Original Article

Utility of multimodal sampling and testing during advanced bronchoscopy for diagnosing atypical respiratory infections in a *Coccidioides*-endemic region

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Background: The role of advanced diagnostic bronchoscopy (ADB) for assessing atypical respiratory infections is unclear. The purpose of this study was to ascertain: (I) the diagnostic utility of ADB-tissue sampling in patients with focal thoracic lesions due to atypical respiratory infections; (II) how multimodal bronchoscopic sampling and testing enhance diagnosis in a *Coccidioides*-endemic region.

Methods: A retrospective observational cohort study analyzing all ADBs performed over a 10-year period in patients with focal thoracic lesions diagnosed with a non-malignant disorder. Only cases which procured lower respiratory tract secretion and tissue samples by ADB, and had both cytohistology and culture results available were included.

Results: Among 403 subjects with non-malignant disease, 136 (33.7%) were diagnosed with atypical respiratory infections, with ADB contributing a diagnosis in 119 (87.5%) of these. Coccidioidal disease was independently associated with a cytohistologic diagnosis [odds ratio =7.64, 95% confidence interval (CI): 2.51–23.26; P<0.001]. Mycobacteria were more effectively identified by culture (overall yield of 8.4%, *vs.* 2.7% by cytohistology; P<0.001). Among subjects for which both respiratory secretion and tissue sampling were dual-tested with culture and cytology/cytohistology, adding ADB-guided transbronchial needle aspiration and/or forceps biopsy (TBNA/TBFB) to bronchoalveolar lavage and/or bronchial washings (BAL/BW) more than doubled the yield for dimorphic fungi, from 7.1% to 15.1% (increase of 8.0%, 95% CI: 5.2–11.9%). For lung lesions, adding tissue culture to dual TBNA/TBFB cytohistology-tested lung samples doubled the proportion diagnosed with atypical infection over using TBNA-cytohistology alone (increase of 15.8%, 95% CI: 10.4–23.1%). Adding lymph node to lung sampling increased the proportion diagnosed with coccidioidomycosis by 8.8% (95% CI: 4.8–15%). Among subjects with atypical respiratory infections, major ADB-related complications occurred in 1.5%.

Conclusions: ADB is useful for diagnosing atypical respiratory infections manifesting as focal thoracic lesions. A multimodal approach to both sampling and testing enhances yield, while maintaining a favorable procedure safety profile. Cytohistology testing and nodal sampling are beneficial for pulmonary coccidioidomycosis, and culture for mycobacterial disease. The approach to ADB-sampling should be

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adjusted according to clinical context and regional infection patterns.

Keywords: Advanced diagnostic bronchoscopy; pulmonary coccidioidomycosis; endobronchial ultrasound; electromagnetic navigation; atypical respiratory infection

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Introduction

Background

Respiratory infections caused by fungi, mycobacteria, and other atypical organisms can present in a variety of clinical circumstances and result in severe disease (1-6). Therefore, efficient and specific diagnosis is essential. Several non-invasive diagnostic methods are available, but perform inconsistently in practice (7-12). For many of these infections, culture and/or cytohistologic identification through direct sampling remains the gold standard.

Highlight box

Key findings
- For atypical respiratory infections manifesting as focal thoracic lesions, a multimodal approach to guided bronchoscopic sampling and testing enhances diagnostic yield, while maintaining a favorable procedure safety profile.
- Cytohistology testing and nodal sampling are beneficial for pulmonary coccidioidomycosis, and culture for mycobacterial disease.

What is known and what is new?
- Traditional bronchoscopic techniques such as bronchoalveolar lavage, and tests such as culture and microscopy, have variable value for diagnosing lower respiratory infections.
- Tissue sampling and cytohistology testing, guided by advanced bronchoscopic modalities, enhance the diagnosis for atypical respiratory infections.

What is the implication, and what should change now?
- The approach to bronchoscopic sampling of a focal thoracic lesion suspected due to atypical infection should utilize advanced guidance modalities, employ multimodal techniques and testing, and be adjusted according to clinical context and regional infection patterns.

Rationale and knowledge gap

Collecting lower respiratory secretions using bronchoscopic bronchoalveolar lavage (BAL) or bronchial washings (BW) for cytologic and/or culture analysis is a well-established means for evaluating infection. However, the value of these techniques depends on microbial factors, clinical context, and the testing methods utilized (6,13,14). For example, *Pneumocystis* is better identified by cytologic microscopy (14-16), while diagnostic yield for both culture and cytology testing is inconsistent for mycobacterial, fungal, and viral infections (16-26). BAL/BW performance also varies considerably in the ICU setting and for immunosuppressed patients (27-31).

Bronchoscopic tissue sampling using forceps biopsy or needle aspiration may complement BAL/BW by directly confirming infection and hastening the diagnosis (14,16,32). Tissue can also be cultured for a comparatively more specific analysis. However, studies evaluating the utility of transbronchial lung biopsy for diagnosing infection are limited. They demonstrate conflicting results, infrequently incorporate tissue culture testing, and mostly pre-date the advanced bronchoscopy era (18,25,26,33-38).

Advances in guidance technologies, such as endobronchial ultrasound (EBUS) and electromagnetic navigation (EMN), have augmented the breadth and accuracy of bronchoscopy for assessing focal thoracic disease. Collectively termed 'advanced diagnostic bronchoscopy' (ADB), it is widely used and considered standard of care for the tissue evaluation of lung cancer (39-45). Infections such as those caused by fungi and mycobacteria may manifest similarly, and some are endemic in a large part of the Americas (46-51). Consequently, they can represent a sizeable proportion of the case-mix evaluated by bronchoscopists. The available literature offers promise on ADB's performance in this

context, but is mostly limited to small case series, studies focusing on convex-probe EBUS, or tuberculosis-prevalent populations (32,52-56). Therefore, the utility of modern bronchoscopic tissue sampling for these infections remains unclear.

Objective

Our goal for this study was 2-fold. The first was to ascertain by what magnitude tissue sampling improved ADB diagnostic performance over BAL/BW in patients with focal thoracic lesions due to infections not caused by routine bacteria. Second, we comprehensively analyzed the performance characteristics of commonly employed techniques and tests to help guide the bronchoscopic approach for such patients in a *Coccidioides*-endemic region. We present this article in accordance with the STROBE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-83/rc).

Methods

Design

This was a retrospective observational cohort study analyzing registry data of all bronchoscopy procedures performed between January 2012 and December 2021, supplemented by electronic medical record. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). It was approved by the institutional review board of Loma Linda University Medical Center (No. 5190131) and individual consent for this retrospective analysis was waived.

Patients eligible were those who (*Figure 1*): (I) received ADB for focal thoracic lesions; (II) had both lower respiratory tract secretion and tissue samples procured by ADB; (III) had both cytohistology and culture results available; (IV) were ultimately diagnosed with a non-malignant disorder. Subjects that had coincident infection and malignancy diagnosed were also included.

All study variables were pre-defined and a data extraction form was created *a priori*. Each subject's medical record was reviewed and compared to the existing database to ensure accuracy. Data relevant to the study but not part of the existing database was collected as needed. Two separate investigators independently performed same-subject data extraction to ensure consistency. Discrepancies were adjudicated by a third investigator.

Outcomes

The primary outcome was the increase in ADB diagnostic yield for atypical respiratory infections with the addition of tissue sampling over BAL/BW alone using testing methods of culture and cytohistology among subjects with non-malignant disease. As secondary outcomes we analyzed the individual and synergistic efficacy of commonly used bronchoscopic techniques and tests, per infection type.

