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Molecular testing on endobronchial ultrasound (EBUS) fine needle aspirates (FNA): Impact of triage

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Abstract

Background: Endobronchial ultrasound (EBUS)-guided fine needle aspiration (FNA) is performed to diagnose and stage lung cancer. Multiple studies have described the value of Rapid On-Site Evaluation (ROSE), but often the emphasis is upon diagnosis than adequacy for molecular testing (MT). The aim was to identify variable(s), especially cytology-related, that can improve MT.

Methods: A search for EBUS-FNAs with ROSE was conducted for lung adenocarcinomas or when this diagnosis could not be excluded. All such cases underwent reflex MT on cell blocks. The impact of cytology-related variables [i.e., number of pass(es), dedicated pass(es) directly into media, cytotechnologist (CT), laboratory technician (LT) and triage with 1 or >1 cytologist] was evaluated. The latter category was divided into Group A [ROSE, triage and slide preparation by cytopathologist (CP) *and* CT *at start* of the procedure] and Group B (ROSE only by CT *or* by CT/CP *after* start of procedure; triage and slide preparation by CT or clinical staff). The impact of all these variables on MT was assessed.

Results: A total of 100 cases were identified, and 79 had sufficient tissue for MT. Of all variables evaluated, MT was positively affected by performing a direct dedicated pass ($P = 0.013$) and ROSE by Group A ($P = 0.033$).

Conclusions: ROSE with appropriate triage, including performing a dedicated pass and proper slide preparation, improves MT, and this is enhanced by having >1 cytologist at the start of the procedure. In the era of personalized medicine, "adequate" should denote sufficient tissue for diagnosis and MT.

KEYWORDS
EBUS FNA, endobronchial ultrasound fine needle aspiration, lung, molecular testing, rapid on-site evaluation, ROSE, triage and adequacy

1 | INTRODUCTION

Endobronchial ultrasound (EBUS)-guided fine needle aspiration (FNA) has become the standard initial procedure in diagnosing centrally located endobronchial, peribronchial, pulmonary, and mediastinal lesions and staging lung cancer[1,2] EBUS-FNA has largely replaced mediastinoscopy, which is more invasive and poses greater risk and morbidity.[3] Added advantages of EBUS-FNA are its accessibility, efficiency, high sensitivity and specificity, and lower cost.[4,5] On the other hand, EBUS-FNA yields smaller specimens relative to than those acquired with more invasive surgical procedures. This can be problematical, as sufficient sample is necessary for morphological diagnoses, immunohistochemical (IHC) studies and/or molecular testing (MT) for appropriately subtyping and evaluating the mutational profile.

In a vast majority of instances, distinction between the two most common primary nonsmall cell lung carcinoma (NSCLC) subtypes—adenocarcinoma and squamous cell carcinoma—can be readily achieved with cytomorphology and/or two slides for IHC, thyroid transcription factor-1 (TTF-1) and p40, respectively.[6–8] As per National

Comprehensive Cancer Network (NCCN) guidelines, for those with lung adenocarcinoma presenting at an advanced stage, personalized therapy is a potential option in the presence of a targetable mutation. Eligibility for the currently approved therapies necessitates detection of either an epidermal growth factor receptor (*EGFR*) mutation or anaplastic lymphoma kinase (*ALK)* rearrangement, which are identified with ancillary testing [i.e., PCR-based method for the former and fluorescent in situ hybridization (FISH) or IHC with FISH confirmation of positive staining for the latter].[9] More extensive MT in the form of next-generation sequencing (NGS) is increasingly being used to detect other potential targets, further increasing the need for tissue.

Despite technological advances in molecular pathology[10] the demands for ancillary testing continue to grow at a rate that has the potential to exhaust available tissue.[11,12] Many have been able to keep pace with the contemporaneous MT panels on small biopsy and cytology specimens,[13–17] while others struggle to achieve the results of their counterparts. Clearly, one or several factors account for the variability, including operator skill, nature of lesion, and laboratory preparation technique, amongst others.

Rapid on-site evaluation (ROSE) has been shown to be effective at increasing diagnostic yield and efficiency in EBUS-FNA samples.[18,19] The purpose of this study was to identify attributes, especially those related to cytology, which improve tissue availability for MT in carcinomas at the time of ROSE.

