Accuracy of Fine Needle Aspiration Cytology in the Pathological Typing of Non-small Cell Lung Cancer

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Background: Histological typing of non-small cell lung cancer (NSCLC) has an increasing clinical relevance due to the emerging differences in medical treatment between squamous and nonsquamous tumors. However, most NSCLCs are diagnosed in an advanced stage, and the diagnosis is often obtained exclusively by cytology either exfoliative or following fine needle aspiration. We investigated the accuracy of fine needle aspiration cytology (FNAC) in NSCLC typing as compared with histology.

Methods: Over the period 2000–2009, 1182 transbronchial needle aspirate or transthoracic needle aspirate samples were obtained from patients with suspicious thoracic lesions. In 474 patients, a cytological diagnosis of primary NSCLC was obtained, and 186 (39%) of them (108 transbronchial needle aspirates and 78 transthoracic needle aspirates) received a parallel or subsequent histologic diagnosis on endoscopic biopsy (112) or surgery (74).

Results: At cytology, 158 (85%) NSCLC cases were typed (89 adenocarcinoma and 69 squamous cell carcinoma), while 28 (15%) were classified as NSCLC not otherwise specified. At histology, 183 (98%) cases were typed (109 adenocarcinoma, 69 squamous cell carcinoma, 3 adenosquamous carcinoma, and 2 large cell carcinoma), and only 3 (2%) were classified as NSCLC not otherwise specified. Cytological and histological typing was concordant in 137 of 156 (88%) cases ($K = 0.755$; $p < 0.001$). The positive predictive value of FNAC in typing NSCLC was 92% for adenocarcinoma and 82% for squamous cell carcinoma.

Conclusion: FNAC in expert hands is fairly accurate for typing NSCLC and can be regarded as an acceptable procedure for diagnostic and medical treatment planning purposes in most NSCLC cases, especially when more invasive approaches are unfeasible. In poorly differentiated and doubtful cases, the use of ancillary techniques, such as immunocytochemistry, may be required to improve the diagnostic yield.

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Lung cancer is the leading cause of cancer death both in men and in women worldwide.[1] Non-small cell lung cancer (NSCLC) accounts for about 80 to 85% of all lung cancers and is classified according to the World Health Organization criteria into three major types: adenocarcinoma (50%), squamous cell carcinoma (30–35%), and large cell carcinoma (5–10%).[2]

Historically, NSCLC typing has not been considered relevant for treatment planning. Therefore, a significant proportion of NSCLCs used to be reported as NSCLC not otherwise specified (NSCLC-NOS), particularly on cytology or small biopsy samples.

More recently, tumor histotype has emerged as a critical variable in clinical decision making.[3] Prospective randomized studies have shown that new chemotherapeutic (i.e., pemetrexed) and molecular-targeted agents (i.e., gefitinib, erlotinib, and bevacizumab) may lead to improved results, as compared with prior standard therapeutic options, in nonsquamous advanced lung carcinoma.[4–11] Therefore, there is an increasing demand for pathologists to differentiate between squamous and nonsquamous NSCLC tumors. As most lung cancer patients present at diagnosis in an advanced unresectable stage, small biopsies or cytological samples are frequently the only available material for diagnosis.

Exfoliative cytology obtained at the time of bronchoscopy significantly increases the sensitivity and accuracy of the diagnosis of lung cancer, but the diagnostic yield is high only for central and endobronchial lesions.[12] Fine needle aspiration cytology (FNAC), either transthoracic needle aspiration (TTNA) or transbronchial needle aspiration (TBNA), is often used for the diagnosis of peripheral lung nodules and hilar-mediastinal lymph nodes. Small cell lung cancer and NSCLC can be consistently and accurately diagnosed by FNAC,[13] whereas its role in correctly typing NSCLC tumors is still debated.[14]

The aim of this study was to evaluate the accuracy of FNAC in differentiating NSCLCs of squamous from nonsquamous histology type.

