InaJCC The Indonesian Journal of Cancer Control

Original Article

Expression of thyroid transcription factor-1, P40, and cytokeratin 5/6 in non-small cell lung carcinoma at Persahabatan Hospital in 2021

Linda Masniari[1], Elisna Syahruddin[2], Romi Beginta[3], Eylin Raharjo[3], Ruth E Sembiring[3]*

e-ISSN 2797-457X
DOI: 10.52830/inajcc.v3i2.77

Received: October 31st, 2023
Accepted: July 17th, 2024

Authors' affiliations:
[1]MRCCC Siloam Hospitals Semanggi, Jakarta
[2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia - National Respiratory Center, Persahabatan Hospital, Jakarta
[3]Department of Anatomic Pathology - National Respiratory Center, Persahabatan Hospital, Jakarta

Corresponding author:
Linda Masniari
E-mail: *lindafauzi1204@gmail.com*

Abstract

Background: Non-small cell Lung carcinoma (NSCLC) comprises 80% of lung cancer and mainly consists of adenocarcinoma and squamous cell carcinoma. Immunohistochemistry plays an important role in the accurate diagnosis of different types of lung cancer.
Aim: To determine the expression of thyroid transcription factor-1 (TTF-1), P40, and cytokeratin 5/6 (CK 5/6) in lung cancer and to determine the differences in the expression of TTF-1, P40, and CK 5/6 in adenocarcinoma and squamous cell carcinoma.
Method: This study is a cross-sectional study with 47 samples, 35 adenocarcinoma and 12 squamous cell carcinoma for immunohistochemistry examination. Immunohistochemistry examination on TTF-1 expression using monoclonal mouse antibody 8G7G3/1, P40 expression using polyclonal rabbit antibody, and CK 5/6 expression using CK5/6.007 monoclonal mouse antibody.
Results: Expression of TTF-1 was found in 25 cases of adenocarcinoma (71.4%) and in four cases of squamous cell carcinoma (33.3%). P40 was expressed in eight cases of squamous cell carcinoma (66.7%) and in six cases of adenocarcinoma (17.1%). Cytokeratin 5/6 was expressed in eight cases of squamous cell carcinoma (66.7%) and in four cases of adenocarcinoma (11.4%). Thyroid transcription factor-1 had a positive correlation with adenocarcinoma, whereas P40 and CK 5/6 negatively correlated with adenocarcinoma, but were more associated with squamous cell carcinoma.
Conclusions: There were significant differences in TTF-1, P40, and CK 5/6 expressions between adenocarcinoma and squamous cell carcinoma, but the three expressions could not be used simultaneously. Thyroid transcription factor-1 expression was more accurate than P40 and CK 5/6 for differentiating adenocarcinoma with squamous cell carcinoma.

***Keywords**: adenocarcinoma, CK 5/6, squamous cell carcinoma, P40, TTF-1*

Abstrak

Latar Belakang: Kanker paru karsinoma bukan sel kecil (KPKBSK) mencakup 80% kanker paru dan terutama terbagi menjadi adenokarsinoma dan karsinoma sel skuamosa. Imunohistokimia berperan penting untuk diagnosis yang akurat pada tipe kanker paru yang berbeda.
Tujuan: Mengetahui prevalensi ekspresi dari TTF-1, P40, dan CK 5/6 pada jaringan kanker paru dan mengetahui perbedaan ekspresi TTF-1, P40, dan CK 5/6 pada adenokarsinoma dan karsinoma sel skuamosa.
Metode: Penelitian ini merupakan penelitian potong-lintang dengan jumlah sampel 47, yaitu 35 sampel adenokarsinoma, dan 12 sampel karsinoma sel skuamosa untuk dilakukan pemeriksaan imunohistokimia. Pemeriksaan Imunohistokimia pada ekspresi TTF-1 menggunakan antibodi tikus monoklonal 867 63/1, pada ekspresi P40 menggunakan antibodi kelinci poliklonal dan pada ekspresi CK 5/6 menggunakan antibodi tikus monoklonal CK/6.007
Hasil: Ekspresi TTF-1 ditemukan pada 25 kasus adenokarsinoma (71,4%) dan pada empat kasus karsinoma sel skuamosa (33,3%). Ekspresi P40 ditemukan pada delapan kasus karsinoma sel skuamosa (66,7%) dan pada enam kasus adenokarsinoma (17,1%). Ekspresi CK 5/6 ditemukan pada delapan kasus karsinoma sel skuamosa (66,7%) dan pada empat kasus adenokarsinoma (11,4%). Ekspresi TTF1 mempunyai korelasi yang positif dengan adenokarsinoma sedangkan ekspresi P40 dan CK 5/6 memiliki korelasi negatif dengan adenokarsinoma, dengan kata lain lebih berhubungan dengan karsinoma sel skuamosa.
Kesimpulan: Terdapat perbedaan bermakna dalam ekspresi TTF-1, P40, dan CK 5/6 pada adenokarsinoma dan karsinoma sel skuamosa, namun ketiga ekspresi tersebut tidak dapat digunakan secara bersamaan. Ekspresi TTF-1 lebih akurat dibandingkan P40 dan CK 5/6 dalam membedakan adenokarsinoma dengan karsinoma sel skuamosa.

