ORIGINAL ARTICLE

EXPRESSION OF TTF-1, NAPSIN-A, P63 AND P40 IN DIFFERENTIAL DIAGNOSIS OF NON-SMALL CELL LUNG CARCINOMA

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ABSTRACT

Background: Non-small cell lung cancer is classified into different subtypes. It is now mandatory for the histopathologists to classify the NSCLC into its exact classification because of the increase in promising personalized therapy against each of its subtypes. In order to better classify non-small cell lung cancers, the current study evaluates the diagnostic value of p40 and Napsin-A along with the comparison with the conventional markers TTF-1 and P63.

Methods: 147 blinded diagnosis of NSCLC were included and classified based on histological findings. After histological review, all the specimens were stained with the conventional markers and new markers i.e., p40, p63 for squamous-cell-carcinoma (SQC) and TTF-1, Napsin-A for adenocarcinoma (ADC).

Results: Regarding baseline data, out of 147 NSCLC samples, 106 (72.10%) were males and 41 (27.90%) were females with the mean age of the patient was 57.6 years. The new markers combination reclassified tumors as 60 (40.8%) as ADC, 80 (54.4%) as SQC and 7.0 (4.8%) as Transdifferentiated (TD). The sensitivity and specificity of newly proposed markers, that is, Napsin-A and p40 were better than the conventional markers TTF-1 and p63.

Conclusion: Napsin-A and p40 are better markers than presently used markers (TTF-1and p63). These better markers are suggested to be added or the existing markers (TTF-1 and p63) should be preferred by Napsin-A and p40 in the histopathological diagnostic workup of NSCLC.

Keywords: Adenocarcinoma; Squamous Cell CarcinomaTTF-1; Napsin-A; p63; p40.

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INTRODUCTION

Lung cancer is one of the foremost causes of death due to cancer. Around 1,350,000 new cases and approximately 1,180,000 deaths are reported every year due to lung cancer[1]. In Pakistan, according to the data generated in January 2014 to December 2017 lung cancer is amongst the top ten malignancies in both males and females[2].

Malignant lung tumors are broadly classified into the two main subtypes (1) SCLC (2) NSCLC. The NSCLC which represents about 85% of all lung cancers is further divided into adenocarcinoma (ADC), squamous cell carcinoma (SQC), large cell carcinoma (LCC), large cell neuroendocrine carcinoma and carcinoids on morphological basis[3]. There is a great therapeutic clinical significance to differentiate ADC and SQC. According to the guidelines of National Comprehensive Cancer Network (NCCN) for NSCLC, there are different clinical regimens for both these subtypes of non-small cell lung cancer[4].

Different chemotherapeutic drugs have varied response in adenocarcinoma and squamous cell carcinoma therefore differentiating the NSCLC into ADC and SQC is mandatory. The ADC is strongly responsive to epidermal growth factor receptor, gefitinib, tyrosine kinase inhibitors and erlotinib whereas SQC does not respond to these chemotherapies. Moreover there are many drugs like

bevacizumab and pemetrexed which are contraindicated in SQC as these cause fatal hemoptysis in these patients[3, 5-7].

About 70% of the lung cancer patients are diagnosed in late stages and are not the candidates for surgery, therefore, mostly the diagnosis is made on small core biopsies [8, 9]. The current WHO classification is based on the morphological features seen on routine hematoxylin and eosin (H&E) stained slides. However, this routine staining remains inconclusive in poorly differentiated cases. A number of adjuvant methods like immunohistochemistry (IHC), western blot , PCR can ascertain the differentiation but up till now IHCis the most appropriate tool to reduce the proportion of cases diagnosed as non-small cell carcinoma-not otherwise specified (NSCLC-NOS) from 30–40% to 4–7% for a better treatment plan3, [10, 11].

Due to limited material in small biopsies, a large panel of IHC markers cannot be applied. Therefore the requirement for highly sensitive and specific IHC markers is needed so that the diagnosis should be made on a small IHC panel, preventing the exhaustion of the biopsy for further molecular characterization[12, 13].

