Lung Cancer Cytology: Can Any of the Cytological Methods Replace Histopathology?

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Abstract

Background: Diagnosis of lung cancer can be made in two ways: histopathological and cytopathological. Cytological methods in the diagnosis of lung lesions are generally thought to be one of the most successful tactics. Aims: This study aimed at comparing the efficiency of selected cytological techniques in lung lesions by correlating them with histopathological diagnosis. In addition, we had answered the question whether any of the cytological methods can replace histopathology. Materials and Methods: The study group consisted of 633 patients and 1085 cytological specimens. Cytology samples included: induced sputum, bronchial washing (BW), bronchial brushing (BB), fine needle aspiration (FNA), and cell block (CB). In every case of CB immunocytochemistry (ICC) was performed. For each cytological method sensitivity, specificity, effectiveness, positive predictive value, and negative predictive value were assessed. Results: BW and BB showed the lowest diagnostic parameters. The most valuable diagnostic procedure was CB based on FNA. Close by CB, FNA had the highest diagnostic rate. However, possibility to evaluate tumor cell structure and apply the ICC, give CB an advantage over FNA. Using only morphologic criteria, we had subclassified nonsmall-cell lung cancer (NSCLC) into squamous cell carcinoma (SCC) and adenocarcinoma (AC) as 60.04% of SCC and 32.52% of AC. The use of CB and ICC decreased the NSCLC diagnoses from 22.1% to 2.8% while the percentage of AC and SCC diagnoses increased from 4.11% to 12.64% and from 6.64% to 11.06%, respectively. Metastatic lung tumors were diagnosed based on both the cell morphology and according to the ICC results. Conclusion: Despite the limitations of the cytological procedures, we recommend using CB and ICC to evaluate cytopathological samples derived from FNA. It can in many cases replace a conventional histopathology.

Keywords: Bronchial brushing, bronchial washing, cell block, cytology, fine needle aspiration, immunocytochemistry, lung cancer

INTRODUCTION

Lung cancer is one of the most common cancer and the leading cause of death in the developed countries. Improving the detection rate of early stage lung cancer is necessary for improving the prognosis of this neoplasm. Diagnosis of lung cancer can be made in two ways: histopathological and cytopathological. Increasingly cytology specimens are being used for primary diagnosis of lung malignancies and to perform ancillary studies. Cytological methods in the diagnosis of benign and malignant lesions of the lungs are one of the most successful tactics. Thanks to the flexible fiberoptic bronchoscope various sampling techniques are available to receive specimens for cytological evaluation.

The aim of our work was to compare the efficiency of selected cytological techniques in the lung lesions by correlating them with histopathological diagnosis. In addition, we had answered the question whether any of the cytological methods can replace histopathology.

MATERIALS AND METHODS

In our study, out of all clinical or radiological suspected cases of lung malignancy from February 2017 to April 2019, 633 patients were selected with at least one of five cytology examinations performed. Histopathological examination was considered as the gold standard method. If the result of histopathological examination was not available, we had considered cell block (CB) based on fine needle aspiration (FNA) as a reference diagnostic method. This decision had been made

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based on the results of our study. The cytology samples were obtained with the help of flexible fiberoptic bronchoscopy. Bronchoscopy specimens included: induced sputum, bronchial washing (BW), bronchial brushing (BB), endobronchial ultrasound-guided or transesophageal FNA, and CB based on FNA. Immunocytochemistry (ICC) was performed in every case of CB. Antibodies for the following molecules were used in the differential diagnosis of non-small-cell lung cancer (NSCLC): p40, p63, cytokeratin (CK) 5/6, CK7, thyroid transcription factor-1 (TTF-1) and napsin A. Neuroendocrine markers (CD56, chromogranin A, and synaptophysin), thyroid transcription factor-1 (TTF-1), and Ki-67 were useful indices of neuroendocrine tumors. If the metastatic tumor was suspected, pathologist had decided which additional antibodies should be used depending upon the individual clinical case. All of the cases were diagnosed by two cytopathologists. Smears and CBs with malignant cells were regarded as positive. Smears and CBs with no tumor cells or only a slight amount of cells with nuclear atypia were regarded as negative. Cases with suspicious tumor cells, but with insufficient quantity to make a diagnosis were not included in our study.

