Role of imprint cytology in thoracic endoscopy suite

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Abstract

Background: Fiberoptic bronchoscopy and medical thoracoscopy are basic interventional modalities for the diagnosis of a wide variety of pleuropulmonary diseases. In some cases, we need fast and accurate results for decision-making. We aimed to evaluate the diagnostic accuracy of imprint cytology and its added value to the pulmonologist.

Results: Multiple biopsies were taken from 54 patients included 31 patients with lung masses subjected to fiberoptic bronchoscopy and 23 patients with undiagnosed exudative pleural effusion subjected to medical thoracoscopy. Imprint cytology was done to all biopsies which are later examined histopathologically. Regarding fiberoptic bronchoscopy biopsies, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of imprint cytology were 93.33, 100, 100, 33.33, and 93.55%, respectively. While in medical thoracoscopy biopsies, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of imprint cytology were 94.74, 100, 100, 80, and 95.65%, respectively.

Conclusion: Imprint cytology is an easy, rapid, and reliable method that has a high sensitivity and specificity in the diagnosis of lung and pleural malignancies compared with histopathology.

Keywords: Imprint cytology, Histopathology, Lung and pleural malignancies

Background

In order to evaluate the wide variety of pulmonary and pleural diseases, we can use different endoscopic modalities as fiberoptic bronchoscopy and medical thoracoscopy which can provide us with valuable specimens for accurate diagnosis.

Different bronchoscopic techniques are used for the diagnosis of lung cancer such as histological examination of specimens obtained by bronchial biopsies biopsy and cytological examination procedures such as bronchial washing, brushing, and needle aspiration. Some combinations of these techniques have been reported to increase the diagnostic sensitivity for lung cancer compared with that of bronchial biopsy alone [1]. Although the diagnostic yield of endobronchial visible lesions is greater than 90%, the yield of solitary lung lesions without endobronchial abnormalities varies from 18 to 75% [2]. Fiberoptic bronchoscopy when combined with cytology enhances the sensitivity significantly to 88% [3–5].

Pleural disease affects more than 300 people per 100,000 of the population every year, and the patients suffering from pleural malignancy are often present by pleural effusion [6]. The need for the usage of medical thoracoscopy (MT) by pulmonologists is increasing for diagnostic and therapeutic purposes. MT allows direct visualization of the pleura and subsequent biopsy of visually abnormal areas, providing a high diagnostic yield [7]. In malignant pleural effusion, MT provides the option to pleurodese at the time of biopsy via talc poudrage in order to reduce the risk of recurrence. Histopathological results of pleural biopsies are not available at the time of the procedure, and therefore, in order to perform talc poudrage, the physician needs high clinical confidence of a malignant diagnosis [8].

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Imprint cytology is a great step in rapid tissue diagnosis. Besides its speed and technical simplicity, it provides excellent cellular details. A technique for examination of imprints of fresh specimens was first described by Dudgeon and Patrick (1927) [9]. Imprint cytology identifies lesions whether it is malignant or not while the patient is still under anesthesia and with these results, the interventional plans can be modified [10]. It provides valuable information on morphological details of the cell with an immediate result with minimal artifacts, it is cheaper, and so it is most commonly used [11].

The aim of this work was to evaluate to the sensitivity and specificity of imprint cytology of bronchial and pleural biopsies in diagnosing lung and pleural lesions, using histopathological results as a gold standard for diagnosis.

Methods

This prospective cross-sectional observational study was enrolled in Chest Department, Tanta University Hospitals, and Tanta International Teaching Hospital on 54 patients; their age ranges from 34 to 74 years during the period between November 2017 to November 2019. The Ethical Committee of Faculty of Medicine, Tanta University, was approved the research protocol and written informed consents were obtained from all subjects.

The 54 patients were classified into two groups: the bronchoscopic group included 31 patients aged more than 18 years who were subjected to fiberoptic bronchoscopy after clinical and radiological suspicion of bronchogenic carcinoma by both clinical and radiological findings. Patients with uncorrectable coagulopathy, who had oxygen saturation < 90% or severe underlying cardiac disorder (unstable angina pectoris, myocardial infarction in the past 30 days, decompensated heart failure, or untreated arrhythmia), were excluded [12].

