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

By

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- **Lung cancer is a leading cause of cancer deaths world wide.**
- **In Egypt, according to statistics released by National Cancer Institute, lung cancer cases represent 8.2% of total cancer cases in men and about 2.4% of total cancer among women .**
- **Cancer lung is related to cigarette smoking.**

Siegel R, et al., 2012 - El-Bolkainy M et al., 2012

- **Many methods are available for pathologic diagnosis of lung cancer important among which are : Sputum cytology, bronchial brushing, invasive procedures such as bronchoscopic biopsy and bronchial lavage are widely used in diagnosis of lung cancers .**

Travis et al., 2012

- Because 70% of lung cancers are unresectable as patients present in advanced stages, small biopsy and cytology specimens are the primary method of diagnosis for the majority of lung cancer patients. All received specimens in this work are small biopsy.

Travis et al., 2011

Immunohistochemistry is a valuable tool in diagnosis of lung cancer especially in small biopsy. The importance of the IASLC 2011 new classification of lung cancer in small biopsies has been implified also. In this study we used minimal panel for diagnosis of lung cancer. This panel included **Napsin A, CK 5/6 & CD 56.**

Aim of work

We aim in this study to apply recommendations of International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS) /European Respiratory Society (ERS) on diagnosis of lung cancer in small biopsies and to define role of immunohistochemical staining in definitive diagnosis and subcategorization of lung cancer .

Patient and method

This is a prospective case series of **86 patients** with lung cancer either central or peripheral.

All cases admitted or referred to chest medicine department, Mansoura University from the period between April 2012 and April 2014.

Fiberoptic bronchoscope biopsy is used for central lung tumors in 70 cases and trams thoracic CT guided for peripheral lung tumor in 16 cases.

Patient consent was done for all cases.

Antigen	Localization of expression	Scoring of tumor intensity	Scoring of % positive tumor cell
Napsin A	Granular cytoplasmic	0 negative 1 weak 2 intermediate 3 strong	Score 1: 1-9% Score 2: 10 – 49% Score 3: > 50%
CK5/6	Cytoplasmic	The same	As napsin-A
CD56	Cytoplasmic or membranous	The same	> 10% tumor cells

Data were analyzed with SPSS version 16.

Results

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

histologic subtypes of tumors after immune staining with Napsin A , CK5/6 , CD56 (n=84) (According to IASLC 2011)

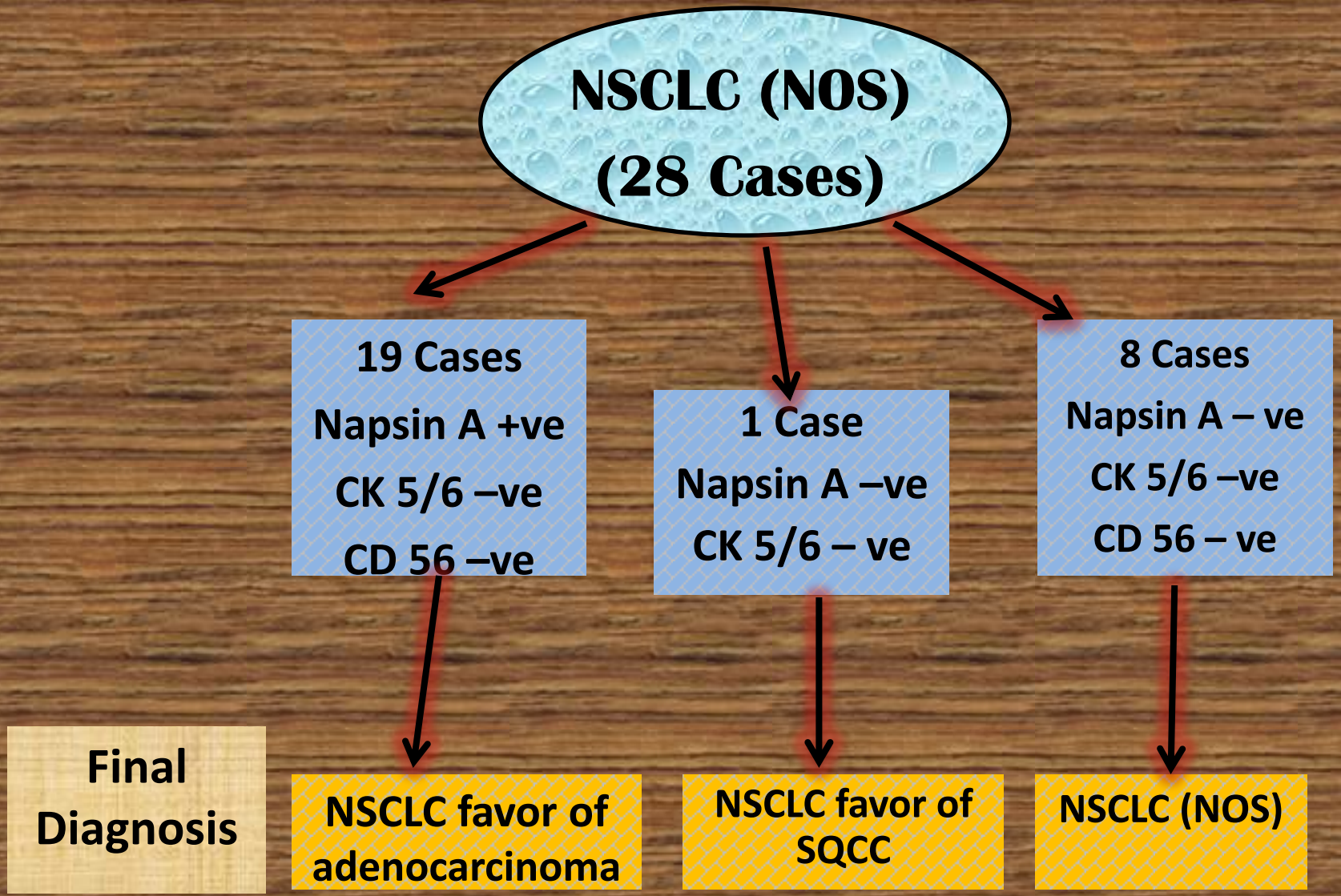
Tumor Cell Types	n	%
ADC	54	64.3
SCC	11	13.1
NSCL (NOS)	8	9.5
Neuroendocrine tumors	11	13.1

