Diagnostic Concordance of Non-Small Cell Lung Carcinoma Subtypes Between Biopsy and Cytology Specimens Obtained During the Same Procedure

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BACKGROUND: The objectives of this study were: 1) to determine the diagnostic concordance of non-small cell lung carcinoma (NSCLC) subtypes in cytology and biopsy specimens taken during the same procedure and evaluate the causes of discordance; and 2) to determine the frequency of immunohistochemistry (IHC) use for subtyping NSCLC. METHODS: Biopsy and cytology specimens that were obtained at the same procedure and diagnosed as NSCLC between January 2011 and December 2014 at the McGill University Health Center were identified (n = 226 pairs). The diagnostic concordance between the 2 methods was evaluated. The slides from discordant cases were reviewed, and final diagnoses were made based on IHC, resection specimens, or pathologist discussion. RESULTS: Concordance in subtype diagnosis was perfect (adenocarcinoma or squamous-cell carcinoma) in 66.2% of cases and was partial (adenocarcinoma or squamous-cell carcinoma vs non-small cell) in 23%; discordance (adenocarcinoma vs squamous-cell carcinoma) was observed in 7.8%. Although subtyping was not possible (ie, the final diagnosis was NSCLC, not otherwise specified) in 12.8% of biopsy specimens and 16.3% of cytology specimens, specific subtyping was not achieved in only 3% of cases when both modalities were considered. IHC was used in 47% of biopsy cases and 13% of cytology cases. CONCLUSIONS: Subtyping of NSCLC can be achieved in most cases (97%) by considering findings in both biopsy and cytology specimens, and concordance in subtyping between cytology and biopsy specimens can be reached in a high percentage of cases (89.2%). Cancer Cytopathol 2016;124:737-43. © 2016 American Cancer Society.

INTRODUCTION

Pulmonary carcinoma is the leading cause of cancer-related death worldwide. In recent years, several new therapeutic strategies have been developed for the treatment of non-small cell lung carcinoma (NSCLC).1-2 Several organizations with expertise in lung disease/cancer, particularly the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society (IASLC/ATS/ERS), have recommended that NSCLC detected on small biopsies and cytology specimens should be classified into a specific histologic type whenever possible.3-6 Although it is frequently possible to achieve this subtyping by hematoxylin and eosin or Papanicolaou staining alone in surgical pathology and cytologic specimens, respectively, immunohistochemistry (IHC) study is sometimes necessary to be more precise, particularly when encountering poorly differentiated tumors. According to the literature, concordance generally is good (range, 88%-97%) between biopsy and cytology for all types of lung cancer.7-12 However, cases of discordance between biopsy and cytology diagnoses do occasionally occur and can cause confusion for clinicians and delays in treatment.

KEY WORDS: biopsy; concordance; cytology; lung; non-small cell lung carcinoma (NSCLC).
Although 1 of the “pathology considerations for good practice” recommended by IASLC/ATS/ERS is that, when there are paired cytology and biopsy specimens, to reach the most specific and nondiscordant diagnosis, they should be reviewed together, at the McGill University Health Center Pathology Department, pulmonary biopsy and cytology specimens are reported by different pathologists, because the division of work and the teaching are done by subspecialties (eg, cytopathology, respiratory pathology). In addition, although cytohistologic correlation is performed periodically for the purpose of quality assessment in a retrospective fashion, no prospective, formal, ongoing routine correlation is performed on a daily basis between biopsy and cytology diagnoses unless a “problem” or difficult case is encountered; in the latter cases, consultation between the cytopathologist and respiratory pathologist does occur. The multisite setting of our institution at the time of the study made consultation more difficult, because most of the cytopathologists and respiratory pathologists were located at different sites. Moreover, different antibodies are used in cytology versus in histology for IHC categorization of poorly differentiated tumors. The current study was undertaken: 1) to determine the diagnostic concordance of NSCLC subtypes in cytology and biopsy specimens taken during the same procedure and evaluate the causes of discordance, and 2) to determine the number of cases in which IHC was used for subtyping NSCLC in cytology and biopsy specimens.

