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Pathology

USE OF A THREE PANEL IHC MARKER- CK 5/6, P63 & TTF-1 IN SUB CLASSIFYING POORLY DIFFERENTIATED LUNG CARCINOMAS

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ABSTRACT The use of immunohistochemistry (IHC) has been well documented as an important ancillary tool in subclassifying lung carcinomas. This study is aimed to assess the expression of a panel 3 IHC markers - CK5/6, p63 and TTF-1, in lung carcinomas and to study their efficiency in subclassifying poorly differentiated carcinomas. It was observed that 57% of poorly differentiated carcinomas included in this study could be subclassified based on their immunoprofile. In conclusion, three panel IHC markers, CK5/6, p63 and TTF-1 when used together and interpreted correctly can be of help for the final diagnosis even in cases in which the morphology is unclear.

KEYWORDS : CK 5/6, p63, TTF-1,Poorly differentiated carcinoma, Squamous cell carcinoma, Adenocarcinoma

INTRODUCTION

Lung cancer is the leading cause of cancer related mortality, both in the United States and worldwide . Clinically, lung carcinomas were classified as non-small cell lung cancer (NSCLC) or small cell lung cancer.

According to the current World Health Organization classification, Epithelial tumors include the following histological types: Squamous cell carcinoma(SCC); Adenocarcinoma(ADC), Neuroendocrine tumors, Large cell carcinoma(LCC) etc. In the same classification, small cell carcinomas (SCLC) are included under neuroendocrine tumors[2]. Clinical staging and identification of the histological type are fundamental to establishing the therapeutic strategy for treating such patients. The identification of the different histological types and of prognostic factors is important and has the purpose of selecting patients for receiving appropriate treatment, in addition to identifying tumors at higher risk of recurrence. These selected patients would potentially benefit from therapeutic approaches directed at the histological type, as well as from adjuvant therapies aimed at avoiding cancer recurrence and fatal outcomes.

This study aims to assess the utility of immunohistochemical markers in subclassifying poorly differentiated carcinomas

Settings and Design: This is a Cross sectional study with a sample size of 21 cases done over a period of 18 months

METHODS AND MATERIAL: The formalin fixed bronchial biopsy of all cases after processing and embedding in paraffin, multiple 3–5 mm sections were taken and each biopsies were stained with hematoxylin and eosin (H&E).

The specimens were processed for immunohistochemical examination. The immunohistochemical analysis was performed on serial sections, using an immunoenzymatic soluble compex method. 4µm thick sections were made on poly L lysine coated slides, kept at 37^0C overnight & 60^0C for 1 hour, dewaxing was done in xylene. Hydration was done followed by antigen retrieval & staining.

Antibodies used for immunohistochemistry were Rabbit Monoclonal Antibody CK 5/6, Mouse Monoclonal Antibody p63 and Mouse Monoclonal Antibody TTF-1 Immunohistochemically, cytoplasmic staining was considered positive for cytokeratin 5/6 and nuclear staining was considered positive for TTF-1 and p63.

Immunoreactivity was scored semiquantitatively by recording percentage of reactive tumor cells. Diffuse reactivity was defined as labeling of >50% tumor cells, and focal reactivity as 1–49%. In addition, intensity of reactivity was recorded as weak (1+, less than normal cells); moderate (2+, same as normal cells); and strong (3+, stronger than normal cells). Pneumocytes served as internal controls for TTF-1 reactivity, and bronchial basal cells for p63 & CK5/6.

For subclassification of poorly differentiated lung carcinomas using immunohistochemical markers an algorithm was proposed based on the study by Mukhopadhyay and Katzenstein[1]:

Table 1

Adenocarcinoma Marker (TTF 1)	Squamous Cell Carcinoma Markers (P63 And CK5/6)	Diagnosis On Small Biopsy
Positive	Both Negative	Poorly differentiated adenocarcinoma
Positive	p63 positive, CK5/6 negative	Poorly differentiated adenocarcinoma
Negative	Both Positive	Poorly differentiated squamous cell carcinoma
Negative	p63 diffusely positive, CK5/6 negative	Poorly differentiated squamous cell carcinoma
Negative	p63 focally positive, CK5/6 negative	Poorly differentiated non-small cell carcinoma/NSCLC, NOS
Negative	Negative	Poorly differentiated non-small cell carcinoma/NSCLC, NOS

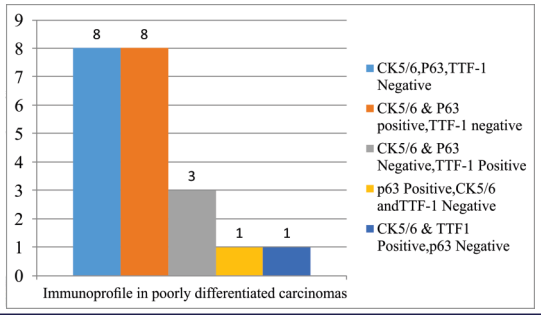
Statistical analysis : Data were collected and entered in Microsoft office Excel 2007 sheet. Statistical analysis was done using SPSS software, version 16.

