Journal of the Egyptian National Cancer Institute (2013) **25**, 209–218

Cairo University

Journal of the Egyptian National Cancer Institute

www.nci.cu.adu.eg
www.sciencedirect.com

Full Length Article

Role of thyroid transcription factor-1 and P63 immunocytochemistry in cytologic typing of non-small cell lung carcinomas

Eman Abu Sinna [a], **Noha Ezzat** [a,*], **Ghada M. Sherif** [b]

[a] *The Department of Pathology, Cytopathology Unit, NCI, Cairo University, Egypt*
[b] *The Department of BioStatistics and Epidemiology, NCI, Cairo University, Egypt*

Received 28 March 2013; accepted 27 May 2013
Available online 19 August 2013

KEYWORDS

FNAC;
NSCLC;
ICC;
TTF-1;
P63

Abstract *Purpose:* Evaluation of the value of thyroid transcription factor (TTF-1) and P63 in subtyping of non-small cell lung cancer in cytologic material.
Patients and methods: This is a retrospective study including 40 cases of primary lung lesions who underwent image guided FNAC from pulmonary nodules. The final histopathologic diagnosis was the gold standard. Cell blocks were stained with anti-TTF-1, and P63. Nuclear immunoreactivity for both markers was considered specific. Sensitivity, specificity, positive and negative predictive values, of the cytologic diagnosis and of the two markers, as well as the accuracy of the combined markers were calculated.
Results: Cytomorphology achieved a sensitivity of 83.3%, specificity of 91%, PPV of 91%, and NPV of 83.3%, for the diagnosis of AC, and 91% sensitivity, 83.3% specificity, 83.3% PPV, and 91% NPV, for the diagnosis of SCC. The concordance between cytologic and histopathologic diagnoses of AC and SCC was 87%. TTF-1 achieved 87.5% sensitivity, 94.7% specificity, 95.5% PPV, and 85.7% NPV for AC, while P63 achieved 94.7% sensitivity, 95.8% specificity, 94.7% PPV, and 95.8% NPV for SCC. TTF-1 enhanced the sensitivity of cytomorphology for AC from 83.3% to 87.5%, and specificity from 91% to 94.7%. Similarly P63 enhanced the sensitivity for SCC from 91% to 94.7%, and specificity from 83.3% to 95.8%.
Conclusion: TTF-1 achieved moderate sensitivity, and high specificity in the diagnosis of AC, while P63 was highly sensitive and specific for the diagnosis of SCC. Immunocytochemistry raised the sensitivity and specificity of FNAC in diagnosing AC and SCC using TTF-1 and P63, respectively.

* Corresponding author. Tel.: +20 0233052404/01221810284.
E-mail address: Noha781973@yahoo.com (N. Ezzat).
Peer review under responsibility of The National Cancer Institute, Cairo University.

Production and hosting by Elsevier

Introduction

Lung cancer is the leading cause of cancer death both in men and women worldwide [1]. NSCLC accounts for about 80–85% of all lung cancers and is classified according to the World Health Organization criteria into three major types:

1110-0362 http://dx.doi.org/10.1016/j.jnci.2013.05.005

adenocarcinoma (50%), squamous cell carcinoma (30–35%), and large cell carcinoma (5–10%) [2].

Prior to lung cancer patient treatment, it is important for an experienced pulmonary pathologist to review each case. After diagnosing the case as lung cancer, the initial and crucial aim is to differentiate small cell lung cancer (SCLC) from non-small cell lung cancer (NSCLC) to decide whether the treatment is medical or surgical respectively.

Next it is important to subtype non-small cell cases into squamous cell carcinoma and adenocarcinoma as the response to certain targeted therapy regimens in patients with advanced or recurrent adenocarcinoma is different from squamous cell carcinoma patient response [3]. For example, the epidermal growth factor receptor inhibitors, gefitinib and erlotinib, are more likely to be effective in ACs than in SCC [4]. The addition of the antifolate agent, pemetrexed, to conventional chemotherapy provides increased efficacy in non-squamous carcinomas but not in SCCs [5,6]. Bevacizumab in combination with carboplatin and paclitaxel improves overall response and survival in patients with advanced or recurrent non-small cell lung carcinoma. However, this drug is not recommended in patients with squamous cell carcinoma or neoplasms with a dominant squamous component as these patients have an increased risk for life-threatening pulmonary hemorrhage, some of which may be fatal. Thus, only patients with non-squamous cell carcinoma or mixed subtypes of NSCLC, if the predominant cell type is non-squamous, are eligible for bevacizumab. Therefore, subtyping of NSCLC has therapeutic implications [7,8].

In many instances, fine needle aspiration cytology (FNAC) is the diagnostic tool of choice for lung cancer. Morphologic assessment with the routine modified Papanicolaou stain still remains the cornerstone in lung cancer classification. In the 2004 World Health Organization classification, cytology was addressed for the first time, with descriptions of the morphological criteria for each type of pulmonary carcinoma [2].

In the new revised proposal, an entire section is dedicated to the classification of lung tumors based on small biopsy material including FNAB. This highlights the importance and recognition of the role that FNAB plays in the diagnosis and management of pulmonary carcinomas. Lung cancer histological subtypes that are morphologically recognizable on cytology specimens are ADC, SCC, and small cell lung cancer (SCLC), as well as carcinoid tumors. Other types of lung carcinoma such as large cell carcinoma and other rare variant as fetal type and colloid adenocarcinoma may be suspected on the basis of pure morphology but usually require evaluation of the surgically resected specimen for the final diagnosis [9].

While in the majority of cases a line of differentiation can be clearly identified morphologically between adenocarcinoma and squamous cell carcinoma, however, routine cytomorphology is limited in the classification of NSCLC into squamous and nonsquamous subtypes in poorly differentiated cases due to overlapping morphologic features [10]. It was previously believed that only histologic material was appropriate for sub-typing of non-small cell carcinoma. However, today's immunohistochemical stains work well in small biopsy and cytology material, allowing the separation of adenocarcinoma from squamous cell carcinoma with an accuracy of 100% [8].

Thyroid transcription factor-1 (TTF-1) is a tissue-specific transcription factor expressed in normal thyroid and lung [11,12]. In the lung, TTF-1 is involved in the regulation of surfactant protein production [13,14]. In the adult lung, TTF-1 is expressed in the noncilliated bronchiolar epithelial cells and in type II cells, and involved in the transcription of the surfactant protein genes in alveolar cells [15]. TTF-1 has been shown to be commonly expressed also in carcinomas of thyroid and lung origin. The immunohistochemical nuclear expression of TTF-1 is considered a useful tool in favor of lung or thyroid origin because of its high specificity (95–100%) [16]. TTF-1 is widely used as a pulmonary adenocarcinoma marker in surgical specimens. However, the utility of TTF-1 has rarely been investigated in cytology [12]. As reported in the literature, TTF-1 immunostaining has a sensitivity ranging from 58% to 76% in the detection of pulmonary adenocarcinoma in tissue biopsy specimens [17]. In cell block cytology material, the sensitivity reaches 90%. TTF-1 staining in primary pulmonary squamous cell carcinoma has been reported to be variable, ranging from 0% to 38% [11].