Many researchers have addressed the importance of IHC markers for differentiating the subtypes of NSCLC[14,15]. However, the clinical problem regarding the IHC panel with limited number of IHC markers in small biopsies is still under debate. Furthermore, it is challenging for the histopathologists to use a minimal amount of tumor tissue while diagnosing the case accurately[13,16].

The immunohistochemical markers, which are currently used in the clinical laboratories, are TTF-1 and p63, which are not reliable markers to differentiate the NSCLC into its subtypes i.e., ADC and SQC leading to a large number of cases diagnosed as non-small cell lung cancer not otherwise specified (NSCLC-NOS) which is insufficient for the treatment strategy. The current series reveals that the proposed markers i.e., Napsin-A and p40 are better diagnostic markers then the current laboratory markers which would help the histopathologists to improve diagnosis leading to better treatment options. Furthermore, the data regarding the sensitivity and specificity of Napsin-A and p40 is very scanty in Pakistan.

In this study, two IHC markers (Napsin A and p40) are evaluated and compared with the conventional markers (TTF-1 and pp63) for the sub-typing of NSCLC in small biopsies. The objective of our study was (a) to characterize the NSCLC on the basis of immunohistochemical markers (b) to determine and compare the sensitivity and specificity (validity) of proposed markers i.e., Napsin-A and p40 with the conventional panel, i.e. TTF-1 and p63. Therefore, the study provides an evidence-based approach for the utilization of the better markers with greater sensitivity and specificity in routine cases of histopathology laboratories.

MATERIAL AND METHODS

Case Collection

This is a cross sectional study for which the specimens were collected during the period of November 2013 to December 2017. The study was carried out at histopathological section of Dow Diagnostics Reference and Research laboratory (DDRRL) at Dow University of Health Sciences, Karachi. Sample size of 144 NSCLC tissue biopsies were calculated with the help of open source calculator of OpenEpi version 2.3, considering 24% prevalence of p63 expression in lung SQC at 95% confidence level[17]. The NSCLC were classified into Adenocarcinoma, Squamous cell carcinoma and NSCLC-NOS based on hematoxylin and eosin staining. Then these cases were further stained with all the four specific markers i.e., TTF-1andp63 (which are currently in clinical use in the laboratories), Napsin-A and p40 (proposed markers) for exact subtyping. The study was approved (IRB Approval No- IRB-747/DUHS/Approval/ 2016/258) by the Institutional Review Board of Dow University of Health Sciences.

Inclusion Criteria

a) All of the primary NSCLC are included
b) All of the tru-cut (small) biopsies are included

Exclusion Criteria

a) All NSCLC with less material
b) All NSCLC with necrotic areas
c) All of the small biopsies with insufficient biopsy material were excluded

Immunohistochemistry (IHC)

Immunohistochemistry was performed by using a panel of TTF1 (Dako, IR056), p63 (Dako, IR662), Napsin-A (Biocare, AC13043C) and p40 (Biocare, AC13066C). Briefly, 3-4µm thick paraffin embedded tissue was cut, deparaffinized and dehydrated in graded alcohol from 100%-50%. Then the targeted antigen retrieval was performed followed by application of the primary antibodies. The two histopathologists examined the stained slides.

With the help of combined scoring system (sum of staining intensity and percentage of quantification positive cells), the semi-quantitative immunoreactivity analysis for the IHC markers was conducted in the neoplastic cells. The intensity of the staining was scored as: 0 for absent, 1 for weak, 2 for moderate, 3 for strong. Positive cells were quantified as percentage of the total number of neoplastic cells and were calculated as: Less than 5% = 0, 5% - 25% = 1, 26% - 50% = 2, 51% - 75% = 3, Greater than 75% = 4. For each case, an immunoreactivity score was generated as percentage of positive tumor cells

and staining intensity generating a score ranging from 0 to 12. A case was considered positive if the score was equal to or greater than 2; otherwise the case was considered negative[18]. In terms of specific staining patterns, cytoplasmic staining was considered positive for Napsin A. Nuclear staining was considered positive for TTF-1, p63 and p40.

