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Role of minimal panel immunostaining in accurate diagnosis of lung cancer using small biopsies

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ABSTRACT

Introduction: In small biopsies standard morphology cannot specifically subtype the tumor. Histologic subtyping of lung cancer is mandatory for treatment. Immunohistochemical staining is a valuable tool for diagnosis of lung cancer.
Aim: The aim of this study was to evaluate the diagnostic accuracy of minimal panel of Napsin A, CK 5/6 and CD 56 versus H&E of lung cancer in small biopsies.
Methods: 84 small sized tissue samples were obtained. Seventy samples were obtained via fiberoptic bronchoscope (FOB) and 14 samples were obtained with transothoracic CT guided trucut needle. All samples were stained with H&E for morphologic diagnosis, then the same samples were stained with immunohistochemical (IHC) staining including 3 antibodies (Napsin A, CK 5/6 and CD 56), then we compared the diagnostic yield of both methods.
Results: After H&E staining, according to WHO 2004 classification: 40 cases were adenocarcinoma (AC), 10 were squamous cell carcinoma (SCC), 22 were large cell carcinoma (LCC) and 12 were neuroendocrine tumors (NET). After IHC; 54 (64.3%) were AC, 11 (13.1%) were SCC, 11 (13.1%) were NET and 8 (9.5%) were non small cell lung cancer not otherwise specified NSCLC NOS (Counterpart of large cell carcinoma in 2004 WHO classification). Napsin A was expressed in 98% (53/54) and CK 5/6 in 90.9% (10/11) of SCC. CD 56 in 100% (11/11) of neuroendocrine tumors.
Conclusion: IHC with Napsin A, CK 5/6 and CD 56 has a more diagnostic value in precise typing of different cell types of lung cancer than H&E in small biopsies.

Introduction

Lung cancer is one of the major causes of cancer deaths worldwide. Lung cancer is the main cause of cancer death cases in men as well as in women, lung cancer is the second leading cause of death cases from cancer globally, following breast cancer [1].

In Egypt, according to The National Cancer Institute lung cancer is the first cause of cancer related death cases in both gender [2].

Seventy percent of lung cancers are presented in advanced stages and can't be resected, small biopsy specimens remain the main method of diagnosis for the majority of lung cancer patients [3].

Based on microscopic picture, main types are: Non-small cell lung cancer (NSCLC), representing 85% of the patients. (NSCLC) include types of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma [4] and small cell lung cancer (SCLC), accounting for about (15%) of the patients. Neuroendocrine tumors are also included among main types of lung cancer.

Recently, classification of lung cancer especially the Non-small variant found to be very important in the field of targeted therapy, so accurate subtyping is important especially in small biopsy [3,5].

IASLC provided recommendation for the use of immune stains as an aid to diagnosis, especially in tumors that do not show established morphologic criteria by H&E stain [6]. Established morphological criteria are as follow: glandular differentiation or mucin for adenocarcinoma, where the criteria for squamous cell carcinoma are intercellular bridges and or keratinization. When all previous established morphologic criteria absent, we do immunohistochemistry (see Table 1).

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Table 1
Detailed antigen retrieval methods and antibody dilution for each primary antibody.

Antigen	Type	Manufacturer	Catalog	Dilution	Procedure	Antibody incubation	Antigen Retrieval
Napsin A	Rabbit Polyclonal	Cell MARQUE	352A-78	Ready to use 7 ml prediluted	Manual	Over night	Heat induced epitope retrieval (HIER)
CK5/6	Mouse monoclonal	DAKO	Clone D5/16B4	Ready to use 6 ml	Manual	Over night	Heat induced epitope retrieval (HIER)
CD56	Mouse monoclonal	DAKO	Clone 123C3	Ready to use 6 ml	Manual	Over night	Heat induced epitope retrieval (HIER)

Napsin A is a lung-specific marker [7] as it is more sensitive and specific than TTF-1 in diagnosis of lung adenocarcinoma [8]. The diagnosis of SCC typically does not require the use of IHC techniques. CK 5/6 is useful in diagnosis of SCC especially in the poorly differentiated cell type [9,10].

CD56 is one of the most helpful neuroendocrine immunohistochemical markers [11,12]. It's the most sensitive marker for SCLC [13].