2 | MATERIALS AND METHODS

Following Institutional Review Board approval, a retrospective computerized search of consecutive EBUS-FNAs over a 32-month period was performed. All cases with final diagnosis (or component) of adenocarcinoma, NSCLC, adenosquamous cell carcinoma, and poorly differentiated carcinomas were selected. The latter included cases in which the primary site (i.e., lung versus other) was uncertain following a limited (e.g., TTF-1, Napsin A, p63, p40) and more elaborate panel of immunohistochemical (IHC) stains (e.g., CK7, CK20, CDX-2, GATA3, PAX-8).

Several variables were analyzed. The sites aspirated [i.e., lymph node (LN), lung, other], number of passes performed/placed directly into media for (potential) MT, nature of lesion (e.g., presence of necrosis) and cytology-related variables [i.e., cytotechnologist (CT) participating in ROSE, cytology preparatory laboratory technician (LT) involved], and impact of 1 or >1 cytology personnel [i.e., CT and/or cytopathologist (CP) performing specimen triage *at start* of procedure] were evaluated. More specifically, to assess the latter variable, the cohort was divided into two groups based on data extracted from the final reports: (Group A) *2* cytology-trained personnel, including a CP and CT were present *at start* of the procedure for ROSE, triage and slide preparation; (Group B) 1 cytology-trained personnel—only a CT present *or* 2-cytology-trained personnel—CT/CP present *after onset* of procedure with triage and slide preparation by CT or clinical staff. **(Table** 1) Participation in ROSE from the start of the procedure is dependent upon preference of the CP rather than other factor(s) (e.g., nature of lesion, suspected diagnosis, etc.). CP assignment to the FNA service is typically every third day during a 4-week rotation and not predetermined or influenced by the types of FNAs that occur on any particular day. In addition, CT coverage of the FNA service includes all of the CTs, who also rotate on the FNA service on a cyclical basis, and is random as regards to the type of lesion being aspirated; LTs, who process the specimens and prepare the cell blocks, are also assigned on a cyclical basis. All CTs included in the study were certified by the American Society for Clinical Pathology with minimum of 6 years in practice. All CPs included in the study were certified in cytopathology by the American Board of Pathology with minimum of 2 years in practice.

TABLE 1 Key differences between Group A and B

	Group A	Group B
Triage *at start* of procedure	+	+/−
>1 cytology personnel	+	+/−
Slides prepared by clinical (non-cytology) staff	−	+/−

2.1 | EBUS-FNA and ROSE

Per mutually established protocol, the clinical staff informs the cytology division approximately 10 minutes prior to start of the procedure allowing the CT time to restock the FNA basket and travel to the intervention suite. The specimen is triaged and slides are prepared in the procedure room; the slides are stained and reviewed in another room where a microscope is stationed permanently. On occasion, the same CT and/or CP may be involved in more than one procedure being performed simultaneously in adjacent suites.

All FNA samples were typically obtained with a 21 or 22-gauge needle by the pulmonologist and had ROSE performed by a CT and/or CP using a previously outlined protocol[20] developed in collaboration alongside the pulmonologists with the objectives of (1) establishing a diagnosis and (2) allocating tissue for cell block or other media [e.g., RPMI in cases of suspected lymphoproliferative disorders, appropriate tube for microbiological cultures] for ancillary testing. Briefly, each aspirated sample was expelled onto a slide and a portion of the sample was prepared for ROSE, with each pass generally limited to two smears. To capture any residual cells, the needle was rinsed into CytoLyt (Hologic, Bedford, MA) by passing saline through it. The remaining specimen was allowed to clot on the slide for one-to-two minutes and then placed directly into formalin. The main purpose of the established protocol is to select minute, tan-white tissue particles for smears and ROSE while allocating the majority for cell block (or other ancillary studies) as outlined in Figure 1.

A cell block was prepared from the material in formalin; a cell block and/or ThinPrep (Hologic, Bedford, MA) were made from the CytoLyt. Cell blocks fixed in formalin and/or CytoLyt were used for MT. Additional passes were requested if deemed necessary for ancillary studies. At the discretion of the cytologist present, the additional passes were either placed directly into media for cell block preparation or ROSE was performed. IHC and special stains were performed on cell blocks. The following techniques were undertaken when preparing cell blocks. After centrifugation of the formalin or CytoLyt specimen in a 50-mL