PATIENTS AND METHODS

Patients and Study Design

Cases were derived from a single-institution database of TBNA and TTNA samples performed for diagnostic purposes in patients with a thoracic lesion. From 2000 to 2009, a total of 1182 thoracic FNACs were performed: 708 cases were not diagnosed as NSCLC (331 negative for neoplastic cells, 114 small cell lung cancers, 76 metastatic lesions, 75 inadequate samples, 19 recurrences of primary lung cancer, 17 lymphomas, 14 carcinoids, 14 mesenchymal tumors, and 48 others), while 474 cases yielded a cytologic diagnosis of NSCLC, with or without an indication of a specific histotype. Among these, 186 cases had a concurrent or subsequent histological diagnosis and were considered for the study. All cytological and histological slides were retrieved and reviewed for the study in a blinded fashion.

Cytological Diagnosis

TBNA was performed in patients with hilar-mediastinal lymph nodes or submucosal and/or peribronchial lesions using a 22-gauge needle connected to a flexible bronchovideoscope. Computed tomography-guided TTNA was performed in patients with peripheral nodules using a 22-gauge needle connected to a pistol for aspiration. Both these procedures were performed with rapid on-site examination of specimens for evaluating the adequacy of sampling. The smears were stained with May-Grunwald-Giemsa staining plus Papanicolaou and/or periodic acid-Schiff (PAS) stainings based on morphology and examined by two experienced cytologists (R.N., A.G.). Differential diagnosis between squamous and nonsquamous tumors was based on the criteria detailed in Table 1.[15,16] The cytologists reviewed the 186 cases independently from the pathologists who evaluated the histology (G.R., E.M.S.) and without any knowledge of their diagnosis. Discrepant interpretations between the cytologists were resolved by consensus.

TABLE 1. Morphological Criteria Used on Fine Needle Aspiration Cytological Samples for Differentiating Between Adenocarcinoma and Squamous Cell Lung Carcinoma[15,16]

	Adenocarcinoma	Squamous Cell Carcinoma
Background	Cell debris, foamy macrophages	Necrosis
Cell distribution	Small aggregates	Individually dispersed (in the background)
Architecture	Glandular, acinar, papillary	Solid, trabecular
Cells groups	Morulae	Pearl formations
Cell membrane	Poorly defined	Well defined
Cytoplasm	Scanty, vacuolated	Large, dense, keratinized
Nuclei	Round-oval, lightly stained	Irregular, hyperchromatic
Nucleoli	Prominent (well differentiated)	Inconspicuous (keratinized) prominent (nonkeratinized)
Nuclear pseudoinclusions	Present	Absent

Histological Diagnosis

Histological samples included 74 surgical resections and 112 endoscopic biopsies. Histology was evaluated on standard Hematoxylin and Eosin (H&E) and PAS-Alcians stains in all specimens. All cases were independently reviewed by two experienced pathologists (G.R., E.M.S.) blinded to the original diagnosis. Discrepant interpretations were observed only for small endoscopic biopsies and were resolved by consensus or with the aid of immunostains. All cases labeled as NSCLC-NOS in the original diagnoses were immunostained.

A panel of immunohistochemical (IHC) markers was used, including TTF-1 (thyroid transcription factor-1), CK-7 (cytokeratin-7), HMW-CKs (high-molecular-weight cytokeratins), and p63, according to standard procedures. TTF-1/CK-7 positive and HMW-CKs/p63 negative cases were considered consistent with adenocarcinoma, whereas the complementary staining pattern favored a squamous cell carcinoma. Divergent or unreliable immunostaining results were not considered for the diagnosis that was exclusively based on morphology.

Statistical Analysis

The diagnostic agreement between cytology and histology was calculated as the ratio of concordant cases to total cases. Kappa statistics was used to assess the level of agreement; Kappa values ranging from 0.61 to 0.8 were assumed to indicate a very good agreement. Statistical significance was considered for p values <0.05.

RESULTS

One hundred eighty-six FNAC cases (108 TBNAs and 78 TTNAs) diagnosed as NSCLC, having a paired concomitant or subsequent histologic diagnosis on endoscopic biopsies (112) or surgical specimen (74), were included into the study.