All analyses were performed using STATISTICA 13 software (Statsoft, USA). Sensitivity, specificity, diagnostic accuracy (effectiveness), positive predictive value (PPV), and negative predictive value (NPV) for each cytological method were assessed. Quantitative data was presented as mean ± SD for variables with normal distribution or median with interquartile range for non-normal distributions. Normality of distribution was assessed using Shapiro–Wilk W-test. Mann–Whitney U-test was performed to compare quantitative variables. P values of <0.05 were considered to be statistically significant.

**Results**

In our study of 633 patients with clinical or radiological suspicion of lung malignancy, 233 (36.8%) were females, whereas 400 (63.2%) were males. Their age ranged between 21 and 87 years. There was no significant differences between mean age of females and males (65.1 ± 8.5 years vs. 65.3 ± 10.7 years). Maximum cases were seen in the seventh decade of life, both among women and men. The study group consisted of 1085 cytological specimens. Distribution of the diagnostic procedures was listed in Table 1. All five techniques were used simultaneously in none of the patients. In 12 patients (1.9%) four types, in 30 patients (4.7%) three types while in 356 patients (56.2%) only two types of specimens were received. The most common combination of techniques was FNA and CB.

In 429 (67.8%) patients, and thus in 643 (59.3%) cytological specimens histopathology was considered as the gold standard method. Among them, 243 (56.6%) patients were positive and 186 (43.4%) patients were negative for malignancy. Among cases positive for malignancy 82 (33.7%) were females and 161 (66.3%) were males. The patients with malignant lesions were significantly older than patients with benign lesions (66.6 ± 7.8 years vs. 61.5 ± 12.1 years) (P < 0.0005).

The presented study showed the highest number of patients were with squamous cell carcinoma (SCC) (34.57%) and adenocarcinoma (AC) (34.57%), followed by small cell carcinoma (SCLC) (9.47%). The negative specimens were diagnosed as acute inflammatory lesions, chronic nonspecific inflammatory lesions, and chronic granulomatous lesions.

Sputum cytology was performed only in eight patients - all results were negative for malignancy, although histopathology revealed three in eight positive diagnoses.

Comparison of the test results of BW, BB, FNA, and CB was presented in Table 2. BW and BB showed the lowest diagnostic parameters, however diagnostic effectiveness of BB was better compared to BW. Interestingly, combination of the above methods did not show more sensitivity as compared to usage of individual method. Among all bronchoscopic procedures, CB based on FNA was the most valuable diagnostic procedure. Its sensitivity was 88.76%, specificity 100.00%, diagnostic accuracy 90.57%, PPV 100.00%, and NPV 62.96%. Due to such high values we had decided to use the CB as a gold standard diagnostic test especially in cases with inaccessible histopathological result. Hence, CB was used as a reference diagnostic method in case of 306 (48.3%) patients and in 338 (43.3%) cytological specimens. Table 3 compares diagnostic rate between various cytological techniques with CB as a reference point. Close by CB, FNA had the highest diagnostic rate among all cytological methods. Interestingly, the evaluated diagnostic parameters were almost the same in the cases of FNA and CB.

| Table 1: Types of cytological specimen under the study group |
|---------------------------------------------------------------|
| **Nature of specimen** | **Total number (n)** | **Percentage (%)** |
| Sputum | 8 | 0.73 |
| BW | 312 | 28.76 |
| BB | 83 | 7.65 |
| FNA | 376 | 34.65 |
| CB | 306 | 28.20 |
| **Total** | **1085** | **100** |

| Table 2: Comparative rate between BW, BB, FNA, and CB diagnosis and final histologic diagnosis |
|-------------------------------------------------------------------------------------------------|
| **BW (%)** | **BB (%)** | **FNA (%)** | **CB (%)** |
| Sensitivity | 23.25 | 32.14 | 87.41 | 88.76 |
| Specificity | 100.00 | 100.00 | 97.50 | 100.00 |
| Diagnostic accuracy | 65.74 | 74.00 | 89.71 | 90.57 |
| PPV | 100 | 100.00 | 99.16 | 100.00 |
| NPV | 61.78 | 70.31 | 69.64 | 63.00 |