P63 is a P53 homologous nuclear protein, which is expressed in basal cells of stratified squamous and glandular epithelia. In the lung, it has been mainly studied in different histologic subtypes of epithelial neoplasms, with the highest expression consistently noted in squamous cell carcinomas. The frequency of expression in pulmonary adenocarcinomas is lower, with most cases showing only focal staining [18]. The different isoforms of P63 are thought to have different functions as well. The truncated forms are thought to inhibit cell cycle arrest and apoptosis driven by transactivating P63/P53 interaction [19]. The truncated isoforms are preferentially expressed in the basal cell compartment of normal epithelium and transactivating forms are more widely distributed in benign and neoplastic epithelium [20]. Consequently, different isoforms of P63 appear to play a role in maintaining the epithelial stem cell population, spurring epithelial differentiation and inducing neoplasia [21]. In the literature, the sensitivity and specificity of P63 for detection of pulmonary squamous cell carcinoma in cytologic material reached up to 100% [22,23].

This study aimed at evaluating the effectiveness of TTF-1 and P63 immunocytochemistry in improving cytologic sensitivity in subtyping of NSCLC cases in fine needle aspiration cytology.

Patients and methods

The current study included 40 cases referred from the Radiology Department to Cytology Unit, Pathology Department, National Cancer Institute, Cairo University in the period from January 2010 to December 2011. The patients presented with either single or multiple pulmonary nodules. Patients' files were reviewed and information about age, sex, site and size of the lesion, radiological findings, and any other relevant data were recorded. Cases having lung nodules without history of primary tumors elsewhere, and without any previous history of chemo or radiotherapy, were selected for the study.

FNAC from pulmonary nodules was performed under CT guidance for all cases included in the study using a 22-gauge needle. Six slide smears and one cell block were made for each case. The slides were immediately fixed in 95% ethyl alcohol for about 30 min, and stained with modified Papanicolaou stain. Immediate on-site assessment was done to detect specimen adequacy, and to give a provisional diagnosis that can segregate cases into positive or negative for cancer. According to the provisional diagnosis given by on-site cytopathologist,

material may be secured in some cases for culture and sensitivity, or TB culture and Zeil-Nielsen (ZN) stain. With further detailed examination in the cytology unit, positive cases were sub-typed and most of the negative cases that were inflammatory in nature were sent for microbiologic assessment. The diagnosis of cytological smears was done based on criteria defined by various authors [24], smears with inadequate cellularity or bad quality were considered inadequate, and excluded from the study. Cases showing morphologic features of non small lung cancer, and having final pathological diagnosis (surgical resections and/or endoscopic biopsies) were included in the study; all cases included in our study were diagnosed as primary lung carcinoma.

For each case, five-micrometer section was cut and the slide was stained with hematoxylin and eosin, all cell blocks were evaluated for cellular adequacy. Additional two sections were prepared for each case on electrostatically charged glass slides, and stained with anti-TTF-1using the mouse monoclonal antibodies from the manufacturer Cell Marque, USA, clone (BG7G3/1) ready to use, and anti-P63 using the mouse monoclonal antibody from the manufacturer Cell Marque, USA, clone (4A4), ready to use, using avidin biotin peroxidase technique, the reaction was detected using Diaminobenzidine (DAB) with hydrogen peroxide (H2O2), appropriate positive (lung adenocarcinoma for TTF-1, and breast sclerosing adenosis for P63) and negative controls (by substituting Phosphate Buffer Saline (PBS) for the primary antibody), were used. All slides were counter stained with hematoxylin, and examined for TT-F1 and P63 expression. In few cases where cell blocks showing hypocellularity, immunocytochemical staining was done on the smears. Results of immunostaining with TTF-1 and P63 were evaluated based on nuclear staining of neoplastic cells. Tumors were considered to be immunopositive for TTF-1 or P63 if the tumor cells demonstrated unequivocal nuclear staining. TTF-1 positive and 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.

Sensitivity, specificity, positive and negative predictive values, of the morphologic cytologic diagnosis and that of the two markers were calculated, concordance, discordance of cytologic assessment, and total accuracy of both markers were assessed on the basis that pathological diagnosis was the gold standard. (When calculating sensitivity, specificity, PPV, and NPV for adenocarcinoma, it was considered the positive component, while SCC was the negative component, and the opposite was true for that of SCC) Because adenosquamous carcinoma cases have two components, adenocarcinomatous components were added to adenocarcinoma cases, and squamous carcinoma components to squamous cell carcinoma cases when different estimates of validity were calculated. *P* values $< .05$ were considered statistically significant.

Results

The present study was conducted on 40 patients with NSCLC, including 28 (70%) males, and 12 (30%) females, with a male to female ratio of 2.3:1. The age ranged from 43 to 81 years, with the median age being 60.5 years. Twenty six (65%) patients presented with a single pulmonary nodule while 14 patients only (35%) presented with multiple nodules.

The cases included in the present study were classified into AC and SCC according to established criteria [25]. The smears were evaluated for cellular arrangement, shape of the cells, size and shape of the nuclei, cell membrane, and cytoplasmic and background characters. Morphologic assessment of cytologic smears allowed tumor typing in 23 (57.5%) cases, 11 (27.5%) cases were diagnosed as AC, 12 (30%) cases as SCC, while 17 (42.5%) cases were diagnosed as NSCLC-NOS. (Table 1). The smears from 12 cases diagnosed as squamous cell carcinoma showed malignant cells arranged in solid groups, loose clusters, and sheets with variable degrees of cellular cohesiveness, the cells had pleomorphic hyperchromatic nuclei, abundant eosinophilic keratinized cytoplasm, and well defined cell membrane. Epithelial pearl, separate spindle-shaped cells (fiber cells) and (tad-pole cells) which are cells with a large head and wispy tails, were present in some cases. Bizarre cells and necrosis were present in the background. (Figures 1 and 3). The smears from 11 adenocarcinoma cases showed malignant tumor cells arranged in papillae, large clusters, syncytial grouping, or acinar formations. The cells exhibited abundant pale or vacuolated cytoplasm with ill defined cell borders, and pleomorphic rather vesicular nuclei, having prominent nucleoli. Mucinous background was noticed in some cases. (Figures 5

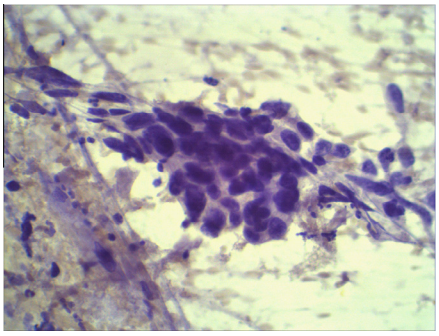

Figure 1 FNAC of a case of squamous cell carcinoma showing sheet of atypical squamous cells with hyperchromatic markedly pleomorphic nuclei in necrotic background (Pap x400).

Table 1 Pathologic and cytologic diagnoses of 40 primary lung cancer cases.