FNAC allowed tumor typing in 158 cases (85%), 89 (56%) adenocarcinomas and 69 (44%) squamous cell carcinomas. Twenty-eight cases (15%) were diagnosed as NSCLC-NOS because cytological features of a specific histotype could not be identified (Table 2). Cytological diagnosis was performed in 58 cases on slides with May-Grunwald-Giemsa routine staining, while 39 cases had also Papanicolaou staining, 65 PAS, and 24 both of them.

TABLE 2. Distribution of NSCLC Typing Diagnosis

	Cytology	Histology
Adenocarcinoma	89 (56)	109 (59)
Squamous cell carcinoma	69 (44)	69 (38)
Large cell carcinoma	—	2 (1)
Adenosquamous carcinoma	—	3 (2)
Total	158 (85)	183 (98)
NSCLC-NOS	28 (15)	3 (2)

Values are given as n (%). A total of 186 patients had cytological and histological diagnosis of NSCLC.

NSCLC-NOS, non-small cell lung cancer not otherwise specified.

At histology, 183 cases (98%) were typed: 109 (59%) adenocarcinomas, 69 (38%) squamous cell carcinomas, 3 (2%) adenosquamous, and 2 (1%) large cell carcinomas (Table 2). Immunostains were performed in 40 (22%) endoscopic specimens, including all cases originally labeled as NSCLC-NOS and cases with discordant cytological and histological diagnoses. Immunostains allowed tumor typing in 28 cases. In nine other cases, the IHC profile was not consistent with a specific histotype or was poorly interpretable and typing was based on morphology. At the end, only three (2%) cases were considered as NSCLC-NOS mainly due to limited or necrotic tissue.

Cytological and histological diagnoses were pair-matched to obtain agreement estimates. The comparison between cytological and histological corresponding types was feasible in 156 cases, excluding 29 cases reported as NSCLC-NOS (28 cases by cytology and 3 by histology, 2 of which were NOS by both methods) and one case diagnosed as adenosquamous carcinoma at histology (Table 3). Notably, one case classified as NSCLC-NOS at histology had been identified as squamous carcinoma at cytology; the main reason for this peculiar result can be ascribed to the lack of cellular material on the small biopsy and the evidence of abundant dense cytoplasm without glandular formation on the cytologic smear.

Agreement between cytological and histological typing was found in 137 of 156 (88%) cases ($K = 0.755$; $p < 0.001$). Concordant diagnoses included 55 squamous cell carcinomas and 82 adenocarcinomas (Table 4). Twelve cases classified as squamous cell carcinoma at cytology were diagnosed as adenocarcinoma at histology (Figures 1*A*, *B*; Tables 3 and 4), whereas seven cases classified as adenocarcinoma at cytology resulted squamous cell carcinoma at histology (Figures 2*A*, *B*; Tables 3 and 4). Of the 28 NSCLC-NOS cases at cytology, histologic typing was obtained in 26 cases, 9 of which with the aid of IHC (Table 3).

TABLE 3. Cytological Diagnosis in 186 FNAs from NSCLC with Corresponding Histological Diagnosis

Cytology/Histology	SQC	ADC	LCC	ADSQC	NOS	Total
SQC	55	12	—	1	1	69
ADC	7	82	—	—	—	89
NOS	7	15	2	2	2	28
Total	69	109	2	3	3	186

SQC, squamous cell carcinoma; ADC, adenocarcinoma; LCC, large cell carcinoma; ADSQC, adenosquamous carcinoma; NOS, not otherwise specified.

TABLE 4. Comparison Between Cytological and Histological Type Definition (Adenocarcinomas vs. Squamous Tumors)

Cytology/Histology	ADC	SQC	Total
ADC	82	7	89
SQC	12	55	67
Total	94	62	156

ADC, adenocarcinoma; SQC, squamous cell carcinoma.

Positive predictive value of FNAC in NSCLC typing was 92% (82 of 89) for adenocarcinomas and 82% (55 of 67) for squamous cell carcinomas (Table 4). Sensitivity, specificity, and positive and negative predictive values of cytology for a diagnosis of adenocarcinomas were 87%, 89%, 92%, and 82%, respectively.

