quarter of 2021. Noting the differences between PCR- and NGS-based EGFR assessment, risks of "false negative" were estimated based on Bayesian analyses. Given the limited scope of PCR tests in terms of variants detected, the post-test, residual risk of "false negative" EGFR was estimated to range ~1:90 in white, Caucasian patients, to ~1:9 in Asian patients.

We observed consistent implementation of EGFR, ALK, and PD-L1 testing during the study period, which was in alignment with 2017 National Comprehensive Cancer Network (NCCN) guideline recommendations. However, the delayed adoption of ROS1 testing and NGS-based profiling, including assays for MET and RET alterations, reflects broader limitations in provincial funding policy and highlights the need for equitable access to comprehensive biomarker testing in Ontario.

Introduction

Clinical management of non-small cell lung cancer (NSCLC) is increasingly guided by biomarker testing, which has become a cornerstone of precision oncology and is now embedded in standard clinical care. The use of broad next-generation sequencing (NGS) panels is routinely recommended for patients with NSCLC to identify oncogenic drivers—including point mutations and gene rearrangements—as reflected in the most recent National Comprehensive Cancer Network (NCCN) guidelines.[1,2] However, the high cost of NGS has been a limiting factor in many jurisdictions, including Canada. In Ontario, the introduction of a "comprehensive" cancer biomarker testing program aimed to expand access to molecular testing for NSCLC, incorporating both NGS and programmed death-ligand 1 (PD-L1) immunohistochemistry (IHC) assessments. In 2021, Ontario Health–Cancer Care Ontario (OH-CCO) endorsed NGS as the preferred initial test at diagnosis, replacing single-gene assays. This policy shift followed a period in which alternative molecular testing approaches were more commonly used in lieu of NGS.

The value of biomarker testing in informing prognosis and guiding targeted therapies is well established. NGS offers the advantage of simultaneously detecting a broad range of actionable alterations, including *MET* exon 14 skipping mutations and *RET* gene rearrangements, providing a more comprehensive molecular profile of each patient's tumour. With consistent provincial funding, patients diagnosed with NSCLC in Ontario are more likely to receive equitable access to molecular diagnostics, enabling clinicians to integrate precision oncology into treatment planning. Robust biomarker testing may be especially important in a diverse metropolitan area such as Toronto, where a large proportion of patients identify as immigrants from East or South Asia, or as members of Indigenous communities. While *EGFR* mutations are known to be more prevalent in certain Asian populations[3], the distribution of targetable oncogenic drivers in North American multi-ethnic cohorts remains incompletely understood.

In this study, we examined biomarker testing practices among patients with NSCLC diagnosed at a single academic centre in Toronto between 2016 and 2021. We describe the transition from predominantly non-NGS testing to implementation of panel-based NGS and assess the potential clinical impact of limited variant detection, including the risk of false-negative results in certain patient subgroups.

Materials & Methods

Study Design and Cohort Selection

This was a single-centre, retrospective cohort study conducted at Unity Health Toronto, an academic tertiary care hospital in Ontario, Canada. A total of 265 consecutive patients diagnosed with NSCLC between 2016 and 2021 were identified through electronic medical records (EMRs) and included for demographic and clinical characterization. Patients diagnosed with neuroendocrine neoplasms (including typical carcinoid, large cell neuroendocrine carcinoma, and small cell carcinoma) or pleomorphic carcinoma were excluded. To analyze biomarker testing patterns, we focused on 215 patients with histologically confirmed adenocarcinoma or adenosquamous carcinoma, as these histologic subtypes are routinely considered for molecular profiling per clinical guidelines. Patients with squamous cell carcinoma (n = 50) were excluded from biomarker testing analysis due to the low prevalence of actionable driver mutations in this subgroup.

Biomarker Testing Methodology

All biomarker testing was performed as send-out assays to external reference laboratories. For *EGFR* testing, PCR-based assays targeting the most common sensitizing mutations (exon 19 deletions and exon 21 p.L858R substitutions) were utilized. *ALK* gene rearrangements were assessed by IHC, typically using the D5F3 clone, with fluorescence *in situ* hybridization (FISH) performed at the discretion of the testing laboratory. PD-L1 testing was generally conducted using either the SP263 or 22C3 clone, depending on institutional protocol and availability. *ROS1* testing by IHC (clone D4D6) was introduced in the first quarter (Q1) of 2020.

NGS was implemented in Q1 2021 using a hybrid capture-based panel covering hotspot mutations, gene rearrangements, and copy number alterations. Prior to that, single-gene testing approaches predominated. Biomarker testing decisions were made at the discretion of treating oncologists or pathologists, generally based on tumour histology, disease stage, and sample availability.