BB - Bronchial brushing, BW - Bronchial washing, CB - Cell block, FNA - Fine needle aspiration
Table 3: Comparative rate between BW, BB, FNA diagnosis, and final CB diagnosis

|                  | BW (%) | BB (%) | FNA (%) |
|------------------|--------|--------|--------|
| Sensitivity      | 13.64  | 12.50  | 95.92  |
| Specificity      | 100.00 | 100.00 | 96.94  |
| Diagnostic accuracy | 38.71  | 46.15  | 96.26  |
| PPV              | 100.00 | 100.00 | 98.43  |
| NPV              | 32.14  | 41.67  | 92.23  |

BW - Bronchial brushing, BB - Bronchial washing, CB - Cell block, FNA - Fine needle aspiration, NPV - Negative predictive value, PPV - Positive predictive value

Among all cytological techniques, we had encountered false positives only in case of FNA, however, they constituted only 1% of all cases. Among cases in which histopathology had been recognized as the gold standard, 34% of cases were false negatives on BW compared to 26% on BB, 10% on FNA, and 9% on CB. Whereas, in cases with CB as a reference point, false negatives had been observed in 61% of cases on BW, 54% on BB, and only 3% on FNA.

Additionally, we had compared results of subtype diagnoses based on CB and ICC with diagnoses based on smears alone. Using only morphologic criteria we had subclassified NSCLC into 32.52% of AC and 60.04% of SCC. The use of CB and ICC decreased the NSCLC diagnoses from 22.1% to 2.8% of the cases while the percentage of AC and SCC diagnoses increased from 4.11% to 12.64% and from 6.64% to 11.06%, respectively. We had diagnosed SCC when tumor cells were positive for p40, p63, and cytokeratin (CK) 5/6, whereas AC when it was positive for CK7, TTF-1 and napsin A.

We could not distinguish metastatic lung tumors from primary lung tumors based only on smears. After CB and ICC had been performed, 12 metastatic tumors had been categorized as five bowel tumors, three bladder cancers, two breast tumors, one chondrosarcoma, and one nasopharyngeal cancer. Metastatic bowel tumors were positive for CDX-2 and CK20. Metastatic bladder cancers were positive for CK7, uroplakin, and GATA3. Metastatic breast tumors were positive for estrogen receptor (ER), progesterone receptor (PR), anti-gross cystic disease fluid protein (GCDFP15), and mammaglobin. Metastatic chondrosarcoma was positive for S-100, and the metastatic nasopharyngeal carcinoma was positive for epithelial membrane antigen (EMA) and CK AE1/AE3. The ICC for TTF-1 and napsin A were negative in all of these metastatic tumors.

**Discussion**

Nowadays, respiratory tract cytology is accessible throughout the world as an important diagnostic procedure in the evaluation of suspected lung lesions. Thanks to the flexible fiberoptic bronchoscope various sampling techniques with different diagnostic efficiency are available to receive specimens for cytological evaluation of lung lesions: exfoliative (induced sputum), abrasive cytology [bronchoalveolar lavage (BAL), BW, BB], and FNA cytology (endobronchial ultrasound guided, transesophageal). The major goal is using the safest, least invasive, and least costly test. The major and minor complications of bronchoscopy are uncommon (0.5% and 0.8%, respectively).

Sputum cytology is the least reliable method that is increasingly losing its importance in diagnostic process. It has lower sensitivity in case of peripherally located masses (49%) in comparison with central lesions (71%).[11] In the study of Tatar et al. from all 30 patients with primary lung cancer only one patient had positive sputum cytology.[10] Our study also showed that this technique is only occasionally used in the diagnosing process and it is the right approach because of their low diagnostic rate.

BW is a non/minimally invasive, widely accepted, safe and simple technique utilized for diagnosis of pulmonary lesions.[11] The sensitivity of BW is reported between 52.63% and 80.5%, specificity between 80% and 96.6%, and accuracy between 62.06% and 87.3%, respectively. PPV and NPV are found to be between 83.33% and 97% and between 47.05% and 78.4%, respectively.[7,8] In our study sensitivity of BW was definitely lower (23.25%) than in the cited research, but other diagnostic parameters were within the ranges mentioned above. However, because of rather low sensitivity, BW plays a limited role in diagnosing pulmonary lesions. In spite of this limitation, due to its high specificity, it still may be a valuable method that provides significant information in the evaluation of lung pathology.