***Kata kunci:** adenokarsinoma, CK 5/6, karsinoma sel skuamosa, P40, TTF-1.*

Introduction

Lung cancer is one of the most common causes of death in the world.[1] There are 2 main types of lung cancer with 80 % non-small cell lung cancer (NSCLC) and 20% small cell lung carcinoma (SCLC). Non-small cell lung carcinoma (NSCLC) mainly consists of adenocarcinoma and squamous cell carcinoma. Adenocarcinoma is the most common NSCLC type in the world. [2,3] Persahabatan Hospital from 2004-2006 showed NSCLC adenocarcinoma was the most common lung cancer with 56.3%.[3] Epidemiological studies from WHO reported NSCLC adenocarcinoma was 40% from all of NSCLC cases.[4] Lung adenocarcinoma incidence increase in Asia and United States mainly in female, young age and non-smokers.[4]

Immunohistochemistry (IHC) plays a significant role in diagnosing different lung tumor accurately.[5] Immuno-histochemistry staining from mucin can identify a subtype of non-small cell carcinoma lung cancer and help to predict final subtype from non-small cell carcinoma lung cancer that cannot be specifically identified. [5,6] Immunohistochemistry staining of TTF-1 was found negative in almost all squamous cell carcinoma cases and 70-85% of adenocarcinoma. Antibody towards CK 5/6 and P40 has been used to identify squamous cell phenotype[7].

Methods

This study is a cross-sectional study. The subject of this study is 51 paraffin blocks of non-small cell carcinoma lung cancer that fulfil immunohistochemistry standards. Of 51 sample, 37 adenocarcinoma and 14 squamous cell carcinoma sample underwent immunohistochemistry examination. A total of 35 adenocarcinomas and 12 squamous cell carcinomas went for further examination due to the heating process in immunohistochemistry process. All research samples were taken from 1st September 2021 to 31st December 2021. This research occurred at the Pulmonology Department and Respiratory Division of Faculty Medicine of the University of Indonesia RSUP Persahabatan National Lung Center.

Immunohistochemistry

Paraffin block were cut into 3 mm thickness, and went to deparaffination with Xylol, antigen retrieval deblocking chamber (heated with deblocking chamber 95^0C degree for 30 minutes, slide chilled and washed in Phosphate Buffered Saline (PBS) and went to deblocking with tissue primer, blocking with background blocker, given primary primer antibody (TTF1, P40 and CK/56 seen on table 1) for 60 minutes, with 1:1000 dilution, washed in PBS and given secondary antibody for 10 minutes for further binding, then carried Host Radish Peroxidase (HRP). This enzyme catalyzed hydrogen peroxide into water and oxygen for 10 minutes, then performed diaminobenzidine tetrahydrochloride (DAB) chromogen 1 ml on 1 drop for 5 minutes, identifying positivity. Suppose the result was positive indicated with brown staining. Afterwards, the slide was washed, given gradual alcohol dehydration for 5 minutes and Xylol. Lastly, the sample underwent mounting and closed with a glass lid.

Statistical Analysis

The numerical and categorical results are presented in table and pie diagram. The analysis consists of univariate and bivariate. The univariate analysis includes proportion distribution from categorical variables and frequency distribution for numerical variables. The univariate analysis includes proportion distribution for categorical variable and frequency distribution for numerical variable. Bivariate analysis of chi square was used to analyze the relation between two categorical variables.

Table 1. Antibody Panel Used in this esearch

Antibodies	Mono/Polyclonal	Source	Clone	Dilution
TTF-1	M	Biocare Medical, USA	8G7G3/1	1: 100
p40	P	Biocare Medical, USA		1: 100
Cytokeratin 5/6	M	Biocare Medical, USA	CK5/6.007	1: 100

Results

A total of 47 subject were successfully retrieved in this research. **Table 2** showed comparison between female and male were similar. The majority of subjects were active and passive smokers (72.3%). Subject with familial history of cancer were higher compared with a history of cancer (23.4 vs 12.8%), although majority of subject did not have a previous history or familial history of cancer. A higher proportion of subjects had stage IV cancer (83%) and had PA diagnosis of adenocarcinoma (74.5%). The median age of subject was 59.1 years old. The positivity rate of TTF-1 was higher compared to negative (61.7%). The positivity of p40 and CK5/6 were lower than the negative (29.8 and 25.5% respectively).