This table shows that adenocarcinoma is the most common histologic subtype of lung cancer representing 64.3% of the total cases .

♠ What is NSCLC (NOS) ?

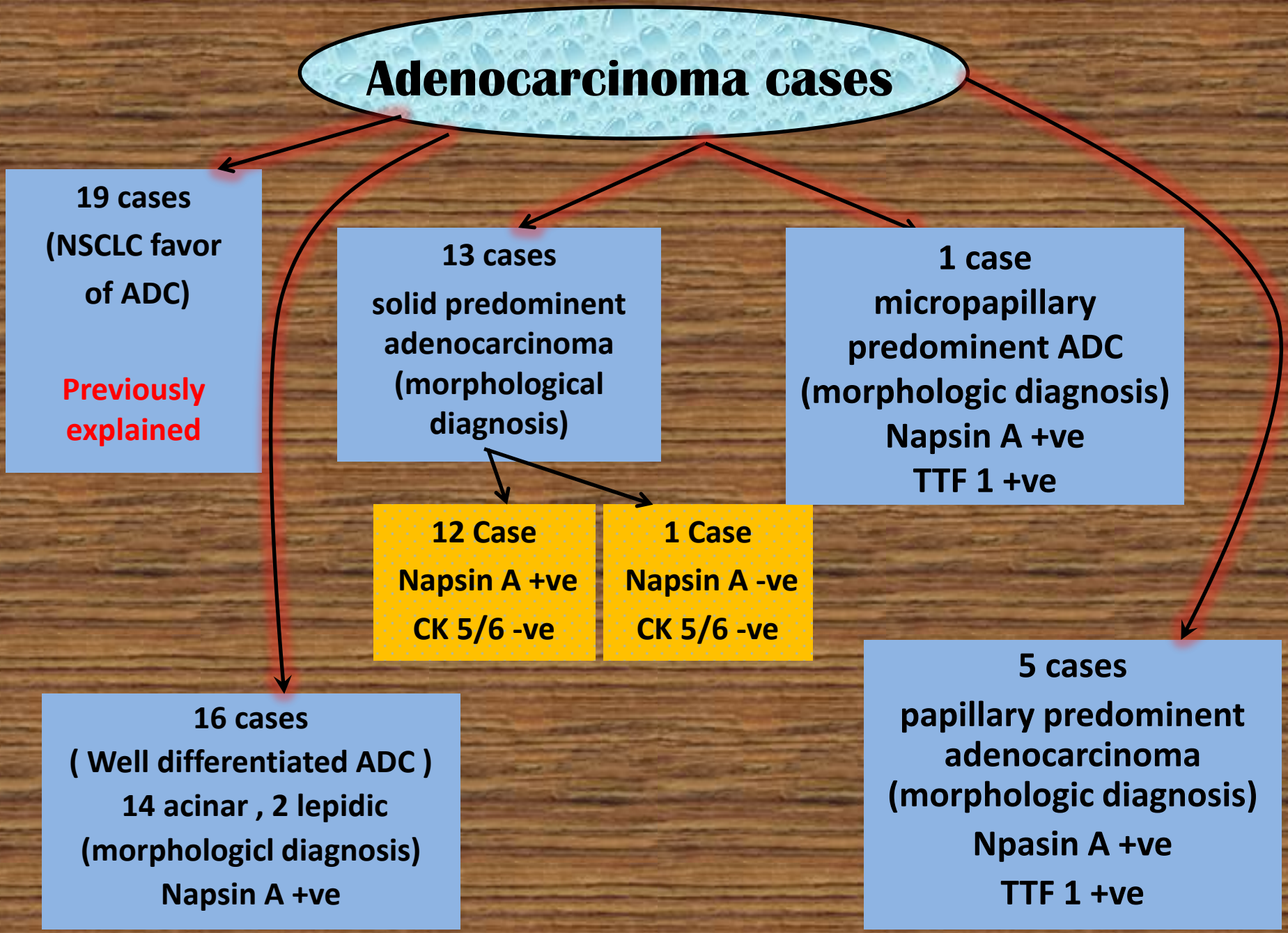
Term present only in IASLC 2011 classification & in 2015 WHO classification of lung cancer.