Demographic Classification

Race and ethnicity were not discretely captured in the EMRs. To approximate *EGFR* mutation prevalence by race, patients were classified as "Asian" or "Non-Asian" using surname inference, supplemented by preferred language and country of birth, where available. The "Asian" category included East, Southeast, and South Asian patients; "Non-Asian" patients were presumed to be predominantly white/Caucasian. This classification was used for subgroup-based modelling of false-negative risk associated with PCR-based *EGFR* testing.

Statistical Analysis

Descriptive statistics were used to summarize cohort characteristics and biomarker testing frequencies. Differences between observed and expected mutation frequencies were assessed using two-tailed Chi-square tests, with a p-value <0.05 considered statistically significant.

Bayesian modelling was applied to estimate the risk of false-negative results associated with PCR-based *EGFR* testing. Published prevalence estimates for *EGFR* mutations in Asian and white populations were used to establish pre-test probabilities. Assuming 90% sensitivity and ~100% specificity for PCR assays, post-test probabilities were calculated using Bayes' theorem. This model allowed estimation of the residual risk of undetected *EGFR* mutations following a negative PCR result, stratified by racial background. All statistical analyses were conducted in R (base version 4.1.1).

Results

Biomarker Testing Patterns in NSCLC Cohort

The mean age at diagnosis was 68 years. The slight majority of patients (137/265, 51.7%) were male. Where cigarette smoking status was available, 144 of 203 patients (70.9%) reported a history of tobacco use. Adenocarcinoma was the most common histologic diagnosis, identified in 211 of 265 patients (79.6%), followed by 50 patients with squamous cell carcinoma, and 4 patients with adenosquamous carcinoma. Most patients (63.9%) were diagnosed at American Joint Committee on Cancer (AJCC) Stage I (8th edition). One patient was diagnosed at Stage 0, 167 at Stage I, 31 at Stage II, 41 at Stage III, and 23 at Stage IV. Staging data were unavailable for two patients. The median follow-up period was two years.

All biomarker studies during the study period were performed as send-out assays to external reference laboratories. For patients with adenocarcinoma or adenosquamous carcinoma (n=215), biomarker testing primarily consisted of: polymerase chain reaction (PCR)-based detection of common epidermal growth factor receptor (*EGFR*) mutations, such as exon 19 deletions and exon 21 p.L858R; immunohistochemistry (IHC) for overexpression of anaplastic lymphoma kinase (*ALK*), used as a surrogate for *ALK* gene rearrangement and performed with or without fluorescence *in situ* hybridization (FISH); and IHC for programmed death-ligand 1 (PD-L1) expression (**Figure 1**). *ROS1* IHC, used as a surrogate marker for *ROS1* gene rearrangement, was implemented in Q1, 2020. Routine panel-based next-generation sequencing (NGS) testing was adopted in Q1, 2021. In comparison, the 2017 National Comprehensive Cancer Network (NCCN) guidelines had already incorporated *ROS1* testing into the main diagnostic algorithm, and included the option of either PCR-based or NGS-based testing for *EGFR* mutations.[2]

Impact Assessment

In constitutional genetics, Bayesian analysis has been employed to calculate pre- and post-test probabilities for pathogenic germline variants. For example, cystic fibrosis risk associated with cystic fibrosis transmembrane conductance regulator (*CFTR*) gene variants differs across ethnic groups, as certain mutations are more prevalent in specific populations; accordingly, assay design can substantially influence the residual risk following a negative test result.[4,5] Although this framework is not routinely applied in cancer genetics, it can offer useful insights into differences in test performance across populations.[6] In this study, we applied Bayesian analysis to estimate the potential impact of relying on non-NGS methods for NSCLC biomarker testing.

EGFR mutations have been reported in approximately 10% of white, Caucasian patients with NSCLC, up to 19% of Black patients, and as high as 50% of Asian patients.[3,7,8] Exon 19 deletions and exon 21 p.L858R variants comprise approximately 85–90% of the *EGFR* alterations.[9] Given that many PCR-based platforms are limited to detecting only these common variants, it can be inferred that 10–15% of *EGFR* mutations would have been missed. Assuming a sensitivity of 90%

