Clinical Utility of Reflex Ordered Testing for Molecular Biomarkers in Lung Cancer

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Background: Clinical testing of molecular biomarkers to guide therapy in non–small cell lung cancer has become routine and increasingly complex. To standardize and expedite molecular biomarker testing in these patients, our institution implemented reflex ordered testing for a group of targeted gene alterations in all newly diagnosed non–small cell lung carcinoma. The aim of our study is to evaluate the clinical utility of reflex ordered molecular testing in lung cancer.

Methods: Lung adenocarcinoma specimens received for molecular testing at our institution over a 6-month period in 2017 were identified with IRB approval. Testing for the entire group of molecular biomarkers was ordered at diagnosis (reflex ordered) or specific biomarkers were tested at request of oncologists (nonreflex ordered). Reflex ordered biomarkers included EGFR, KRAS, BRAF, and ERBB2 mutations; ALK, RET, and ROS1 gene rearrangements; MET exon 14 skipping (MET14); MET gene amplification; and PDL-1 expression.

Results: The cohort included 160 patients with non–small cell lung carcinoma with 49% (n = 78) males, 51% (82) females, and median age of 70 years (age range 43–90 years). There were 73% (117) Caucasians, 19% (30) African Americans, and 8% (13) Asians. Nineteen percent (31) of cases had nonreflex testing, and 81% (129) had reflex testing. The mean number of days from anatomic pathology reporting to molecular reporting was 23 days for reflex testing vs 52 days for nonreflex. Reflex testing had a higher variant detection rate than nonreflex (47% vs 19%, P < 0.05). Specific variant detection rates were EGFR = 13% (n = 20), KRAS = 26% (33), BRAF = 1.5% (2), ERBB2 = 0% (0), ALK = 5% (8), RET = 1.5% (2), ROS1 = 2.7% (4), MET14 = 0.8% (1), and MET amplification = 2.8% (3); 76% of cases had PD-L1 expression in <50% of tumor.

Conclusions: The detection rates and types of pathogenic variants identified within our cohort were similar to those reported in literature. The mean turnaround time of molecular result reporting was significantly reduced with reflex testing. The variant detection rate was 2.5 times higher for reflex testing than nonreflex due to a larger number of genes covered on the reflex panel. These findings show that a standardized comprehensive strategy for molecular testing increases timely opportunities for personalized therapy in patients with non–small cell lung carcinoma.

A Probabilistic Model for PT/PTT Mixing Studies With Error Propagation

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Background: A mixing study is a commonly used test in determining the etiology of a prolonged PT and/or PTT. Briefly, the patient’s plasma is mixed with control plasma replete with all coagulation factors, and the PT and PTT values in this mixed sample are compared to the patient’s straight (ie, unmixed) sample. Multiple algorithms exist for interpretation of mixing studies, yet these algorithms fail to take into account the error inherent to PT/PTT measurements and calculations and typically provide only a binary result that may provide false confidence in identifying an underlying mechanism for a patient’s coagulopathy. In addition, an informal survey of current interpretation protocols at our institution identified a heterogeneous and error-prone approach, possibly resulting in interpretation error of mixing studies that could impact patient care.

Methods: We developed a probabilistic model for interpreting mixing study results, which is sensitive to several sources of analytical error: (1) that from the instrument itself, (2) the variability of activity in the control plasma, and (3) errors that arise in the calculation of the mixing study results themselves. We derived instrument error from daily quality control (QC) testing runs and calculated the error of the standard curve relating PT/PTT and factor level using a resampled exponential curve-fitting approach. Our probabilistic model samples from these distributions and outputs a mixing study result with an associated probability distribution.

Results: We first analyzed 1,402 historical mixing studies from two large academic hospital laboratories. Evaluation of linear and random-forest based models using PT/PTT values measured in seconds found that 97% of PT abnormalities but only 85% of PTT abnormalities were concordant with the pathologist’s interpretations originally entered in the EHR. Next, our probabilistic model was used to reinterpret the 1,078 mixing studies with PTT abnormalities. On average, the median coefficient of variance for the mixing study was 10.9% (90% CI, 6%-34%). In total, 703 interpretations were found to be unchanged, 323 were resulted as indeterminate (defined as >5% chance of stochastic error changing the interpretation), and 25 were changed with high confidence as a result of our automated approach.

Conclusions: Taken together, our results suggest that mixing study interpretations have been approached with some heterogeneity in methodology, resulting in suboptimal accuracy. We developed a new approach that provides a robust, error-aware model of mixing studies that reports a confidence level for more accurate interpretation and clinical utility. Surprisingly, our model identified that one-third of mixing studies were likely indeterminate. The methods and probabilistic model developed here can be applied to other laboratory results and can be extended to a more general Bayesian model when clinical predictors and downstream results are included.