It's counterpart to large cell carcinoma in 2004 WHO classification of lung cancer.


NSCLC (NOS)
(28 Cases)
19 Cases
Napsin A +ve
CK 5/6 –ve
CD 56 –ve
1 Case
Napsin A –ve
CK 5/6 – ve
8 Cases
Napsin A – ve
CK 5/6 –ve
CD 56 – ve
Final Diagnosis
NSCLC favor of adenocarcinoma
NSCLC favor of SQCC
NSCLC (NOS)

So,

after use of immunestain number of NSCLC (NOS) decreased from 33.3% to 9.5% .


Adenocarcinoma cases
19 cases
(NSCLC favor
of ADC)
Previously
explained
13 cases
solid predominent
adenocarcinoma
(morphological
diagnosis)
1 case
micropapillary
predominent ADC
(morphologic diagnosis)
Napsin A +ve
TTF 1 +ve
12 Case
Napsin A +ve
CK 5/6 -ve
1 Case
Napsin A -ve
CK 5/6 -ve
16 cases
(Well differentiated ADC)
14 acinar , 2 lepidic
(morphologicl diagnosis)
Napsin A +ve
5 cases
papillary predominent
adenocarcinoma
(morphologic diagnosis)
Npasin A +ve
TTF 1 +ve

So,

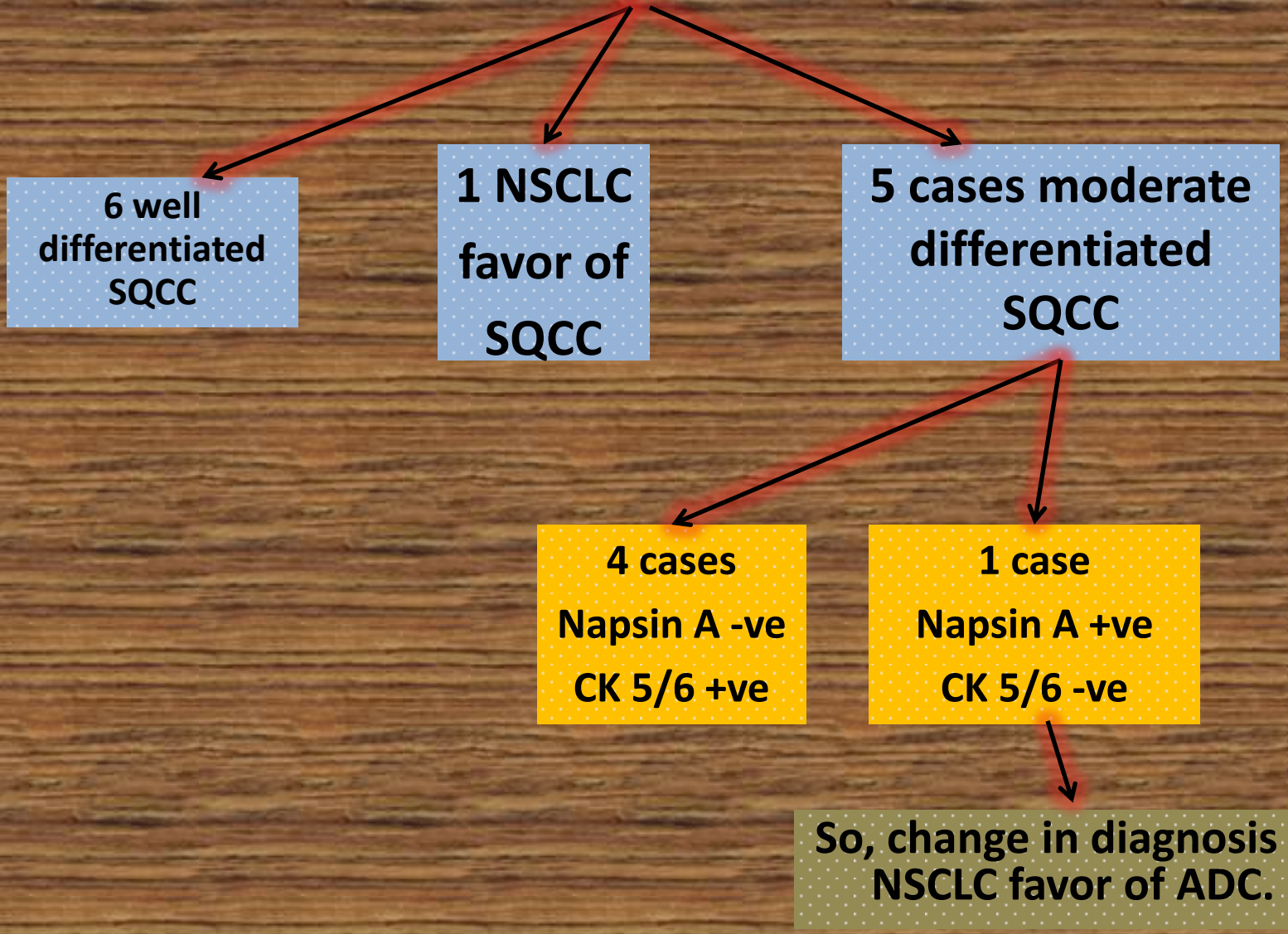
cases of adenocarcinoma increased after use of immunestain from 47.6% to 64.3% .