MATERIALS AND METHODS

After we received ethical approval for this study from the Research Ethics Board, the Cerner database from the Pathology Department at McGill University Health Center was searched retrospectively for lung biopsy specimens (transthoracic, transbronchial, bronchial) that also had corresponding cytology specimens, including transthoracic fine-needle aspirations (TTFNAs), bronchial washings, bronchial brushings, and bronchoalveolar lavages, taken during the same procedure and diagnosed as NSCLC by both modalities. Cases were retrieved from January 2011 to December 2014. Because our objective was to compare concordance of subtyping on NSCLC diagnosed by both cytology and biopsy, in this study, we included only the 226 cytologic-histologic pairs for which an NSCLC diagnosis (not otherwise specified [NOS] or with more specific subtyping) was reached on both the cytology and biopsy specimens (N = 226 pairs).

To compare the diagnoses between biopsy and cytology specimens, we categorized them into the following 5 groups: 1) definite adenocarcinoma (ADC), 2) NSCLC favor ADC (FADC), 3) definite squamous cell carcinoma (SQCC), 4) NSCLC favor SQCC (FSQCC), and 5) NSCLC unclassified (NSCLC-NOS). The paired samples were then matched in a comparison table. We recorded the original diagnosis given by the individual pathologists during the initial sign-out of the case without modification. In general, for the purpose of this study, a “definite diagnosis” means that morphologic features with or without IHC were diagnostic of a tumor type, ie, ADC or SQCC. The “NSCLC FADC” or the “NSCLC FSQCC” category was used when morphology with or without IHC was suggestive but insufficient for a definitive diagnosis. We note that this approach is slightly different from that suggested by Travis et al, because this study antedates the implementation in our department of those particular guidelines.

The diagnostic concordance based on the original diagnoses between the cytology and biopsy specimen was evaluated by a pathology resident (M.E.). The slides from discordant cases were reviewed independently by a cytopathologist (M.A.) and a pulmonary pathologist (S.J.) who were blinded to the original diagnoses. Final diagnoses were made based on IHC, resection specimens, and/or pathologist consensus (M.E., M.A., S.J., and R.S.F.).

The following antibodies were used for IHC: 1) thyroid transcription factor 1 rabbit monoclonal primary antibody (SP141; Ventana Medical Systems, Oro Valley, Ariz); 2) tumor protein p63 (P63) mouse monoclonal primary antibody (4A4; Ventana Medical Systems); and 3) cytokeratin 5/6 (D5&16B4; Cell Marque Corporation, Rocklin, Calif).

RESULTS

Of 226 cytology specimens, 102 (45%) were exfoliative (bronchial washings, bronchial brushings, and bronchoalveolar lavages), and 124 (55%) were TTFNAs. Ninety-nine cytology specimens (44%) had cell blocks, most of which were prepared from TTFNAs (96%). Of 226 biopsy specimens, 115 (51%) were bronchial biopsies, and 111 (49%) were transthoracic needle biopsies. There were 12 cases for which subsequent resection specimens were...
available. Definite diagnoses (ie, ADC or SQCC) were given more often in cytologic specimens than in biopsies (59% vs 45%). ADC and SQCC had almost the same distribution in both methods: 100 ADCs were diagnosed on biopsy versus 109 on cytology, and 87 SQCCs were diagnosed by biopsy versus 80 by cytology (Table 1). Concordance on subtype diagnoses, including definite and favored diagnoses, was perfect (ie, ADC-ADC or SQCC-SQCC) in 66.2% of cases and was partial (ie, ADC or SQCC by 1 modality vs NSCLC-NOS by the other modality) in 23%. Discordance (ADC vs SQCC) was observed in 18 pairs (7.8%). Although subtyping was not possible (ie, the final diagnosis was NSCLC-NOS) in 12.8% of biopsies and 16.3% of cytology specimens, specific subtyping was not achieved in only 3% of cases when both modalities were considered (Table 2). Table 3 lists the follow-up diagnoses of resection specimens for the 12 patients who had such specimens available.