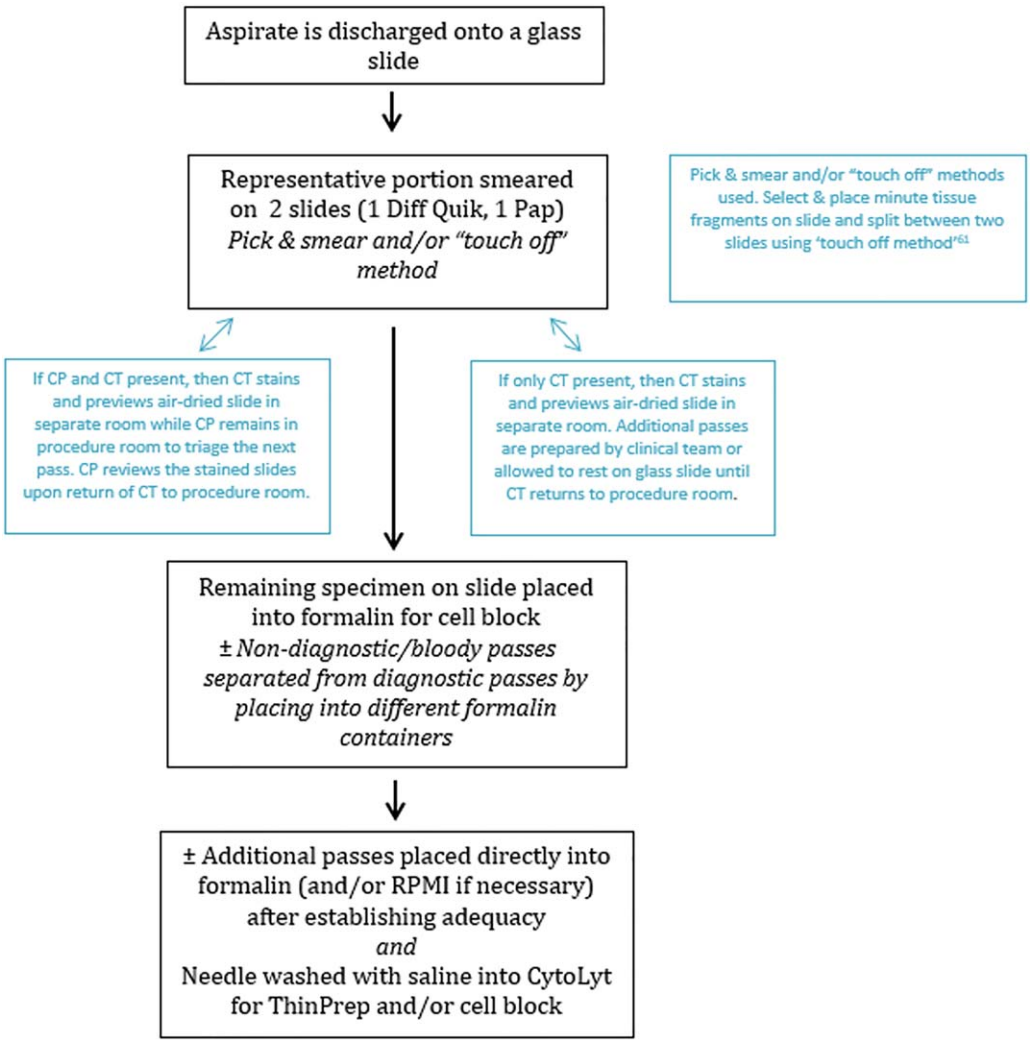

FIGURE 1 FNA tissue triage protocol [Color figure can be viewed at wileyonlinelibrary.com]

tube for 5 minutes, the supernatant was removed. Well-formed clots were placed directly in Bio-Wrap (Leica Biosystems, Buffalo Grove, IL). For the remainder, HistoGel (Thermo Fisher Scientific, Waltham, MA) was added to the cell pellet post centrifugation following removal of the supernatant and solidified in the refrigerator at 4°C. Once solidified, the pellets were placed in Bio-Wrap. The Bio-Wrapped specimens were fixed in 10% neutral buffered formalin, processed and embedded in paraffin.

Preliminary assessment communication to the pulmonologist varied based on the cytology personnel performing ROSE. For Group A, adequacy was reported (i.e., "adequate", "inadequate", "more needed") with additional descriptions describing the lesion and quantity of the FNA sample (e.g., adenocarcinoma, scant). For Group B, generally only adequacy was reported without description of lesion and/or quantity.

2.2 | Corresponding biopsies

We also noted if a corresponding biopsy [i.e., transbronchial (Tbx) or endobronchial (Ebx)] was performed, and if it could be used for MT in case of insufficient tissue in the cell block.