Cytologic diagnosis	Total	Pathologic diagnosis		
		Adenocarcinoma	Squamous cell carcinoma	Adeno-squamous carcinoma
Adenocarcinoma	11	10 (91%)	1 (9%)	0 (0%)
Squamous cell carcinoma	12	2 (16.7%)	10 (83.3%)	0 (0%)
NSCLC-NOS	17	9 (53%)	5 (29.4%)	3 (17.6%)
Total	40	21	16	3

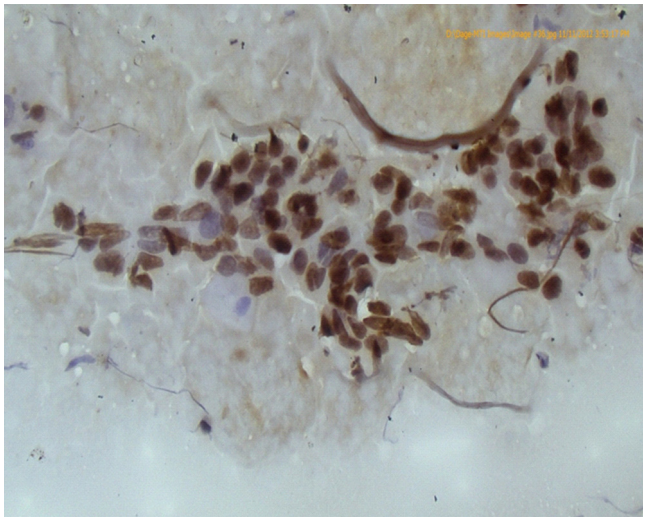

Figure 2 Stained smear of the same case showing positive immunocytochemical nuclear expression for P63 (X200).

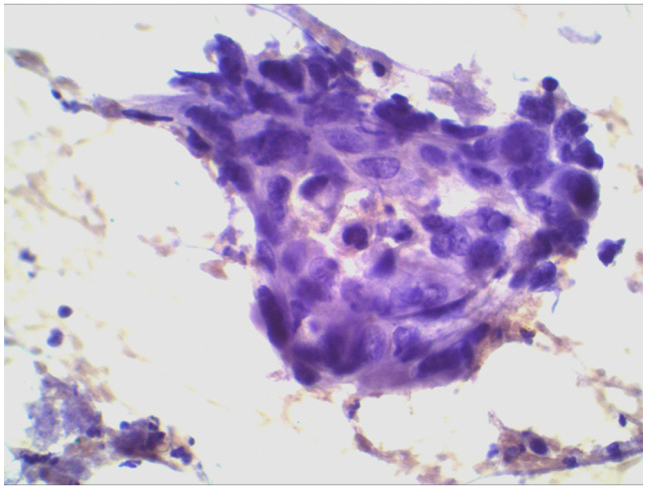

Figure 3 FNAC of a case of squamous cell carcinoma showing sheet of atypical squamous cells showing abundant eosinophilic cytoplasm and hyperchromatic pleomorphic nuclei (Pap x400).

and 7). The smears from cases of NSCLC-NOS did not show cytologic features belonging to either AC or SCC (Figure 9).

By referring to the final histopathologic diagnosis of corresponding cases, 21 (52.5%) cases were finally diagnosed as adenocarcinoma, 16 (40%) as squamous cell carcinoma, and 3 (7.5%) as adenosquamous (AS) carcinoma. Of 17 cases of NSCLC-NOS, 9 cases (53%) were confirmed to be AC, 5 (29.4%) as SCC and 3 (17.6%) as adenosquamous (AS) carcinoma (Table 1).

The results of cytologic and pathologic diagnosis considering adenocarcinoma and squamous cell carcinoma were compared, of the 12 cases confirmed as AC, 10 (83.3%) cases were diagnosed cytologically, while of 11cases proved to be SCC, 10 (91%) were cytologically diagnosed (Table 2). Thus, the sensitivity, specificity, PPV, and NPV of cytologic diagnosis for AC were 83.3%, 91%, 91%, and 83.3%, respectively, and those for SCC were 91%, 83.3%, 83.3%, and 91%, respectively. The concordance rate between cytologic and

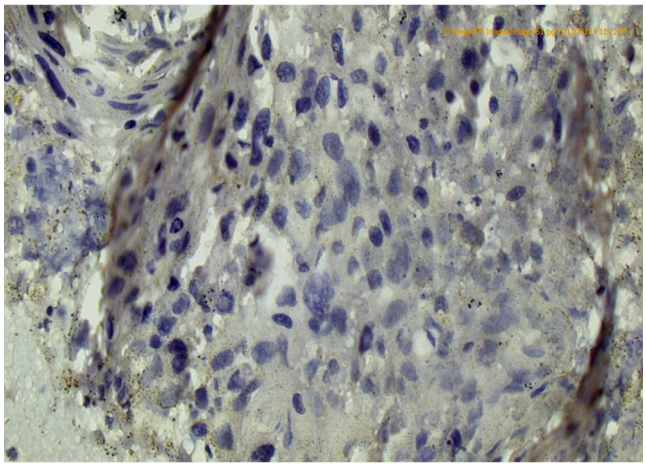

Figure 4 Cell block of the same case showing negative nuclear staining for P63(x400).

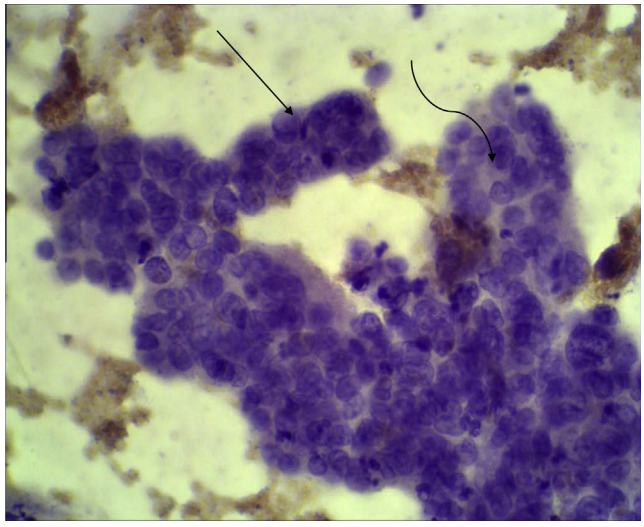

Figure 5 FNAC of a cases of adenocarcinoma showing atypical glandular cells with pleomorphism, focal acinar formations (curved arrow), and papillary pattern (straight arrow) (Pap x400).