The thoracoscopic group included 23 patients with undiagnosed exudative pleural effusion (according to Light’s criteria [13] after cytological, chemical, and bacteriological examination of pleural fluid) subjected to medical thoracoscopy. Patients with cardiovascular instability, PaO2 less than 60 mmHg or PaCO2 more than 50 mmHg contralateral pneumonectomy, severe chronic obstructive pulmonary disease (FEV1 < 1 L or < 35% predicted), pyogenic cutaneous lesion, or extensive pleural adhesions were excluded [14].

| Table 1 Demographic, clinical, and radiological characteristics of the studied patients |
|-----------------|-----------------|-----------------|
|                 | Bronchoscopic group (N = 31) | Thoracoscopic group (N = 23) |
| Age (years)     | 61.51 ± 7.92     | 54.78 ± 7.57     |
| Male/female     | 23/8             | 9/14             |

Clinical presentations, N (%)

- Dyspnea: 27 (87.09%) vs. 23 (100%)
- Hemoptysis: 13 (41.93%) vs. –
- Wheezes: 18 (58.06%) vs. –
- Fever: 3 (9.68%) vs. 7 (30.43%)
- Chest pain: – vs. 13 (41.93%)

CT finding, N (%)

- Lung mass: 14 (45.16%) vs. –
- Lobar collapse: 10 (32.25%) vs. –
- Consolidation: 7 (22.57%) vs. 4 (17.39%)
- Pleural effusion: – vs. 23 (100%)
- Parenchymal infiltrates: – vs. 8 (34.87%)
- Pleural nodularity and masses: – vs. 11 (47.81%)

Fig. 1 Biopsy techniques in the bronchoscopic group
Bronchoscopy
All the bronchoscopies were performed after the administration of IV midazolam 2–2.5 mg. Supplemental doses were given if required: 1 mg at 2–10-min intervals, with a usual maximum total dose of 3.5–7 mg to achieve adequate sedation and spraying lidocaine (2% solution) in the nose and mouth. Arterial oxygen saturation (SaO2) and heart rate were continuously monitored by pulse oximetry. Oxygen was administered through a nasal cannula, and the flow was adjusted upward from 1 L/min to maintain SaO2 greater than 90%. Bronchoscope (Pentax FB 19 TV, Tokyo, Japan, with a 3.2-mm working-channel diameter and 60-cm working length) equipped with biopsy forceps were used. After visualization of the vocal cords, lidocaine (1 mL of a 2% solution) was sprayed as needed. All segments of the bronchial tree were visualized. Five to six biopsies were taken by forceps or cryoprobe (Erbokryo, ERBE Medizintechnik GmbH, Tuebingen, Germany) and were obtained from each patient. From each bit of tissue, an imprint smear was prepared and the rest of the tissue was sent for histopathology [15].

Thoracoscopy
All thoracoscopies were performed in the endoscopy suite using rigid thoracoscopy (Karl Storz; Tuttlingen, Germany) and its equipment and videoscopy unit (camera (Karl Storz Endoskope Telecam Pal 20211020) with light source (Pentax LH-150 PC). Pethidine 100 mg (50 mg IM, 50 mg IV injection) was used to ensure proper control of pain. The vital signs of the patients were monitored (blood pressure, heart rate respiratory rate) during the procedure; also, oxygen saturation was measured by pulse oximetry with spontaneous ventilation during the procedure. Oxygen was provided to maintain oxygen saturation above 90%. The patient was placed in the lateral decubitus position side that will be operated upon up and the arms above the head. The lateral chest wall at the side of entry (4th or 5th intercostals space, midaxillary line) was sterilized with iodopovidone solution. The skin, subcutaneous tissues, periosteum of the ribs, and parietal pleura at the site of entry were infiltrated by lidocaine 2% as local anesthesia. Skin incision about 1 cm was made at the planned thoracoscopic insertion point. Blunt dissection with round-ended scissors was carefully performed through the chest wall.