For the 18 discordant pairs that were reviewed by 3 pathologists and a pathology resident, a final diagnosis of ADC or SQCC was reached by consensus based on the biopsy specimens in 6 cases and on the cytologic specimens in 5 cases; 7 cases could not be classified beyond NSCLC-NOS (Fig. 1). The method for reaching a final review diagnosis was IHC in 4 cases, histologic features of the follow-up surgical resection (as opposed to the original biopsy material) in 3 cases, and pathologist consensus in 11 cases. Significant factors that we believed had contributed to diagnostic discrepancies included scant cellularity, absence of cell block in cytology, poor differentiation, necrosis, and equivocal IHC results (Table 4).

IHC was used for diagnostic purposes in 47% of biopsy specimens, 13% of cytology specimens, and 7.5% of both paired specimens. It was used to reach a diagnosis of ADC or SQCC more often in biopsy specimens than in cytologic specimens (37% vs 12%) (Table 5). Cytokeratin 5/6 and TTF1 were the 2 most common IHC stains used in biopsies, whereas TTF1 and P63 were the most commonly used in cytologic specimens. Table 6 provides more details about the availability of cell blocks and the use of IHC in the cytologic specimens. It is noteworthy that 5 cytologic specimens in which IHC was performed were diagnosed as NSCLC-NOS either because there were no residual, relevant diagnostic cells in the slides used for IHC or because the IHC results were equivocal.

**DISCUSSION**

The discovery of genetic abnormalities, especially related to epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), have revolutionized the treatment and diagnostic approach to NSCLC. In approximately 70% of lung cancers, small biopsies or cytologic specimens are the source of tissue for diagnosis. To date, few studies have compared NSCLC subtyping in paired small biopsies versus cytologic specimens. Diagnostic concordance in subtyping NSCLC in our study was 89.2% (Table 2), similar to the 93% concordance achieved in another published study. A review by Paech et al revealed that agreement between pathologists in subtyping NSCLC on histology varied from 77% to 94.2%. Considering that, in our department, different pathologists

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**TABLE 1. Correlation of Tumor Subtypes to the Degree of Diagnostic Certainty for Biopsy and Cytology**

| Diagnosis       | Biopsy Total | ADC | SQCC | Cytology Total | ADC | SQCC |
|-----------------|--------------|-----|------|----------------|-----|------|
| Definite        | 101 (44.7)   | 45  | 56   | 133 (59.0)     | 70  | 63   |
| Favored         | 96 (42.4)    | 65  | 31   | 56 (24.7)      | 39  | 17   |
| Unclassified    | 29 (12.9)    | —   | —    | 37 (16.4)      | —   | —    |
| Total           | 226 (100)    | 110 | 87   | 228            | 109 | 80   |

Abbreviations: ADC, adenocarcinoma; SQCC, squamous cell carcinoma.

**TABLE 2. Correlation of Tumor Subtypes Diagnosed by Biopsy Versus Cytology**

| Cytology        | Biopsy ADC | Biopsy SQCC | Biopsy NSCLC, NOS | Total |
|-----------------|------------|-------------|--------------------|-------|
| ADC             | 82         | 11          | 16                 | 109   |
| SQCC            | 7          | 67          | 6                  | 80    |
| NSCLC, NOS      | 21         | 9           | 7                  | 37    |
| Total           | 110        | 87          | 29                 | 226   |

Abbreviations: ADC, adenocarcinoma; NSCLC, NOS, non-small cell lung carcinoma not otherwise specified; SQCC, squamous cell carcinoma.