2.3 | DNA mutation analysis

All MTs were performed on cell blocks. MT protocol for lung carcinomas varied over time as new tests became available and as guidelines were modified.[15] Reflex MT was prompted either by a diagnosis of lung adenocarcinoma, adenosquamous carcinoma, or when the possibility of lung adenocarcinoma could not be excluded. Initially, the reflex testing consisted of Kirsten rat sarcoma (*KRAS)* mutational analysis, but it was subsequently expanded to include *EGFR* mutation analysis and then again to incorporate *ALK* rearrangement by fluorescence in situ hybridization. *KRAS* mutation analysis was initially performed with the *KRAS* codon 12/13 Amplification Refractory Mutation System/Scorpion assay (Qiagen); later a polymerase chain reaction-based method to amplify regions within *KRAS* exon 2, which were then sequenced via ABI BigDye Terminator kit V1.1 (Applied Biosystems, Carlsbad, Calif), was implemented. *EGFR* mutation analysis was also a polymerase chain reaction-based with amplification of *EGFR* exons 18 to 21. This protocol included direct di-deoxyterminator (Sanger) sequencing for EGFR and KRAS. Sanger sequencing requires at least 50% tumor purity with a sensitivity of 25% for EGFR or 40% tumor for KRAS, and 10 ng of

DNA. Later, the sequencing platform was modified to NGS using TruSeq panel on the Illumina MiSeq platform with reversible fluorescent terminators. This protocol included EGFR, KRAS, GBAR, PIK3CA, MET, and ERBB2 genes for standard lung cancer panel and requires 20% of lesional cells and 50 ng of DNA. For this study, sufficiency for molecular analysis was determined as having adequate tissue to perform the contemporaneous reflex testing protocol. Microdissection was utilized when necessary to enhance tumor proportion.

2.4 | Slide review

For the purposes of this study, the slides for cases that failed MT (i.e., insufficient for MT) were retrieved and reviewed by 3 pathologists for tumor cellularity and classified on a 4-level scale: absent, scant, moderate and high. For each case, the Diff-Quik smears, evaluated at the time of ROSE, were evaluated separately from the remaining slides (Papanicolaou-stained smears, cell block sections and/or ThinPrep). The reasons for examining them in this manner were to assess (1) if the specimen was appropriately triaged (e.g., if the vast majority of the specimen was allocated for cell block rather than smeared for Diff-Quik or alcohol-fixed, Papanicolaou stain per protocol outlined in Figure 1), (2) if the cellular content on the Diff-Quik smears accurately reflected ROSE (e.g., an "adequate" assessment was in fact "adequate" for diagnosis and potential MT), and (3) if the background lymphocytes inflated the adequacy assessment of lymph node(s) with metastatic carcinoma (i.e., a lymph node with scant carcinoma and numerous lymphocytes was deemed "adequate").

2.5 | Statistical analysis

Statistical analysis using χ^2 test was performed to assess the influence of sites aspirated (i.e., LN, lung, other) and cytology-related variables (i.e., CT participating in ROSE, number of passes performed, number of passes placed directly into media for (potential) MT and LT involved) on MT. The "*N*–1" $\chi^2 d$ test was used to test the null hypothesis that differences in availability of >1 or 1 cytology personnel (i.e., Group A and Group B, respectively) for specimen triage during ROSE would not change the success of MT. The level of significance used for all tests was 0.05.

3 | RESULTS

During the study period, a total of 100 EBUS-FNA with ROSE cases were identified that met the inclusion criteria. The patients ranged from 43- to 93-years-old and comprised 62 (62%) women and 38 (38%) men. Of 100 cases, 78 were LNs, 12 were lungs, and 10 were other (hilar, perihilar, paratracheal). Fifty-nine of 100 (59%) cases had a final diagnosis of adenocarcinoma, 19 (19%) were NSCLC, 19 (19%) were poorly differentiated carcinoma, 2 were combined small cell with NSCLC, and 1 was diagnosed as suspicious for carcinoma. A subsequent biopsy of the latter was diagnosed as adenocarcinoma.