The rigid thoracoscopy was passed through the trocar port (using 6 mm blunt tip trocar with 8.5 cm working length). The parietal, visceral pleurae and lung were examined. The cupped biopsy forceps were used to obtain multiple (5–10 pleural biopsies) from abnormal pleural nodules, pleural congestion, thickening, adhesion, or ulcers.

At the end of the procedure, a chest tube connected to an underwater seal was inserted and fixed to the skin of the chest wall by a suture [6].

Table 2 Size of biopsies in the bronchoscopic group

| Size (cm) | Biopsy technique | T test | P value |
|-----------|------------------|--------|---------|
| Range     | 0.3–0.9          | 0.9–2.1| −7.319  | <0.001* |
| Mean ± SD | 0.600 ± 0.211    | 1.441 ± 0.384 |        |

Table 3 Imprint and histopathological examination results (HPE) in the bronchoscopic group

| Imprint cytology | HPE+ | HPE− |
|------------------|------|------|
| Imprint cytology+| 28 (93.33%) | 0 |
| Imprint cytology−| 2 (6.67%) | 1 (3.22%) |
| Total (31)       | 30 (96.77%) | 1 (3.22%) |

Final diagnosis
- Squamous cell carcinoma, 17 (54.83%)
- Adenocarcinoma, 11 (35.48%)
- Atypical carcinoid, 2 (6.45%)
- Endobronchial tuberculosis, 1 (3.22%)

Fig. 2 Imprint cytology for bronchogenic adenocarcinoma

Fig. 3 Imprint cytology for bronchogenic squamous cells carcinoma
Imprint cytology

The imprint smear was prepared by imprinting the tissue on a clean surface of a glass slide without compressing the tissue to avoid distorting the shape of the cells. The smears were fixed in 95% alcohol for 5–6 s and then stained by the Papanicolaou stain [16].

The tissue specimens were then placed in formaldehyde solution (10% formalin) for histopathological examination. The smears were read by a pathologist and categorized as negative and positive for malignant cells. Definite differentiation of the type of cells was not done based on smears. Two independent pathologists reported the histopathology and cytology [9].

Statistical analysis

All quantitative data were expressed as mean ± standard deviation. Data were tested by the Mann-Whitney test to evaluate the significance of difference among both groups using SPSS version.20 (Statistical Package for Social Sciences, version 20, IBM). A P value < 0.05 was considered to be statistically significant. In addition, receiver operator characteristic (ROC) curves were designed to assess sensitivity and specificity for the estimated parameters, “Detector Performance Analysis Using ROC Curves - MATLAB & Simulink Example.” www.mathworks.com. Retrieved 11 August 2016.

Results

Demographic, clinical, and radiological characteristics of the studied patients are shown in Table 1. No statistically significant difference was detected between both groups as regard age and sex.

Forcep biopsies were taken from 14 (45.16%) patients, and cryo biopsies were taken from 17 (54.83%) patients (Fig. 1). There was a significant statistical difference between the size of forcep biopsies and the size of cryo biopsies in the bronchoscopic group (Table 2).

Histopathological examination of the biopsy specimens revealed malignant pathology in 30 (96.77%) patients and benign in 1 (3.22%) patient who had endobronchial tuberculosis. Out of 30 malignant cases, 28 (93.33%) biopsies were positive for malignancy on imprint cytology while two (6.67%) biopsies were negative (Table 3; Figs. 2 and 3).

