The utility of TTF-1, Napsin A, CK5 and p63 staining in the sub-classification of non-small cell carcinoma of the lung

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Background:
The potentially curative and/or palliative therapy for unresectable lung cancer has evolved significantly.
With the availability of targeted therapies the need for precise sub-typing of the non-small cell lung carcinoma (NCSLC) has become paramount.

Objectives:
We aimed to determine utility of TTF-1, Napsin A, p63 and CK5 immunostaining on fine needle aspiration (FNA) cell block and formalin fixed paraffin embedded (FFPE) tissue biopsy specimens in subtyping NSCLC as adenocarcinoma and squamous cell carcinomas.

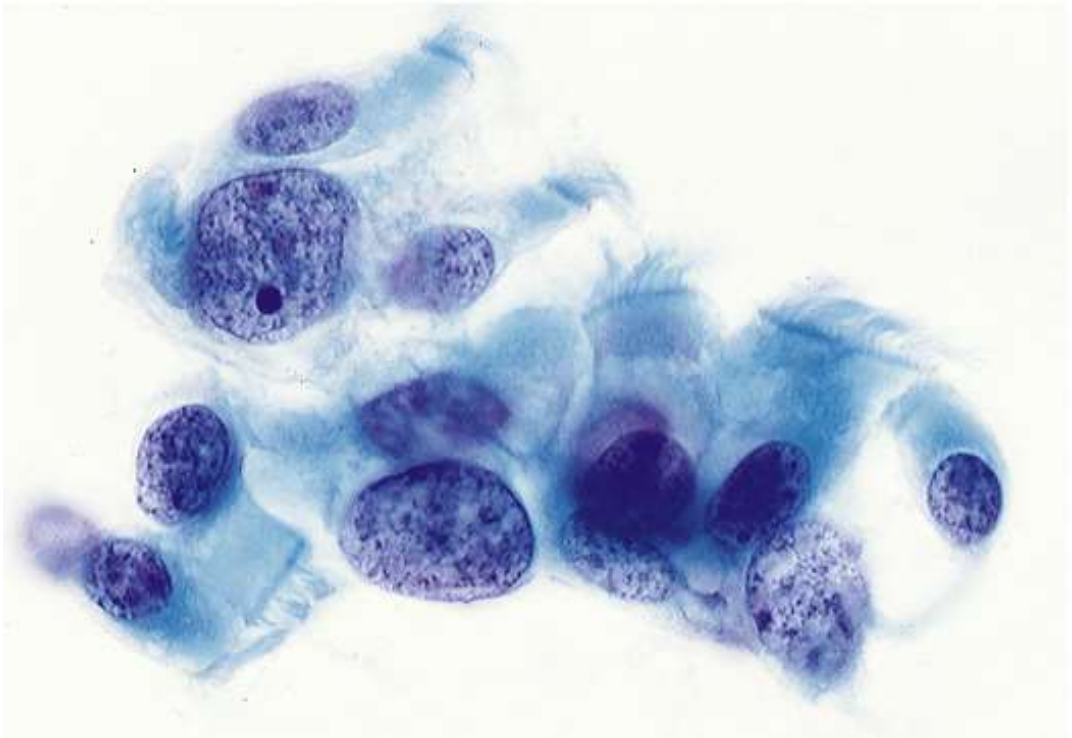
Methods:

All cases of NSCLC diagnosed during a three-year period were retrospectively identified. All FNA biopsy and FFPE cases that were stained with TTF-1, Napsin A, CK5 and p63 were collected. A lung cancer registry was used to access and correlate clinical and radiological data.

Immunocytochemistry

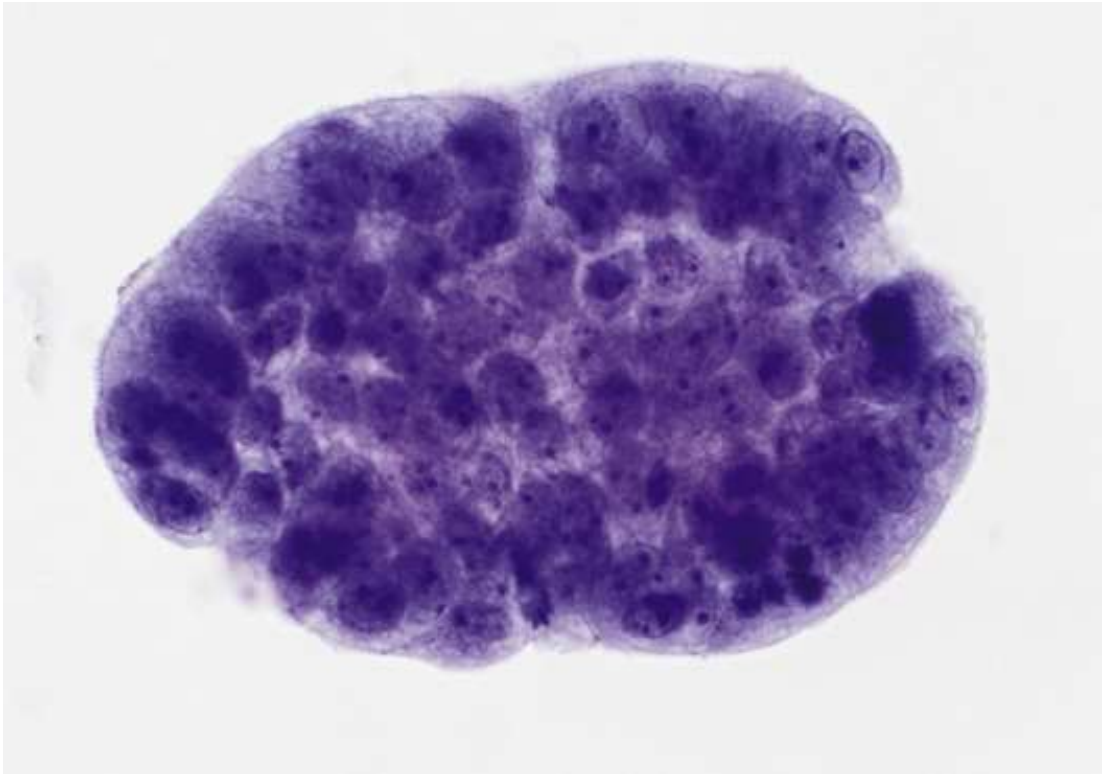
FNAB cell blocks and core needle biopsies were analysed separately. All immunostains were performed on formalin fixed tissue (tissue biopsies and cytology cell block preparation) and the Leica Bond III automated immunostainer (Leica Biosystems, Wetzlar, Germany) was used in the standardised manner for various tissue samples.