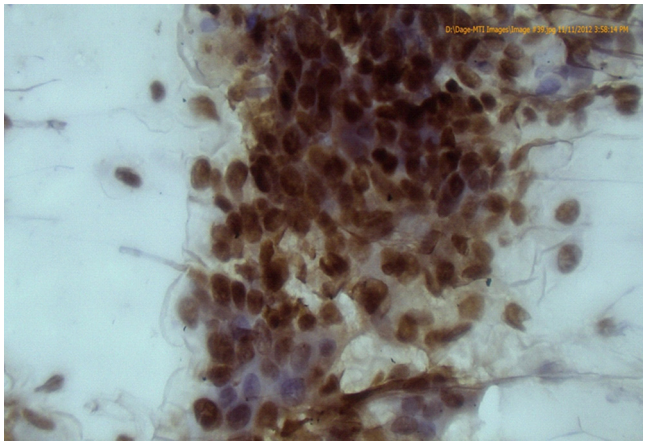

Figure 6 Stained smear of the same previous case showing positive nuclear immunocytochemical expression for TTF-1 (x400).

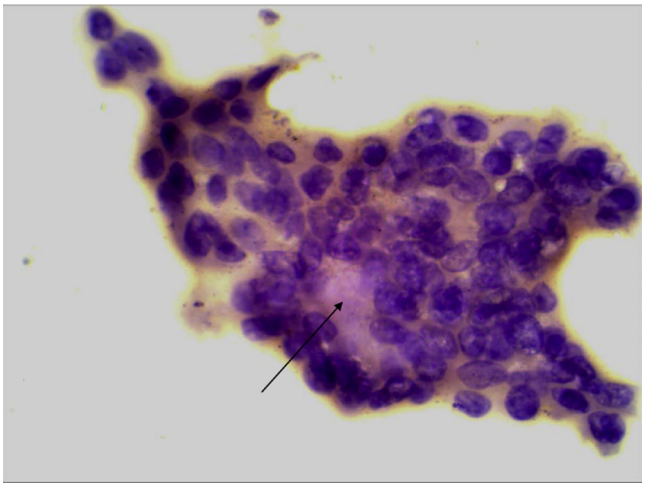

Figure 7 FNAC of a cases of adenocarcinoma showing group of atypical glandular cells, having pleomorphic vesicular nuclei, with acinar formations (arrow) (Pap x400).

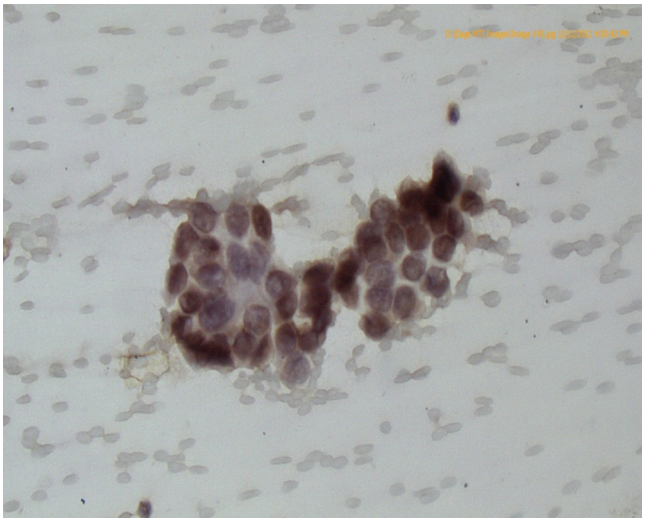

Figure 8 stained smear of the previous case showing positive nuclear immunocytochemical expression for TTF-1 (x400).

histopathologic diagnosis for AC and SCC was 87%, including 10 cases of AC, and 10 cases of SCC, disconcordance rate was 13%, and kappa value was 0.740, indicating good agreement, (Standard error ± 0.14).

When the two primary antibodies were applied, it was found thatTTF-1immunostaining was observed in 18/21 cases (85.7%) of lung adenocarcinoma (Figures 6 and 8), and in all 3 cases (100%) of adenosquamous carcinoma, where it was positive in the area showing adenocarcinomatous differentiation (Figure 10), TTF-1 was expressed in 21/24 (87.5%) of adenocarcinoma cases. TTF-1 was not expressed in 15/16 cases of SCC, and in squamoid component in all three cases (100%) of adenosquamous carcinoma (Table 3). Thus the sensitivity, specificity PPV, and NPV of TTF-1 were 87.5%, 94.7%, 95.5%, and 85.7%, respectively.

P63 was expressed in 16 cases (100%) of SCC (Figures 2 and 4), and in squamoid area in 2 out of the 3 (66.7%) cases of adenosquamous carcinoma, thus P63 was expressed in 18/19 (94.7%) of SCC. The marker was negative in 20/21 (95.2%) of adenocarcinoma, and in adenocarcinoma component of the 3 cases (100%) of adenosquamous carcinoma (Table 4), thus P63 achieved 94.7% sensitivity, 95.8% specificity, 94.7% PPV, and 95.8% NPV.

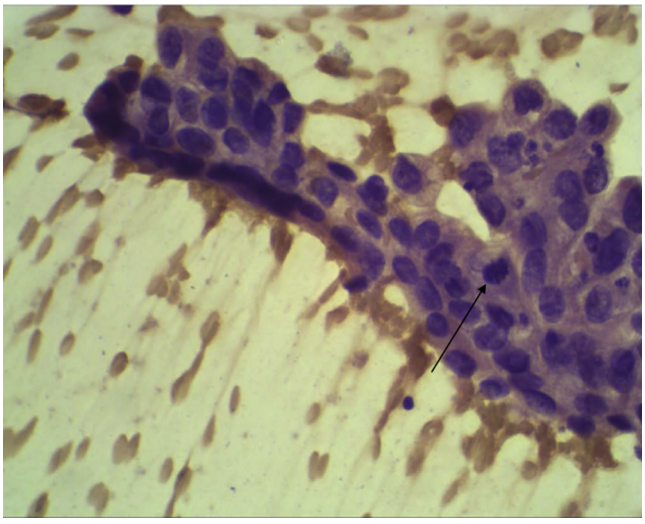

Figure 9 FNAC of a case of NSCLC-NOS showing group of atypical epithelial tumor cells, having pleomorphic hyperchromatic nuclei, abundant eosinophilic cytoplasm, and mitotic figure (arrow), with no specific features or patterns characteristic of AC or SCC (Pap x400).

Unlike the sensitivity and specificity of cytomorphologic examination for AC which were 83.3%, and 91%, respectively, they were 87.5%, and 94.7%, respectively with TTF-1 immunocytochemistry. And unlike sensitivity (91%) and specificity (83.3%) of cytomorphologic examination for squamous cell carcinoma, they were 94.7% and 95.8%, respectively with P63 immunocytochemistry.

When TTF-1 and P63 immunocytochemical results were combined it was found that both markers allowed an accurate diagnosis of 17/21 (81%) cases of adenocarcinoma, 15/16 (93.8%) cases of squamous cell carcinoma, and 2/3 (66.7%) cases of adenosquamous carcinoma.

Of the 17 cases diagnosed cytomorphologically as NSCLC-NOS, 9 (53%) cases were subtyped histopathologically as AC, 5 (29.4%) cases as SCC, and 3 (17.6%) cases as adenosquamous carcinoma. When the expression of the markers was evaluated in the previously mentioned cases, it was found that TTF-1 was expressed in 8/9 of AC cases, and in the adenocarcinomatous area of all the 3 cases of adenosquamous carcinoma, so the marker was expressed in 11/12 (91.7%) cases, and was negative in all SCC, so in these cases, TTF-1 showed 91.7% sensitivity, and 100% specificity. Table 5. On the other hand, P63 was expressed in all the 5 cases of SCC, and in 2/3 cases of adenosquamous carcinoma, while it was not expressed in all AC cases, thus P63 achieved a sensitivity of 87.5%, and a specificity of 100% as in Table 6.