The key immunohistochemical stains useful for SCLC diagnosis including keratin, chromogranin, CD56, Ki-67, TTF-1 [14].

Aim of the study

The aim of the study was to evaluate the diagnostic accuracy of minimal panel of Napsin A, CK 5/6 and CD 56 versus H&E of lung cancer in small biopsies.

Materials and methods

Prospective case series study included 84 patients with lung cancer; 70 cases were central tumors diagnosed with biopsies obtained via FOB (pentax FB-18V or FB 19 TV) and 14 cases with peripheral tumors diagnosed with transthoracic CT or ultrasound guided biopsies.

All biopsies were formalin fixed paraffin embedded, processed and from which 4-μm-thick sections were obtained and stained by H&E then examined at pathology department, Faculty of medicine, Mansoura university by 2 pathologists.

Immunostaining

For IHC staining, we routinely deparaffinized 4-m-thick sections from paraffin block. The sections were incubated with 3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. We used EDTA and citrate buffer. The sections were incubated overnight at 37 °C, and stained with a HRP (horseradish peroxidase) method. The following antibodies were applied (Napsin-A–C K5/6–CD56). We used 3-3′-diaminobenzidine, harris hematoxylin as the chromogen and counter stain, respectively.

Positive immunostaining for CD56 required 10% or more cells with an intensity of at least 2+ on the relevant subcellular localization [membranous for CD56/Neural Cell Adhesion Molecule (NCAM)].

CK5/6 was considered positive for when it was cytoplasmic. For Napsin A, stain was considered positive when it revealed cytoplasmic granularity.

Statistics

Data were analyzed with SPSS version 16. The normality of data was first tested with one-sample Kolmogorov-Smirnov test.

Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test. When 25% of the cells have expected count less than 5, Fisher exact test was used.

Continuous variables were presented as mean ± SD (standard deviation). Analysis Of Variance (ANOVA test) used for comparison of means of more than two groups.

Level significance

For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value).

The results were considered highly significant when the probability of error is less than 0.1% ($p < 0.001$).

Results

According to established morphologic criteria, adenocarcinoma represented 47.6% of the cases (Table 2). However, after application of immunostain adenocarcinoma was the most prevalent variant among the histologic subtypes of lung cancer representing 64.3% of the total cases (Table 3). It was found that adenocarcinoma represented 47.6% of the cases. The term NSCLC (NOS) was present only in the IASLC 2011 classification and it is the counterpart to large cell carcinoma to WHO 2004 classification.

After the application of immunostain, NSCLC (NOS) cases decreased from 33.3–9.5% (Table 3).

Twenty eight cases initially diagnosed as NSCLC (NOS) where established morphologic criteria of squamous cell carcinoma and adenocarcinoma were absent. With application of three immunestain (Napsin A, CK 5/6 and CD 56) the result were as follow: The immuneprofile of nineteen cases was Napsin A +ve, CK 5/6 −ve, CD 56 −ve. So, diagnosis changed to NSCLC favour adenocarcinoma. One Case was Napsin A −ve, CK 5/6 +ve, CD 56 −ve. So, diagnosis changed to NSCLC favour squamous cell carcinoma (Photo 2). Eight cases were Napsin A −ve, CK 5/6 −ve, CD 56 −ve. So, the diagnosis remained NSCLC (NOS).

Thirteen cases were initially diagnosed as solid predominant adenocarcinoma and stained with Napsin A and CK 5/6. The results were as follow: Twelve cases were Napsin A +ve, CK 5/6 −ve and one case was Napsin A −ve, CK 5/6 −ve with positive internal con-

Table 2
Histologic subtype of lung cancer among the studied cases according to WHO 2004 classification of lung cancer (morphological diagnosis by H&E) (n = 84).

Types		n	%
ADC		40	47.6
	Acinar pattern	9	
	Solid pattern with mucin production	8	
	Papillary pattern	1	
	Non mucinous bronchioalveolar	2	
	Mucinous type bronchioalveolar	1	
	Mixed pattern	19	
SCC		10	11.9
LCC		22	26.2
Neuroendocrine tumors	Small Cell Carcinoma	10	14.3
	Atypical carcinoid	2	

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