Definitions

We considered an infection 'atypical' if it was not caused by routine extracellular bacteria. A focal thoracic lesion was any lesion within the thorax with clearly identifiable borders.

ADB diagnostic yield for the primary and secondary outcomes was based on final culture, cytology, or cytohistology results. For atypical infections, ADB was diagnostic if an organism was identified in the proper clinical context. Typical bacterial infection was diagnosed if organisms were seen within tissue samples or if cultures met accepted yield thresholds for a given testing method (57,58). Time to infection diagnosis was from specimen collection to direct organism identification (cytohistology) or first speciation (culture).

The final clinical diagnosis was determined by combination of ADB-obtained data, other invasive and non-invasive testing, and clinical course. This assessment was recorded and then compared to the subject's final clinical diagnosis within the medical record. A lesion without a specific etiology after initial evaluation that had remained stable or decreased in size after one year of imaging surveillance and did not have an explanatory clinical diagnosis was classified as 'non-specific'.

Bronchoscopy procedures

After a routine pre-bronchoscopy risk assessment, the decision to proceed was at the discretion of the attending bronchoscopist. Per our protocol, target coagulation parameters for bronchoscopic biopsy included a platelet count of over 50,000/μL and prothrombin time and international normalized ratio levels below two times the upper limit of normal.

During the study period, four interventional pulmonologists performed ADB consistent with established technique (59,60) and using one or a combination of the

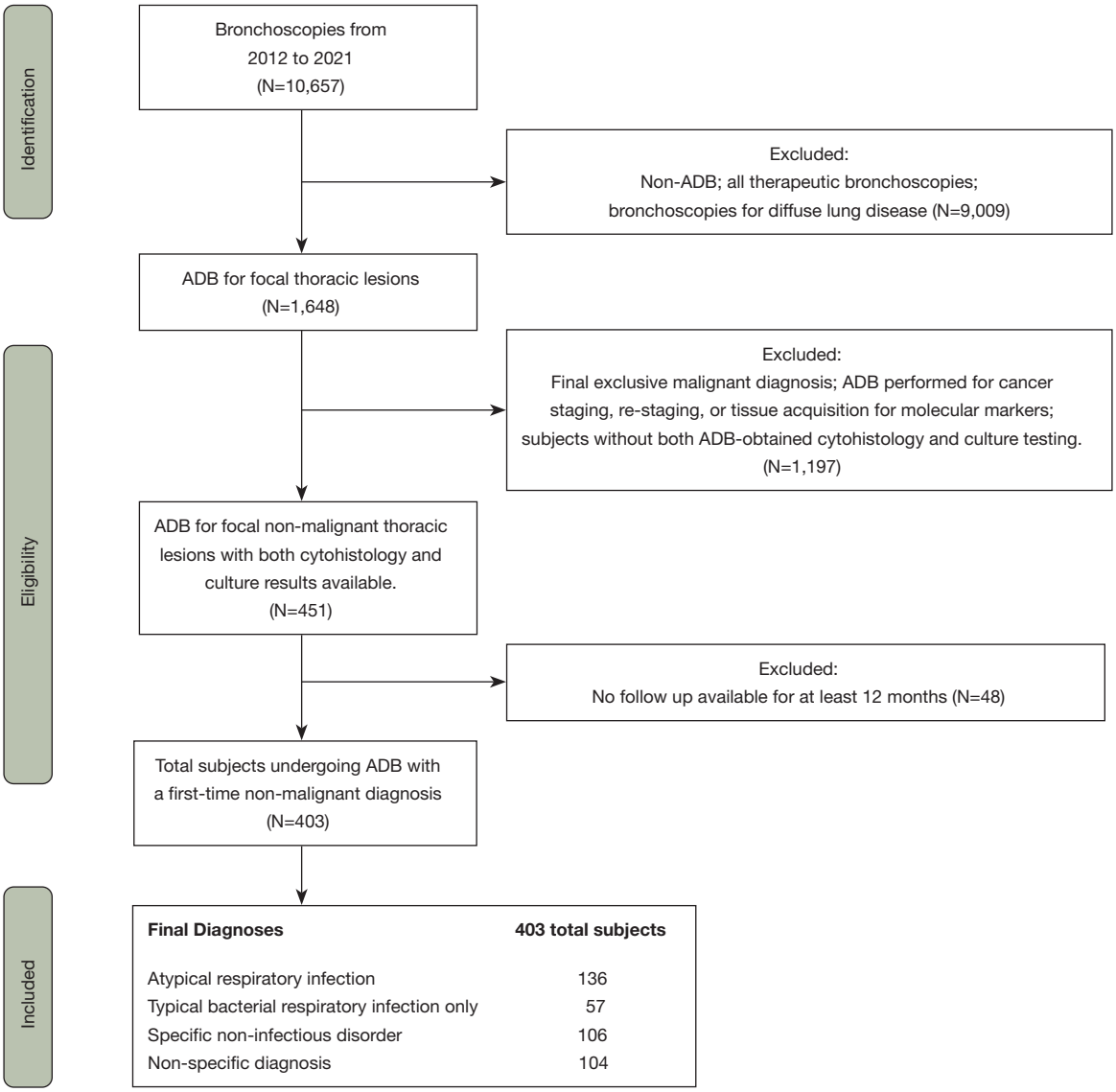

Figure 1 Summary of subject selection. ADB, advanced diagnostic bronchoscopy.

following advanced-guidance modalities: convex-probe endobronchial ultrasound (cEBUS; BF-UC-180/190 F, Olympus America Inc., Cypress, USA), radial-probe endobronchial ultrasound (rEBUS; UM-S20-17S, Olympus America Inc., Cypress, USA), and electromagnetic navigation (EMN; Veran Medical Technologies, St. Louis, USA).

Routine sampling techniques included bronchoalveolar lavage (BAL), bronchial washings (BW), transbronchial needle aspiration (TBNA), and transbronchial forceps biopsy (TBFB). While sampling order and number of specimens procured were individualized per case, our approach followed commonly used methods (18,60-62). If an infection was suspected, 2–3 additional tissue samples from the target lesion were obtained using TBNA and/or TBFB and sent for culture. All lung lesions received directed BAL. For cases with only nodal sampling, BW collected during the procedure was analyzed. Other accessory tools were infrequently utilized in practice, and not included in our primary evaluation.

Specimen processing

We considered testing of a specimen as 'cytohistology' if it was processed in formalin fixative. These were either

centrifuged cells obtained by TBNA ('cell block') or biopsied tissue using TBFB. BAL/BW fluid collected for cytology was preserved in CytoLyt solution (Hologic, Inc., Marlborough, MA). Any specimen obtained for culture (tissue, cells, fluid) was placed in non-bacteriostatic saline. Results of rapid on-site cytologic evaluation (ROSE) were not incorporated into the analysis.

Statistical analysis

Diagnostic yield for a test, technique, or combination thereof was the proportion of a given diagnosis achieved among the sample being analyzed. T-tests and chi-square tests were used to evaluate for differences between continuous and categorical variables of independent samples, respectively. Comparisons of diagnostic performance between two different techniques or tests (e.g., culture *vs.* cytohistology) were conducted using McNemar's tests. Logistic regression was used to ascertain independent associations with diagnosis by either cytohistology or culture testing in both the overall cohort and in subjects with a final diagnosis of atypical infection. Variables for this model were chosen by clinical relevance.