DISCUSSION

For clinical purposes, lung cancer has been historically classified into small cell and non-small cell types; this time-honored practice is no longer tenable, given the advent of new drugs with indications to specific NSCLC types.[4–11]

Differentiating between squamous and nonsquamous tumors may be challenging when limited material is available such as in FNAC samples or in bronchoscopic biopsies. However, as only 25 to 30% of tumors are resected, these types of specimen represent the source of tissue diagnosis in most patients.[16,17] In this setting, the issue of accuracy of the cytological diagnosis is critical.

Several studies have addressed the diagnostic agreement between endoscopic biopsies and resection specimens and have provided reassuring figures of concordance for most histotypes.[17–20] The literature is scarcer when it comes to the accuracy of cytology.[14,21,22]

The results of this study indicate that FNAC allows typing of NSCLCs with high accuracy, considering the agreement with histology (88%) and the positive predictive value for squamous and nonsquamous histotype (82% and 92%, respectively). These figures compare favorably with those reported in similar studies that indicated a value of 45%[17] and 60%.[21] In our series, the accuracy of FNAC may be especially high due to the experience of the cytologists and their habitude to work in a multidisciplinary team.

The diagnostic yield of FNAC should be compared with that of the corresponding histology that allowed typing in 98% of cases in the study. The high proportion (40%) of surgical specimens included in the series and the use of IHC staining to aid in the morphological interpretation are likely explanation for these results. It should also be considered that for the purpose of the study, the pathologists were asked to express a judgment on the NSCLC histotypes also in the nine cases in which the IHC profile was not entirely consistent or was poorly interpretable. Adding the latter cases to the three NSCLC-NOS, a 6% (12 of 186) proportion of NSCLC-NOS is obtained that is consistent with a recent study on bronchial biopsies.[20]

The reasons to explain discrepancies between cytological and histological diagnosis (19 of 156 cases) are manifold. Sampling bias and tumor heterogeneity are a likely cause for the misdiagnosis of adenocarcinoma as squamous cell carcinoma due to the failure of detecting keratinized cells. The sampling or smearing techniques and the tumor necrosis may also induce artifactual vacuolization or pseudoacinar structures that contribute to a proportion of misdiagnoses. Poor cell differentiation with large eosinophylic cytoplasms, sheet-like aggregation of cells, and necrosis are the most likely causes of adenocarcinomas being diagnosed as squamous cell

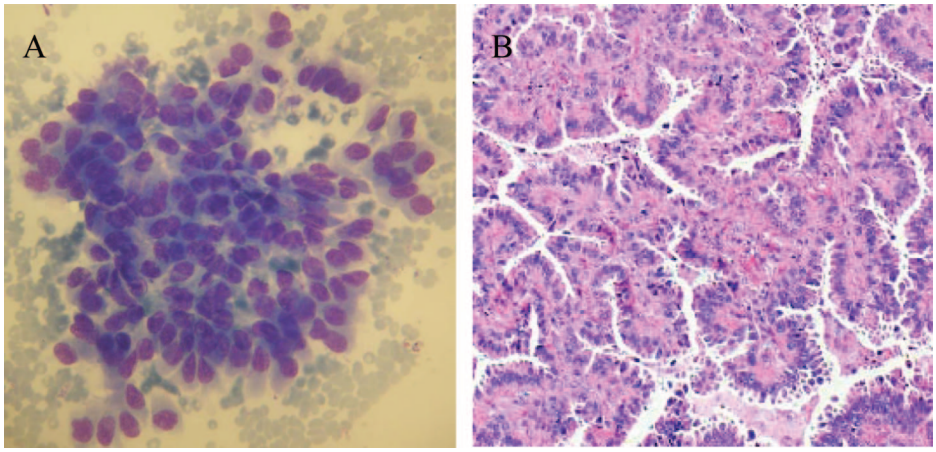

FIGURE 1. A case of misleading squamous differentiation on fine needle aspirate (*A*, May-Grunwald-Giemsa; ×10) resulted adenocarcinoma on corresponding tissue section (*B*, Hematoxylin and Eosin; ×20).