Twenty-one of 100 total cases (21%) failed MT, and the remaining 79 cases (79%) had successful MT. The reason for failed MT for all 21 cases was insufficient malignant cells in cell block(s). The sites aspirated (e.g., LN, lung mass, other) did not impact MT ($P = 0.94$). All of the specimens in the study are carcinomas, so the nature of the lesion (i.e., carcinoma vs. spindle cell neoplasm vs. benign vs. other) did not impact this study. Similarly, the presence of necrosis (2 with sufficient and 1 with insufficient material for MT) did not influence outcome. The number of passes did not affect availability of tissue for MT. The average numbers of passes performed for Groups A and B were 4.5 and 4.3, respectively ($P = 0.92$) and for cases with sufficient and insufficient material for MT were 4.2 and 5, respectively ($P = 0.35$).

Placing a dedicated pass directly into media impacted MT. Of the 100 cases, 43 had >1 pass(es) (range 0–5) placed directly into media for (potential) MT, including 39/79 (49%) and 4/21 (19%) from cases with sufficient and insufficient material for MT, respectively ($P = 0.013$). For Groups A and B, the number of passes placed directly into media was 10/22 (45%) and 33/78 (42%), respectively.

A subset (15) of cases had concurrent Tbx and Ebx. Of the 15, 5 had carcinoma, 7 were benign/nondiagnostic, and 3 were atypical. Only 1 (of 5) was used/had sufficient material to pursue MT; for the remaining 4, there was scant tissue on the biopsy or greater tissue on the cell block.

Seven CTs were involved in the EBUS FNAs; there was no statistical difference between the CT involved and MT ($P = 0.74$). During the timeframe of the study, the department did not specifically record the name of the LT processing the specimens, including preparing the cell blocks; however, as the LTs are assigned to different tasks in the laboratory on a rotation schedule, similar to CTs and CPs, it is unlikely to have impacted MT.

There was a difference in availability of sufficient tissue for MT on cell blocks between Group A and Group B. One case from Group A ($n = 1/22$; 4.5%) and 20 from Group B ($n = 20/78$; 25.6%) had insufficient malignant cells in cell block(s) for MT. Because the smallest expected cell count in the resulting contingency table is smaller than 5, the classic Pearson-Fisher χ^2d test is not recommended for these data. Instead, following the recommendations of Campbell, the "*N*–1" $\chi^2 d$ test was used and showed that the difference between the rate of failure for MT in Group A and the rate of failure for MT in Group B is statistically significant with *P* values = 0.033.[21]

Group A included 22 cases, age ranged from 55 to 86 years (mean 69.5) with 12 (54.5%) women and 10 (45.5%) men. Diagnosis during ROSE included 17 (77.3%) "adequate" with comments such as malignant/neoplastic cells present and 5 (22.7%) with limited cellularity or rare suspicious cells. Group B included 78 cases, age ranged from 43 to 93 years (mean 69) with 50 (64.1%) women and 28 (35.9%) men. Diagnosis during ROSE included 65 (83%) "adequate" and 13 (17%) "inadequate" with comments such as "more needed" in 4 cases.

A key difference between Group A and Group B was specimen triage. This was influenced by (1) the number of cytology personnel involved, (2) presence *at the start* of procedure, and (3) personnel triaging and preparing smears. (Table 1). There were no systematic factors that determined whether patients were in Group A or Group B that could have biased the results.

The slides from the cases with insufficient material for MT were reviewed with the exception of 1 case from Group B for which the

slides were unavailable. Overall, 11 of 20 had moderate-to-high cellularity on smears and/or ThinPrep, but the corresponding cell blocks had either no or scant carcinoma. Of the 20, 12 were deemed "adequate" during ROSE; the smears/ThinPrep showed 8 with moderate-to-high tumor content, 3 with scant tumor and many lymphocytes, and 1 with scant tumor and scant lymphocytes. ROSE for 5 was inadequate; smears/ThinPrep of 1 had moderate tumor and 4 had scant tumor; however, moderate numbers of lymphocytes were present in one. The case with an "atypical" diagnosis and 1 with "suspicious" cells on ROSE had moderate tumor on the smears/ThinPrep. Finally, the case with "rare suspicious cells" had scant tumor on all of the preparations.

4 | DISCUSSION

There is a paradigm shift in managing lung cancer with a personalized approach, making MT a crucial part of diagnostic work-up. FNAs and small biopsies comprise the vast majority of samples from those with advanced stage lung cancer for whom targeted therapy, rather than surgery, is an option, and studies have proven that such samples can provide sufficient tissue for MT.[15,20] In the current analysis, 79 (of 100; 79%) had sufficient material for MT. The central focus was to investigate which factor(s), particularly cytology-related variables, preclude(s) achieving greater success.