BB permits sampling of the tracheobronchial tree — visualized mucosal abnormalities or systemic sampling of all segmental bronchi. The advantage of BB is that the surface of the suspicious lesion is scraped using a brush. It manages to remove the cells from the surface of those lesions, which do not exfoliate cells easily. That’s why, the chances of getting adequate diagnostic specimen by BB remarkably increase in comparison to BW samplings.[9] Additionally, BB is better than BW and BAL because the surface of the lesion is scraped and the cells have better preserved morphological details in comparison to the cells, which have already exfoliated into the bronchial cavity.[7,9,10] All these factors contribute in the increased diagnostic efficiency of BB samplings.

In various studies from literature the values of sensitivity, specificity, and overall accuracy of BB are 63.0%—87.3%, 75%–100%, and 65.67%–93.9%, respectively.[14,15] PPV and NPV vary from 95.65% to 100% and from 12% to 80.77%, respectively.[4,10,11] Our results confirmed the better diagnostic effectiveness of BB compared to BW. However, as in the case of BW, in our study the sensitivity of BB (32.14%) was lower than in the cited publications while other diagnostic parameters were consistent with those from the above research.

Some studies suggest that a combined method of BB and BW has shown more sensitivity as compared to the usage of individual method.[7,9,10] This observation is not in concordance.
with ours—we had not observed that the combination of both methods gives better diagnostic results.

FNA involves sampling the lesion with the help of a fine needle. The samples can be obtained by ultrasonography, computed tomography-guided transthoracic or transbronchial approach. In the literature FNA shows sensitivity, specificity, and overall accuracy as 84%-96.29%, 95.45%-100%, and 89.55%-95.91%, respectively.[10,12,17,18] The PPV is reported close to 100% (91.66%-100%) and NPV between 36.36% and 84%.[4,10,11] The sensitivity and specificity for detecting lung tumors by FNA cytology is better than that of BW/BAL and BB cytology. This is due to the reason that BB and BW aspirate superficial exfoliated cells, which often show extensive necrosis while FNA obtains material from the depth of the lesion showing greater amount of viable cells.[11] In our study almost all diagnostic parameters of FNA were close to 90%-100% and they were significantly higher than these of BW and BB. This high diagnostic rate of FNA cytology in many situations can approach that of conventional histopathology in providing an unequivocal diagnosis, however it still cannot replace it.

Interestingly, the evaluated diagnostic parameters were almost the same in the case of FNA and CB. This is due to the fact that cytological material obtained from FNA is then used to prepare the CB. However, possibility to evaluate the tumor cell structure and apply the ICC are added values of CB and that give it an advantage over FNA.

The cytomorphologic diagnosis of lung malignancies is fraught with numerous mimics and pitfalls, which may lead to false positive or false negative diagnoses. A zero false positive rate may be unobtainable as a false positive rate of approximately 1% is observed even with experienced cytopathologists.[19] We encountered a false positive rate of 1% only in the case of FNA while none of false positives were obtained in cases of BW, BB, and CB. False positives in cytology can be reported due to misinterpretation of smears due to cellular changes in chronic inflammatory disorders, tuberculosis, bronchiectasis, or lung fibrosis.[10] It is essential to know potential pitfalls and mimics in respiratory cytology because false positive diagnoses may result in a significant morbidity or even mortality.[2,21] Because of very unfortunate consequences for patients after false positive diagnosis, some authors advise to undergo repeat instead of overreport of suspicious cases.[20] The best decision if cytology is suspicious for malignant cells, seems to be a repeat biopsy with a clinical correlation with radiological/bronchoscopic findings to rule out malignancy.[10]

On the other hand false negative diagnoses may result in delayed diagnosis and treatment. The reasons of false negatives could have been superadded inflammation, nonrepresentative material, or hypocellular aspirate. The number of cells is the key factor that determines cytology samples success. The absence of malignant cells in smears can be caused by inability to dislodge them from the epithelial surface during bronchoscopy.