Classification of each case into its subtype was based on the immunohistochemical profile. The case which showed positive expression for TTF-1 and/ or for Napsin A, and negative for p63 and p40 was classified as ADC. The case which showed positive expression for p63 and/ or p40 and negative for Napsin A and TTF-1 was classified as SQC. Case which showed positive expression for Napsin A and/ or TTF-1 and positive for p40 and/ or p63 was taken as TD.

Statistical Analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated based on the final histological diagnosis.

RESULTS

In the present series, out of 147 NSCLC biopsy specimens, 60 (40.8%) were adenocarcinoma, 80 (54.4%) were squamous cell carcinoma and 07(4.8%) were transdifferntiation. These cases include 106 (72.10%) males and 41 (27.90%) females aged 22-97 years (mean = 57.6 years, SD=12.94).

The current study revealed that most of the cases of NSCLC can be classified on the basis of H&E with the help of the morphological features, however about 30% to 40% of cases need IHC for the accurate diagnosis. Among these 147 cases, 99 cases were diagnosed same on IHC as they were initially diagnosed on H&E. Whereas, 48 cases differ on IHC from the diagnosis which was made initially on H&E. Out of these 48 cases, 7 cases were diagnosed as transdifferntiation (TD) which were initially diagnosed in favor of ADC (5/7) or NSCLC-NOS (2/7), whereas 21out of 48 cases were diagnosed as SQC which were initially diagnosed in favor of ADC (11/21) or NSCLC-NOS (10/21). Twenty cases were diagnosed as ADC, which were initially diagnosed in favor of SQC (7/20) or NSCLC-NOS (13/20).

Sensitivity and Specificity of ADC Markers

The histological features of adenocarcinoma shows two prominent morphological features: glandular differentiation in the form of tubules or papillae formation and mucin secretion. Immunostaining patterns of TTF-1, Napsin A, p63 and p40 in ADCs are shown in Figure 1.

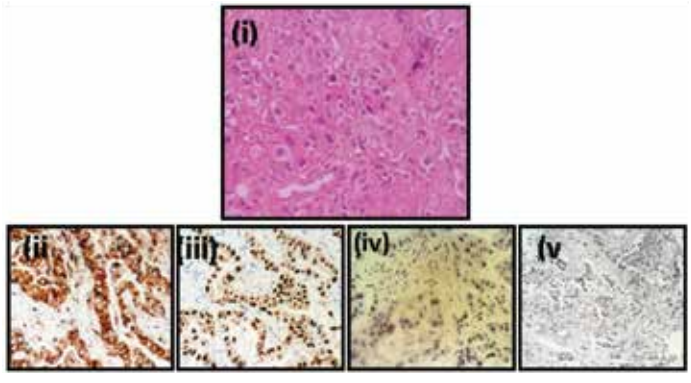

Figure 1: Representative microphotograph at x40 of Adenocarcinoma(i) H and E (ii) Napsin-A (iii) TTF-1 (iv) p40 (v) p63.

TTF-1 was positive in 35 of 40 pulmonary ADC compared with 36 of 40 that were positive for Napsin-A.There were 11 lung SQC cases in which TTF-1 showed false positive results and 5 lung ADC cases in which TTF-1 showed false negative results. On the other hand all squamous cell carcinoma cases were negative for Napsin-A, whereas Napsin-A showed false negative results in 4 cases. The results of IHC makers for ADC are summarized in Table 1.

Table 1: Sensitivity and specificity of IHC markers for ADC (n = 99)(a = true positive, b = false negative, c = false positive, d = true negative).