ROSE is recognized as an effective and valuable component of FNA that ensures adequate sample for diagnosis as illustrated in numerous earlier[22] and more recent studies.[20,23–28] However, the reported impact of ROSE varies. In a meta-analysis, ROSE increased specimen adequacy rate by 12% overall.[26] For pulmonary-related specimens, one study showed that the adequacy rate ranged from 44% to 88% without ROSE and 79% to 100% with ROSE.[26] The end point of many prior ROSE studies was to assess "adequacy" to render a diagnosis rather than "adequacy" for ancillary studies, such as MT.[29,30]

Identifying areas for improvement can be difficult, because several pre-analytical variables are involved, including in acquiring and processing a specimen. Several noncytology factors (e.g., size of target, accessibility of target) cannot be controlled and may influence MT. For instance, prior studies have examined characteristics of LNs, including size, shape, distinct margins, central nodal vessel and echogenicity, and these may influence the presence of malignancy and thus availability for MT.[31–33] Though some describe greater likelihood of metastatic disease in LNs measuring >10 mm, others note that smaller ones may also harbor disease in a significant (43.46%) proportion.[31] Some malignant nodes have high neoplastic content whereas others have greater stroma and vessels interspersed; the former may have higher cellular yield than the latter.[34] Thus, factors other than size may also impact MT.

Optimizing ROSE can positively affect MT. Investigating this in greater depth demonstrated that triage, including performing and placing a dedicated pass directly into media and having >1 cytologist *at start* of the procedure for proper slide preparation, had significant impact. Though noncytology factors (e.g., size of lymph node) may also influence MT, the results of the study would not be invalidated given the statistically significant differences. First, placing a pass into media without ROSE, rather than the total number of passes, was associated with successful MT—a finding also reported by Collins et al.[35] There is literature addressing the "optimal" number of passes. Much of this focuses on the need to enhance cellular yield, especially when ROSE is unavailable. Further, Fielding and colleagues found that the number of passes is less important than the cellularity of individual aspirates.[34] Second, our results showed that of the cases that failed MT, 55% had moderate-to-high tumor content (all from Group B) on smears/TP, which if triaged differently, would have theoretically resulted in greater cellularity of cell blocks and improved the overall results of Group B.

Specific components of triage are often overlooked and underaddressed with limited reports calling attention to proper specimen acquisition, triage, and management.[12,36] These are significant elements of ROSE that could increase MT adequacy.[37] Having >1 person (e.g., Group A) from cytology participate in ROSE *at start* of procedure was advantageous for a few reasons. Optimal ROSE began with qualified cytology service personnel being present at the time that the first pass was obtained in order to perform proper triage. (Figure 1) Otherwise, individuals not appropriately trained proceed with suboptimal specimen preparation (e.g., thick smears, bloody smears, air-dried smears, large smears, insufficient material placed in formalin or CytoLyt for adequate cell block, etc.). At other times, to avoid specimen clotting or drying, untrained noncytology personnel prepare slides, which may happen if the one and only CT is occupied staining and reviewing slide(s) in another room. This is particularly likely when multiple passes are rapidly obtained, when multiple lesions are sampled (e.g., multiple lymph node stations and lung masses), when multiple procedures are being performed in adjacent suite(s) and/or when the stains and microscope are in another room. Managing multiple tasks in such scenarios by a single person is difficult.

After confirming the presence of lesion on the slides, the CP triaging the specimen(s) evaluated whether the formalin and/or CytoLyt had sufficient material for an adequate cell block. This was achieved by correlating the results from a diagnostic smear with the gross appearance/proportion of viable lesional cells and extrapolating that the remainder of such fragments will yield the same on the cell block. In fact, gross examination, a practice commonly associated with surgical specimens, has been used for FNAs to predict adequacy by cytologists and radiologists successfully.[36,38–40] Mayall et al. have shown that it is possible to assess adequacy of FNA specimens based on the gross appearance of the aspirated material, including both its appearance suspended in fluid and its appearance on the direct smears/glass slides.[38,39] For example, in FNA of keratinizing squamous cell carcinoma, "snowflakes" of white keratin can be seen suspended in the fluid, and the direct smears show a granular film.[38] Although there is currently no known method of establishing with 100% certainty whether FNA material placed in formalin and/or CytoLyt is sufficient for an adequate cell block, for Group A we employed a similar approach used by Mayall et al. using combined microscopic and gross examination to assess adequacy for successful cell block preparation and subsequent ancillary studies.