When assessing the increase in diagnosis from using a single technique (e.g., BAL/BW) to a combination of techniques (e.g., BAL/BW and TBNA/TBFB), confidence intervals are provided. McNemar's tests are not appropriate since for a given subject a combined diagnosis also implies a diagnosis with at least one of the two methods. Confidence intervals for the probability of diagnosis using both methods for individuals who were undiagnosed using the first were also calculated. Statistics were performed using the R software package (R Core Team, 2021).

Results

A total of 403 subjects met inclusion criteria, with characteristics summarized in *Table 1*. A mix of ADB modalities was used to sample a total 1,054 thoracic lesions (2.6±1.4 per bronchoscopy). All subjects had received BAL/BW-culture and TBNA/TBFB-cytohistology testing. BAL/BW-cytology and TBNA/TBFB-culture were also each performed in 319 (79%). Acuity was high, with 184 bronchoscopies (46%) performed in the inpatient setting—59 (15%) in the ICU.

Overall, 140 atypical infections were discovered in 136 subjects using all methods, with ADB contributing a diagnosis in 119 (87.5%) of these. Subjects with atypical infection were more likely to receive simultaneous lung and nodal sampling (*Table 1*). Their lung lesions were smaller and more likely to be nodules, masses, and cavitary. Immediate bronchoscopy-related major complications occurred in 2 (1.5%): 1 pneumothorax requiring chest tube (0.7%) and 1 respiratory failure requiring endotracheal intubation (0.7%). Bleeding requiring intervention beyond routine bronchoscopic measures did not occur in any with atypical infection.

Coccidioidomycosis was the most frequently identified infection (n=49) and was the only atypical infection more likely to be discovered in immunocompetent subjects [odds ratio (OR) =4.57, 95% confidence interval (CI): 1.77–11.83; P=0.002]. All other atypical infections except mycobacteria were more frequently diagnosed in the immunosuppressed. A specific etiology explaining thoracic lesions could not be identified in 104 subjects, despite comprehensive diagnostics for underlying infection (*Table 2*).

Performance of ADB testing methods

Among the entire cohort (N=403), ADB yielded a specific diagnosis of atypical infection in 119 subjects (29.5%; *Table 3*). Mycobacteria was the only subgroup more frequently identified by culture than cytohistology (8.4% *vs.* 2.7%; P<0.001).

To determine independent associations with either cytohistology or culture diagnosis, we performed multivariate analysis controlling for patient, lesional and procedure-related factors (*Tables 4,5*). Among the entire cohort, immunosuppressed status was associated with diagnosis by both cytohistology (OR =3.46, 95% CI: 1.77–6.78; P<0.001) and culture (OR =2.12, 95% CI: 1.20–3.74; P=0.010). However, in those with an ultimate diagnosis of atypical infection, this association persisted only with cytohistology (OR =5.71, 95% CI: 1.64–19.82; P=0.006). In addition, Coccidioidal disease was strongly associated with diagnosis by cytohistology (OR =7.64, 95% CI: 2.51–23.26; P<0.001) and less likely to be diagnosed by culture (OR =0.23, 95% CI: 0.07–0.78; P=0.018). Lung cavitation predicted yield with culture in both models.

Overall average time to organism identification by cytology/cytohistology was 3.4±1.7 days, compared to 19.2±16.7 days with culture (P<0.001). The diagnostic interval difference was largest for mycobacteria (4.1 *vs.* 33.2 days; P<0.01) and smallest for opportunistic fungi

Table 1 Sample characteristics: overall, subjects diagnosed with atypical infection, and all other non-malignant diagnoses

Characteristic	Overall (N=403)	Atypical infection (N=136)	All other non-malignant diagnoses[†‡] (N=267)	P value
Patient				
Age, years, mean ± SD	57.8±16.3	57.4 ±16.8	57.9 ±16.1	0.764
Female gender, n (%)	181 (44.9)	61 (44.9)	120 (44.9)	1.000
Immunosuppressed status, n (%)[§]	126 (31.3)	57 (41.9)	69 (25.8)	0.002
Inpatient status, n (%)	184 (45.7)	66 (48.5)	118 (44.2)	0.471
ICU status, n (%)	59 (14.6)	21 (15.4)	38 (14.2)	0.767
Lung lesions				
Number of patients with lung lesion	308	120	188	
Number sampled per patient, mean ± SD	0.97±0.73	1.12±0.64	0.90±0.76	0.003
Size of primary lesion, mm, mean ± SD	44.0±24.0	38.1±20.3	47.7±25.4	<0.001
Upper lobe location, n (%)	184 (59.7)	75 (62.5)	109 (58.0)	0.503
Nodule/mass (*vs.* consolidation/infiltrate), n (%)	117 (38.0)	62 (51.7)	55 (29.3)	<0.001
Solid attenuation, n (%)	103 (33.4)	39 (32.5)	64 (34.0)	0.691
Cavitation present, n (%)	70 (22.7)	42 (35.0)	28 (14.9)	<0.001
Lymph nodes				
Number of patients with lymph node	263	83	180	
Number sampled per patient, mean ± SD	1.6±1.5	1.4±1.3	1.7±1.5	0.033
Size of largest node, mm, mean ± SD	17.3±8.0	16.9±8.3	17.4±7.9	0.631
Bronchoscopy procedure				
cEBUS only, n (%)	138 (34.2)	22 (16.2)	116 (43.5)	<0.001
rEBUS or EMN only, n (%)	136 (33.8)	47 (34.6)[¶]	89 (33.3)	0.893
Combined modalities, n (%)	129 (32.0)	67 (49.3)[¶]	62 (23.2)	<0.001
Moderate sedation (*vs.* GA), n (%)	280 (69.5)	90 (66.2)	190 (71.2)	0.361
Trainee involved, n (%)	249 (61.8)	74 (54.4)	175 (65.5)	0.039
Duration, min, mean ± SD	63.1±27.0	65.7±29.7	61.8±25.4	0.191
Bronchoscopy complications, n (%)				
Any	55 (13.7)	15 (11.0)	40 (15.0)	0.348
Minor	44 (10.9)	14 (10.3)	30 (11.2)	0.896
Major[††]	14 (3.5)	2 (1.5)	12 (4.5)	0.199

[†], includes typical respiratory bacterial infections =57, specific non-infectious disorders =106, and cases with a non-specific final clinical diagnosis =104. [‡], non-infectious diagnoses include: sarcoidosis =62; pneumonia/pneumonitis unspecified =62; granulomatous disease (nodule, pneumonitis or adenopathy) =18; non-specific benign nodule =14; reactive adenopathy =10; rheumatologic-associated lung disease =9; cryptogenic organizing pneumonia =7; drug-induced lung disease =4; hypersensitivity pneumonitis =3; radiation-pneumonitis =3; eosinophilic pneumonia =2; pneumoconiosis =2; IgG-4 related disease =2; Castleman disease =2; hemophagocytic lymphohistiocytosis =2; other =8. [§], 'Immunosuppressed' was defined by presence of at least one of the following at time of initial evaluation: acquired immunodeficiency syndrome; neutropenia; post-transplantation immunosuppressive therapy of any type; at least 2 weeks therapy with greater than 20 mg prednisone-equivalent per day for any reason; any cytotoxic therapy within the month prior to evaluation; primary immunodeficiency of any type; active lympho-hematogenous malignancy; poorly controlled diabetes mellitus (hemoglobin A1c >9.0%). [¶], among the atypical infection cohort, radial probe EBUS and electromagnetic navigation were used during 80 and 34 procedures, respectively, and individually each provided a specific cytohistologic diagnosis in 50% of cases. [††], major complications overall: pneumothorax requiring a chest tube =9 (2.2%), respiratory failure requiring endotracheal intubation =6 (1.5%), bleeding requiring intervention beyond routine measures =2 (0.5%), escalation of care =6 (1.5%). No cardiac arrest or death occurred. All other complications were considered minor. SD, standard deviation; cEBUS, convex-probe endobronchial ultrasound; rEBUS, radial-probe endobronchial ultrasound; EMN, electromagnetic navigation; GA, general anesthesia.