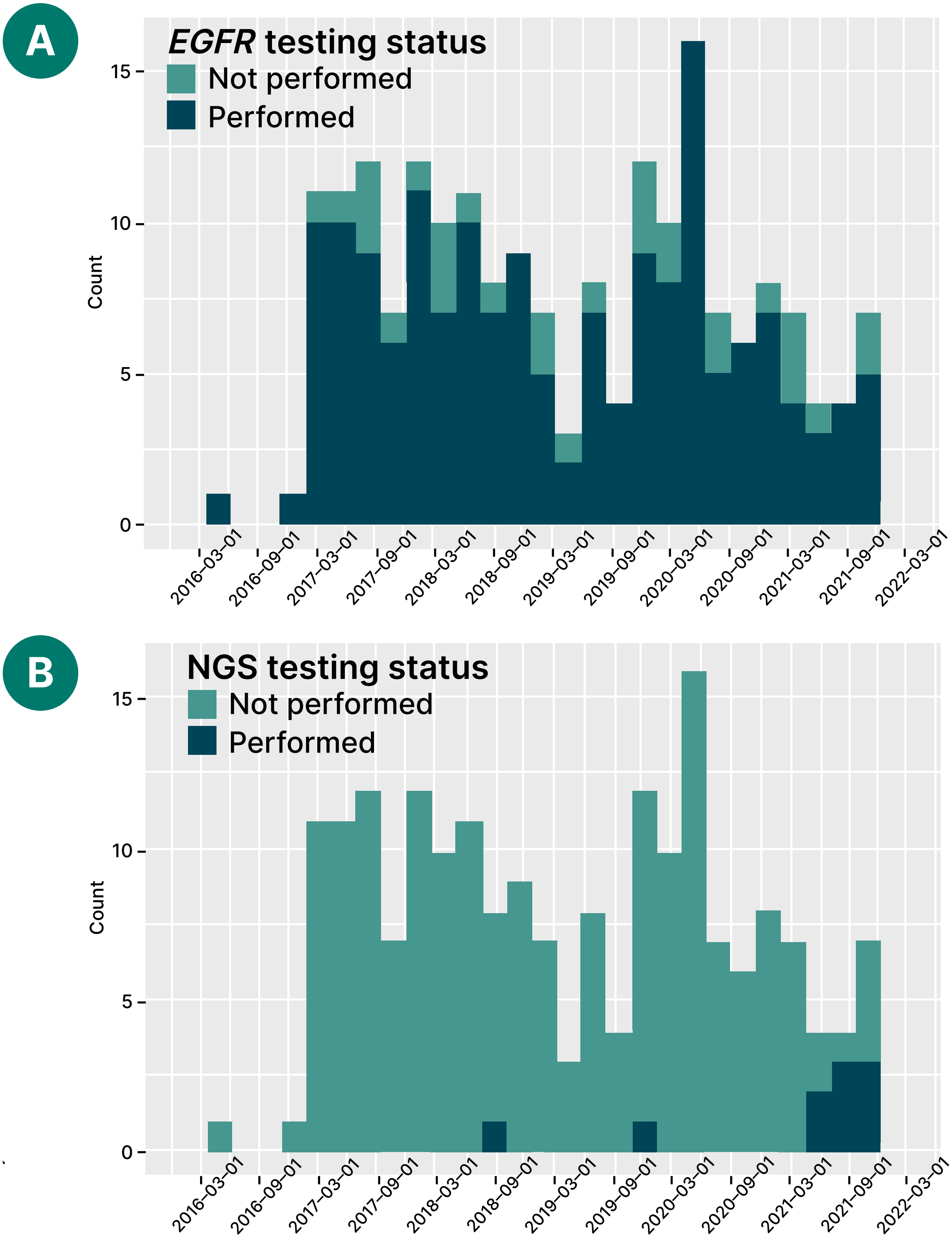

Figure 1. A) *EGFR* and **B)** next-generation sequencing (NGS) testing patterns during our study period; *courtesy of Yunting Liu, Steven Shen, Manav Shukla, Janet Malowany, Shaheed Hakim, Zared Aziz, David N. Parente, Victoria Cheung, Suneil Khanna, Yoo-Joung Ko, Wondwossen Kidanewold, Michael A. Ko, Kelsie L. Thu, and Ju-Yoon Yoon.*

	Asian patient		White, Caucasian	
***EGFR* gene status**	Mutant	Wild-type	Mutant	Wild-type
Pre-test probability	0.5	0.5	0.1	0.9
Negative PCR	0.1	~1	0.1	~1
Joint probability	0.05	~0.5	0.01	~0.9
Posterior probability	~0.09	~0.91	~0.01	~0.99
(Residual) Risk	~1:9		~1:90	

Table 1. Risk of false-negative *EGFR* results in a patient with NSCLC, based on ethnicity; *courtesy of Yunting Liu, Steven Shen, Manav Shukla, Janet Malowany, Shaheed Hakim, Zared Aziz, David N. Parente, Victoria Cheung, Suneil Khanna, Yoo-Joung Ko, Wondwossen Kidanewold, Michael A. Ko, Kelsie L. Thu, and Ju-Yoon Yoon.*

Abbreviations: EGFR: epidermal growth factor receptor; **NSCLC:** non-small cell lung cancer; **PCR:** polymerase chain reaction.

and near-perfect specificity for *EGFR* PCR assays, the risk of a false-negative result is estimated to be ~1:9 for an Asian patient and ~1:90 for a white, Caucasian patient (**Table 1**).