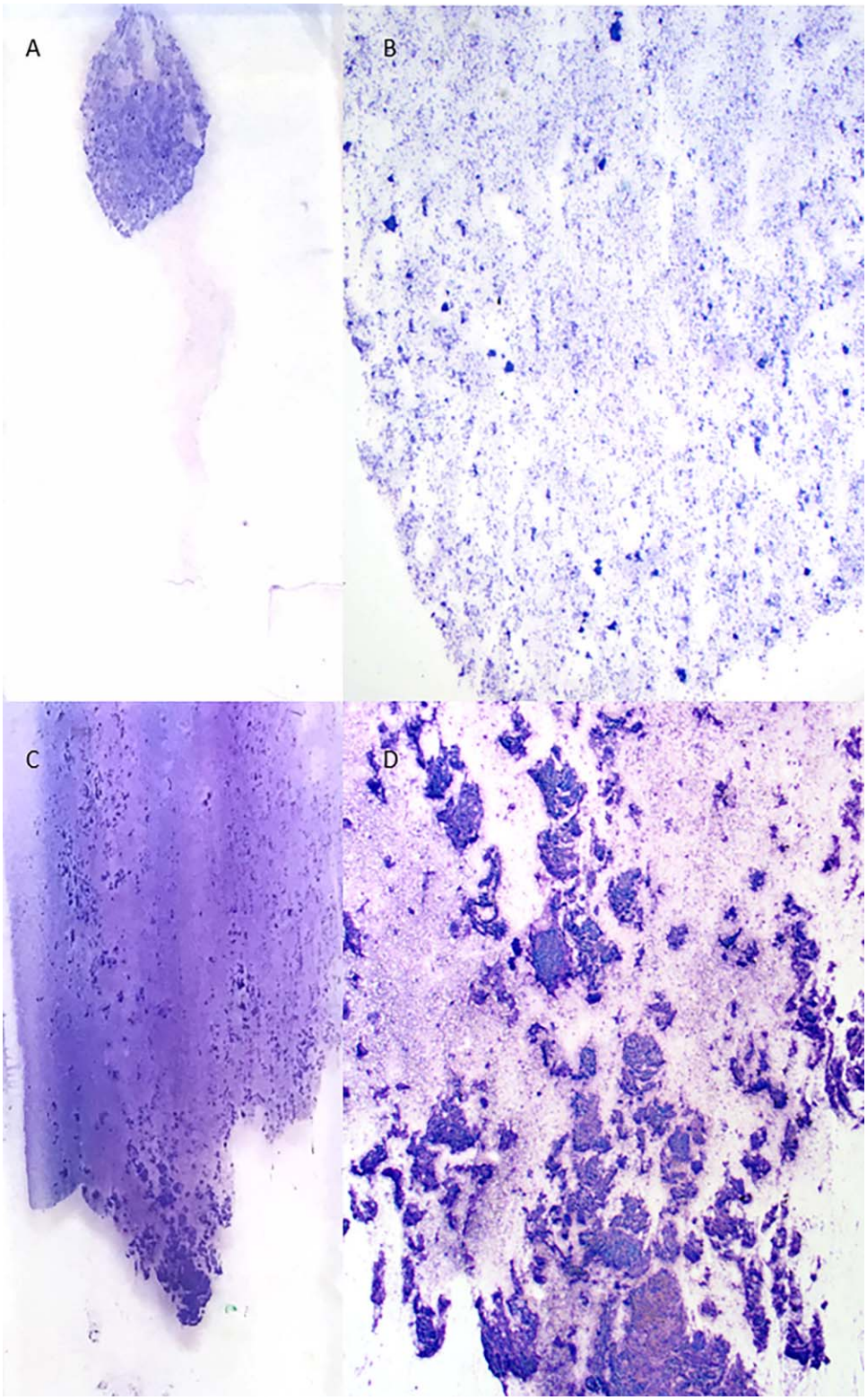

FIGURE 2 (A and B) Slide demonstrating well triaged sample. Only a small portion of the aspirate is selected and smeared. The resulting smear (A) is small with material localized to mostly the top of the slide (adjacent to label). It has sufficient diagnostic cells to render a diagnosis. [Diff-Quik-stained smear, low-magnification and (2×)]. (C and D) Low magnification (C) of slide demonstrating specimen with sub-optimal triage. The smear is large, and thicker areas correspond to the diagnostic tissue. Allocation of aspirate to predominantly smears results in sparsely cellular cell block. Thicker smears may preclude evaluation of cellular detail also. [Diff-Quik-stained smear, low-magnification and (2×)] [Color figure can be viewed at wileyonlinelibrary.com]