Table 2 Evaluation profile of subjects with a final non-specific clinical diagnosis (N=104)

Evaluation	Pneumonia, unspecified (N=62)	Granulomatous disease (N=18)	Other benign nodule and/or adenopathy (N=24)	Total (N=104)
ADB cytohistology result, n (%)				
Non-specific inflammatory tissue	43 (69.4)	1 (5.6)	21 (87.5)	65 (62.5)
Supportive cytohistology[†]	16 (25.8)	15 (83.3)	0	31 (29.8)
Fibrous tissue	1 (1.6)	1 (5.6)	1 (4.2)	3 (2.9)
Non-lesional	3 (4.8)	1 (5.6)	1 (4.2)	5 (4.8)
ADB-ancillary testing performed (PCR or antigen), n (%)				
At least one test	39 (62.9)	9 (50.0)	6 (25.0)	54 (51.9)
Mycobacterium tuberculosis PCR	29 (46.8)	7 (38.9)	2 (8.3)	38 (36.5)
Coccidioides PCR	13 (21.0)	4 (22.2)	2 (8.3)	19 (18.3)
Other	17 (27.4)	5 (27.8)	5 (20.8)	27 (26.0)
Serum or urine testing performed (serology or antigen), n (%)				
At least one test	54 (87.1)	17 (94.4)	14 (58.3)	85 (81.7)
Coccidioides serology	51 (82.2)	17 (94.4)	14 (58.3)	82 (78.8)
Histoplasma serology or antigen	37 (59.7)	11 (61.1)	8 (33.3)	56 (53.8)
Cryptococcus serology	28 (45.2)	11 (61.1)	5 (20.8)	44 (42.3)
Aspergillus antigen	30 (48.4)	2 (11.1)	2 (8.3)	36 (34.6)
Other	37 (59.7)	11 (61.1)	8 (33.3)	56 (53.8)
Additional tissue sampling, n (%)				
Percutaneous biopsy	4 (6.5)	3 (16.7)	1 (4.2)	8 (7.7)
Surgery	1 (1.6)	0	1 (4.2)	2 (1.9)
Autopsy	1 (1.6)	0	0	1 (1.0)
Received only imaging surveillance after bronchoscopy	9 (14.5)	0	7 (29.1)	16 (15.4)

[†], granulomas, necrosis, organizing pneumonia, acute lung injury, or cell-specific inflammation. ADB, advanced diagnostic bronchoscopy; PCR, polymerase chain reaction.

(2.9 *vs.* 11.1 days; P<0.01).

Performance of ADB techniques

Individual and combined performance of techniques and tests were analyzed in the 252 subjects for which both respiratory secretion and tissue sampling were subjected to dual culture and cytology/cytohistology testing. Specific diagnostic yields for atypical infection of individual technique-test combinations are summarized in *Figure 2*. Overall, BAL/BW-cytology was inferior to all others (P<0.001), providing a diagnosis in only 15 subjects (6.0%).

Figure 3 summarizes the diagnostic yields of bronchoscopic techniques among dual-tested samples, separately and combined, for atypical infection subgroups (N=252). Overall, adding TBNA/TBFB to BAL/BW diagnosed an additional 30 subjects with atypical infection (increase in proportion =11.9%, 95% CI: 8.5–16.5). Multimodal sampling was most beneficial for dimorphic fungi: adding TBNA/TBFB to BAL/BW more than doubled the yield, from 7.1% to 15.1% (increase =8.0%, 95% CI: 5.2–11.9%).

Of the 127 subjects for which both TBNA and TBFB were used to sample the same lung lesion (*Figure 4*), TBNA provided a specific cytohistologic diagnosis of

Table 3 Specific diagnostic yield of advanced bronchoscopy testing methods for atypical infection among the total cohort of non-malignant disease (N=403)

Atypical infection	Culture[¶¶], yield n (%)	Specific cytohistology, yield n (%)	Culture or specific cytohistology, yield n (%)	Increase in diagnostic yield when adding specific cytohistology to culture (95% CI)
Any atypical infection[†]	96 (23.8)*	75 (18.6)	119 (29.5)	5.7 (3.8–8.4)
Dimorphic fungi[‡]	28 (6.9)	34 (8.4)	43 (10.7)	3.8 (2.3–6.1)
Opportunistic fungi[§]	29 (7.2)	23 (5.7)	33 (8.2)	1.0 (0.4–2.5)
Mycobacteria[¶]	34 (8.4)**	11 (2.7)	34 (8.4)	0 (0–0.9)
Other extracellular organisms[††]	5 (1.2)	6 (1.5)	8 (2.0)	0.8 (0.2–2.2)
Intracellular organisms[‡‡]	0	1 (0.2)	1 (0.2)	0.2 (0–0.7)

[†], 140 total atypical infections diagnosed in 136 subjects using all methods; [‡], 51 total infections: *Coccidioides* =49, *Histoplasma* =2; [§], 36 total infections: *Aspergillus* =18, *Cryptococcus* =10, *Mucor/Rhizopus* sp. =6, *Candida* =2; [¶], 34 total infections: non-tuberculous =21, *M. tuberculosis* =13; [††], 10 total infections: *Nocardia* =8, *Actinomyces* = 1, *Pneumocystis* =1; [‡‡], 9 total infections: *Legionella* =2, metapneumovirus =2, SARS-CoV-2 =2, *Coxiella* =1, herpes simplex virus =1, *Mycoplasma* =1; [¶¶], within each group, culture yield was compared to cytohistology yield using McNemar's tests; *, P<0.05; **, P<0.001. CI, confidence interval.