When the two markers were combined, it was found that 8/9 (89%) of AC cases, all 5 (100%) of SCC, and 2/3 (66.7%) of AS carcinoma cases could be diagnosed. So both markers allowed accurate diagnosis of 15/17 (88.2%) of cases included in our study that were diagnosed as NSCLC-NOS,

Table 2 Cytologic accuracy in diagnosing squamous cell carcinoma and adenocarcinoma of primary lung cancer.

Cytologic diagnosis	Pathologic diagnosis		Total
	Adenocarcinoma	Squamous cell carcinoma	
Adenocarcinoma	10 (83.3%)	1 (9%)	11
Squamous cell carcinoma	2 (16.7%)	10 (91%)	12
Total	12	11	23

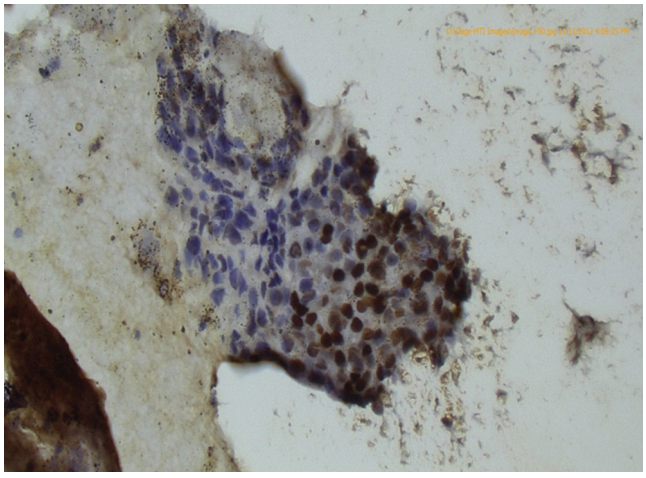

Figure 10 Cell block from the same previous case showing focally positive nuclear staining for TTF-1(x200).

Table 3 TTF-1 immunocytochemical expression in primary lung cancer cases.

TTF-1	Histopathologic diagnosis			Total
	AC	SCC	AS carcinoma	
Positive	18	1	3 Adenoca component*	
Negative	3	15	3 Squamoid component**	
Total	21	16	3***	40

AC: adenocarcinoma, SCC: squamous cell carcinoma, AS: adenosquamous.
* The adenocarcinoma component of 3/3 cases of AS carcinoma was TTF-1 positive.
** The squamous component of 3/3 cases of AS carcinoma was TTF-1 negative.
*** The total number of AS carcinoma cases.

Table 4 P63 immunocytochemical expression in primary lung cancer cases.

P63	Diagnosis			Total
	AC	SCC	AS carcinoma	
Positive	1	16	2 Squamoid component*	
Negative	20	0	3 Adenoca component**	
Total	21	16	3***	40

AC: adenocarcinoma, SCC: squamous cell carcinoma, AS: adenosquamous carcinoma.
* The squamous component of 2/3 AS carcinoma cases was P63 positive.
** The adenocarcinomatous component of 3/3 AS carcinoma cases was P63 negative.
*** Total number of AS ca. cases.

Table 5 TTF-1 immunocytochemical expression in cytologically diagnosed NSCLC-NOS cases in relation to their corresponding histopathologic diagnoses.

TTF-1	Histopathologic diagnosis			Total
	AC	SCC	AS carcinoma	
Positive	8	0	3 Adenoca component*	
Negative	1	5	3 Squamoid component**	
Total	9	5	3***	17****

AC: adenocarcinoma, SCC: squamous cell carcinoma, AS: adenosquamous carcinoma.
* The adenocarcinoma component of 3/3 cases of AS carcinoma was TTF-1 positive.
** The squamous component of 3/3 cases of AS carcinoma was TTF-1 negative.
*** Total number of AS carcinoma cases.
**** Total number of NSCLC-NOS cases.

into either adenocarcinoma, squamous cell carcinoma, or adenosquamous carcinoma, while 2 (11.8%) were enabled to be classified.

Discussion

Historically, NSCLC typing has not been considered relevant for treatment planning. More recently, tumor histotype has emerged as a critical variable in clinical decision making [4]. Prospective randomized studies have shown that new chemotherapeutic and molecular-targeted agents may lead to improved results, as compared with prior standard therapeutic options [26,27]. 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 [28]. Cytology specimens provide several advantages over surgical specimens for the subtyping of NSCLC. Fine needle aspiration biopsy is a simple, relatively safe, rapid, and reliable technique for obtaining tissue samples that can help the diagnosis of pulmonary mass, it is less invasive than open and closed surgical biopsies, both of which involve a larger incision in the skin and require local or general anesthesia, the results are as accurate as when a tissue sample is removed surgically, in addition, recovery time is brief and patients can soon resume their usual activities. FNAC helps in tumor typing of lung cancer, so initiation of specific therapy like chemotherapy or surgery is possible without unnecessary delay [29].

Table 6 P63 immunocytochemical expression in cytologically diagnosed NSCLC-NOS cases in relation to their histopathologic diagnoses.

P63	Histopathologic diagnosis			Total
	AC	SCC	AS carcinoma	
Positive	0	5	2 Squamoid component*	
Negative	9	0	3 Adenoca component**	
Total	9	5	3***	17****

AC: adenocarcinoma, SCC: squamous cell carcinoma, AS: adenosquamous carcinoma.
* The squamous component of 2/3 cases of AS carcinoma was P63 positive.
** The adenocarcinoma component of 3/3 cases of AS carcinoma was P63 negative.
*** The total number of AS carcinoma cases.
**** The total number of NSCLC-NOS cases.

On-site cytopathology interpretation has been previously shown to improve the diagnostic yield of FNA. On-site assessment helps to ensure that cytopathological samples are both representative of the target organ and adequate for diagnosis [30]. This is particularly important when trying to differentiate between a suspected malignancy and a benign/inflammatory process, where in negative cases TB etiology or other specific infections can be predicted by microbiologic studies. Preliminary assessment of specimen also allows the cytopathologist to prospectively identify cases that would benefit from additional aspirates for molecular testing, flow cytometry, or performing cell blocks for confirmatory immunocytochemical stains [31].

The key morphologic criteria for AC versus SCC are glandular architecture versus keratinization, respectively. The Papanicolaou (Pap) stain has exquisite sensitivity for even minimal keratinization aiding in the distinction of SCC from AC. The morphologic patterns which emerge in tumor smears provide a clue to a tumor subtype which may not be apparent in surgical specimens. In addition, due to immediate fixation, cytology provides greater nuclear and cytoplasmic resolution than histology [32].