Many pathologists cite time constraints, workflow disruption, and little reimbursement as reasons for not participating in ROSE.[41–43] Even more, some may view the presence of 2 individuals as poor utilization of resources, but this approach is not unlike frozen sections, where there are typically two or more individuals participating in accessioning, gross specimen preparation, cutting and staining of the frozen section and histological interpretation. All of these steps can be undertaken by a single person but with >1, there is improvement in efficiency, less stress and likely lower incidence of error. In fact, a team approach, similar to that in Group A, has been described by Collins et al. and was associated with improved results, including a 32% reduction in the number of sites sampled.[44] Whether or not the second person in Group A being a CP rather than a CT was influential in achieving a higher percentage of adequate cell blocks cannot be extrapolated from our data (i.e., it is possible that two other cytology trained personnel could have achieved similar results as a CP and CT, such as 2 CTs or a CT and cytopathology fellow).

Certainly MT can be performed from smears or ThinPrep slides, which have been reported to have excellent DNA preservation and yield.[45–49] Currently, there is no standardized protocol, and either preparation—cell blocks or smears—can be used per the recommended guidelines. Cell blocks are preferred, though some preferentially prepare dedicated smears for MT and perform a microscopic assessment of unstained slides to confirm diagnostic material.[50–53] In such a scenario, a delicate balance has to be struck between smears for MT and cell blocks for IHC, as recently IHC companion testing for detecting *ALK* and PD-L1 (currently for histology specimens but being evaluated on cytology specimens[54]) was approved and may become routine. The DNA yield in both smears and cell blocks is equivalent.[10] The factors reportedly associated with MT failures on cell blocks are low cellularity and percentage of tumor[10,55] prompting use of smears. Based on our series of cases that had moderate-to-high cellularity on smear(s) but failed MT, adequate samples were obtained but likely much of the tissue was rationed to the smears rather than prioritized for cell block (Figure 2). In some instances, a corresponding Tbx or Ebx may provide an additional source of tissue for ancillary studies.

The data described in the literature[56–59] demonstrate high concordance between CT[59,60] and final diagnosis, but correlation between ROSE and specimen adequacy for ancillary testing has not specifically been addressed. All our cases with failed MT had only scant tumor on the cell blocks. These results highlight two points. First, using "adequate" as a diagnostic term could have contributed to the lack of sufficient material for ancillary testing. Regulations constrain the specificity of diagnosis a CT can communicate to the interventionist. CTs are limited to using "adequate" or "inadequate",[60] and though this has sufficed for decades, the guidelines may need to be revisited and modified, so CTs also specify to the interventionist the quantity of tissue obtained to request additional for MT, if needed. Furthermore, being limited to "adequate" and "inadequate" can be challenging. Having many lymphocytes and scant tumor from a lymph node suggests a good aspirate but not necessarily an adequate one for ancillary testing—a scenario that may have occurred in the instances of scant tumor but many lymphocytes. Second, CPs routinely order ancillary testing and are accustomed to evaluating specimens for MT, including tumor proportion, whereas CTs may not be exposed to these aspects.

Currently, to render an "adequate" immediate assessment, the individual performing ROSE must not only identify diagnostic cells on a Diff-Quik stained slide but also have an understanding of how microscopic cellularity and quantity of grossly obtained material correlate with adequacy for a cell block to pursue potential or necessary ancillary studies and communicate this to the interventionist. The importance of well-trained, skilled individuals participating in all aspects of specimen processing, including proper triage, presence *at the onset* of the procedure and appropriate smear preparation, in today's era of precision medicine cannot be overstated. In cases of inadequate cellularity for MT on cell blocks, smears should be an option. Whether material should be triaged to smears at the onset is a consideration but requires careful balance to avoid compromising the cell block for IHC. In summary, optimizing successful tissue triage for MT on EBUS-FNA is an important part of clinical practice that deserves attention given the benefits of early initiation of appropriate treatment, avoiding repeat invasive procedures and reducing overall healthcare cost.[3,42]

CONFLICT OF INTEREST

All authors have read and approved the manuscript and do not have conflict of interest pertaining to the content of the manuscript. The manuscript is not under consideration elsewhere. No funding was used for the research.

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