*This category includes both definite and favored diagnoses.*
report the biopsy and the cytology specimens, a 89.2% concordance between biopsy and cytology specimens is very good. This high concordance despite no formal prospective comparison between cytology and biopsy specimens may be because all of pathologists who report the cytologic specimens have subspecialty training in cytopathology; also, discussions do occur between the cytopathologist and the pulmonary pathologist whenever a challenging or problematic case is encountered. It is noteworthy that definitive diagnoses were issued more often in cytologic than in biopsy specimens (59% vs 45%, respectively). IHC was used in 47% of biopsies and in only 13% of the cytologic specimens, a range similar to that reported in another study (31% vs 7%). Despite this less frequent use of IHC in cytology, approximately the same percentage of NSCLC could be subtyped by cytology as in the biopsy specimens. It is possible that better preservation of morphology and the use of more stains—the Papanicolaou stain in addition to the hematoxylin and eosin stain used for the cell blocks—in cytologic specimens may explain the lower need for IHC to identify the tumor type when using this method; in addition, the frequent lack of cell block in the exfoliative cytologic specimens contributed to this less frequent use of IHC in cytology specimens.

### TABLE 3. Diagnoses of the 12 Follow-Up Resection Specimens

| Case | Diagnosis on Biopsy | Diagnosis on Cytology | Diagnosis on Follow-Up Surgical Resection |
|------|---------------------|-----------------------|-------------------------------------------|
| 1    | NSCLC               | FADC                  | Large cell carcinoma, rhabdoid type       |
| 2    | ADC                 | ADC                   | Metastatic ADC in brain                   |
| 3    | SQCC                | SQCC                  | SQCC*                                    |
| 4    | FSQCC               | SQCC                  | Poorly differentiated SQCC               |
| 5    | Adenosquamous       | FADC                  | Poorly differentiated ADC                 |
| 6    | ADC                 | ADC                   | ADC                                      |
| 7    | ADC                 | ADC                   | Moderately differentiated ADC             |
| 8    | NSCLC               | FSQCC                 | Poorly differentiated SQCC               |
| 9    | FSQCC               | SQCC                  | SQCC                                      |
| 10   | SQCC                | SQCC                  | Poorly differentiated SQCC               |
| 11   | SQCC                | SQCC                  | Moderately differentiated SQCC            |
| 12   | FSQCC               | SQCC                  | SQCC                                      |

Abbreviations: ADC, adenocarcinoma; FADC, non-small cell lung carcinoma favor adenocarcinoma; FSQCC, non-small cell lung carcinoma favor squamous cell carcinoma; NSCLC, non-small cell lung carcinoma; SQCC, squamous cell carcinoma.

*The follow-up resection specimen for this case was from the autopsy.

### TABLE 4. Review of the 18 Cases With Discrepant Biopsy and Cytology Diagnoses

| Case | Biopsy Method | Cytology Method | Biopsy Dx | Cytology Dx | Final Dx | Modality With Correct Dx | Method of Final Dx | Contributing Factors to Discrepancy |
|------|---------------|-----------------|-----------|-------------|----------|--------------------------|-------------------|-------------------------------------|
| 1    | BB            | BW              | FADC      | FSQCC       | FADC     | Biopsy                   | IHC               | Scant cellularity, absent CB        |
| 2    | TTB           | FNA             | ADC       | FSQCC       | ADC      | Biopsy                   | Consensus         | Interpretation                      |
| 3    | TTB           | FNA             | ADC       | SQCC        | SQCC     | Cytology                 | Consensus         | Interpretation                      |
| 4    | BB            | BW              | SQCC      | FADC        | SQCC     | Biopsy                   | IHC               | Interpretation                      |
| 5    | TTB           | FNA             | SQCC      | ADC         | ADC      | Cytology                 | Resection         | Poorly differentiated, necrosis     |
| 6    | TTB           | FNA             | FADC      | SQCC        | SQCC     | Cytology                 | Resection         | Poorly differentiated, necrosis     |
| 7    | BB            | BW              | FSQCC     | FADC        | FSQCC    | Biopsy                   | IHC               | Scant cellularity, no CB            |
| 9    | TTB           | FNA             | FADC      | SQCC        | ADC      | Biopsy                   | IHC               | Interpretation                      |
| 10   | BB            | BW              | FSQCC     | ADC         | NSCLC    | None                     | Consensus         | Scant cellularity, noncontributory IHC |
| 11   | BB            | BW              | FADC      | FSQCC       | NSCLC    | None                     | Consensus         | Scant cellularity, noncontributory IHC |
| 12   | BB            | BW              | SQCC      | FADC        | SQCC     | Biopsy                   | Consensus         | Scant cellularity, no CB            |
| 13   | TTB           | FNA             | FADC      | SQCC        | NSCLC    | None                     | Consensus         | Poorly differentiated, noncontributory IHC |
| 14   | BB            | BW              | FSQCC     | ADC         | NSCLC    | None                     | Consensus         | Scant cellularity, no CB, noncontributory IHC |
| 15   | BB            | BW              | FSQCC     | ADC         | ADC      | Cytology                 | Consensus         | Noncontributory IHC                 |
| 16   | BB            | BW              | FSQCC     | ADC         | ADC      | Cytology                 | Consensus         | Scant cellularity, noncontributory IHC |
| 17   | BB            | BW              | SQCC      | ADC         | NSCLC    | None                     | Consensus         | Scant cellularity, no CB            |
| 18   | BB            | BW              | SQCC      | ADC         | NSCLC    | None                     | Consensus         | Poorly differentiated, no CB        |