The distinction of squamous and nonsquamous cell carcinoma, including adenocarcinoma and large cell carcinoma, can be made in the majority of patients in daily cytology practice. However, in certain patients, the distinction cannot be made by an assessment of morphology alone for a variety of reasons, such as sampling error, poor specimen preparation, and tumor differentiation. Poorly differentiated carcinomas are particularly difficult to classify, because they may lack specific architectural and cytomorphologic characteristics of either adenocarcinoma or squamous cell carcinoma. In this situation, the profile of IHC markers in tumor cells may provide additional differential diagnostic information [33].

Several studies have evaluated the diagnostic agreement between endoscopic biopsies and resection specimens and have revealed high figures of concordance for most histotypes [34]. The literature, however, is fewer when it compares the sensitivity, specificity, and accuracy of morphologic assessment of cytology in typing of NSCLC in relation to pathologic diagnosis [35].

The present study showed that FNAC allowed tumor typing in 23 out of 40 (57.5%) cases included, where 11 (47.8%) cases were diagnosed as AC, and 12 (52.2%) cases as SCC, while it was enabled in 17 (42.5%) cases that were classified as NSCLC-NOS. By comparing the results with those of corresponding tissue sections, it was found that the sensitivity, specificity, PPV and NPV of morphologic and cytologic diagnoses for AC in the present study were 83.3%, 91%, 91%, and 83.3%, respectively, and those for SCC were 91%, 83.3%, 83.3%, and 91%, respectively. Rita et al. reported a higher figure of sensitivity 87%, a lower specificity 89%, and nearly similar PPV & NPP, 92% & 82%, respectively, for AC, while they showed a lower sensitivity and NPV 88.7%, 87.2%, respectively, a higher specificity, 92.1%, and nearly similar PPV, 82%, for SCC [28].

The overall accuracy of cytology for diagnosing both AC, and SCC was 87%, our finding was similar to that reported in other 2 studies done by Rita et al. and Piaton et al. where they showed figures of 88%, and 88.4%, respectively [28,36]. Edwards et al. reported a lower figure where they revealed a total accuracy of 54%. [37].

The reasons for different results of interpretation of AC or SCC between cytologic and pathologic assessment in different studies could be due to a different number of cases, small sized biopsy material obtained by FNAC and fewer number of tumor cells compared to that present in tissue section, the presence of cellular degeneration, and poor preparation of the smear.

Thyroid transcription factor-1 (TTF-1) is a nuclear protein that is selectively expressed in normal epithelial cells of thyroid and lung origin and in adenocarcinomas derived from these cells [38,39]. TTF-1 is a recently introduced monoclonal antibody; several authors have found that TTF-1 was a promising marker for pulmonary adenocarcinoma in histologic specimens [40]. There was only limited information on the utility of anti-TTF-1 in cytologic material [41].

P63 is a homologous of P53 that is consistently expressed by epithelial cells of stratified epithelia, myoepithelial cells of breast, and prostatic basal cells [42]. P63 nuclear expression is seen in SCC, and other tumors with the capacity or potential to undergo squamous differentiation [43–45]. In the lung, it has been mainly studied in different histologic subtypes of epithelial neoplasms, with the highest expression consistently noted in squamous cell carcinomas [46]. In the literature, few studies have evaluated the usefulness of P63 in subtyping lung NSCLC into SCC and neoplasms with no squamous differentiation in paraffin-embedded tissue, and in cytologic material, as in many instances cytology is the only available material for diagnosis [10].

In the current study, TTF-1 was positive in 18/21 (85.6%) of confirmed AC cases, and in adenocarcinomatous area of 3 (100%) cases of adenosquamous carcinoma, thus the marker was positive in 21out of 24 (87.5%) of AC, giving a sensitivity of 87.5%. Our result was nearly similar to that obtained by Hecht et al. [47] where they achieved a sensitivity of 89%. On the other hand, our figure was higher than that observed by Stoll et al. [33], Loreto et al. [40], Chhieng et al. [48], Harlamert et al. [49], Fabbro et al. [50], and Kulshrestha1 and Vijayan [51], where they showed figures of 81%, 62.5%, 71%, 70%, 42%, and 66.7%, respectively.

In the present work, TTF-1 achieved a specificity of 94.7%, our finding correlates with the previous study by Chhieng et al. [48] where they showed a specificity of 95%. On the other hand, our figure was lower than that observed by Hecht et al. [47] who achieved a specificity of 98%. While our results were higher than those detected by Stoll et al. [33], and Loreto

et al. [40] where the specificity achieved in their studies were 81%, and 62.5%, respectively.

In the current work, TTF-1 was positive in 6.3% of SCC cases. Our finding agreed with the false positive rate observed by other studies, where it ranged from 0% to 38% [41,47,52,53].

P63 immunocytochemical expression was detected in all 16 cases (100%) confirmed to be squamous cell carcinoma, and in 2/3 (66.7%) cases of adenosquamous carcinoma, where it was positive in the area showing squamoid differentiation. The overall sensitivity for P63 in our study was 94.7%. This figure was nearly similar to that achieved by Kim and Kwon [54], where they showed a sensitivity of 92.3%. while our finding was higher than that observed by Jorda et al. [10], and Terry et al. [55] who achieved a sensitivity of 88% and 84%, respectively for P63 immunocytochemical expression, On the other hand, our finding was lower than that detected by Uke et al. [22] where they showed 100% sensitivity. The specificity of P63 obtained in the current work was 95.8%, our finding was lower than Kim and Kwon [54] who showed 100% specificity. On the other hand, our result was higher than Jorda et al. [10], Uke et al. [22] and Terry et al. [55], where they showed a specificity of 84%, 90.4%, and 85%, respectively.

The false positive rate for P63 in the previous study ranged from 0% to 15%, (Pelosi et al. [46] and Sheikh et al. [56]), the false positive rate observed in current study fell within that range where P63 immunoreactivity was detected in 4.8% of adenocarcinoma cases.

The reasons for the discrepancy in results between our study and the literature can be explained by the use of different clones of markers, different types of specimens (tissue, FNA biopsy, brushings, and fluids cytology) and variations in the method of fixations.

In the current work, the sensitivity of the cytologic assessment for the diagnosis of AC increased from 83.3% to 87.5% after using TTF-1, and from 91% to 94.7% for SCC, when P63 immunocytochemistry was applied, although, the difference was not statistically significant. Similarly Khayyata et al. [35] showed that the sensitivity for the cytologic diagnosis of adenocarcinoma was 66% and that for squamous cell carcinoma was 53% when cytomorphologic criteria were used alone, but increased when an immunocytochemical panel including TTF-1, and P63 was included in their study.

The sensitivity of cytology for diagnosing SCC increased from 91% to 94.6%, and the specificity from 83.3% to 95.8% after using P63 immunostaining. Similarly Jorda et al. [10] noticed that the sensitivity of cytologic methods for the detection of NSCLC with squamous differentiation increased from 35% to 88% using P63 immunocytochemistry, however, the specificity decreased from 100% to 84% due to the presence of high false positive cases by P63.