Why we added TTF-1 to Napsin A in diagnosis of paillary and the micropapillary variants of adenocarcinoma ?

Change in diagnosis of adenocarcinoma after immunohistochemistry

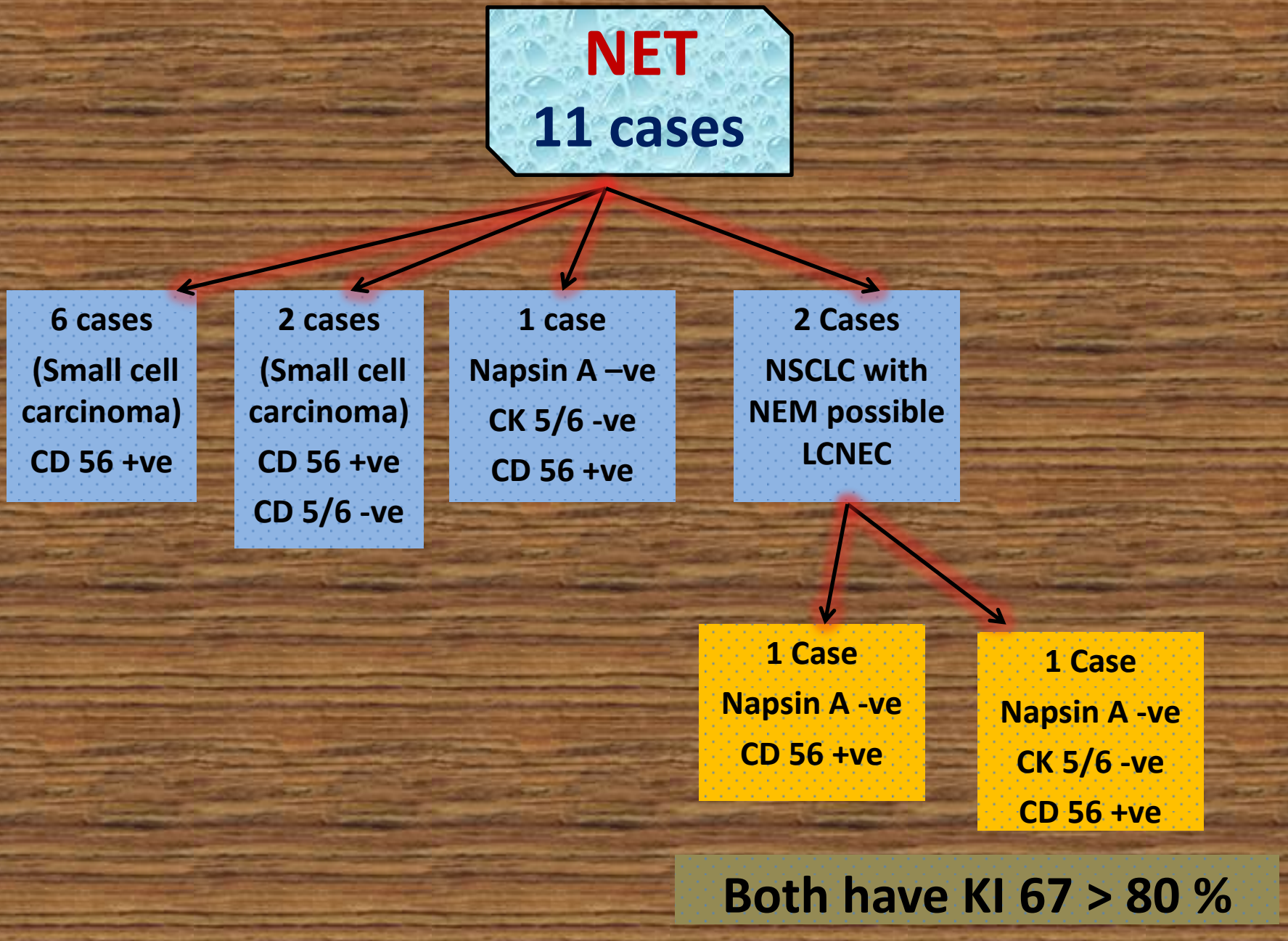
	All cases n = 54	Solid	Acinar	Papillary	Lepidic	Micro papillary
1 stain	Napsin A +ve		14		2	
	Napsin A –ve					
2 stains	Napsin A +ve – CK5/6 –ve	12				
	Napsin A -ve – CK5/6–ve	1		5		
	Napsin A +ve – TTF1+ve					1
3 stains	Napsin A +ve – CK5/6 -ve – CD56-ve	19				