Abbreviations: ADC, adenocarcinoma; BB, bronchial brushing; BW, bronchial washing; CB, cell block; Dx, diagnosis; FADC, favor adenocarcinoma; FNA, fine-needle aspiration; FSQCC, favor squamous cell carcinoma; IHC, immunohistochemistry; LCC, large cell carcinoma; NSCLC, non-small cell lung cancer; SQCC, squamous cell carcinoma; TTB, transthoracic biopsy.
Discrepancies between cytology and biopsy are important to recognize and review, because they can lead to confusion in the decision-making process for patient management. In our study, the significant contributing factors that we identified after review of the discrepant cytologic-histologic pairs (as listed in decreasing order of frequency) were scant cellularity, absence of cell block in cytologic specimens, equivocal IHC, poor differentiation, and necrosis. Factors that reportedly contributed to discrepancies in other studies included sampling problems,
tumor heterogeneity, tumor necrosis, artifactual cytoplasmic vacuolization in poorly differentiated SQCC, and poor differentiation.\textsuperscript{10,11,15}

The rate of unclassified NSCLC when taking into consideration both cytology and biopsy was very low (3%) in our study, a value very close to that in a similar study by Sigel et al (4%).\textsuperscript{11} In contrast, it is important to note that, in our series, subtyping of NSCLC was not possible in 12.8% of biopsy specimens and 16.3% of cytology specimens when considered alone. These results highlight the importance of obtaining both cytologic and biopsy specimens when investigating patients who have suspected lung cancer, because both perform better together than either alone.

We anticipate that our rate of unclassified NSCLC will likely be reduced in the future, because the results obtained from our study made us realize that cell block preparation was underused in our department for exfoliative cytology in contrast to fine-needle aspirates. Indeed, since this study was concluded, cell block preparation for pulmonary exfoliative specimens has been encouraged in the cytopathology preparatory laboratory, already resulting in a notably increased number of cell blocks in exfoliative specimens. However, we have not accrued a sufficient number of cases to generate meaningful data to include in the current study; therefore, this will be the subject of a future study.

Conclusions

Subtyping of NSCLC can be achieved in most cases by considering findings in both biopsy and cytology specimens, highlighting the importance of obtaining both types of specimens when investigating patients with suspected lung cancer. Concordance in subtyping between cytology and biopsy specimens can be reached in a high percentage of cases. Scantiness of malignant cells, necrosis, poor differentiation, absence of a cytology cell block, and equivocal IHC are the most common reasons for discordance in subtyping. In poorly differentiated or suboptimal specimens, IHC and comparison of cytology and biopsy specimens can help in subclassification.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Mojgan Ebrahimi: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft, and visualization. Manon Auger: Conceptualization, methodology, validation, investigation, writing—original draft, writing—review and editing, supervision, and project administration. Sungmi Jung: Conceptualization, methodology, investigation, and writing—review and editing. Richard S. Fraser: Conceptualization, methodology, validation, investigation, writing—original draft, writing—review and editing, and supervision.

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