Statistic	Formula	TTF-1(95% CI)	Napsin-A(95% CI)	Combined
Sensitivity	$\frac{a}{a+b}$	87.5% (73.2% - 95.8%)	90.0% (76.3% - 97.2%)	100% (91.1% - 100%)
Specificity	$\frac{d}{c+d}$	81.3% (69.0% - 90.3%)	100% (93.94% - 100.0%)	81% (69.0% - 90.3%)
Disease prevalence	$\frac{a+b}{a+b+c+d}$	40.4% (30.6% - 50.7%)	40% (30.66% - 50.74%)	40.0% (30.6%-50.7%)
Positive predictive value	$\frac{a}{a+c}$	76.1% (64.8% - 84.6%)	100%	78.4% (68.0% - 86.1%)
Negative predictive value	$\frac{d}{b+d}$	90.5% (80.7% -95.6 %)	93.6% (85.34% -97.39%)	100%

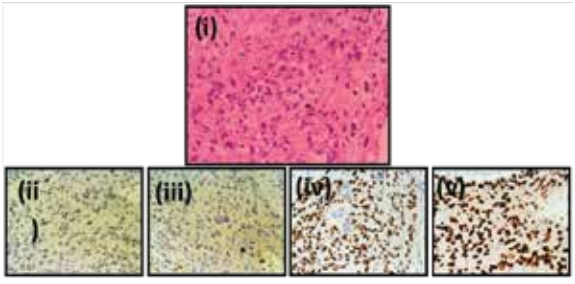

Figure 2: Representative microphotograph at x40 of Squamous cell carcinoma(i) H and E (ii) Napsin-A (iii) TTF-1 (iv) p40 (v) p63.

Sensitivity and Specificity of SQC Markers

The histological features of squamous cell carcinoma show keratinization, intercellular bridges and pearl formation. Immunostaining patterns of TTF-1, Napsin A, p63 and p40 in SQCs are shown in Figure 2.

The IHC marker p63 was positive in 55 out of 59 pulmonary SQC compared with 55 out of 59 that were positive for p40. There were 7 lung ADC cases in which p63 showed false positive results and 4 lung SQC cases in which p63 showed false negative results. On the other hand, all ADC cases were negative for p40 whereas p40 showed false negative results in 4 cases. The results of IHC makers for SQC are summarized in Table 2.

Table 2: Sensitivity and specificity of IHC marker p63 (n = 99)(a = true positive, b = false negative, c = false positive, d = true negative).

Statistic	Formula	P63 (95% CI)	P40 (95% CI)	Combined
Sensitivity	$\frac{a}{a+b}$	93.2% (83.5% - 98.1%)	93.2% (83.5% -98.1%)	100% (93.9% - 100%)
Specificity	$\frac{d}{c+d}$	82.5% (67.2% to 92.7%)	100% (91.2% - 100%)	82.5% (67.2% -92.6%)
Disease prevalence	$\frac{a+b}{a+b+c+d}$	59.6% (49.2% to 69.3%)	59.6% (49.3% - 69.3%)	59.6% (49.3% -69.3%)
Positive predictive value	$\frac{a}{a+c}$	88.7% (79.9% to 93.9%)	100%	89.4% (81.1% - 94.3%)
Negative predictive value	$\frac{d}{b+d}$	89.2 % (76.0% to 95.5%)	90.9% (79.5% - 96.3%)	100%

DISCUSSION

Precise subtyping of NSCLC into squamous and non-squamous type is very important as both these subtypes have different treatment strategy. The exclusion of SQC is compulsory before treating these patients with bevacizumab, as this drug can cause fatal hemoptysis if given in squamous cell carcinoma patients[3, 5].

For accurate classification of NSCLC on small biopsy specimen, this study used conventional makers (TTF-1 and p63) and newly proposed markers (Napsin-A and p40). Among these makers; TTF-1 is a

highly specific (97-100%) marker for lung cancers whereas, Napsin-A is a moderately sensitive (79-85%) and highly specific (100%) for lung ADC. The p63 shows positive expression for lung SQC and bronchial epithelial dysplasia and p40 is highly specific for SQC[19-22].

The current study showed that the sensitivity of Napsin-A was better than TTF-1 which is in accordance with Sharma et al., Gurda et al. and many other researchers[11, 20, 23, 24]. On contrary, one of the studies revealed that the sensitivity of Napsin-A and TTF-1 were similar[25]. However, there are few studies which concluded that TTF-1 has better sensitivity than Napsin-A[26, 27].