In the bronchoscopic group, receiver-operating characteristic (ROC) curve analysis (Table 4) showed that the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of imprint cytology were 93.3, 100, 100, 33.33, and 95.65%, respectively. These results were in concordant with results of Kawaraya et al. who evaluated various cytological examinations to biopsies obtained by fiberoptic bronchoscopy in the diagnosis of peripheral lung cancer. The sensitivity of the imprint cytology with histological examination was 84.8% [1]. Also, Bhat et al. who studied the correlation between

- Table 4 Receiver operating characteristic (ROC) curve of imprint cytological results in the bronchoscopic group.

| Imprint cytological results | Sensitivity | Specificity | PPV | NPV | Accuracy |
|----------------------------|-------------|-------------|-----|-----|----------|
| 93.3%                      | 100%        | 100%        | 33.33% | 93.55% |

PPV positive predictive value, NPV negative predictive value

- Table 5 Relation between the size of the biopsy specimen and the sensitivity in the bronchoscopic group.

| Size (cm) | Results of imprint cytology | T test | P value |
|-----------|-----------------------------|--------|---------|
| Range     |                             |        |         |
| 0.3–0.9   | Negative (n = 3)            |        |         |
| 0.3–2.1   | Positive (n = 28)           |        |         |
| Mean ± SD | 0.567 ± 0.306               | 1.114 ± 0.523 |        |

- Table 6 Imprint and histopathological examination (HPE) in the thoracoscopic group.

| HPE+     | HPE−  |
|----------|-------|
| Imprint cytology + | 18    | 0     |
| Imprint cytology − | 1     | 4     |
| Total (23) | 19    | 4     |

Final diagnosis

- Metastatic adenocarcinoma, 16 (84.21%)
- Tuberculosis pleurisy, 3 (15.78%)
- Mesothelioma, 3 (15.78%)
- Pleural lipomatosis, 1 (4.34%)

Discussion

The present work aimed at the evaluation of the sensitivity and specificity of imprint cytology of bronchial and pleural biopsies in diagnosing lung and pleural lesions, comparing the results with that of histopathology. In this work, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of imprint cytology in the bronchoscopic group were 93.33, 100, 100, 33.33, and 93.55%, respectively. These results were in concordant with results of Kawaraya et al. who evaluated various cytological examinations to biopsies obtained by fiberoptic bronchoscopy in the diagnosis of peripheral lung cancer. The sensitivity of the imprint cytology with histological examination was 84.8% [1]. Also, Bhat et al. who studied the correlation between
imprint cytology and histopathology in endobronchial growths using fiberoptic bronchoscopy found that sensitivity, specificity, PPV, and NPV of 83.6, 100, 100, and 79.4%, respectively [17]. Bodh et al. who correlated different cytohistological methods in the diagnosis of lung tumors by using fiberoptic bronchoscopy also found that sensitivity and specificity of imprint smear were 81.35 and 78.12%, respectively [18]. Goyal et al. who demonstrated the usefulness of imprint smear cytology of bronchial biopsies in the diagnosis of lung tumors. The imprint smears were positive for malignancy in 84.6% cases with a diagnostic accuracy of 98.08% [19]. These results also were comparable with that of Chowdhury et al. who reported sensitivity and specificity of imprint cytology 84.9 and 72.4%, respectively, when compared to histopathology [20]. In the present study, there was no significant relation between the size of the biopsy specimen and the sensitivity of imprint cytology in the bronchoscopic group as we used two types of biopsies (forceps and cryo ones) and this add a point to the advantages of the imprint cytology, so we can recommend its use as a diagnostic tool even in small-sized samples. This conclusion was reported by Dutta et al. who found that imprint cytology could be superior to conventional histopathology in the identification of a small proportion of cancer cells against a background of non-malignancy [21].

In the thoracoscopic group of the present study, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of imprint cytology were 94.74, 100, 100, 80, and 95.65%, respectively. These results were in concordant with those of Grosu et al. [22] who aimed to assess the role of pleural touch preparations during medical thoracoscopy for the diagnosis of malignancy. A finding of malignant cells on touch preparation cytology during thoracoscopy increases the likelihood of malignancy significantly and gives a chance for immediate pleurodisis. Porfyridis and colleagues [23] also who evaluated the value of touch preparation cytology in medical thoracoscopy in pleural diseases concluded that touch cytology was highly accurate in predicting malignancy. Additionally, a survey made by Hallifax and colleagues [24] in the current practice of medical thoracoscopists at predicting malignancy found that MT provides the option to pleurodese (to reduce risk of fluid recurrence) at the time of biopsy via talc poudrage, with high success rate.