In addition, advances in imaging techniques result in the detection of small lesions, and understandably, FNA of these small lesions has a significant risk of missing them, leading to potentially false negative results. Therefore, it is common to get false negative diagnoses on cytology due to the lack of cells in cytological smears.[10] Sareen et al.[10] reported false negatives of 22.22% on BB and 12.50% on FNA, which is comparable with our results with histopathology as a gold standard method (26% and 10%, respectively). Whereas, in cases with CB as a reference point false negatives on BW were significantly higher (54%) while on FNA were lower (3%) than in the cited research.

It should be emphasized that pathological diagnosis depends upon many factors, not only on the pathologist’s knowledge and experience, but also on preanalytical factors starting from clinician’s experience in bronchoscopy, their ability to obtain a representative cytological material in sufficient quantity and quality, thorough receiving and preparing this material in pathology laboratory until the submission of cytopathology slides for interpretation.

Accurately determining lung cancer subtype based only on morphological findings can be difficult due to the anaplasia of lung cancer cells. Due to the therapeutic progress in the lung cancer field and advancement in the understanding of molecular mechanism of lung cancer, pathological diagnosis has increasingly more significant consequences for clinicians. The first step is distinguishing between NSCLC and SCLC. However, nowadays this classification into NSCLC and SCLC is not adequate for management. The second important step is subclassifying NSCLC, especially into SCC and AC. It can be simply made on cytological smears in well differentiated tumors when the characteristic cytomorphologic features are present, but may be a real challenge in poorly differentiated tumors. Unfortunately, most of lung cancer cells are poorly differentiated.[2,21] In our study we had been able to subclassify NSCLC into AC and SCC based on cytological smears alone as 32.52% of AC and 60.04% of SCC. According to different researchers, the diagnosis of AC or SCC could be determined in 50%–70% of patients based only on cytological specimens,[21‑23] however Rekhtman et al.[24] subtyped approximately 93% lung cancer cases into SCC and AC using morphologic criteria alone. If necessary, preferably CB should be used as an adjuvant method to determine the correct diagnosis. CB can facilitate observation of the tumor cell structure in the resected specimens, is helpful for preserving cytological material, and allows for genetic testing and prognostic assessment via polymerase chain reaction (PCR) or fluorescence in situ hybridization (FISH).[25‑28] In doubtful cases ancillary studies, especially ICC stains, may be essential for the subclassification. However, sometimes it may be impossible to accurately subclassify poorly differentiated NSCLC without ICC. Dong et al.[21] showed that after the CBs had been assessed, the rate of positive diagnosis increased from 79.28% to 84.41%, whereas the rate of suspected carcinoma decreased from 6.08% to 1.90%. The use of CB and ICC decreased their NSCLC...
diagnoses from 30.61% to 6.27%. In our study NSCLC diagnosis based on FNA cytology was obtained in 22.12% of cases, whereas it decreased to 2.84% after using CB and ICC. While the percentage of AC and SCC diagnoses increased from 4.11% to 12.64% and from 6.64% to 11.06%, respectively. It confirms the fact that the most important step in avoiding misdiagnosis is to correlate morphological findings with CB and ICC results.

Similarly, differentiating metastatic tumors from primary tumors of the lung based only on smears is difficult, and ancillary ICC can be necessary to assist in narrowing down the primary site, especially, when there is no previous pathology material available for comparison. However, in a few cases, it is almost impossible to distinguish primary lung cancer from metastatic carcinoma without the use of ancillary tests. Similar to us, Dong et al. could not distinguish metastatic lung tumors from primary lung tumors based only on smears. They diagnosed metastatic lung tumors based on the cell morphology and according to the ICC results. We also performed an appropriate ICC panel for each metastatic tumor, which enabled us to identify the primary tumor site of all 12 metastatic neoplasms. Antibodies selection was dependent on the past medical history of each patient.

Although histopathology remains the gold standard diagnostic method, the cytological techniques are safer, economical, and provide quick results. They can be used as a first line diagnostic and management tool. Despite the limitations of the cytological procedures, they can enable diagnosing the lung malignancies at the earliest possible stage before the lesion has reached the stage of a visible and palpable tumor. Especially, CB based on FNA with ICC can provide a precise diagnosis. Thus, we recommend using CB and ICC to evaluate cytological samples derived from FNA. It can in many cases replace a conventional histopathology, facilitate current treatments regimes of lung cancer and thereby decrease its mortality. This will ultimately affect patient’s survival.

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Conflicts of interest
There are no conflicts of interest.

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