The current study concluded that Napsin-A had better specificity than TTF-1 which was in accordance with Zhao et al[27]. On contrary, Ikeda, et al. revealed that TTF-1 was better than Napsin-A in terms of sensitivity and specificity[26].

The current study concluded that all of the squamous cell carcinoma were negative for Napsin-A, whereas 11 squamous cell carcinoma showed positive expression for TTF-1. These findings were in accordance with another study, which documented that, all the 31 squamous cell carcinoma were Napsin-A negative, whereas 4 out of 31 squamous cell carcinoma showed positivity for TTF-128. In contrast to the currents study, Sharma et al. revealed focal positivity for TTF-1 in some cases of SQC (2/35 cases) and Napsin-A showed positive expression in 1/35 cases13. The current study also revealed that TTF-1 was negative in 5 out of 40 ADC cases which were positive for Napsin-A. These results were in accordance with Mukhopadhyay et al. who also concluded that few of the ADC cases, which showed negative expression for TTF-1, were Napsin-A positive[29].

On the basis on the current study, Napsin-A is a better marker for lung ADC, as compared to TTF-1. The major pitfall of TTF-1 is that it also shows positive expression for small cell lung cancer and neuroendocrine carcinomas[23,27,30]. Caution has to be taken while interpreting TTF-1 and should be correlated with the cyto-morphological findings. If TTF-1 is positive with the cytological features of neuroendocrine tumor then it is advised to perform some additional neuroendocrine markers, such as CD56, chromogranin A, synaptophysin, and Ki-67 to confirm the diagnosis of neuroendocrine tumors as well as SCLC.

While interpreting TTF-1 and Napsin-A, it should be kept in mind that alveolar macrophages are immunoreactive for both TTF-1 and Napsin-A. Alveolar macrophage should not be confused with the malignant cells. However, macrophages appear as isolated structure with round shape whereas malignant cells are found in large irregular clusters[31].

In the current study the sensitivity of p63 was similar to that of p40 (93.2%) which was in accordance with Zhao et al. and Bishop et al.[19]. In contrast to the current study, some researchers concluded that p63 had better sensitivity as compared to p4020,32. Some literature showed that the sensitivity of p40 was better than p6332. The current study concluded that p40 is more specific as compared to p63 which is in accordance with the studies conducted by Zhao et al. and Ma et al[27,32]. In accordance with the current study in which p40 was negative in all the ADC whereas p63 showed reactivity in 17.5% (7 out of 40) ADC, Bishop et al. and Zhao et al. also observed that p40 was entirely specific for SQC and was negative in all ADC whereas p63 was positive in some ADC[19,27]. Conclusively the discrepancies, which are found in the literature, might be due to different patients' selection, racial differences, sample size, antibody used (monoclonal/polyclonal). Another explanation for differences might be technical variability, which includes tissue fixation, processing, antigen retrieval and detection system.

CONCLUSION

In the current study, the expressions of TTF-1, Napsin-A, p63 and p40 were evaluated and compared in lung adenocarcinoma and squamous cell carcinoma, concluding that TTF-1 and Napsin-A are the makers of adenocarcinoma lung whereas p63 and p40 are the makers of squamous cell carcinoma lung. However, Napsin-A is more sensitive and specific when compared with TTF-1.Squamous cell carcinoma markers p63 and p40 are same but the specificity of p40 is better than that of p63. Thus, these two emerging markers can better classify the NSCLC into ADC and SQC, which will help the physicians, surgeons and patients. Thus, the current data might be used in the meta-analysis of the expressions of these markers in lung cancer.