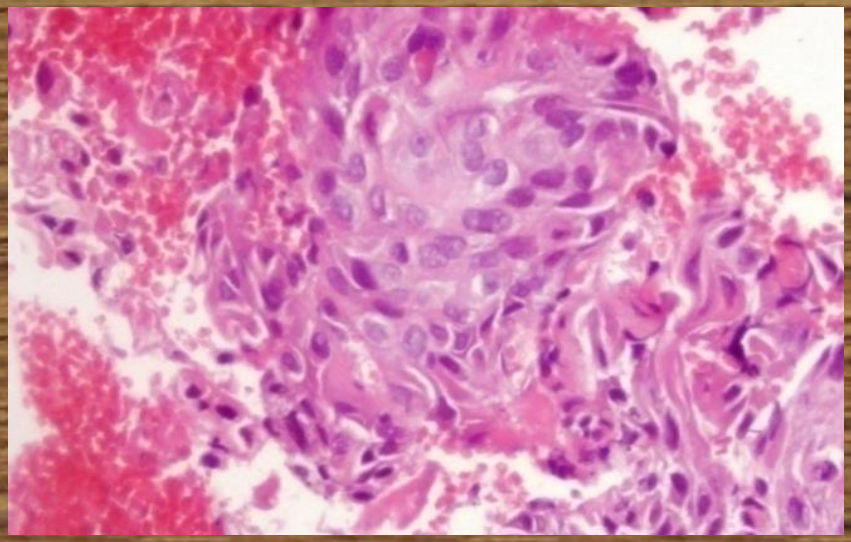
Squamous cell carcinoma (SQCC)



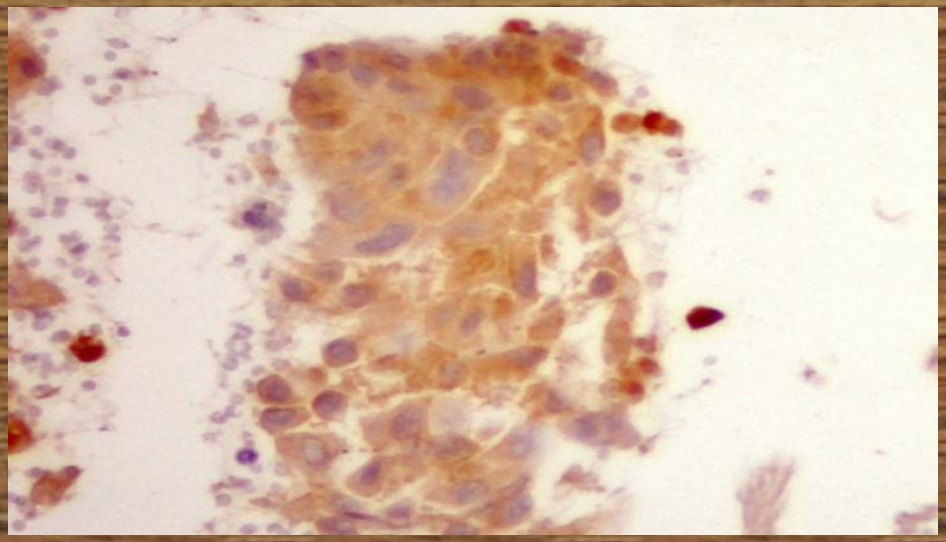
Why we added Napsin A to CK 5/6 in SQC cases ?