As noticed from the present study that the NPV of imprint cytology was 100% in both studied groups and that means that it is a good positive test,

| Table 7 | Receiver operating characteristic (ROC) curve of imprint cytological results in the thoracoscopic group |
|---------|------------------------------------------------------------------------------------------|
| | Sensitivity | Specificity | PPV | NPV | Accuracy |
| Imprint cytological results | 94.74% | 100% | 100% | 80% | 95.65% |

PPV positive predictive value, NPV negative predictive value
accordingly, we can take the decision to stop sampling and ending the bronchoscopic procedure and also pleurodesis at the time of thoroscopic maneuver. And this will shorten the time of the thoroscopic procedure and avoid exposing our patients to another invasive procedure.

The present study had several limitations such as a limited number of the study population and a limited time of the study. And it only depended upon limited tools of diagnosis (bronchoscopic or thoroscopic forceps and bronchoscopic cryo biopsy); other tools should be included as transthoracic core biopsy or transbronchial biopsy.

Conclusion
Imprint cytology is an easy and reliable method that can be used to provide quick diagnosis of both pulmonary and pleural pathology. Also, it decreases the procedure time so it reduces the complication rate. Diagnosis becomes clear at least 7 to 10 days earlier, which helps in planning management faster.

Abbreviation
MT: Medical thoracoscopy; PaO2: Partial pressure of arterial oxygen; PaCO2: Partial pressure of arterial carbon dioxide; PPV: Positive predictive value; NPP: Negative predictive value

Acknowledgements
The authors thank the patients who subjected to the study.

Authors’ contributions
MEH conceived the research concept and strategies and performed bronchoscopic and thoroscopic techniques. SAG performed bronchoscopic and thoroscopic techniques, and wrote and reviewed the manuscript. All authors discussed the results and read and approved the final manuscript.

Funding
There was no funding of the study.

Availability of data and materials
All data generated or analyzed during this study are included in this published article

Ethics approval and consent to participate
The Ethics Committee of Faculty of Medicine, Tanta University, was approved the research protocol Reference No. is (33830) and written informed consents were obtained from all subjects.

Consent for publication
Not applicable

Competing interests
The authors declared that they did not have any conflict of interest.

Received: 1 June 2020 Accepted: 21 August 2020
Published online: 09 September 2020