In the current work, the combined use of both markers achieved a total accuracy of 81% for AC, 93.8% for SCC, and 66.7% for adenosquamous carcinoma. To our knowledge, the previous few studies that used TTF-1 and P63 on cytologic material were either limited to a single antibody in each study, or the combined total accuracy of the two antibodies were not assessed when both markers were used in a panel.

In the current work 17/40 (42.5%) cases were diagnosed as NSCLC-NOS using cytomorphologic criteria alone, when TTF-1 was applied, 11/12 (91.7%) cases were re-classified as AC, while 7/8 (87.5%) cases were diagnosed as SCC using P63 immunostaining, when the two markers were combined, it was found that 8/9 (89%) AC cases, 5/5 (100%) SCC cases, and 2/3 (66.7%) AS carcinoma cases could be diagnosed, thus the marker combination allowed an accurate classification of 15/17 (88.2%) cases of NSCLC-NOS into either AC, SCC or AS carcinoma, while 2 (11.8%) were enabled to be classified. In a similar manner, Nicholson et al. [57] showed that 53% of their cases were classified as NSCLC-NOS after the initial microscopic examination, while after using a panel including TTF-1, and P63, 81% of cases were correctly diagnosed, and only 19% of cases remained unclassified.

From this work, we finally concluded that TTF-1 achieved moderate sensitivity, and high specificity in the diagnosis of AC, while P63 was both a highly sensitive and specific marker for diagnosis of SCC. The use of immunocytochemistry raised the sensitivity and specificity of FNAC in the diagnosis of AC and SCC using TTF-1 and P63, respectively. While 17/40 (42.5%) cases were cytomorphologically diagnosed as NSCLC-NOS, the panel used in the present study allowed the classification of 15/17 (88.2%) of these cases into either AC, SCC, or AS carcinoma.

References

[1] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. CA Cancer J Clin 2010;60:277–300.

[2] Travis WD, Muller-Hermelink HK, Harris CC. World health organization classification of tumours. In: Pathology and genetics of tumours of the lung, pleura, thymus and heart. Lyon, France: International Agency For Research On Cancer; 2004.

[3] David C, Hoak MD. Distinguishing adenocarcinoma from squamous cell carcinoma of the lung. Incyte Diagnostic 2011. Available from: http://www.incytepathology.wordpress.com.

[4] Hirsch FR, Spreafico A, Novello S, Wood MD, Simms L, Papotti M. The prognostic and predictive role of histology in advanced nonsmall cell lung cancer: a literature review. J Thorac Oncol 2008;3:1468–81.

[5] Azzoli CG, Baker Jr S, Temin S, Pao W, Aliff T, Brahmer J, et al. American Society of Clinical Oncology clinical practice guideline update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol 2009;26:6251–66.

[6] Johnson DF, Fehrenbacher L, Novotny WF, Herbst RS, Nemonaitis JJ, Jablons DM. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic nonsmall-cell lung cancer. J Clin Oncol 2004;22:2184–91.

[7] Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegoid C. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage nonsmall-cell lung cancer. J Clin Oncol 2008;26:3543–51.

[8] Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: utility of an immunohistochemical panel containing TTF-1, Napsin A, p63, and CK5/6. Am J Surg Pathol 2011;35(1):15–25.

[9] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe R. International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6(2):244–85.

[10] Jorda M, Gomez-Fernandez C, Garcia M, Mousavi F, Walker G, Mejias A, et al. P63 differentiates subtypes of nonsmall cell