Among the 181 patients in our cohort with known *EGFR* status, alterations were identified in 45 (24.9%). Based on the racial composition of our cohort—and assuming that non-Asian patients were predominantly white—the expected prevalence of *EGFR* alterations would be approximately 14.4% (26/181). NGS was performed in 20 patients, with *EGFR* alterations detected in five patients. Among the 161 patients who did not undergo NGS, PCR testing identified *EGFR* mutations in 7 of 21 (33.3%) patients of Asian background, a rate not statistically different from the expected 50% (two-tailed Chi-square p=0.1899).

ALK rearrangements have been reported in approximately 5% of NSCLC cases.[10] In our cohort, *ALK* gene rearrangements were identified in 3 of 176 patients (1.7%) who underwent *ALK* IHC testing, which was significantly lower than the expected frequency (two-tailed Chi-square *p*=0.0401). Previous studies have reported a sensitivity of roughly 90% for detecting *ALK* rearrangements by IHC;[11,12] thus, some rearrangements may have been missed by using IHC alone as a screening modality. *ROS1* gene rearrangement was identified in 1 of 42 tested patients (2.4%), a frequency consistent with published estimates of 1–2%.[13,14]

Discussion

We observed robust implementation of *EGFR*, *ALK*, and PD-L1 biomarker testing during our study period, primarily through PCR-based assays and IHC with or without FISH. However, *ROS1* IHC testing was only introduced in the latter half of the study window. Broad molecular profiling using NGS panels was limited to the final year of the study period. In 2021, Ontario Health–Cancer Care Ontario (OH-CCO) expanded biomarker testing at diagnosis to include NGS as the first line platform, replacing single-gene testing. The pattern of biomarker testing observed at our institution closely mirrors the provincial funding model in Ontario for NSCLC. Although our testing for *EGFR*, *ALK*, and PD-L1 aligned with the 2017 NCCN recommendations, those guidelines also included *ROS1* and NGS testing, highlighting a significant delay in the implementation of comprehensive biomarker strategies in Ontario compared to U.S. centres. Of the 215 patients in our adenocarcinoma/adenosquamous cohort, based on known prevalence of *ROS1* (~1–2%),[13,14] *MET* exon 14 skipping (~3–4%),[15,16] *RET* rearrangements (~1–2%),[17] and given that 195 patients did not receive NGS testing during the study period, these targetable genetic alterations may have been missed in roughly ~10–16 (~5–8%) of patients in the cohort.

An important consideration when selecting a biomarker testing modality is the difference in analytic sensitivity. PCR-based detection of

EGFR mutations is highly sensitive and can also be applied to liquid biopsy samples.[18,19] While differences between PCR and NGS platforms have been well described, we did not observe overt evidence of negative impact in our limited cohort. However, the lack of statistical significance is likely attributable to sample size constraints. The potential risk of false-negative results remains, particularly among patients of Asian ancestry, in whom *EGFR* mutation prevalence is higher.

Our findings related to *ALK* rearrangement suggest a lower-than-expected detection rate, raising the possibility that test sensitivity may have contributed. While the reported sensitivity of *ALK* IHC is high (~90%),[11,12] the use of IHC alone—as opposed to upfront FISH or RNA sequencing—may not fully account for the discrepancy.

The estimated risks of false-negative *EGFR* results presented in **Table 1** are based solely on racial background; however, these risks are further modulated by additional clinical factors, such as smoking history. Moreover, driver mutations in lung adenocarcinoma are generally mutually exclusive.[20] For example, a patient whose NGS-based tumour testing identifies a *KRAS* p.G12C mutation would have a near-zero probability of also harbouring an *EGFR* mutation. The primary advantage of NGS lies in its ability to comprehensively identify mutually exclusive oncogenic drivers, thereby minimizing the risk of false-negative or false-positive results. This also underscores the importance of re-testing in cases where initial diagnostic material is inadequate for NGS.

Conclusion

In summary, this retrospective study outlines real-world patterns in NSCLC biomarker testing at a Canadian academic centre during a period of evolving provincial funding policy. While guideline-concordant testing for *EGFR*, *ALK*, and PD-L1 was well established, the delayed implementation of *ROS1* and NGS testing reflects systemic barriers to comprehensive molecular profiling. Our findings highlight the importance of equitable access to broad-panel testing and underscore the limitations of single-gene assays, particularly in ethnically diverse populations. Ongoing efforts to standardize testing practices across jurisdictions will be critical for optimizing precision oncology in lung cancer care.

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