Benign cellular changes:



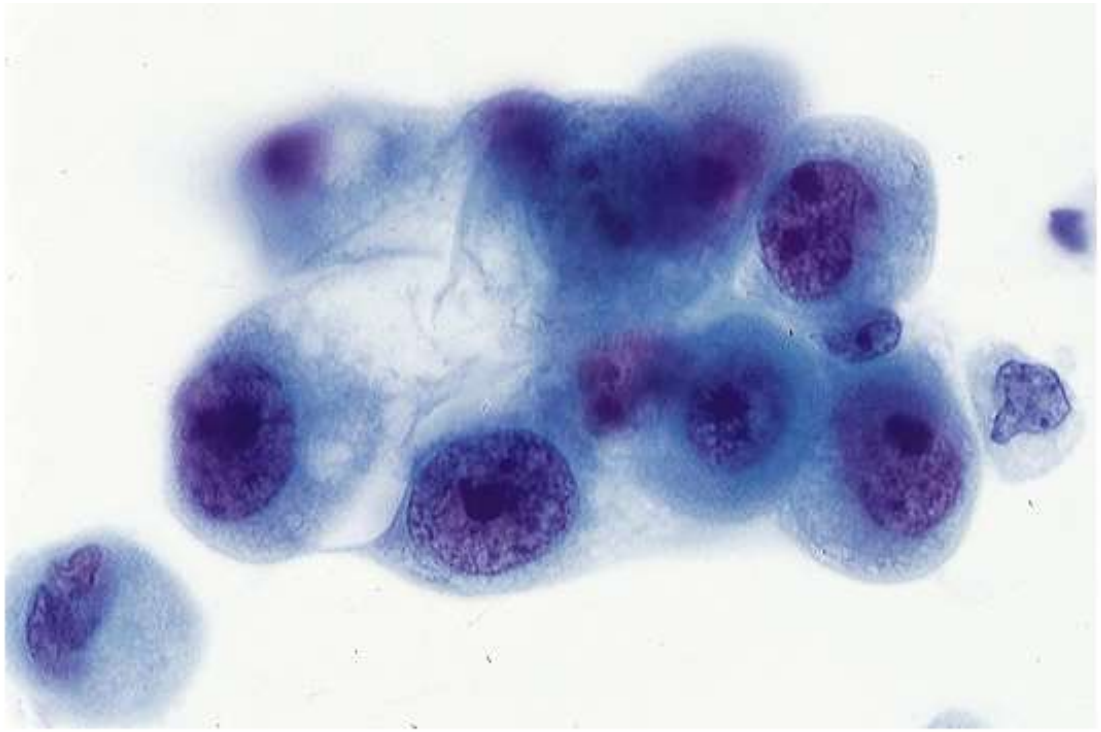
Reactive bronchial cells can show marked nuclear size variation.
Note that cilia—evidence of their benign nature—are retained.

Benign cellular changes:



Reactive bronchial cells (Creola body; bronchial washing).
In chronic lung diseases, as in this case of asthma, clusters of reactive bronchial cells can assume a spherical shape and resemble the cells of an adenocarcinoma.
Normal nuclear features and cilia indicate their benign nature.

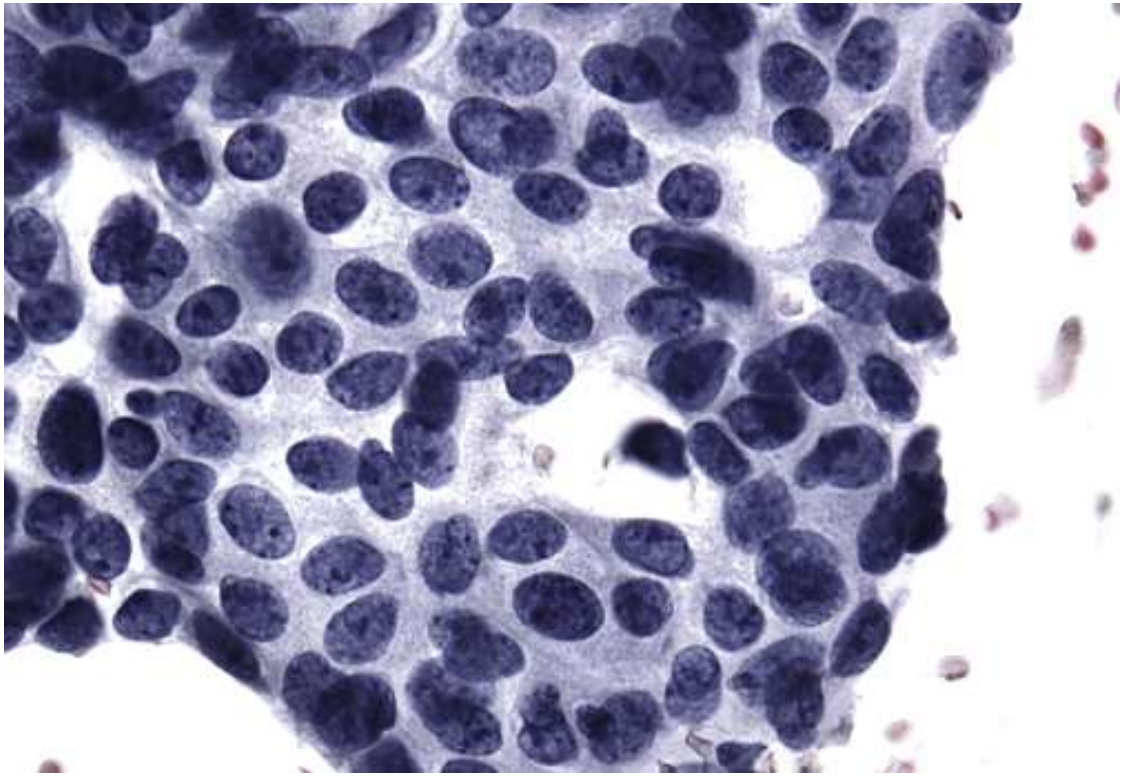
Benign cellular changes:



Cytomorphology of type II pneumocyte hyperplasia

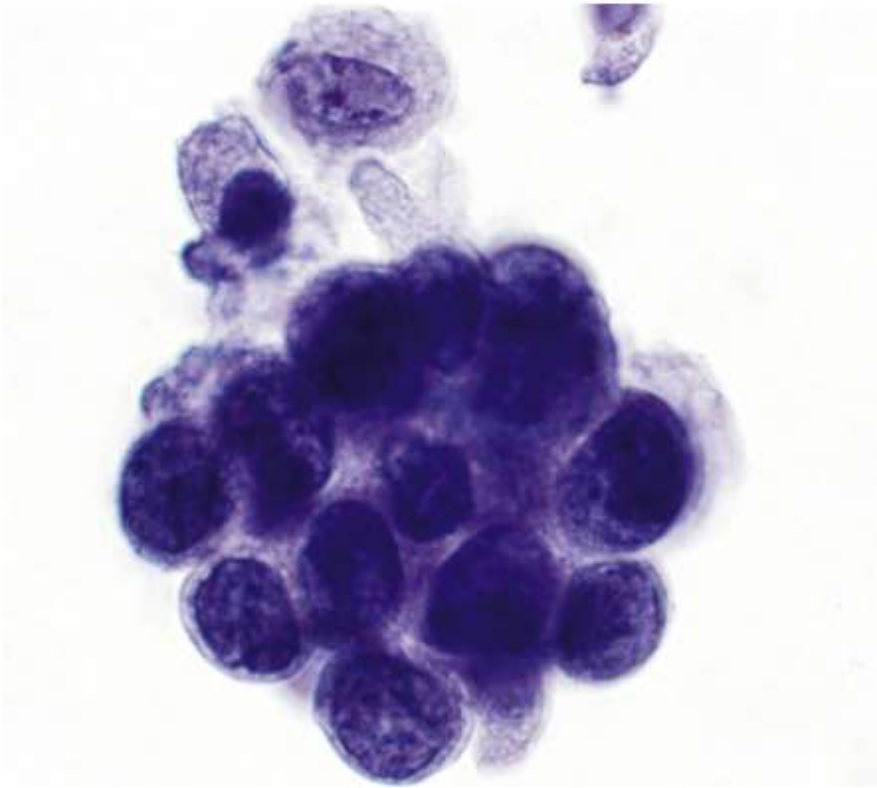
- isolated cells and three-dimensional clusters
- large nuclei
- coarse chromatin
- prominent nucleoli
- scant to abundant cytoplasm

Adenocarcinoma:

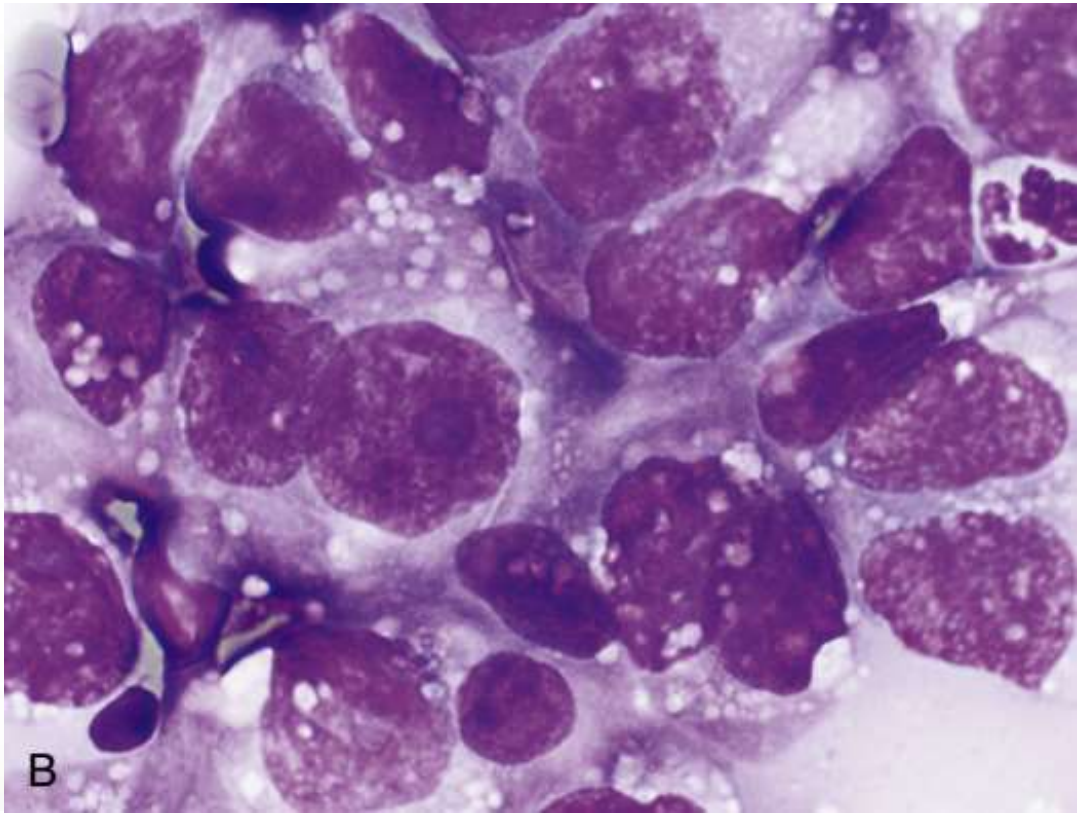


Cytomorphology of adenocarcinoma
- honeycomb like sheets, acini
- eccentrically placed, round or irregular nuclei
- finely textured chromatin
- large nucleoli

Adenocarcinoma:

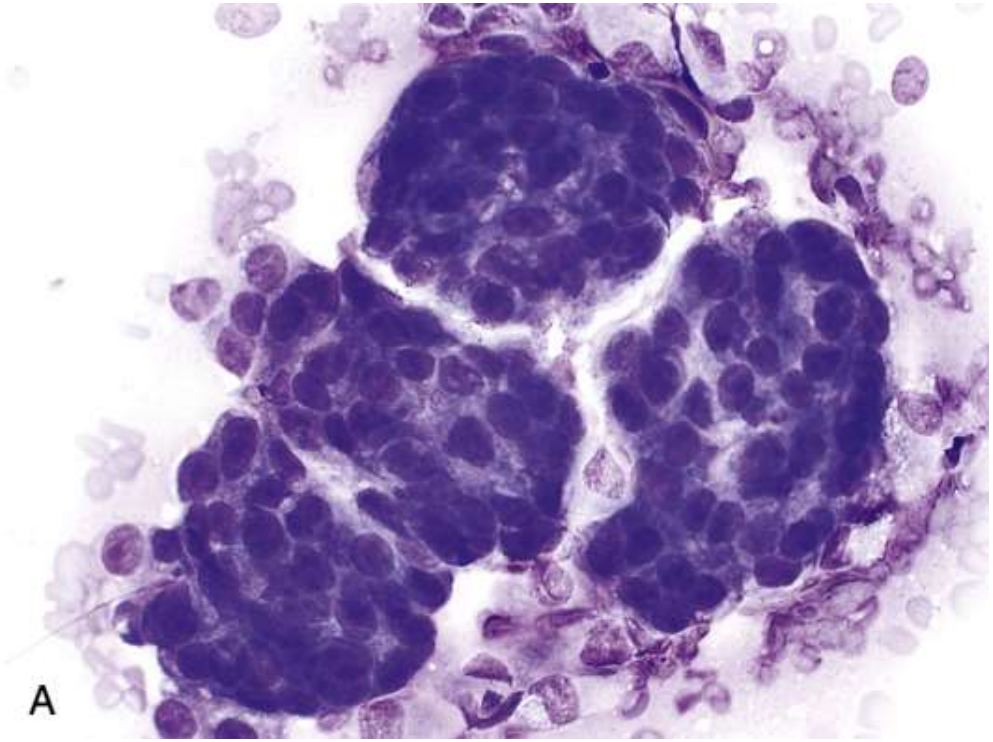


Three dimensional clusters

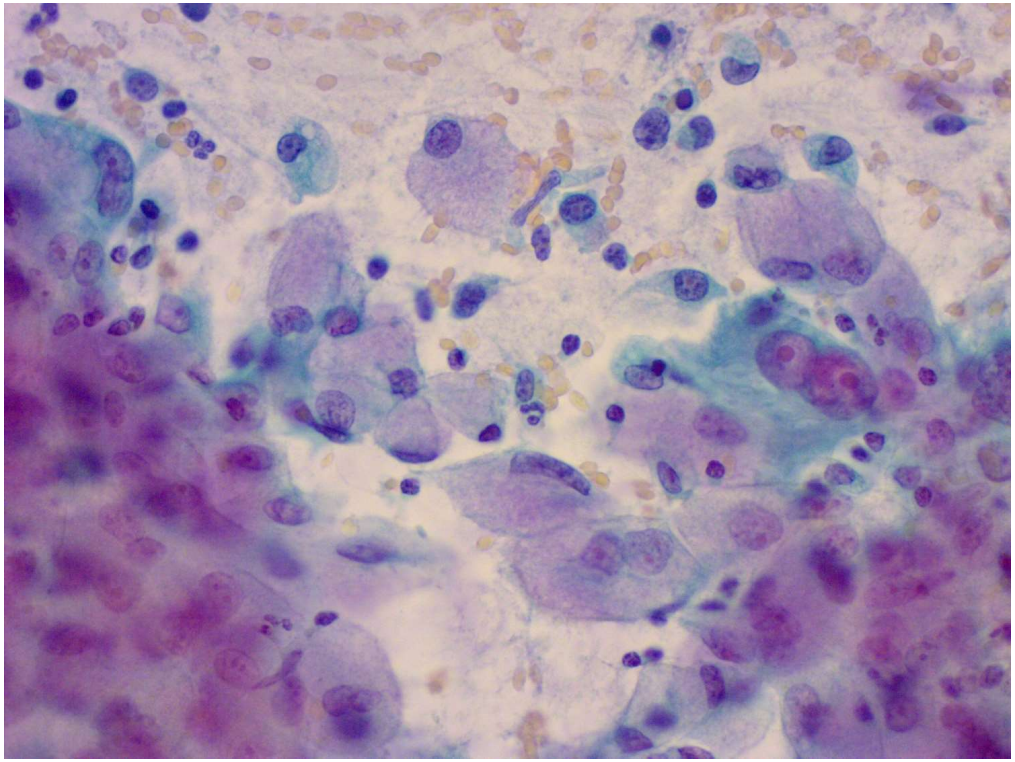


Irregular nuclear membranes, large nucleoli,
mucin vacuolated cytoplasm

Adenocarcinoma:

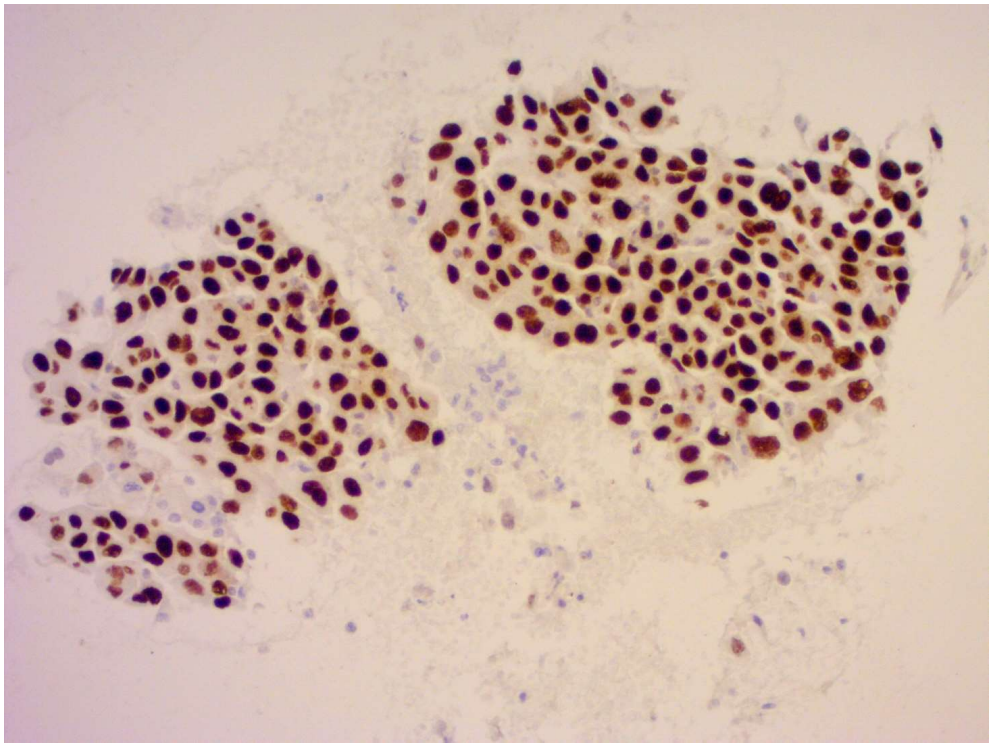


Micro papillae

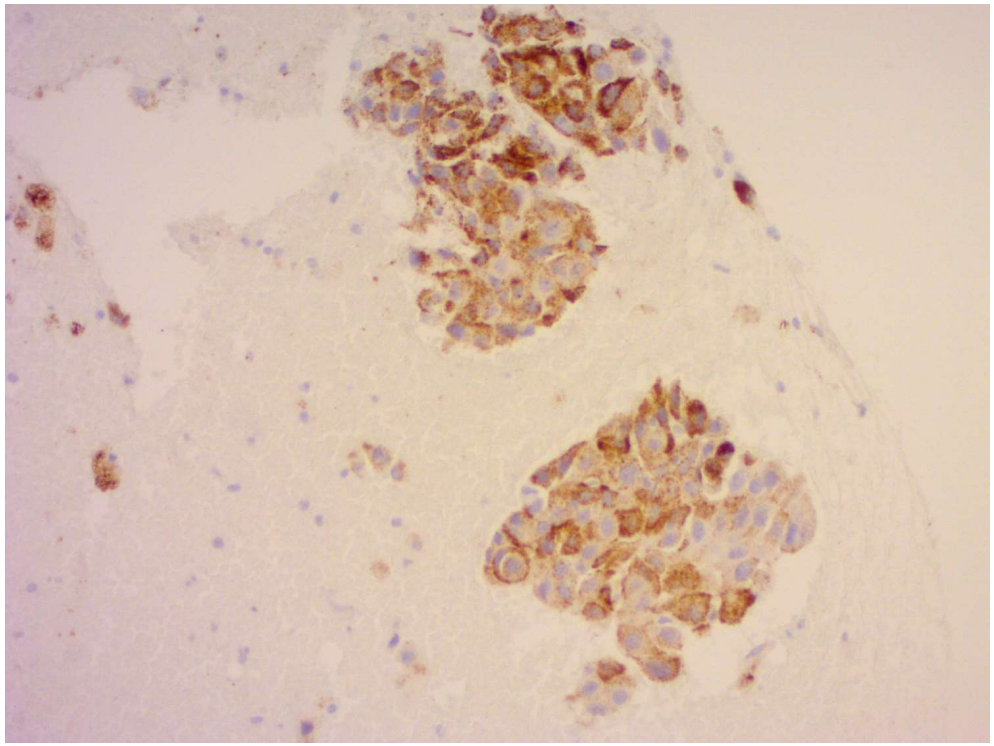


Translucent foamy cytoplasm

Immunostaining Adenocarcinoma:

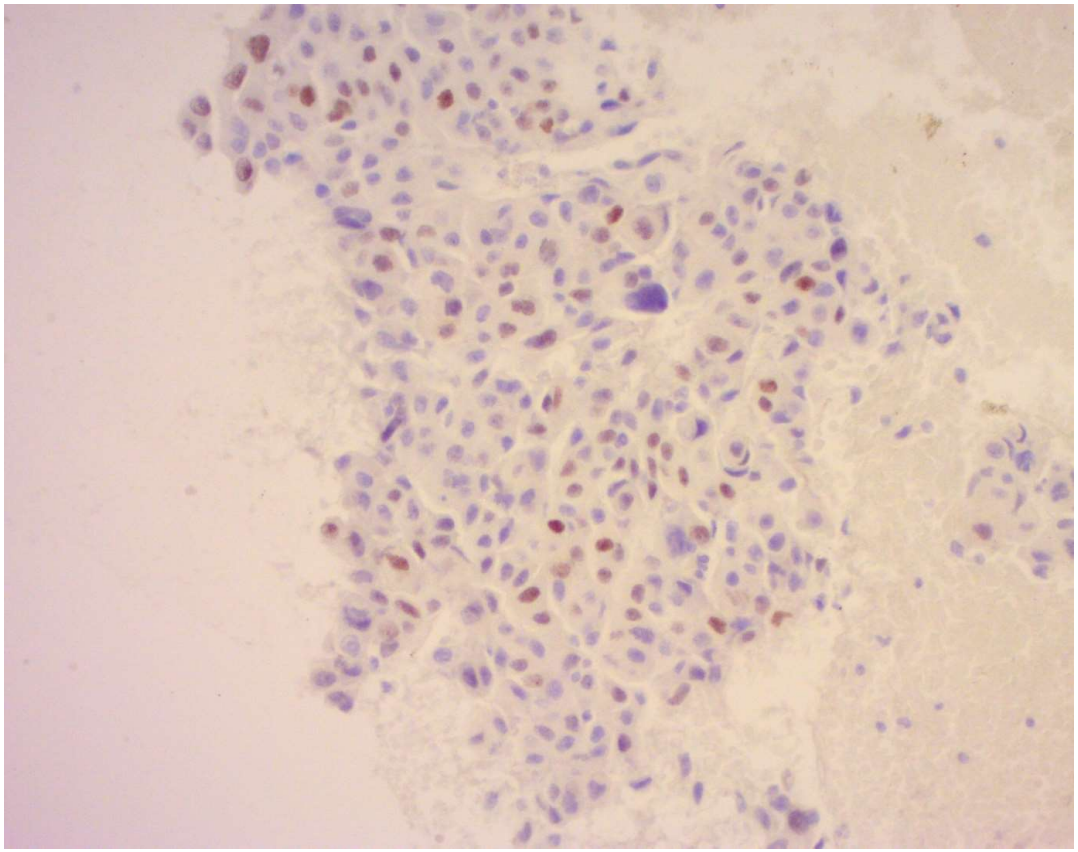


Strong nuclear staining TTF1



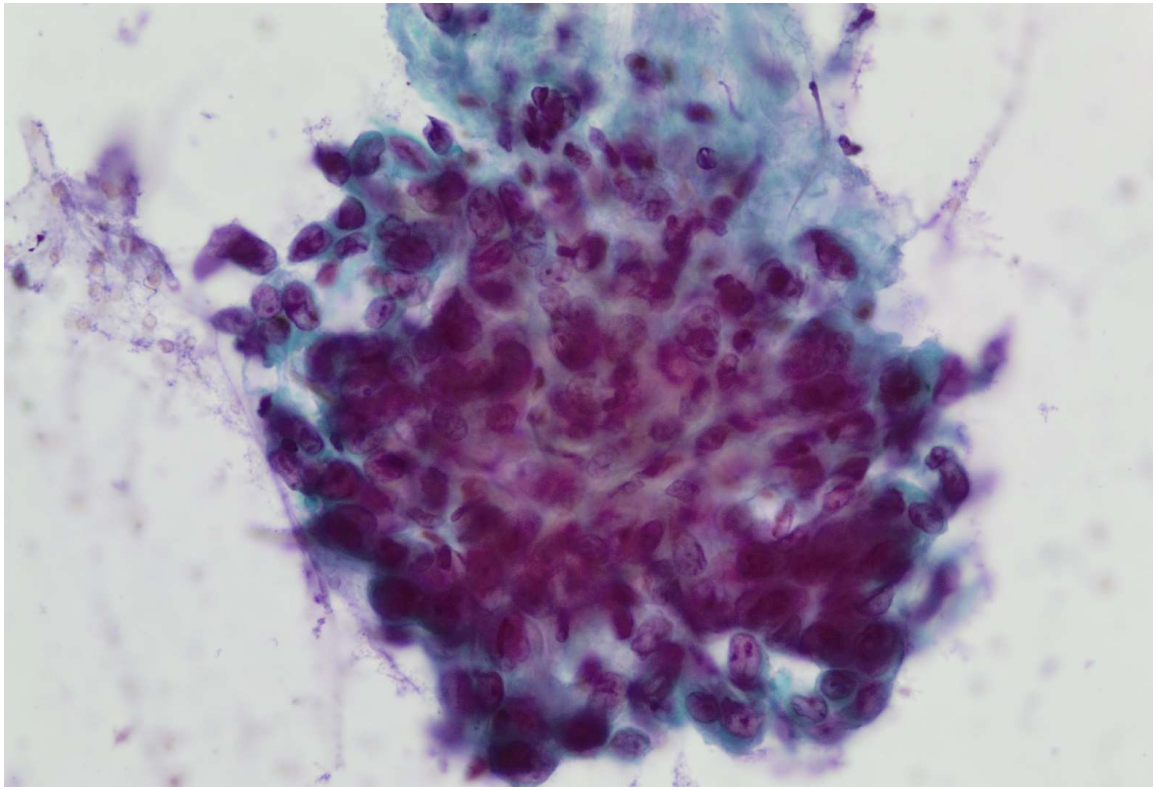
Granular cytoplasmic staining NapsinA

Immunostaining Adenocarcinoma:



P63 nuclear staining in some tumour cells

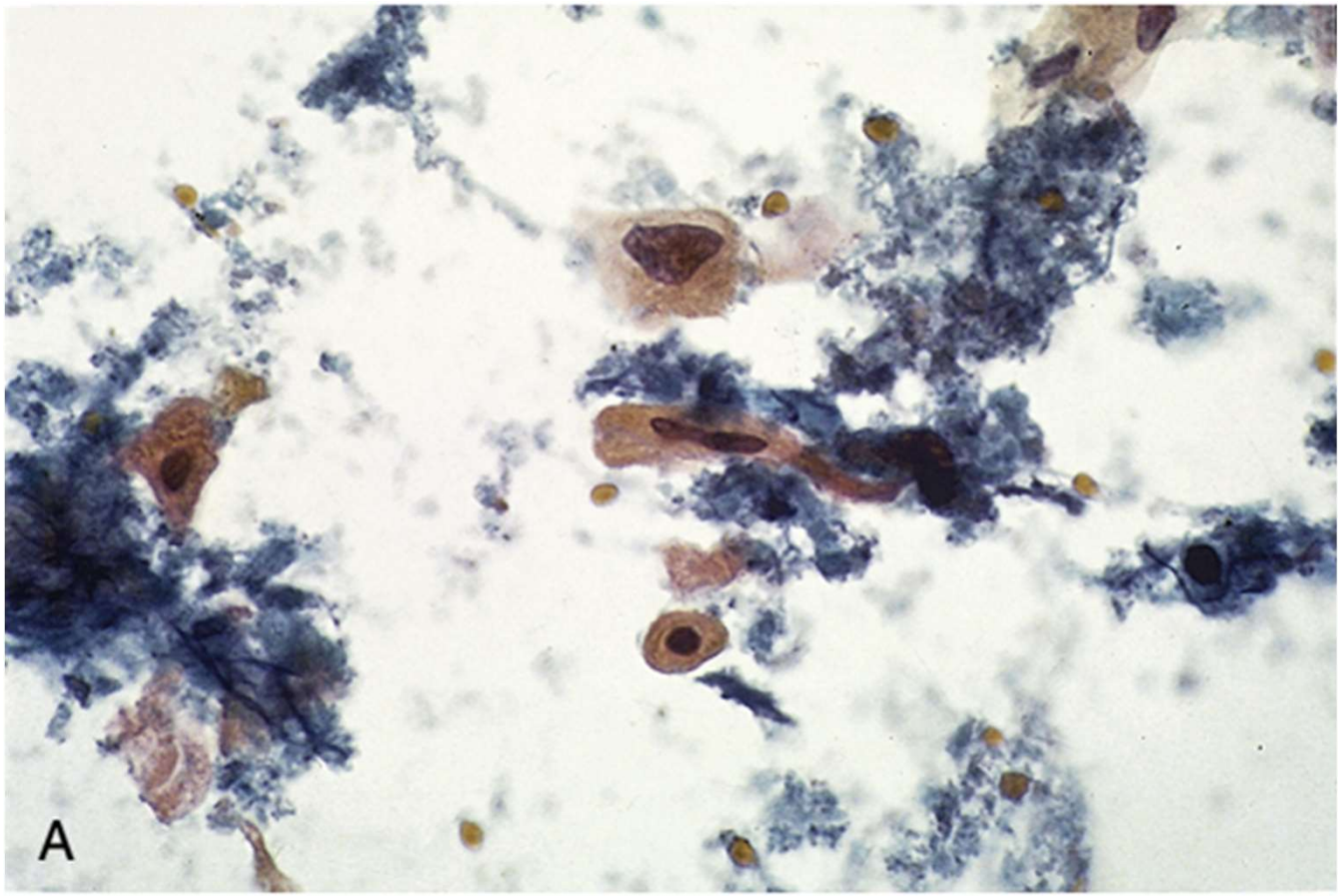
Squamous cell carcinoma:



Cytomorphology of well-differentiated squamous cell carcinoma

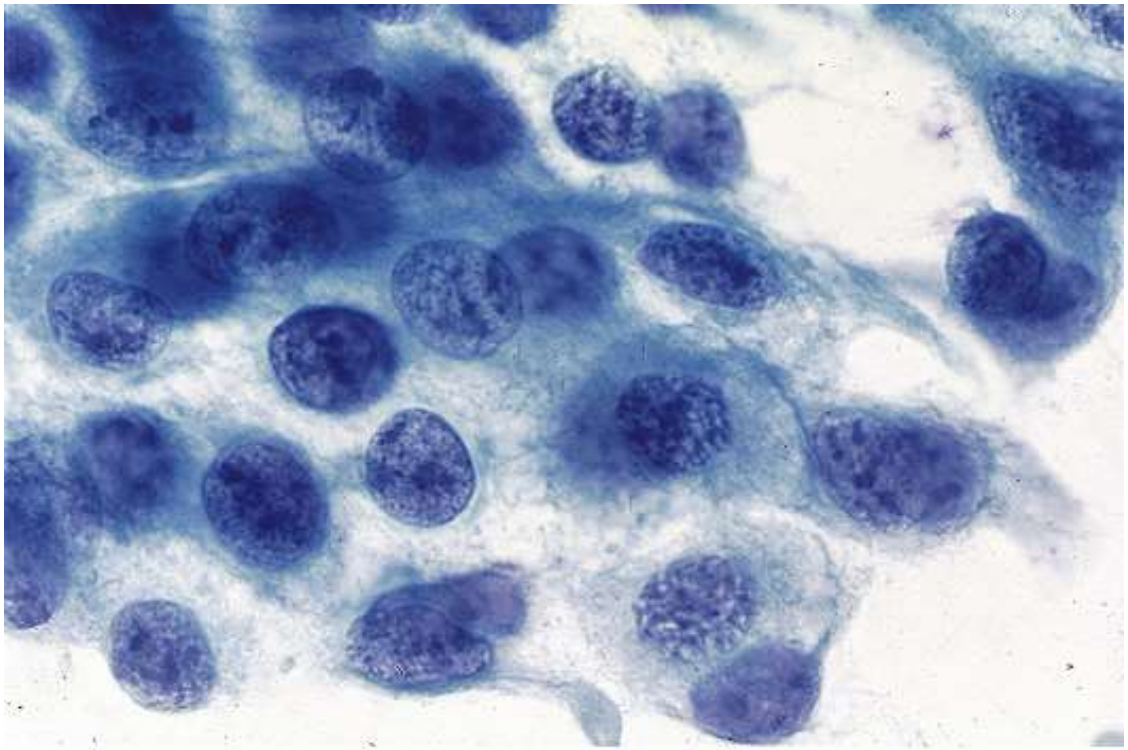
- abundant non cohesive cells
- polymorphic cell shapes: polygonal, rounded, elongated, tadpole-shaped
- dense cytoplasmic orangeophilia (Papanicolaou stain)
- pyknotic nuclei
- frequent anucleate cells

Squamous cell carcinoma:



Elongated tadpole shaped cells

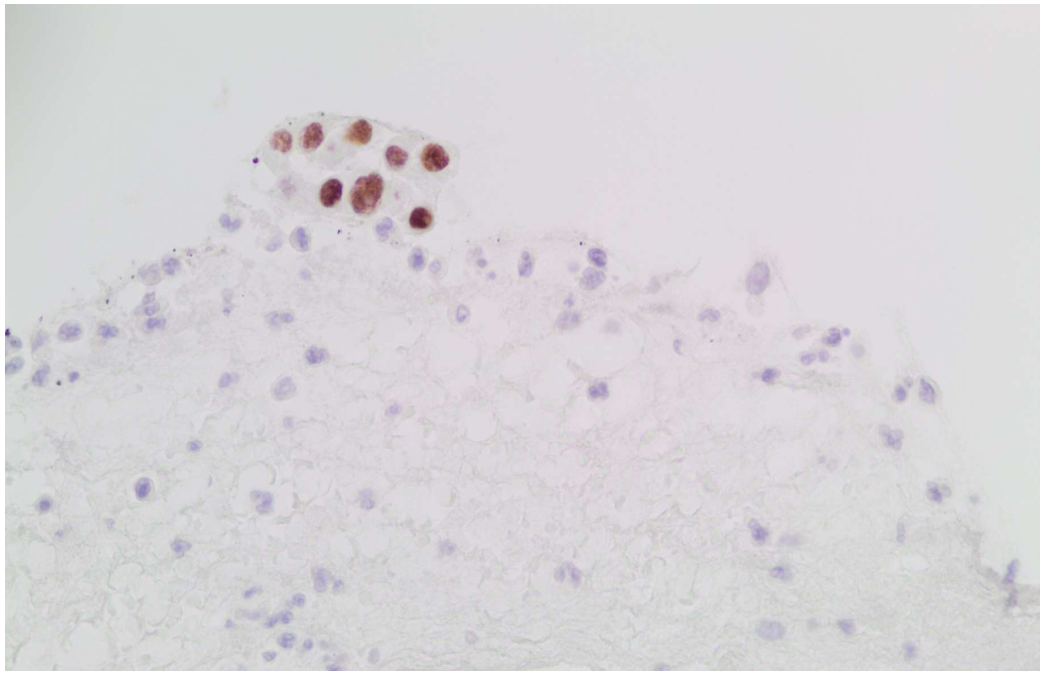
Squamous cell carcinoma:



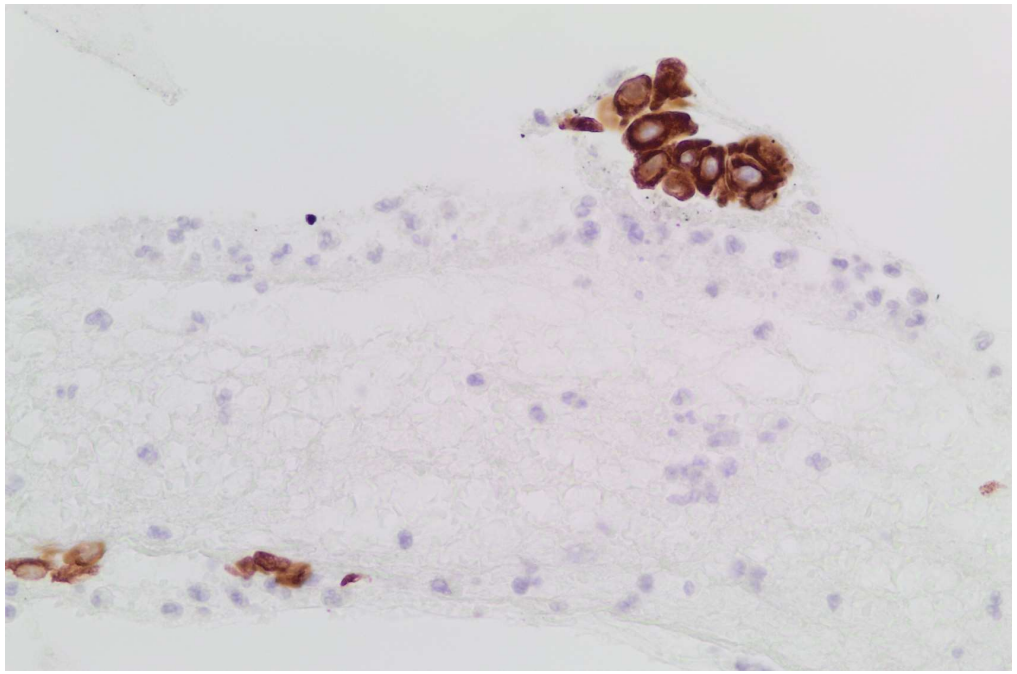
Cytomorphology of moderately and poorly differentiated squamous cell carcinomas

- large, cohesive clusters of spindle cells
- rare to absent keratinization
- large nuclei
- coarse chromatin texture
- ± prominent nucleoli

Immunostaining Squamous cell carcinoma:



Nuclear staining p63



Membranous and cyto-plasmic staining CK5

TABLES

Table 1: The utility of TTF-1 in the diagnosis of adenocarcinoma of the lung (n=201)

	Sensitivity	Specificity	PPV	NPV	Accuracy
All (n=201) (95% CI)	99.0% (96.4-99.9)	54.6% (38.9-69.6)	90.8% (87.7-93.2)	92.3% (74.7-98.0)	91.0% (86.6-94.2)
Cell blocks (n=143) (95% CI)	98.6% (95.0-99.8)	62.8% (46.7-77.0)	87.7% (85.5-92.8)	93.1% (77.0-98.2)	90.2% (85.0-94.1)

95% CI, 95% confidence interval; PPV, positive predictive value; NPV negative predictive value

Table 2: The utility of Napsin A in the diagnosis of adenocarcinoma of the lung (n=201)

	Sensitivity	Specificity	PPV	NPV	Accuracy
All (n=201) (95% CI)	91.9% (86.9-95.4)	90.2% (76.9-97.3)	97.7% (94.3-99.1)	71.2% (60.1-80.2)	91.0% (87.1-94.8)
Cell blocks (n=143) (95% CI)	90.9% (84.7-95.2)	90.6% (75.0-98.0)	97.6% (93.2-99.2)	70.7% (58.2-80.7)	90.9% (85.3-94.8)

95% CI, 95% confidence interval; PPV, positive predictive value; NPV negative predictive value

Table 3: The utility of p63 in the diagnosis of squamous cell carcinoma of the lung (n=53)

	Sensitivity	Specificity	PPV	NPV	Accuracy
All (n=53) (95% CI)	97.9% (88.7-100.0)	61.2% (46.2-74.8)	70.8 (62.9-77.5)	96.8% (80.0-99.5)	79.2% (69.7-86.8)
Cell blocks (n=43) (95% CI)	97.9 (88.7-100.0)	67.7% (49.5-82.6)	80.7% (72.2-87.2)	95.8% (76.5-99.4)	85.2% (75.6-92.1)

95% CI, 95% confidence interval; PPV, positive predictive value; NPV negative predictive value

Table 4: The utility of CK5 in the diagnosis of squamous cell carcinoma of the lung (n=53)

	Sensitivity	Specificity	PPV	NPV	Accuracy
All (n=53) (95% CI)	95.4% (84.2-99.4)	84.4% (73.1-92.2)	80.4% (69.8-87.9)	96.4% (87.4-99.1)	88.8% (79.0-93. 7)
Cell blocks (n=43) (95% CI)	93.2% (81.3-98.6)	82.2% (68.0-92.0)	83.7% (73.1-90.6)	92.5% (80.4-97.4)	87.6% (81.2-94.1)

95% CI, 95% confidence interval; PPV, positive predictive value; NPV negative predictive value

In Conclusion:

Our data confirms recent observations that suggests that p63 is less specific for squamous cell carcinoma than previously assumed. [1] In fact, p63 was positive in 36% of adenocarcinomas in our cohort, suggesting that co-expression of TTF-1 and p63 should be regarded as an adenocarcinoma.

TTF-1 and Napsin A both had a high PPV and diagnostic accuracy for adenocarcinoma of the lung, whereas CK5 had an equally high PPV and diagnostic accuracy for squamous cell carcinoma of the lung. The specificity of Napsin A for adenocarcinoma was higher than that of TTF1 of the lung. The specificity of CK5 for squamous cell carcinoma of the lung was higher than p63. Collecting cytology material in BD Cyto Rich Red® medium with dual fixation for cell block preparations has no negative impact on the overall staining accuracy.

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