ACKNOWLEDGEMENTS

The authors would like to thank Dow Diagnostics Reference and Research laboratory (DDRRL) at Dow University of Health Sciences, Karachi for all the help during the experiments.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Werutsky G, Hochhegger B, Lopes de Figueiredo Pinto JA, Martinez-Mesa J, Zanini ML, Berdichevski EH, et al. PET-CT has low specificity for mediastinal staging of non-small-cell lung cancer in an endemic area for tuberculosis: a diagnostic test study (LACOG 0114). BMC cancer. 2019;19(1):5.
2. Shaukat Khanum Memorial Cancer Hospital and

Research Centre (SKMCH&RC) https://shaukatkhanum.org.pk/health-care-professionals-researchers/cancer-statistics/.
3. Ezzat Nel S, Tahoun N. The role of Napsin-A and Desmocollin-3 in classifying poorly differentiating non-small cell lung carcinoma. J Egypt Natl Canc Inst. 2016;28(1):13-22.
4. Huang T, Li J, Zhang C, Hong Q, Jiang D, Ye M, et al. Distinguishing Lung Adenocarcinoma from Lung Squamous Cell Carcinoma by Two Hypomethylated and Three Hypermethylated Genes: A Meta-Analysis. PloS one. 2016;11(2):e0149088.
5. da Cunha Santos G, Shepherd FA, Tsao MS. EGFR mutations and lung cancer. Annu Rev Pathol. 2011;6:49-69.
6. Loo PS, Thomas SC, Nicolson MC, Fyfe MN, Kerr KM. Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens. J Thorac Oncol. 2010;5(4):442-7.
7. Cagle PT, Dacic S. Lung cancer and the future of pathology. Arch Pathol Lab Med. 2011;135(3):293-5.
8. Nishino M, Hoang MP, Della Pelle P, Morales-Oyarvide V, Huynh TG, Mark EJ, et al. Napsin A/p40 antibody cocktail for subtyping non-small cell lung carcinoma on cytology and small biopsy specimens. Cancer Cytopathol. 2016;124(7):472-84.
9. da Cunha Santos G, Lai SW, Saieg MA, Geddie WR, Pintilie M, Tsao MS, et al. Cyto-histologic agreement in pathologic subtyping of non small cell lung carcinoma: review of 602 fine needle aspirates with follow-up surgical specimens over a nine year period and analysis of factors underlying failure to subtype. Lung Cancer. 2012;77(3):501-6.
10. 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.
11. Turner BM, Cagle PT, Sainz IM, Fukuoka J, Shen SS, Jagirdar J. Napsin A, a new marker for lung adenocarcinoma, is complementary and more sensitive and specific than thyroid transcription factor 1 in the differential diagnosis of primary pulmonary carcinoma: evaluation of 1674 cases by tissue microarray. Arch Pathol Lab Med. 2012;136(2):163-71.
12. Travis WD, Rekhtman N, Riley GJ, Geisinger KR, Asamura H, Brambilla E, et al. Pathologic diagnosis of advanced lung cancer based on small biopsies and cytology: a paradigm shift. J Thorac Oncol. 2010;5(4):411-4.
13. Sharma R, Wang Y, Chen L, Gurda GT, Geddes S, Gabrielson E, et al. Utility of a novel triple marker (combination of thyroid transcription factor 1, Napsin A, and P40) in the subclassification of non-small cell lung carcinomas using fine-needle aspiration cases. Hum Pathol. 2016;54:8-16.
14. Fatima N, Cohen C, Lawson D, Siddiqui MT. TTF-1 and Napsin A double stain: a useful marker for diagnosing lung adenocarcinoma on fine-needle aspiration cell blocks. Cancer cytopathol. 2011;119(2):127-33.
15. Yung RC, Otell S, Illei P, Clark DP, Feller-Kopman D, Yarmus L, et al. Improvement of cellularity on cell block preparations using the so-called tissue coagulum clot method during endobronchial ultrasound-guided transbronchial fine-needle aspiration. Cancer cytopathol. 2012;120(3):185-95.
16. Gurda GT, Zhang L, Wang Y, Chen L, Geddes S, Cho WC, Askin F, Gabrielson E, Li QK. Utility of five commonly used immunohistochemical markers TTF-1, Napsin A, CK7, CK5/6 and P63 in primary and metastatic adenocarcinoma and squamous cell carcinoma of the lung: a retrospective study of 246 fine needle aspiration cases. Clin Transla Med. 2015 Dec 1;4(1):16.
17. Shankar S, Thanasekaran V, Dhanasekar T, Duvooru P. Clinicopathological and immunohistochemical profile of non-small cell lung carcinoma in a tertiary care medical centre in South India. Lung India. 2014;31(1):23-8.
18. Bari MF, Brown H, Nicholson AG, Kerr KM, Gosney JR, Wallace WA, et al. BAI3, CDX2 and VIL1: a panel of three antibodies to distinguish small cell from large cell neuroendocrine lung carcinomas. Histopathology. 2014;64(4):547-56.
19. Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. p40 (DeltaNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol. 2012;25(3):405-15.
20. Ao MH, Zhang H, Sakowski L, Sharma R, Illei PB, Gabrielson E, et al. The utility of a novel triple marker (combination of TTF1, napsin A, and p40) in the subclassification of non-small cell lung cancer. Hum Pathol. 2014;45(5):926-34.
21. Lilo MT, Allison D, Wang Y, Ao M, Gabrielson E, Geddes S, et al. Expression of P40 and P63 in lung cancers using fine needle aspiration cases. Understanding clinical pitfalls and limitations. J Am Soc Cytopathol. 2016;5(3):123-32.
22. Koh J, Go H, Kim MY, Jeon YK, Chung JH, Chung DH. A comprehensive immunohistochemistry algorithm for the histological subtyping of small biopsies obtained from non-small cell lung cancers. Histopathology. 2014;65(6):868-78.
23. Bishop JA, Sharma R, Illei PB. Napsin A and thyroid transcription factor-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. Hum Pathol. 2010;41(1):20-5.
24. Noh S, Shim H. Optimal combination of immunohistochemical markers for subclassification of non-small cell lung carcinomas: A tissue microarray study of poorly differentiated areas. Lung Cancer. 2012;76(1):51-5.
25. Rekhtman N, Ang DC, Sima CS, Travis WD, Moreira AL. Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. Mod Pathol. 2011;24(10):1348-59.
26. Ikeda S, Naruse K, Nagata C, Kuramochi M, Onuki T, Inagaki M, et al. Immunostaining for thyroid