carcinomas of lung in cytologic samples: implications in treatment selection. Cancer 2009;117(1):46–50.
[11] Jonathan LH, Jack LP, Lisa JW, Geraldine SP. The value of thyroid transcription factor-1 in cytologic preparations as a marker for metastatic adenocarcinoma of lung origin. Am J Clin Pathol 2001;116(4).
[12] Khoor A, Byrd-Gloster AL, Nicosia SV. Expression of thyroid transcription factor-1 in malignant pleural effusions. Pathol Oncol Res 2011 Jun;17(2):263–7.
[13] Bohinski RJ, Di Lauro R, Whitsett JA. The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. Mol Cell Biol 1994;14:5671–81.
[14] Kelly SE, Bachurski CJ, Burhans MS, Glasser SW. Transcription of the lung-specific surfactant protein C gene is mediated by thyroid transcription factor 1. J Biol Chem 1996;271:6881–8.
[15] Bejarano PA, Baughman RP, Biddinger PW, Miller MA, Fenoglio-Preiser C, Al-Kafaji B, et al. Surfactant proteins and thyroid transcription factor-1 in pulmonary and breast carcinomas. Mod Pathol 1996;9:445–52.
[16] Eva C, Fan Z, Cedric P, Thierry M, Pierre M, Beatrice M, et al. Variable sensitivity and specificity of TTF-1 antibodies in lung metastatic adenocarcinoma of colorectal origin. Mod Pathol 2005;18:1371–6.
[17] Di Loreto C, Puglisi F, Di Lauro V, Damante G, Beltrami CA. TTF-1 protein expression in pleural malignant mesotheliomas and adenocarcinomas of the lung. Cancer Lett 1998;124:73–8.
[18] Sheikh HA, Fuhrer K, Cieply K, Yousem S. p63 expression in assessment of bronchioloalveolar proliferations of the lung. Mod Pathol 2004;17:1134–40.
[19] Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dotsh V. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol Cell 1998;2:305–16.
[20] Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garrawy L. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000;157:1769–75.
[21] Nylander K, Vojtesek B, Nenutil R, Lindqren B, Roos G, Zhanxiang W. Differential expression of p63 isoforms in normal tissues and neoplastic cells. J Pathol 2002;198:417–27.
[22] Uke M, Rekhi B, Ajit D, Jambhekar NA. The use of p63 as an effective immunomarker in the diagnosis of pulmonary squamous cell carcinomas on de-stained bronchial lavage cytological smears. Cytopathology 2009;21(1):56–63.
[23] Kim D, Seon Kwon M. Role of fine needle aspiration cytology, cell block preparation and CD63, P63, and CD 56 immunostaining in classifying the specific tumor type of the lung. Acta Cytol 2010;54:55–9.
[24] Orell SR, editorOrell SR, Sterrett GF, Walters MN, Whitakar D, editors. Manual and atlas of fine needle aspiration cytology. New Delhi: Churchill-Livingstone; 2005. p. 125–64.
[25] Sturgis CD, Nassar DL, D'Antonio JA, Raab CC. Cytologic features useful for distinguishing small cell from non-small cell carcinoma in bronchial brush and wash specimens. Am J Clin Pathol 2000;114:197–202.
[26] Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for non-squamous non-small cell lung cancer: AVAiL. J Clin Oncol 2009;27:1227–34.
[27] Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958–67.
[28] Rita N, Marcello T, Francesco G, Marco B, Maria M, Lilia F, et al. Accuracy of fine needle aspiration cytology in the pathological typing of non-small cell lung cancer. J Thorac Oncol 2011;6:489–93.
[29] Mondal SK, Nag D, Das R, Mandol PK, Biswas PK, Osta M. Computed tomogram guided fine-needle aspiration cytology of lung mass with histological correlation: a study in Eastern India. South Asian J Cancer 2013;2:14–8.
[30] Eloubeidi MA, Tamhane A, Jhala N, Chhieng D, Jhala D, Crowe DR, et al. Agreement between rapid onsite and final cytologic interpretations of EUS-guided FNA specimens: implications for the endosonographer and patient management. Am J Gastroenterol 2006;101:2841–7.
[31] Bhutani MS, Logrono R. Endoscopic ultrasound-guided fine-needle aspiration cytology for diagnosis above and below the diaphragm. J Clin Ultrasound 2005;33:401–11.
[32] Sigel CS, Moreira AL, Travis WD, Zakowski MF, Thornton RH, Riely GJ. Subtyping of non-small cell lung carcinoma (NSCLC): a comparison of small biopsy and cytology specimens. J Thorac Oncol 2011;6:1849–56.
[33] Stoll LM, Johnson MW, Gabrielson E, Askin F, Clark DP, Li QK. The utility of Napsin-A in the identification of primary and metastatic lung adenocarcinoma among cytologically poorly differentiated carcinomas. Cancer Cytopathol 2010;118:441–9.
[34] Loo PS, Thomas SC, Nicolson MC, Fify MN, Kerr KM. Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens. J Thorac Oncol 2010;5:442–7.
[35] Khayyata S, Yun S, Pasha T, Jian B, McGrath C, Yu G, et al. Value of P63 and CK5/6 in distinguishing squamous cell carcinoma from adenocarcinoma in lung fine-needle aspiration specimens. Diagn Cytopathol 2009;37:178–83.
[36] Piaton E, Grillet-Ravigneaux M, Saugier B, Pellet H. Prospective study of combined use of bronchial aspirates and biopsy specimens in diagnosis and typing of centrally located lung tumours. BMJ 1995;310:624.
[37] Edwards SL, Roberts C, McKean M, Cockburn S, Effrey R, Kerr K. Preoperative histological classification of primary lung cancer: accuracy of diagnosis and use of the non-small cell category. J Clin Pathol 2000;53:537–40.
[38] Ordonez NG. Thyroid transcription factor-1 is a marker of lung and thyroid carcinomas. Adv Anat Pathol 2000;7:123–7.
[39] Jagirdar J. Application of immunohistochemistry to the diagnosis of primary and metastatic carcinoma to the lung. Arch Pathol Lab Med 2008;132:384–96.
[40] Di Loreto C, Di Lauro V, Puglisi F, Damante G, Fabbro D, Beltrami CA. Immunocytochemical expression of tissue specific transcription factor-1 in lung carcinoma. J Clin Pathol 1997;50:30–2.
[41] Khoor A, Whitsett JA, Stahlman MT, Olson SJ, Cagle PT. Utility of surfactant protein B precursor and thyroid transcription factor 1 in differentiating adenocarcinoma of the lung from malignant mesothelioma. Hum Pathol 1999;30:695–700.
[42] Rosai J. Rosi and ackerman's surgical pathology. 9th ed. Edinburgh: Mosby; 2004.
[43] Wang BY, Gil J, Kaufman D, Li G, Kohtz DS, Burstein DE. P63 in, pulmonary epithelium, pulmonary squamous neoplasm, and other pulmonary tumors. Hum Pathol 2002;33:921–6.
[44] Wu M, Wang B, Gil J, Sabo E, Miller L, Li G, et al. P63 and TTF1 immunostaining: a useful marker panel in distinguishing small cell carcinoma of lung from poorly differentiated squamous cell carcinoma of lung. Am J Clin Pathol 2003;119:696–702.
[45] Di Como CJ, Urist MJ, Babayan I, Drobnjac M, Hedvat CV, Teruya-Feldstein J, et al. P62 expression profiles in human normal and tumor tissues. Clin Cancer Res 2002;8:491–501.
[46] Pelosi G, Pasini F, Olsen Stenholm C, Pastorino U, Maisonneuve P, Sonzogni A, et al. p63 immunoreactivity in lung cancer: yet another player in the development of squamous cell carcinomas? J Pathol 2002;198:100–9.

[47] Hecht JL, Jack L, Pinkus JL, Weinstein LJ, Pinkus GS. The value of thyroid transcription factor-1 in cytologic preparations as a marker for metastatic adenocarcinoma of lung origin. Am J Clin Pathol 2001;116:483–8.
[48] Chhieng DC, Cangiarella JF, Zakowski MF, Goswami S, Cohen J-M, Yee HT. Use of thyroid transcription factor 1, PE-10, and cytokeratins 7 and 20 in discriminating between primary lung carcinomas and metastatic lesions in fine-needle aspiration biopsy specimens. Cancer 2001;93:330–6.
[49] Harlamert HA, Mira J, Bejarano PA, Baughman RP, Miller MA, Whitsett JA, et al. Thyroid transcription factor-1 and cytokeratins 7 and 20 in pulmonary and breast carcinoma. Acta Cytol 1998;42:1382–8.
[50] Fabbro D, Di Loreto C, Beltrami CA, Belfiore A, Di Lauro R, Damante G. Expression of thyroid-specific transcription factors TTF-1 and PAX-8 in human thyroid neoplasms. Cancer Res 1994;54:4744–9.
[51] Kulshrestha1 R, Vijayan VK. Immunohistochemical staining on fine needle aspiration biopsy-cell block specimens in the differential diagnosis of lung cancers. Indian J Chest Dis Allied Sci 2009;51:21–5.
[52] Kaufmann O, Dietel M. Thyroid transcription factor-1 is the superior immunohistochemical marker for pulmonary adenocarcinomas and large cell carcinomas compared to surfactant proteins A and B. Histopathology 2000;36:8–16.
[53] Puglisi F, Barbone F, Damante G, Bruckbaurer M, Di Lauro V, Beltrami CA, et al. Prognostic value of thyroid transcription factor-1 in primary, resected, non-small cell lung carcinoma. Mod Pathol 1999;12:318–24.
[54] Hoon Kim D, Seon Kwon M. Role of fine needle aspiration cytology, cell block preparation and CD63, P63, and CD 56 immunostaining in classifying the specific tumor type of the lung. Acta Cytol 2010;54:55–9.
[55] Terry J, Leung S, Laskin J, Leslie KO, Gown AM, Ionescu DN. Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell carcinomas in small tumor samples. Am J Surg Pathol 2010;34(12):1805–11.
[56] Sheikh HA, Fuhrer K, Cieply K, Yousem S. p63 expression in assessment of bronchioloalveolar proliferations of the lung. Mod Pathol 2004;17:1134–40.
[57] Nicholson AG, Gonzalez D, Shah P, Pynejar MJ, Deshmukh M, Rice A, et al. Refining the diagnosis and EGFR status of non-small cell lung carcinoma in biopsy and cytologic material, using a panel of mucin staining, TTF-1, cytokeratin 5/6, and P63, and EGFR mutation analysis. J Thorac Oncol 2010;5:436–41.