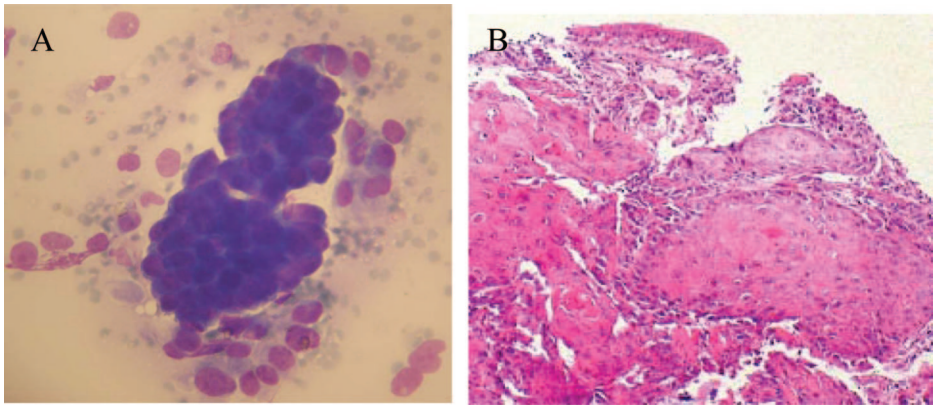

FIGURE 2. A case of glandular-like feature on fine needle aspirate (*A*, May-Grunwald-Giemsa; ×10) resulted squamous carcinoma on corresponding tissue section (*B*, Hematoxylin and Eosin; ×10).

carcinomas. The same features are also challenging for diagnosis in small biopsy samples where IHC is most indicated.

Histology was considered as the gold standard; however, it should be acknowledged that its diagnostic reproducibility is suboptimal. Figures of interobserver agreement in the diagnosis of squamous versus nonsquamous histology using the 2004 World Health Organization criteria were recently reported.[23] In this study, 12 expert pulmonary pathologists and 12 community pathologists were asked to evaluate the same digital images from 96 primary NSCLC resections. The *K* value for overall agreement among all pathologists was 0.55, while for expert pathologists $K = 0.64$ and for community pathologists $K = 0.41$, evidencing a relevant risk of misdiagnosis using morphology alone.

The diagnosis of large cell carcinoma deserves specific discussion as this is a controversial and likely heterogeneous entity in terms of morphological and immunophenotype features.[16,24]

To improve diagnostic accuracy of FNAC, especially in poorly differentiated and doubtful tumors, it might be useful to integrate morphology with validated ancillary techniques, such as immunohistochemistry.[16,25] An increasing number of reports has addressed the use and validation of antibody markers, such as TTF-1, CK-7, p63, HMW-CKs, and desmocollin-3, in the subtyping of NSCLCs.[20,21,26,27]

Khayyata et al. investigated a IHC panel made of TTF-1, CK-7, CK-20, p63, and CK-5/6 in differentiating NSCLC types from 53 FNA specimens compared with surgical samples. Using only cytomorphologic criteria, the concordance between cytologic and histologic diagnosis was 66% for adenocarcinoma and 53% for squamous cell carcinoma but increased when IHC was included in the diagnostic algorithm.[21] Nicholson et al. showed that 53% of the cases (17 of 32 small biopsies or cytological samples) were classified as NSCLC-NOS after the initial light microscopic review, while after special stains (TTF-1, p63, CK-5/6, and PAS), only 6 of the 32 (19%) remained unclassified.[26]

In conclusion, our data indicate that FNAC, in expert hands, is fairly sensitive and accurate in typing NSCLCs and can be regarded as an acceptable procedure for diagnosis and treatment planning, especially when other more invasive approaches are unfeasible. Poorly differentiated and doubtful cases might benefit of ancillary techniques, such as validated immunocytochemical panels, to refine type diagnosis. The role of immunocytochemistry to improve sensitivity and accuracy of conventional cytology should be further investigated.

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