transcription factor 1, Napsin A, p40, and cytokeratin 5 aids in differential diagnosis of non-small cell lung carcinoma. Oncol Lett. 2015;9(5):2099-104.
27. Zhao W, Wang H, Peng Y, Tian B, Peng L, Zhang DC. DeltaNp63, CK5/6, TTF-1 and napsin A, a reliable panel to subtype non-small cell lung cancer in biopsy specimens. Int J Clin Exp Pathol. 2014;7(7):4247-53.
28. Ueno T, Elmberger G, Weaver TE, Toi M, Linder S. The aspartic protease napsin A suppresses tumor growth independent of its catalytic activity. Lab Invest. 2008;88(3):256-63.
29. Mukhopadhyay S, Katzenstein AL. Comparison of monoclonal napsin A, polyclonal napsin A, and TTF-1 for determining lung origin in metastatic adenocarcinomas. Am J Clin Pathol. 2012;138(5):703-11.
30. Ueno T, Linder S, Elmberger G. Aspartic proteinase napsin is a useful marker for diagnosis of primary lung adenocarcinoma. Br J Cancer. 2003;88(8):1229-33.
31. Aikawa E, Kawahara A, Hattori S, Yamaguchi T, Abe H, Taira T, et al. Comparison of the expression levels of napsin A, thyroid transcription factor-1, and p63 in nonsmall cell lung cancer using cytocentrifuged bronchial brushings. Cancer Cytopathol. 2011;119(5):335-45.
32. Ma Y, Fan M, Dai L, Kang X, Liu Y, Sun Y, et al. Expression of p63 and CK5/6 in early-stage lung squamous cell carcinoma is not only an early diagnostic indicator but also correlates with a good prognosis. Thoracic Cancer. 2015;6(3):288-95.