Table 4 Multivariate model evaluating associations with diagnosis by cytohistology and culture, among all subjects with non-malignant disease (N=403)

Independent variable	Cytohistology		Culture	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Male gender	0.98 (0.56–1.71)	0.937	0.78 (0.48–1.25)	0.302
Age	1.01 (0.99–1.02)	0.398	1.02 (1.00–1.04)	0.011
Immunosuppressed state	3.46 (1.77–6.78)	<0.001	2.12 (1.20–3.74)	0.010
Hospitalized	1.30 (0.64–2.62)	0.470	0.91 (0.50–1.66)	0.758
ICU location	0.94 (0.41–2.15)	0.884	1.33 (0.64–2.76)	0.448
General anesthesia	1.67 (0.90–3.11)	0.105	0.51 (0.29–0.91)	0.022
Trainee assisting procedure	0.67 (0.38–1.17)	0.158	0.77 (0.47–1.25)	0.291
Number of overall samples obtained	1.04 (0.79–1.36)	0.783	0.87 (0.68–1.11)	0.273
Lung lesion sampled	0.89 (0.22–3.55)	0.864	1.65 (0.53–5.16)	0.391
Lung lesion size	1.00 (0.99–1.02)	0.744	1.00 (0.99–1.02)	0.603
Upper lobe location	1.18 (0.64–2.17)	0.593	0.69 (0.23–2.05)	0.543
Nodule or mass (*vs.* consolidation or infiltrate)	1.36 (0.61–3.03)	0.457	0.86 (0.43–1.73)	0.681
Solid lung lesion (*vs.* part-solid or ground glass)	1.00 (0.47–2.14)	0.993	1.98 (1.04–3.79)	0.039
Cavitation present	1.85 (0.83–4.13)	0.130	7.56 (3.63–15.74)	<0.001
Lymph node sampled	1.69 (0.59–4.82)	0.324	2.08 (0.82–5.29)	0.125
Lymph node size	1.05 (1.01–1.09)	0.015	1.02 (0.98–1.06)	0.355

CI, confidence interval.

Table 5 Multivariate model evaluating associations with diagnosis by cytohistology and culture, among subjects with a final clinical diagnosis of atypical respiratory infection (N=136)

Independent variable	Cytohistology		Culture	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Male gender	0.4 (0.16–1.01)	0.053	0.34 (0.11–0.98)	0.046
Age	1.01 (0.99–1.04)	0.322	1.05 (1.02–1.09)	0.004
Immunosuppressed state	5.71 (1.64–19.82)	0.006	1.92 (0.52–7.14)	0.33
Hospitalized	1.65 (0.56–4.87)	0.366	0.44 (0.13–1.55)	0.203
ICU location	1.16 (0.32–4.18)	0.823	0.63 (0.16–2.45)	0.509
General anesthesia	2.45 (0.94–6.37)	0.067	0.53 (0.19–1.49)	0.228
Trainee assisting procedure	0.81 (0.34–1.91)	0.630	0.65 (0.25–1.73)	0.39
Number of overall samples obtained	1.11 (0.68–1.82)	0.668	1.08 (0.63–1.86)	0.774
Lung lesion sampled	0.26 (0.02–3.74)	0.320	5.98 (0.18–198.39)	0.317
Lung lesion size	1.05 (1.02–1.09)	0.004	1.01 (0.97–1.05)	0.577
Upper lobe location	1.23 (0.47–3.22)	0.678	0.69 (0.23–2.05)	0.505
Nodule or mass (*vs.* consolidation or infiltrate)	1.08 (0.47–3.22)	0.903	0.93 (0.22–3.87)	0.925
Solid lung lesion (*vs.* part-solid or ground glass)	0.93 (0.26–3.31)	0.917	1.48 (0.37–5.89)	0.58
Cavitation present	0.9 (0.24–3.33)	0.875	5.68 (1.16–27.81)	0.032
Lymph node sampled	0.71 (0.12–4.21)	0.709	0.08 (0.01–0.65)	0.018
Lymph node size	1.07 (0.99–1.16)	0.069	1.16 (1.05–1.28)	0.003
Coccidioidomycosis	7.64 (2.51–23.26)	<0.001	0.23 (0.07–0.78)	0.018

CI, confidence interval.

atypical infection in 20 (16%). Adding TBFB diagnosed an additional 8 subjects (6.3% increase, 95% CI: 3.2–11.9). Adding tissue culture to dual TBNA/TBFB cytohistology-tested lung samples further enhanced the overall yield to 40 subjects (32%), doubling the proportion diagnosed over using TBNA cytohistology alone (increase of 15.8%, 95% CI: 10.4–23.1).

Among the 114 subjects which received simultaneous lung and lymph node sampling (*Table 6*), lung and nodal tissue provided a diagnosis of atypical infection in 26 and 24 subjects, respectively (22.8% *vs.* 21.2%; P=0.72). The techniques combined for a yield in 40 subjects (35.1%). Adding nodal to lung sampling was most beneficial for coccidioidal infections, increasing the proportion diagnosed from 10% to 18%, and also identifying 11% of cases undiagnosed by lung sampling (95% CI: 5.4–17%).

Ancillary testing

A heterogeneous complement of serum and ADB-ancillary testing data was available. The diagnostic contribution to atypical infection is summarized and compared to ADB-culture and cytohistology testing in *Tables 7*,*8*.

Discussion

Herein, we found that a multimodal approach to both sampling and testing during advanced-guidance bronchoscopy augmented the yield for atypical infection over the use of individual techniques, while maintaining a favorable procedure safety profile. The magnitude of gain varied across infection types, and patients with coccidioidomycosis benefitted most from cytohistology testing and nodal sampling.

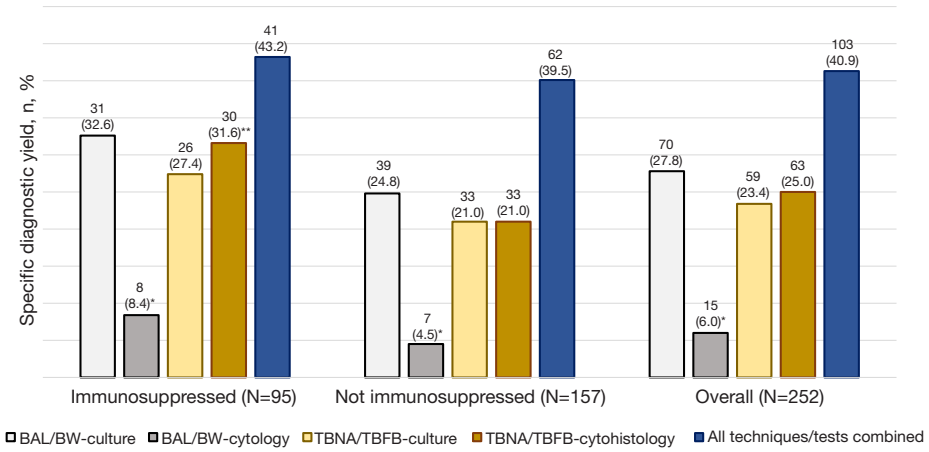

Figure 2 Specific diagnostic yield for atypical infection of individual ADB technique-test combinations among subjects for which both BAL/BW and TBNA/TBFB were used and dual culture and cytohistology testing performed (N=252), per immune status and overall. BAL/BW-cytology was compared to the other technique-test combinations within each group using McNemar's tests, *P<0.001. BAL/BW-culture, TBNA/TBFB-culture and TBNA/TBFB-cytohistology were compared to one another within each group using McNemar's tests; no significant differences found. Each technique-test combination was compared between the 'Immunosuppressed' and 'Not immunosuppressed' groups using the chi-square test, **, P=0.06. 'Immunosuppressed' defined by presence of at least one of the following at time of initial evaluation: acquired immunodeficiency syndrome; neutropenia; post-transplantation immunosuppressive therapy of any type; at least 2 weeks therapy with greater than 20 mg prednisone-equivalent per day for any reason; any cytotoxic therapy within the month prior to evaluation; primary immunodeficiency of any type; active lympho-hematogenous malignancy; poorly controlled diabetes mellitus (hemoglobin A1c >9.0%). ADB, advanced diagnostic bronchoscopy; BAL, bronchoalveolar lavage; BW, bronchial washings; TBFB, transbronchial forceps biopsy; TBNA, transbronchial needle aspiration.