To exclude squamoid variant of adenocarcinoma.


NET
11 cases
6 cases
(Small cell carcinoma)
CD 56 +ve
2 cases
(Small cell carcinoma)
CD 56 +ve
CD 5/6 -ve
1 case
Napsin A –ve
CK 5/6 -ve
CD 56 +ve
2 Cases
NSCLC with NEM possible LCNEC
1 Case
Napsin A -ve
CD 56 +ve
1 Case
Napsin A -ve
CK 5/6 -ve
CD 56 +ve
Both have KI 67 > 80 %

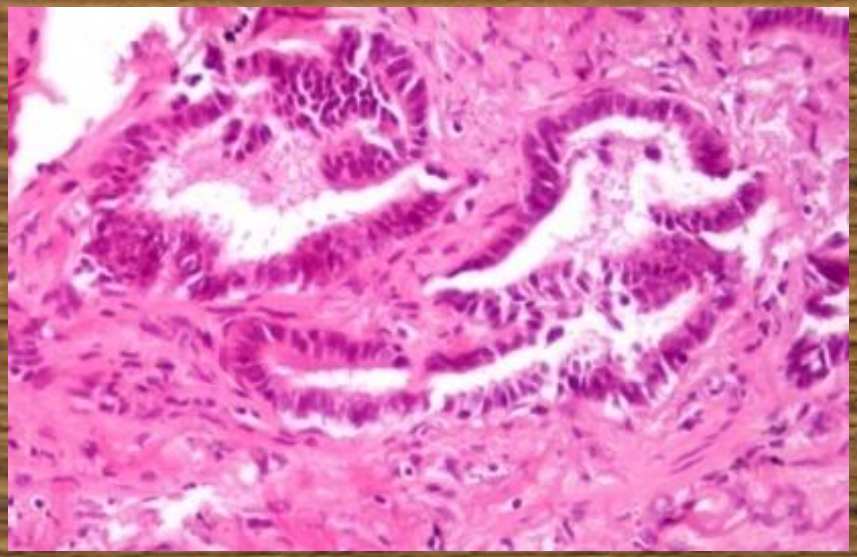


H&E 200x

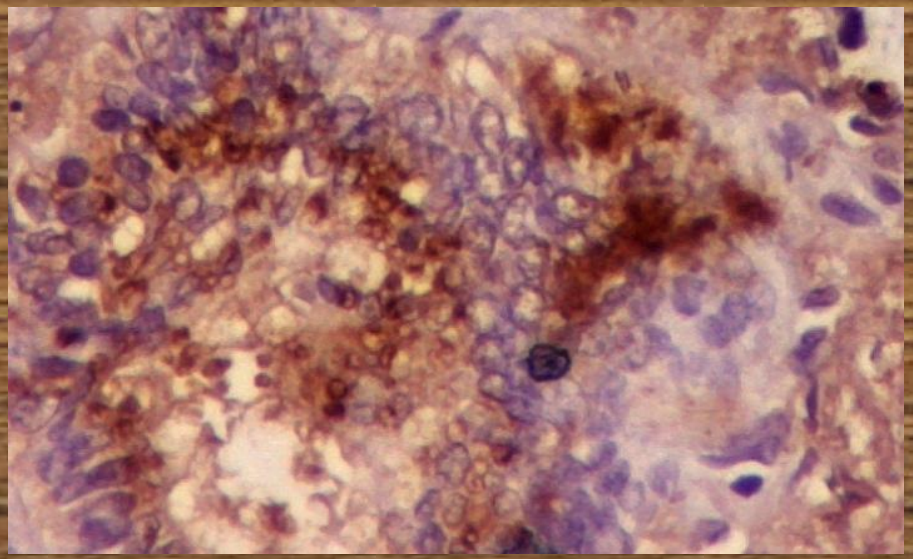


CK 5/6 400x

A case of NSCLC(NOS) proved to be squamous cell carcinoma. A) H&E shows malignant cells with abundant cytoplasm, vesicular nuclei and marked atypia x200. B) Positive cytoplasmic staining of tumor cells for ck5/6 ImmunoPeroxidase-DAB x400. C,D) Negative staining.

