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ORIGINAL ARTICLE

Molecular testing of different cytologic preparations in patients with advanced lung adenocarcinoma: which yields the best results?

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Introduction This study constitutes the first systematic comparison of molecular results between different cytology preparations in patients with lung adenocarcinoma undergoing testing for *EGFR, KRAS,* and *BRAF* mutations.

Materials and methods 115 archival cytology preparations (direct smears, ThinPrep preparations [TP], and cell blocks [CB]) from lung adenocarcinomas with known *EGFR, KRAS*, or *BRAF* mutations were tested and compared with clinical testing results. Results were compared between preparations and analyzed in relation to tumor purity and tumor cell content.

Results 82 (77%) of 106 informative cases were concordant with clinical testing results. There was no significant difference in the concordance rate between CB, TP, air-dried smears, or alcohol-fixed smears ($P = 0.3803$), nor between preparations with <25%, 25% to 50%, or >50% tumor purity ($P = 0.1147$). Concordance rates were lower in preparations with ≤100 tumor cells ($P = 0.0002$).

Conclusions Smears, TP, and CB are all valid substrates for molecular testing. Although tumor purity did not significantly affect results, low tumor content showed poorer performance. Recording tumor purity and content is recommended.

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Introduction

The treatment of non-small cell lung carcinoma (NSCLC) is increasingly directed toward patient- and tumor-specific genomic alterations that drive oncogenesis, tumor progression, and resistance to traditional and targeted therapies.[1,2] Many of these alterations have been identified over the past two decades as a result of advances in polymerase chain reaction (PCR)-based molecular techniques and the conceptualization of lung adenocarcinoma as a heterogeneous group of tumors with regard to molecular and histologic features. Clinical use of gene sequencing for patient management has evolved in parallel with the discovery of targetable gene alterations in NSCLC, and testing for *EGFR* mutations, among others, has become common practice to determine eligibility for tyrosine kinase inhibitor (TKI) therapy.[3,4] Approved TKIs (e.g., erlotinib, crizotinib) have been shown to exhibit significant anti-tumor activity in appropriately selected patients and are becoming standard first-line and second-line therapy for patients with advanced NSCLC.[4]

Molecular testing of patient samples for clinical use requires DNA of sufficient quality and quantity to generate robust and accurate sequencing results, with minimum requirements being methodology-dependent. In general, massively parallel next-generation sequencing methods and targeted pyrosequencing assays require less DNA than long-established but less-sensitive conventional sequencing methods.[5-7] Patients with advanced (stage IV) NSCLC frequently have tumor DNA extracted from paraffin-embedded biopsy material from primary or metastatic sites; however, this population is often medically vulnerable and unable to undergo invasive surgical procedures to obtain material for molecular testing. Accordingly, there has been considerable interest in using cytology preparations that can be obtained via fine-needle aspiration or during therapeutic or diagnostic thoracentesis for molecular testing in this patient population.

Current guidelines for *EGFR* testing in patients with lung cancer recommend using cell blocks rather than smears or liquid-based preparations to derive DNA because of concerns over preservation of diagnostic material and ability to correlate results with malignant cell content.[4] Nevertheless, a significant proportion of cases show more abundant material on smears than in cell block preparations, suggesting that, in some situations, testing non−cell block cytology material may be preferable.[8,9] Recent studies have demonstrated that archival smears and liquid-based preparations up to 10 years old can be used to derive sufficient high quality DNA and RNA for molecular testing.[8,10-12] Criteria to determine adequacy for molecular testing in smears and liquid-based preparations, however, have not been established and have ranged widely among studies, with recommended cutoffs of >20% to >80% for tumor purity and >100 cells to >1,000 cells for tumor cell quantity.[7,13-18] Furthermore, back-to-back comparisons of cell blocks, smears, and liquid-based preparations with correlation to molecular testing performed on clinical specimens have not been reported to date. Finally, a majority of studies assessing the feasibility of molecular testing in cytology preparations enrich the material tested for tumor cells either by using laser-capture microdissection, macrodissection, or by selecting only cases with high tumor purity.[8,9,16,19,20] Micro- or macrodissection is time-consuming and may not be feasible at all centers, and selection of cases with high tumor purity may not be representative of day-to-day practice. The aims of the current study, therefore, were to compare molecular testing results between cell blocks, smeared slides, and liquid-based preparation slides and to better define cutoffs for specimen adequacy of lung cytology specimens in daily practice using widely available molecular techniques that do not require micro- or macrodissection, in a variety of cytology preparations.

Materials and methods

Medical record and cytopathology review

This study was performed with the approval of the institutional review board at the Brigham and Women's Hospital, Boston, Mass. Archival cytology materials were retrieved from the files of Brigham and Women's Hospital's Cytopathology division, corresponding to consecutive patients with lung adenocarcinoma and positive molecular testing results obtained as part of routine clinical practice between 2008 and 2014. Cytology specimens were obtained either before or after material was submitted for clinically driven molecular testing, which was performed on formalin-fixed paraffin-embedded material (biopsies, resections, and cell blocks). Only patients with *EGFR* exon 19 deletion or L858R mutations, *KRAS* codon 12 or 13 mutations, and *BRAF* V600E mutations were included in the study, in order to simplify and standardize the molecular tests used for data analysis. Clinical information was abstracted from the electronic medical record. Age, sex, treatment history, and sites from which clinical and study specimens were obtained were recorded. For purposes of comparison between clinical and study specimens, pleural fluids and pleural biopsies were considered to pertain to the same site, as were bronchial brushings, bronchoalveolar lavages, lung fine-needle aspirates (FNAs), and lung biopsies or resections.

All available cytology preparations, including hematoxylin and eosin−stained cell blocks (CB, prepared from needle rinses), alcohol-fixed Papanicolaou-stained smears (PAP), air-dried Hemacolor-stained smears (AD), and ThinPrep liquid-based preparations (TP) were retrieved for each patient sample and reviewed to confirm the diagnosis and assess specimen cellularity. At least one slide with