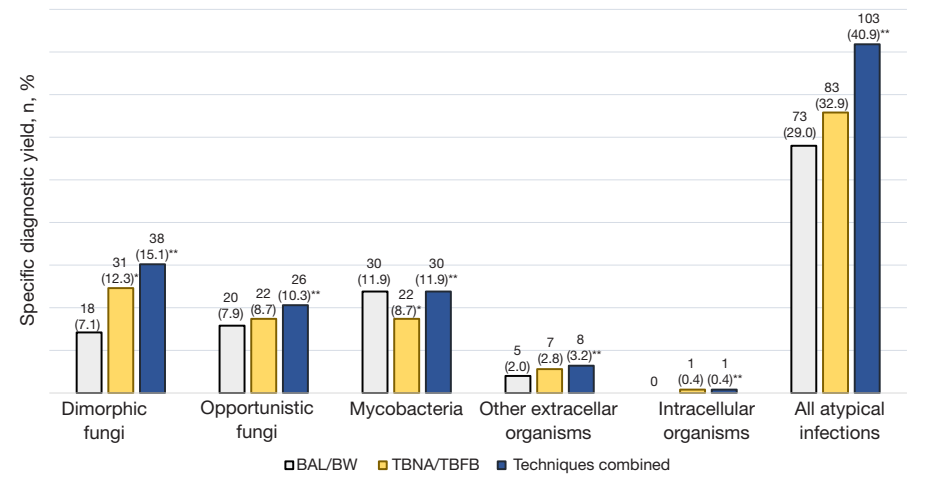

Figure 3 Specific diagnostic yield for atypical infection[†] of bronchoscopic techniques among dual-tested[‡] samples (N=252), per infection subgroup. [†], either culture or cytology/cytohistology specifically diagnostic. [‡], both culture and cytology/cytohistology testing performed on all samples. *, TBNA/TBFB was compared to BAL/BW within each group, using McNemar's tests, P<0.05. **, increase in diagnostic yield proportion by adding TBNA/TBFB to BAL/BW (95% confidence interval): dimorphic fungi =8.0% (5.2–11.9%); opportunistic fungi =2.4% (1.1–5.1%); mycobacteria =0 (0–1.5%); other extracellular organisms =1.2% (0.4–3.4%); intracellular organisms =0.4% (0.1–2.2%); overall =11.9% (8.5–16.5%). BAL, bronchoalveolar lavage; BW, bronchial washings; TBFB, transbronchial forceps biopsy; TBNA, transbronchial needle aspiration.

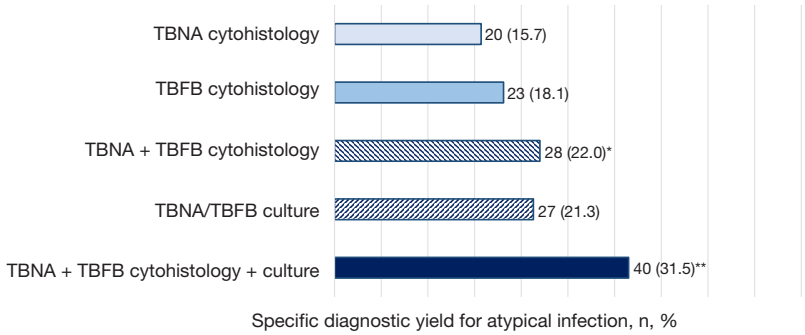

Figure 4 Synergy of tissue sampling techniques and testing for diagnosing atypical infection of the same lung lesion (N=127). Both TBNA and TBFB performed on same lung lesion, with dual cytohistology and culture testing available (N=127). Culture testing on lung tissue obtained by either TBNA or TBFB. *, increase in diagnostic yield proportion by adding TBFB cytohistology to TBNA cytohistology =6.3% (95% confidence interval: 3.2–11.9). **, increase in diagnostic yield proportion by adding TBNA/TBFB cytohistology and tissue culture to TBNA cytohistology alone =15.8% (95% confidence interval: 10.4–23.1). No significant differences between TBNA and TBFB cytohistology, or tissue cytohistology and culture using McNemar's tests. TBFB, transbronchial forceps biopsy; TBNA, transbronchial needle aspiration.

Table 6 Synergy of advanced guidance bronchoscopy modalities for diagnosing atypical infection in subjects that received simultaneous sampling of lung lesions and lymph nodes (N=114)[†]

Infection	Lung sampling[‡§], yield n (%)	Nodal sampling[¶], yield n (%)	Lung or nodal sampling, yield n (%)	Increase in yield proportion (%) by adding lung to nodal sampling [95% CI]	Increase in yield proportion (%) by adding nodal to lung sampling [95% CI]	Proportion (%) diagnosed with nodal sampling that were negative with lung sampling [95% CI]
Any atypical infection	26 (22.8)	24 (21.2)	40 (35.1)	14 [8.8–22]	12 [7.5–20]	17 [10–25]
Dimorphic fungi[††]	11 (9.7)	17 (14.9)	21 (18.4)	3.5 [1.4–8.7]	8.8 [4.8–15]	11 [5.4–17]
Mycobacteria	7 (6.1)	4 (3.5)	10 (8.8)	5.3 [2.4–11]	2.7 [0.9–7.5]	4.4 [1.0–7.9]
Opportunistic fungi	5 (4.4)	2 (1.8)[‡‡]	7 (6.1)	4.3 [1.9–9.9]	1.7 [0.4–6.2]	3.5 [0.5–6.4]
Other atypical infections	3 (2.6)	1 (0.9)	3 (2.6)	1.7 [0.5–6.2]	0 [0–3.2]	–

[†], at least one cytohistology or culture result available from both sampling modalities in a given subject. [‡], transbronchial needle aspiration and/or transbronchial forceps biopsy of lung lesions guided by radial-probe EBUS and/or electromagnetic navigational bronchoscopy. [§], no significant differences comparing lung sampling to nodal sampling using McNemar's tests. [¶], transbronchial needle aspiration and/or intranodal forceps biopsy guided by convex-probe endobronchial ultrasound bronchoscopy. [††], all coccidioidomycosis. [‡‡], both diagnostic nodal samples were from subjects with cryptococcal infection. CI, confidence interval.

To our knowledge, to date this is the most comprehensive assessment of the utility of ADB for assessing atypical respiratory infections in a *Coccidioides*-endemic region. Our study also adds value to the existing literature by assessing only focal disease, for which conventional bronchoscopy is less reliable. We examined both the individual contribution and synergy of common bronchoscopic techniques, only included subjects that had dual culture and cytohistology testing, and used definitive identification of an atypical organism as the diagnostic criterium since this most decisively directs therapy. Within this context we address several common questions encountered during the bronchoscopic evaluation of atypical respiratory infection.

When should guided bronchoscopic tissue sampling be performed in addition to BAL/BW?