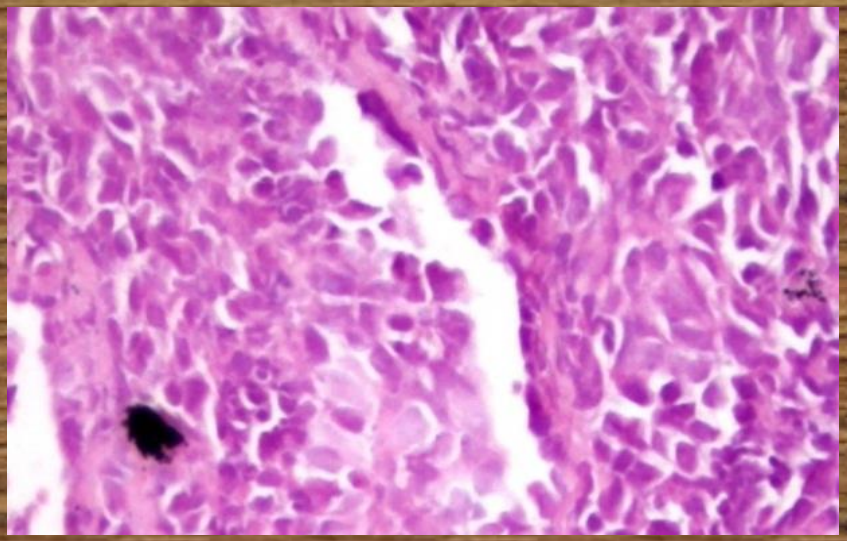


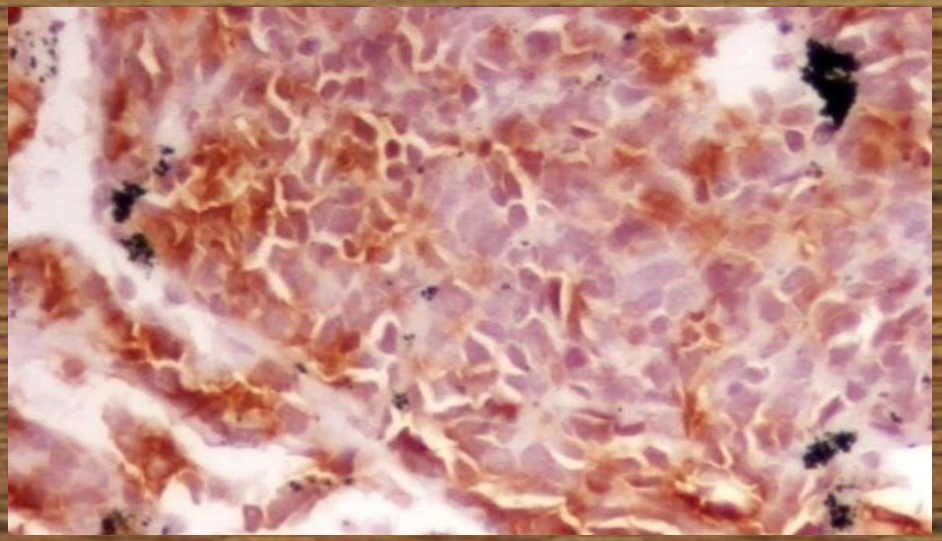
H&E 200x



Napsin A 400x

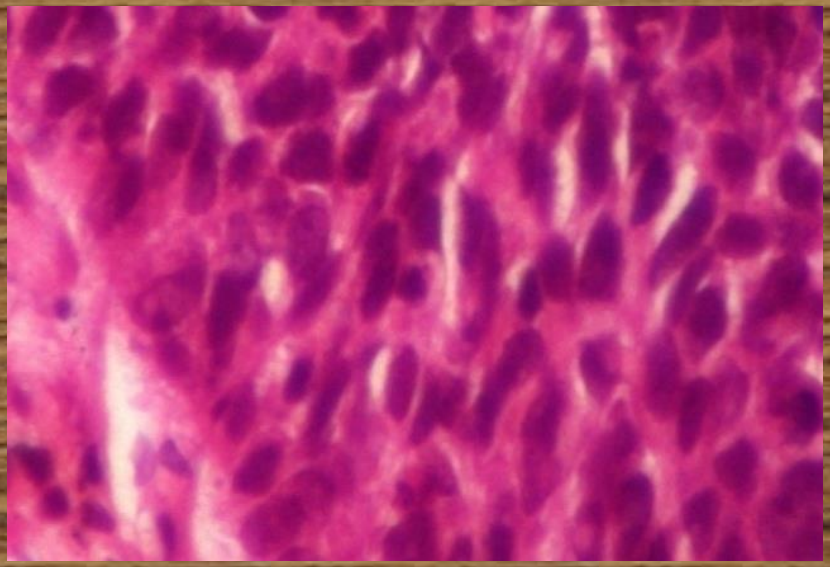
Acinar predominant adenocarcinoma : A) H&E shows tumor cells consists of round to oval-shaped malignant glands separated by desmoplastic reaction x200. B) Strong intensity staining of tumor cells for napsin A, ImmunoPeroxidase-DAB x400