RESULTS:

Based on the modifications in the algorithm proposed in a study by Mukhopadhyay et al[1], for subclassification of poorly differentiated carcinomas according to their immunoprofile, 12 out of the 21 cases (57%) included in this study could be subclassified.

Eight cases showed an immunoprofile consistent with Squamous cell carcinoma i.e, CK5/6, p63 positive and TTF-1 negative were classified as Poorly differentiated squamous cell carcinoma. Also, 1 case which showed positivity for only p63 with CK5/6 and TTF-1 negative was also named the same. Three cases which showed an immunoprofile of that of adenocarcinoma i.e, TTF-1 positive, CK5/6 and p63 negative were classified as poorly differentiated adenocarcinoma.

Immunoprofile in poorly differentiated lung carcinomas (21 cases)
Chart 1



Analysis of all the cases which could be sub-classified showed that the immunoreactivity pattern was highly variable ranging from focal to diffuse and weak to strong.

Rest of the nine cases could not be subclassified as majority did not show any expression for the three immunohistochemical markers and one case showed expression of both CK5/6 and TTF-1.

DISCUSSION:

Lung cancer is one of the cause of cancer-related mortality in men and women worldwide. Several recent studies have addressed the issue of using immunohistochemistry panels to correctly sub classify lung carcinomas. Two recent articles on this subject are the studies of Mukhopadhyay and Katzenstein[1] and Terry et al[3]. One observation regarding the use of IHC to sub-classify lung carcinomas has been that the clinical trials validating the various histologic-specific treatment modalities were based on H&E classification.

In the current study a panel of three immunohistochemical markers, namely CK5/6, p63 and TTF-1 were used for subclassifying poorly differentiated lung carcinomas.

Based on the immunoprofile of lung carcinomas analysed in the current study and by the algorithm proposed by Mukhopadhyay et al[1] in their study for subclassification of poorly differentiated carcinomas, 57% of poorly differentiated carcinomas (12 out of 21 cases) could be subclassified using three-panel IHC markers, CK5/6, TTF-1 and p63.

Accordingly, if glandular marker (TTF-1) is positive and both squamous markers (CK5/6 and p63) are negative, and in cases with glandular marker negative and both squamous markers are positive diagnosis of poorly differentiated adenocarcinoma and poorly differentiated squamous cell carcinoma respectively, can be made. However, interpretation of isolated p63 positivity depends on the proportion of tumor cells stained. As occasional poorly differentiated squamous cell carcinomas are p63 positive (diffuse) but CK5/6 negative. Hence, diffuse p63 positivity is diagnostic of poorly differentiated squamous cell carcinoma. However, isolated focal p63 positivity is non-specific, and such tumors should be considered unclassifiable. Other staining combinations such as a positive CK5/6 with positive TTF-1 do occur occasionally but are uncommon, and there is insufficient evidence to subclassify them on small biopsies.

Furthermore, observations by Wei Zhao et al[4], Downey et al[5] and Kenneth Whithaus et al[6] showed that CK5/6 had high sensitivity and specificity in squamous cell carcinomas. Wang et al, also detected p63 in 65% of pulmonary adenocarcinomas, but positivity was focal, in contrast with extensive p63 immunostaining found in squamous cell carcinomas.

Based on these observations, in the present study eight cases which showed an immunoprofile consistent with Squamous cell carcinoma i.e, CK5/6, p63 positive, TTF-1 negative and one case that showed positivity for only p63 (diffuse and strong) with CK5/6 and TTF-1 negative were classified as Poorly differentiated squamous cell carcinoma.

Based on the observations by Mukhopadhyay and Katzenstein ,Wei Zhao et al and Kargi et al TTF1 was found to be highly specific for Adenocarcinomas [1,7,8].Accordingly, three cases which showed an immunoprofile of that of adenocarcinoma i.e, TTF-1 positive, CK5/6 and p63 negative were classified as poorly differentiated adenocarcinoma.

CONCLUSIONS:

57% of poorly differentiated carcinomas included in this study could be subclassified based on their immunoprofile. Immunoreactivity observed in poorly differentiated carcinomas varied from weak to strong and focal to diffuse.

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