Existing guidelines favor BAL as the primary bronchoscopic technique for evaluating most suspected respiratory infections, especially those manifesting with diffuse disease

Table 7 Diagnostic yield of ADB cytohistology/culture testing compared to non-invasive testing[†] obtained within one month before or after bronchoscopy

Infection being evaluated (No. subjects for which both tests available)	No. diagnosed in cohort	No. diagnosed by either test	Only ADB-culture/ cytohistology diagnostic	Only non-invasive testing[‡] diagnostic	Both tests diagnostic	Neither test diagnostic
Coccidioides (N=335)	48 (14.3)[§]	47 (14.0)	7 (2.1)	4 (1.2)	36 (10.7)	288 (86.0)
Aspergillus (N=141)	16 (11.3)[¶]	15 (10.6)	5 (3.5)	1 (0.7)	9 (6.4)	126 (89.4)
Cryptococcus (N=159)	8 (5.0)[§]	7 (4.4)	3 (1.9)	0	4 (2.5)	152 (95.6)
Histoplasma (N=47)	1 (2.1)	1 (2.1)	0	0	1 (100.0)	46 (97.9)

Data are presented as n (% of total dual tests). [†], ADB-culture/cytohistology compared to ancillary testing for all infection groups using McNemar's tests; no significant differences found. [‡], *Coccidioides* and *Cryptococcus* serology, *Aspergillus* serum antigen, *Histoplasma* urine antigen. [§], additional infection diagnosed by tissue sampling via transthoracic approach. [¶], additional infection diagnosed by antigen testing of bronchoalveolar lavage fluid. ADB, advanced diagnostic bronchoscopy.

Table 8 Diagnostic yield of cytohistology/culture testing compared to ancillary testing performed on samples obtained during same-session ADB[†]

Infection being evaluated (subjects for which both tests available)	Diagnosed in cohort	Diagnosed by either test	Only ADB-culture/ cytohistology diagnostic	Only ADB-ancillary testing[‡] diagnostic	Both tests diagnostic	Neither test diagnostic
Coccidioides (N=61)	13 (21.3)[§]	12 (19.7)	4 (6.6)	0	8 (13.1)	49 (80.3)
Tuberculosis (N=116)	10 (8.6)	10 (8.6)	3 (2.6)	0	7 (6.0)	106 (91.4)
Aspergillus (N=43)	3 (7.0)	3 (7.0)	0	0	3 (100.0)	40 (93.0)
Pneumocystis (N=24)	1 (4.2)	1 (4.2)	0	0	1 (100.0)	23 (95.8)

Data are presented as n (% of total dual tests). [†], ADB-culture/cytohistology compared to ancillary testing for all infection groups using McNemar's tests; no significant differences found. [‡], all ancillary tests performed on bronchoalveolar lavage/bronchial washings fluid; all tests polymerase chain reaction expect *Aspergillus* antigen. [§], additional infection diagnosed by high *Coccidioides* antibody titer. ADB, advanced diagnostic bronchoscopy.

(9,16,63,64). Advice regarding tissue sampling is more restrained due to variability of reported yields, differences in clinical circumstance, concerns over procedure-related risks, the emergence of ancillary tests, and a source data pool mostly pre-dating the ADB era.

The enhanced scope and accuracy of ADB-tissue sampling would be most complementary for infections presenting as lung nodules and lymphadenopathy, to which BAL/BW may have imprecise access. Dimorphic fungi fit this profile, as they often manifest with focal thoracic lesions. Furthermore, BAL/BW and other traditional bronchoscopic methods have a comparatively low diagnostic sensitivity for histoplasmosis and coccidiodomycosis (25,26). Our results concurred, showing that the addition of ADB-guided tissue sampling most benefited this subgroup by more than doubling the yield over BAL/BW. Adjusted analysis also revealed that coccidioidal disease was independently associated with a cytohistologic diagnosis. Tissue examination may be particularly useful in cases of locally contained infection which may limit organism shedding and thus suppress the yield of BAL/BW culture and/or polymerase chain reaction (PCR) testing (*Figure 5A*).

The added diagnostic value of ADB-tissue sampling over BAL/BW was more modest for opportunistic fungi. Nevertheless, TBNA/TBFB may also help distinguish invasive disease from simple colonization by directly demonstrating fungal elements within tissue (*Figure 5B*). This clinically vital distinction, not easily made with less-specific BAL/BW-culture, PCR, or antigen tests, is commonly required with *Aspergillus* species and has therapeutic ramifications (20).

Based on these findings, when performing bronchoscopy for clinically suspected fungal disease, we consider BAL/BW to be suboptimal as the only investigative technique.

We also found tissue cytohistology established diagnosis by an average of 16 days earlier than culture testing. Those

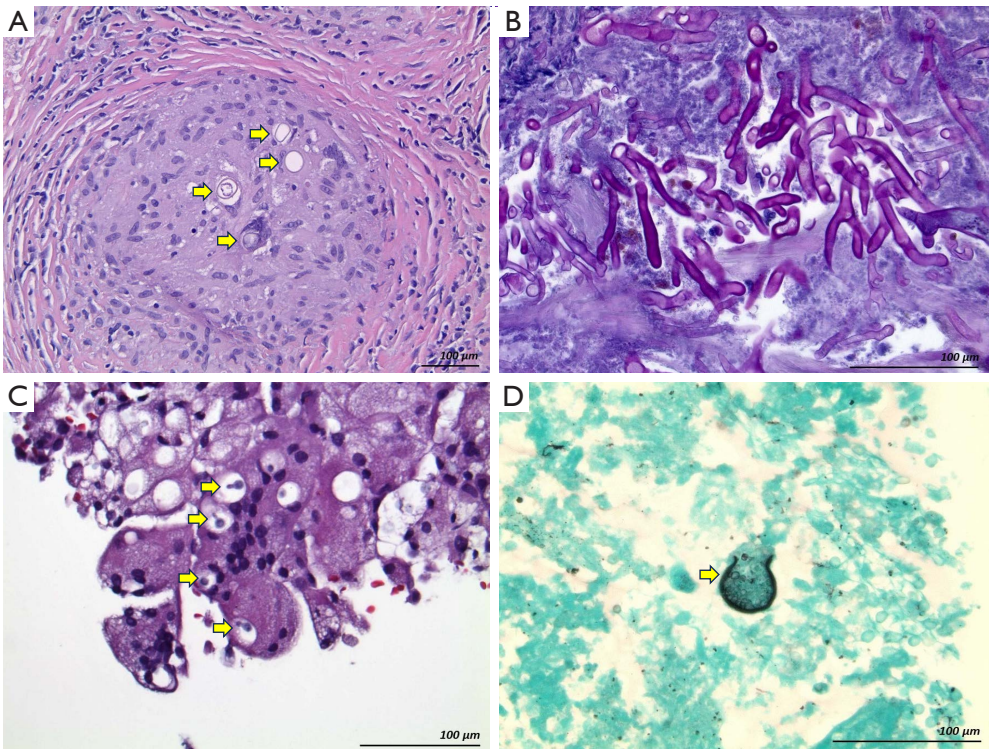

Figure 5 Cytohistologic representation of various atypical infections identified by advanced diagnostic bronchoscopy. (A) Coccidioidal organisms (yellow arrows) within granulomatous inflammation. Obtained by rEBUS-guided TBFB of a solid right lower lobe lung nodule. BAL was unrevealing. Hematoxylin-Eosin stain. (B) Abundant broad, aseptate, variably branching fungal hyphae among necrotic lung tissue. Obtained by EMN-guided TBFB of a cavitating middle lobe nodule in a diabetic patient with dual invasive pulmonary mucormycosis and aspergillosis. Periodic Acid-Schiff stain. (C) Narrow-based budding cryptococcal organisms (yellow arrows) within lung tissue. Obtained by EMN-guided TBFB of a right upper lobe subsolid nodule in an immunocompetent patient. TBNA cytohistology demonstrated multinucleated giant cells, but no organisms. Tissue and BAL cultures grew *Cryptococcus gattii*. Hematoxylin-Eosin stain. (D) A rupturing coccidioidal spherule (yellow arrow) within lymph tissue. Obtained by cEBUS-TBNA of a subcarinal thoracic lymph node in an immunocompetent patient with disseminated coccidioidomycosis. A primary solid left lower lobe lung nodule was simultaneously accessed by rEBUS-sampling, yielding caseous granulomatous inflammation. Lymph node culture grew *Coccidioides immitis*. Grocott's Methenamine Silver stain. BAL, bronchoalveolar lavage; cEBUS, convex-probe endobronchial ultrasound; EMN, electromagnetic navigation; rEBUS, radial-probe endobronchial ultrasound; TBFB, transbronchial forceps biopsy; TBNA, transbronchial needle aspiration.