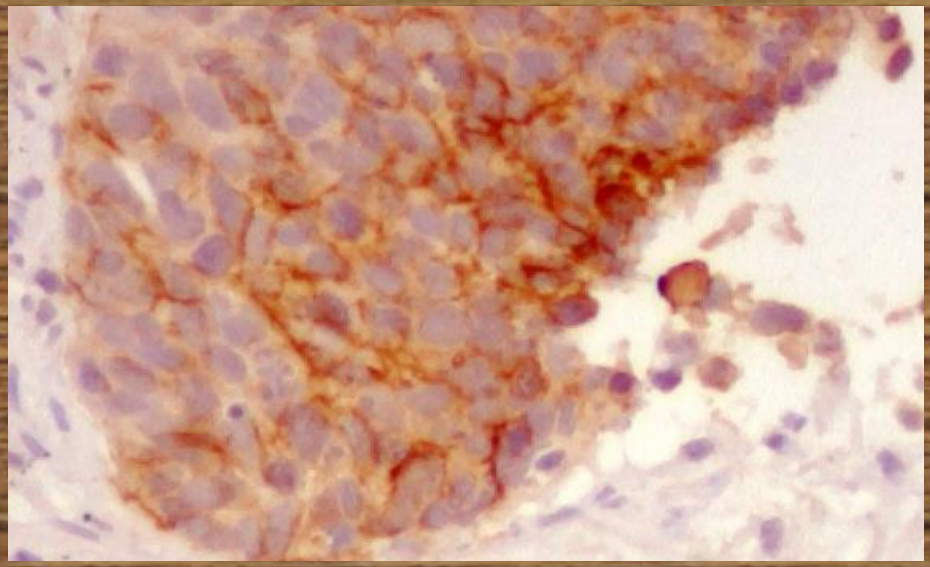


H&E 400x **CD 56 400x**

A case of small cell carcinoma. A) H&E showing malignant round to fusiform cells growing in sheets and nests. The nuclei appears ovoid with moulding and hyperchromasia x400. B) Positive cytoplasmic staining of tumor cells for CD56 tumor cells (score 3) Immunoperoxidase-DAB x400.



H&E 400x



CD 56 200x

A case Non small cell lung carcinoma with neuroendocrine morphology possible large cell neuroendocrine carcinoma (NSCLC with NEM possible NCLEC). A) H&E solid pattern of tumor growth morphologically similar to photo 1 x400. However by doing immunostains proved the following: B) Positive cytoplasmic staining of tumor cells for CD56Immunoperoxidase-DAB x\200

Conclusion & home message

Immunohistochemistry has valuable role in precise typing of different pulmonary tumors, a minimal panel of Napsin A, CK 5/6 & CD 56 is helpful in accurate and final diagnosis of lung cancer in small biopsies .

Conclusion & home message

The use of the three antibodies is done in this work aiming to minimize the term NSCLC – NOS on small samples providing as specific a histologic classification as possible to facilitate treatment approach of medical oncologist.

(Mok et al., 2009 & Boyle and Levin, 2008)

Conclusion & home message

In this study adenocarcinoma was the commonest subtype in young male .

Conclusion & home message

The increasing number of adenocarcinoma cases may be due to the increase of the cases of (Non small cell lung carcinomafavour adenocarcinoma) after use of immunostain and this goes with other reports. This was in agreement with Righiet al., 2011 .

Conclusion & home message

Although adenocarcinoma is still a morphological diagnosis but the variant (NSCLC favour ADC) need the panel done in this study

Conclusion & home message

In the squamous cell carcinoma either well or moderate cases we are not in need to do immunohistochemistry.

Conclusion & home message

The category Non Small Cell Lung carcinoma favour squamous cell carcinoma is diagnosed only by using immunohistochemistry.

Conclusion & home message

Both Napsin A and CK 5/6 have pivotal role in diagnosis and differential diagnosis of adenocarcinoma and squamous cell carcinoma respectively .

Conclusion & home message

As regard neuroendocrine tumors diagnosis of small cell carcinoma not needed immuno-histochemistry.

However we applied CD56 to confirm neuroendocrine features and to exclude small cell variant of squamous cell carcinoma, lymphoma and other blue round cell tumor.

Conclusion & home message

The diagnosis of LCNEC is difficult to establish based on small biopsies or cytology. This is related to difficulty to do immunohistochemistry and to detect neuroendocrine pattern in such small biopsy.

Conclusion & home message

As a whole, immunohisto-chemichal stains should be interpreted with caution especially in the setting of markedly crushed biopsy with otherwise un interpretable morphology .

Thank You