

Pleural Fluid for the Detection of Actionable Somatic Mutations – have we struck oil yet?

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Lung cancer is the leading cause of cancer related mortality in both men and women with an estimated 1.69 million deaths worldwide in 2015.^{1, 2} Up to 15% of patients with NSCLC have MPE at presentation and require pleural fluid drainage for management of dyspnea, in addition to diagnostic and staging purposes, and over 30% develop MPE at some stage during their illness.^{3, 4}

A significant percentage of lung tumors have been demonstrated to carry actionable somatic mutations each of which represents an opportunity for novel targeted or “personalized” therapy for NSCLC. This has created hope for a disease that carries a high morbidity and mortality. Cutting-edge sequencing modalities provide a unique opportunity to investigate potentially targetable molecular aberrations in tumor

suppressor genes and oncogenes. Current American Society of Clinical Oncology guidelines recommend molecular analysis for all actionable somatic mutations to target therapy in patients with NSCLC. Pleural fluid drainage through thoracentesis is among the least invasive and most cost-effective methods of tissue acquisition for staging and diagnosis, when compared to bronchoscopic or surgical biopsy of the primary tumor or adjacent and distant tumor-infiltrated organs. For this reason, MPE has been considered as a potential source for oncogenic driver mutation testing – but key questions about its utility in this context remain.

An important factor to successful molecular analysis is sufficient cellularity for immunohistochemistry (IHC) to confirm diagnosis. The decision to perform molecular testing is based on adequacy of tumor cellularity. The largest study to date to compare epidermal growth factor receptor (EGFR) mutation analysis between pleural effusion and lung tissue samples by Guen et al included 50 patients.⁵ Despite higher mutation rates detected in lung samples, no statistically significant difference in mutation rate was found between the two sources – however, optimal volume of pleural fluid for molecular analysis was not assessed. In a study of 50 NSCLC-associated MPEs, Carter and colleagues showed no significant relationship between the volume of pleural fluid and overall cellularity and tumor cell content, and the decision to perform molecular testing was not influenced by fluid volume used.⁶ Of the 27 specimens that underwent molecular testing, a genetic abnormality (EGFR, KRAS, PIK3CA, ALK, BRAF, AKT, ERBB2, and NRAS) was detected in 59%. Molecular testing was not performed in 14 cases due to inadequate tumor cellularity – this, in addition to a suboptimal yield of pleural fluid cytology for the diagnosis of MPE, are the two most important factors in the clinical utility of pleural fluid in oncogenic driver mutation analysis.

In this issue of the journal, DeMaio and colleagues report on their centers' experience in the detection of oncogenic driver mutations in lung adenocarcinoma-associated MPE in a retrospective study. They sought to investigate simultaneous testing for multiple mutations including EGFR, KRAS, ALK, BRAF and ROS1 in MPE compared to histopathology specimens obtained during surgical, bronchoscopic or transthoracic core needle biopsy in a separate cohort. Specimens were considered sufficient for mutation analysis when all ordered oncogenic drivers could be performed. In addition, the reported diagnostic yield of mutation analysis was compared between fluid volumes of >100 ml and ≤ 100 ml. Interestingly, the authors compared median survival based on treatment with targeted tyrosine kinase inhibitors (TKI) using the Kaplan-Meier method.

The study demonstrated that pleural fluid was sufficient for complete mutation analysis in 40/56 (71.4%) patients, in comparison to a separate cohort of patients, in which mutation analysis performed on histopathology specimens provided a detection rate of 85.7% (72/84). Comparing >100 ml pleural fluid vs ≤ 100 ml, the authors showed no significant difference in satisfactory mutation analysis. Patients with MPE with TKI targeted therapy had significant improved median survival (25 vs 11 months, hazard ratio: 0.4, P= 0.002).

The authors should be congratulated on a highly relevant and clinically applicable study, teasing out the true comparative ability of pleural fluid, and addressing the important question of volume. However, there are important limitations in the study which mean that some vital questions remain. The authors set out to investigate the diagnostic yield of adenocarcinoma-associated MPE for detection of actionable mutations, however, they do not compare the results of pleural fluid molecular testing to a defined gold standard. Guan et al showed that among 50 patients, EGFR mutation was found in 17 tissue samples but only 15 of the same patients had EGFR mutation in pleural fluid. Although the differences were not statistically significant, they should be considered as false negative results when calculating diagnostic performance of the test in question. Whether surgical tissue biopsy or other sources of tissue are used as gold standard, accurate definition is required for diagnostic yield measurement. Describing the prevalence of these mutations will also play a significant role in diagnostic yield assessment. Assuming that this center's patient population is similar to the majority of US population, it is surprising that ALK rearrangement was detected in 16.6% of MPEs, compared to the wide literature reported rate of 4-5% in NSCLC adenocarcinomas.^{7, 8} This finding may be secondary to a small sample size but should be taken in to account given other mutation rates fall within literature reported rates, and may be due to a different disease/mutation prevalence in the studied population. Other findings such as an unusually high median survival rate of 25 months in advanced stage NSCLC with pleural metastasis, limits the generalizability of the results, as is common in most single-centered studies in high-volume referral academic institutions.

The lack of concurrent mutation studies on non-fluid tissue limits the interpretation of the current results. The authors' report of molecular testing in non-fluid tissue from a separate population with MPE attempts to create a comparison group. This group had additional biopsy procedures, hence pleural effusion was not used for mutation analyses. Although the authors suggest that all consecutive patients with lung adenocarcinoma-associated MPE were included in the study, this comparison group presents a significant selection bias. Other limitations, such as change in molecular testing during the study period from PCR amplification to next generation sequencing, are inherent to the nature of the advancing field of oncogenic driver mutation analysis.

Ongoing demand for better understanding of driver genomic aberrations requires continued and relentless effort. DeMaio et al highlight the importance of molecular analysis in pleural fluid samples, obtained in a least invasive approach. The time has come for a direct comparison of pleural fluid and non-pleural fluid samples in the same patients, using modern molecular techniques to directly address if pleural fluid should be considered a gold mine in the diagnosis of actionable driver mutations.

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