References
1. Kawaraya M, Gembka K, Ueoka H, Nishii K, Kiura K, Kodani T, Tabata M, Shibuya T, Kitajima T, Tanimoto M (2003) Evaluation of various cytological examinations by bronchoscopy in the diagnosis of peripheral lung cancer. Br J Cancer 89:1885–1888
2. Kang JY, Kim JW, Kim YH, Park SA, Moon HS, Lee SH, Rhee CK, Kang HH (2010) Diagnostic yield of flexible bronchoscopy without fluoroscopic guidance in evaluating peripheral lung lesions. Bronchol Intervent Pulmonol 17:317–322
3. Schreiber G, McCorry DC (2003) Performance characteristics of different modalities for diagnosis of suspected lung cancer: summary of published evidence. Chest 123:115S–128S
4. Ibusugo I, Tongbram C, Paley T, Prameshwari N, Ningthoujam D (2016) Diagnostic yield of bronchoscopic biopsy and bronchial washing in endoscopically visible lung malignancies. Int J Med Health Res 2(9):21–24
5. Roth K, Hardie JA, Andreassen AH, Leh F, Eagan TM (2008) Predictors of diagnostic yield in bronchoscopy: a retrospective cohort study comparing different combinations of sampling techniques. Pulmonary Med 26(8):2. https://doi.org/10.1155/2017/2465122
6. Du Rand I, Maskell N (2010) Introduction and methods: British Thoracic Society pleural disease guideline. Thorax 65:i1–i3
7. Froudarakis ME (2011) New challenges in medical thoracoscopy. Respiration 82:197–200
8. Dresler CM, Olak J, Herndon JE, Richards WG, Scalzi E, Fleshman SB, Kerstine KH, Dimmey T, Jakobs DM, Kohen L, Daniel TM, Haider GB, Sugabaker DJ, Cooperative Groups Cancer and Leukemia Group B, Eastern Cooperative Oncology Group, North Central Cooperative Oncology Group, Radiation Therapy Oncology Group (2005) Phase III intergroup study of talc poudrage vs talc slurry sclerosis for malignant pleural effusion. Chest 127:909–915
9. Satoh S, Shihavimagal SC, Shinawatima DK, Marunjath G (2016) A study of imprint cytology in computerized tomography-guided coaxial core biopsy of the lung and mediastinal lesions. Clin Cancer Invest J 5:230–235
10. Kamatchi V, Babu NA, Sankari SL, Rajesh E (2015) Imprint cytology. J Pharm Bioal lied Sci 7(1):5207–5208
11. Okubo K, Kato T, Hara A, Yoshimi N, Takeda K, Liaco F (2004) Imprint cytology for detecting metastasis of lung cancer in mediastinal lymph nodes. Ann Thorac Surg 78:1190–1193
12. Bolliger CT, Mathur PN, Beams JW, Becke JD, Cavaillere S, Carl H et al. (2002) ERS/ATS statement on interventional pulmonology. European Respiratory Society/American Thoracic Society. Eur Respir J 19(2):356–373
13. Light RW, Macgregor MJ, Lushchinger PC (1972) WC. Bull pleural effusions: the diagnostic separation of transudates and exudates. Ann Intern Med 77:507–513
14. Dhanya TS (2009) C. Ravindran Medical thoracoscopy: minimally invasive diagnostic tool for a trained pulmonologist. Calicut Med J 1:4
15. Rubio ER, Le SR, Whately RE, Boyd MB (2013) Cytobiospy: should this be used in place of endobronchial forceps biopsies? Biomed Res Int 4–6
16. Suen KC, Wood WS, Syed AA, Clement PB (1978) Role of imprint cytology in intraoperative diagnosis: value and limitations. J Clin Pathol 31:328–337
17. Bhat G, Muffi S, Kumar T, Shah S, Koul P, Shah P, Khan A, Jan R (2013) Correlative study of imprint and crush cytology with histopathology in endobronchial growths. J Cytology Histology 4(3):177. https://doi.org/10.4172/2157-7099.1000177
18. Bodh A, Kaushal V, Kashyap S, Gulati A (2013) Cytohistological correlation in diagnosis of lung tumors by using fiberoptic bronchoscopy: Study of 200 cases. Indian J Pathol Microbiol 56:84–88
19. Goyal S, Mohan H, Handa U, Saini V (2012) Rinse fluid and imprint smear cytology of bronchial biopsies in diagnosis of lung tumors. Diagn Cytopathol 40(9):10–103
20. Chowdhury A (2019) Evaluation of imprint smears of bronchoscopic biopsy in lung tumors: a cytohistological correlation. J Cytol 36(3):157–159
21. Dutta SK, Dasgupta S, Bhattacharya NY, Jain P, Bose D, Biswas PK (2017) Comparative study of imprint cytology and histopathology of soft tissue tumors. J Indian Soc Med Paediatric Oncol. 38(4):461–465
22. Grosu HB, Vial-Rodriguez M, Valik E, Casal RF, Espen GA, Morice R, Stewert J, Sarkiss M, Ozt D (2017) Pleural touch preparations and direct visualization of the pleura during medical thoracoscopy for the diagnosis of malignancy. Ann ATS. 14(8):1326–1331
23. Porfyridis I, Georgiadis G, Michael M, Frangopoulos F, Vagiasinos P, Papadopoulos A, Kara P, Chanlampilous C, Georgiou A (2016) Rapid on-site evaluation with the hemacolor rapid staining method of medical thoracoscopy biopsy specimens for the management of pleural disease. Respirology 21:1106–1112
24. Halifax RJ, Corcoran JP, Paasilas I, Rahaman NM (2016) Medical thoracoscopy: survey of current practice—how successful are medical thoracoscopists at predicting malignancy? Respirology 21:958–960

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