with mycobacterial infection benefited most, with final diagnosis accelerated by 29 days. This can have important clinical implications, particularly for tuberculosis, since delays may postpone treatment or prolong exposure to potentially toxic empiric therapies (2,9,65). Furthermore, while molecular testing may be useful for guiding initial therapeutic decisions, culture-based analysis remains the gold standard for evaluating antimicrobial sensitivity (65,66). Therefore, we agree with current guidelines that tissue sampling with culture testing may be valuable as both an efficient and comprehensive approach for selected cases of suspected mycobacterial disease (66).

In summary, our results support that unless contraindicated, ADB-tissue sampling should supplement BAL/BW for evaluating most cases of focal thoracic lesions in the setting of suspected atypical infection. In a *Coccidioides*-endemic region, we suggest this approach be routine and not necessarily depend on a patient's immune status, since we detected these infections more frequently in immunocompetent hosts.

When sampling a focal lung lesion suspected due to an atypical respiratory infection, should the bronchoscopist use TBNA, TBFB, or both? Should tissue culture also be tested?

The value of multimodal sampling during ADB is well-established for lung cancer patients (67-69). However, such data for infectious respiratory disease is limited and the utility of cytohistology testing ranges widely (70-73). This heterogeneity is likely due in part to organism-specific influences that dictate culture growth patterns and success of histologic identification (74-76). In support, we found both culture and cytohistology yield varied across infection types despite a fairly uniform bronchoscopic approach during the study period. Furthermore, in our practice the ADB-cytohistologic yield for malignancy is higher than that for infection, despite source lesions being comparatively smaller (77). These considerations highlight the importance of diversifying lung tissue procurement and testing methods when evaluating atypical respiratory infection.

We analyzed the utility of adding TBFB to TBNA, rather than in the reverse order, since in practice the typical approach is to first perform TBNA, particularly when ROSE is utilized (69). We found that by combining culture and cytohistology testing of TBNA/TBFB-obtained lung samples, diagnostic yield doubled compared to using TBNA-cytohistology alone. Cytohistologic synergy of the two sampling techniques most aided fungal diagnosis (*Figure 5C*), and tissue culture that of mycobacteria.

In summary, when sampling a lung lesion due to a suspected atypical infection, a reasonable approach is that if ROSE of the TBNA sample does not reveal malignant cells, extra specimens should be obtained using TBFB and tested for both cytohistology and culture. If ROSE is not available, both techniques should be utilized.

For patients suspected of atypical respiratory infection who have concomitant lung and lymph node lesions, should sampling of both be performed?

Nodal biopsy in the setting of non-malignant disease often yields only reactive, diagnostically non-contributory lymph tissue (78-80). However, sampling directly infected nodes may increase the likelihood of visualizing organisms or a characteristic inflammatory response (i.e., granuloma), and could also augment culture yield.

Dimorphic fungi and mycobacteria can infect lymph nodes and yield prominent, caseous, and/or partially calcified adenopathy (3-5,78). As these infections may manifest with small, difficult to access lung lesions, the coexisting nodal disease offers an alternative diagnostic target using cEBUS guidance (32,52,54-56,81-84). In support, our data demonstrated synergy between lung and nodal tissue assessment. Not surprisingly, this result was primarily driven by *Coccidioides* (and to a lesser extent mycobacteria and *Cryptococcus*), for which nodal sampling almost doubled the proportion diagnosed and identified infection in 11% of cases for which lung sampling was not specifically diagnostic (*Figure 5D*). Conversely, *Aspergillus*, *Mucor*, and *Candida* species were not identified in lymph tissue, suggesting their relative lack of proclivity for nodal spread.

In summary, based on our findings, when *Coccidioides* (and possibly mycobacteria and *Cryptococcus*) is suspected, cEBUS-guided sampling of thoracic adenopathy should be performed and tissue tested for both cytohistology and culture.

Limitations and other considerations

The main limitation of our study is its retrospective and single institution design, though the data originated from a prospectively maintained database.

Our study should not be interpreted as insight into the absolute diagnostic sensitivity of ADB for atypical infection. Even though we believe ADB diagnostic sensitivity is high in this setting, establishing a reliable reference standard necessary for such analysis can be problematic for non-malignant disease. Even with the thorough evaluation of our 'non-specific' cohort (*Table 2*), given the characteristics of disease and limitations of retrospective design, definitively excluding all self-limiting atypical infections such as dimorphic fungi—which are prevalent in our region—is not possible. Nevertheless, our assessment of the synergistic impact of ADB techniques on the diagnostic yield of atypical infection among patients with non-malignant disease provided similarly useful general conclusions.

We did not incorporate into our primary hypothesis the value of antigen and PCR analysis of respiratory samples, and we acknowledge the individualized benefit of these ancillary tests. However, despite advances in their applications, practical considerations may limit their availability and use, and concerns persist over clinical utility (9,11,85-94). Within our limited sample of histologically and/or culture-proven disease, these tests performed inconsistently (*Table 8*). Culture and cytohistology testing

remain the cornerstone of routine bronchoscopy practice, especially when prospectively evaluating an undiagnosed focal thoracic lesion. Thus, we thought the generalizability of our analysis was maximized by focusing on these methods.

We also did not study the impact of bronchial brushings (BB) as this technique is not routine in our practice. A previous internal quality review found BB adds little to the overall bronchoscopic yield for infection, consistent with existing guidelines and other reports (18,36).

Finally, our findings are most relevant for practice in a *Coccidioides*-endemic region. However, because other dimorphic fungi such as *Histoplasma* and *Blastomyces* have similar clinical characteristics, our results could be reasonably applied in corresponding endemic areas.

Conclusions

We found that multimodal evaluation using commonly utilized techniques and tests during advanced-guidance bronchoscopy enhances specific diagnostic yield for patients with atypical respiratory infections. Cytohistology testing and nodal tissue sampling are beneficial for pulmonary coccidiodomycosis, and culture for mycobacterial disease. The value of emerging advanced bronchoscopic modalities, such as robotics, augmented real-time guidance, and transbronchial cryobiopsy, are yet unexplored in this setting. Our findings could provide a basis for future investigation with these approaches.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-83/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-83/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-83/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-83/coif). AAC has received consulting and speaker fees from Intuitive Surgical, Inc. BF has received speaker fees from Insmed, Inc. and consulting fees from Intuitive Surgical, Inc., and STERIS Life Sciences. EH has received consulting fees from Biodesix Inc., Intuitive Surgical, Inc., and Olympus Corp. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). It was approved by the institutional review board of Loma University Medical Center (No. 5190131) and individual consent for this retrospective analysis was waived.

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