Table 2. Subject Characteristics

Characteristics	N	%
Gender		
Male	26	55.3
Female	21	44.7
Age		
Median		61
Mean		59.1
SD		11.7
Smoking		
Yes	34	72.3
No	13	27.7
Family history of cancer		
Yes	11	23.4
No	36	76.6

Table 3. Subject's Clinical Characteristics

Characteristics	n	%
Cancer Cell Type		
Adenocarcinoma	35	74.5
Squamous Cell Carcinoma	12	25.5
Staging		
I-II	0	0
IIIA/B/C	8	17.0
IVA/B	39	83.0
Previous History of Cancer		
Yes	6	12.8
No	41	87.2

Table 4. Result of TTF-1,p40, and CK5/6 expression on NSCLC

PA		N	%
TTF 1	Positive	29	61.7
	Negative	18	38.3
P40	Positive	14	29.8
	Negative	33	70.2
CK 5/6	Positive	12	25.5
	Negative	35	74.5

Gender, smoking habit, history and familial history of cancer, staging, and PA diagnosis are categorical variables. These variables were examined to determine whether they related to TTF-1, p40, and CK5/6 expression using Chi- Square test. Several variables that did not fulfill the Chi-Square requirement were analyzed using Fisher's exact test.

Bivariate

Table 5. Relation between age and TTF1, P40 and CK/56 expression

		TTF1				P (Chi-square)	P40				P (Chi-square)	CK 5/6				P (Chi-square)
		positive		negative			positive		negative			positive		negative		
		n	%	n	%		n	%	n	%		n	%	n	%	
Gender	Female	13	61.9	8	38.1	0.9	5	23.8	16	76.2	0.4	4	19	17	81	0.5*
	Malei	16	61.5	10	38.5		9	34.6	17	65.4		8	30.8	18	69.2	
Age	Mean	59.8		57.8		0.6	61.9		57.8		0.3	62.6		57.8		0.2
	SD	12.1		11.1			9.9		12.3			10.5		11.9		
Smoking	Yes	21	61.8	13	38.2	0.9	12	35.3	22	64.7	0.3*	10	29.4	24	70.6	0.5*
	No	8	61.5	5	38.5		2	15.4	11	84.6		2	15.4	11	84.6	
History of cancer	Yes	3	50	3	50	0.7*	2	33.3	4	66.7	1*	1	16.7	5	83.3	1*
	No	26	63.4	15	36.6		12	29.3	29	70.7		11	26.8	30	73.2	
Family history of cancer	Yes	7	63.6	4	36.4	1*	3	27.3	8	72.7	1*	3	27.3	8	72.7	1*
	No	22	61.1	14	38.9		11	30.6	25	69.4		9	25	27	75	
Stage	III	3	37.5	5	62.5	0.23*	3	37.5	5	62.5	0.7*	3	37.5	5	62.5	0.4*
	IV	26	66.7	13	33.3		11	28.2	28	71.8		9	23.1	30	76.9	
Histopatology	KSS	4		8	66.7	0.04*	8	66.7	4	33.3	0.03*	8	66.7	4	33.3	0.001*
	Adeno Ca	25		10	28.6		6	17.1	29	82.9		4	11.4	31	88.6	

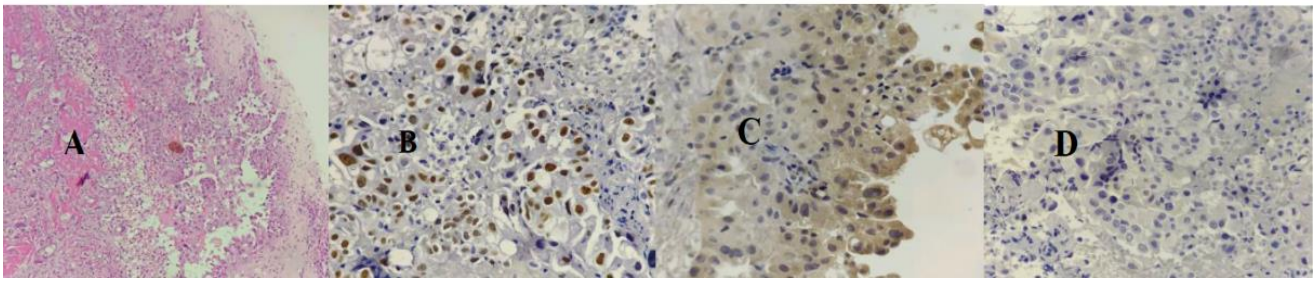

Figure 1. A. Adenocarcinoma, B. Positive staining of TTF1 expression showed as yellow or brown particle expressed in nucleus, C. Negative staining of P40 expression, D. Negative staining of CK/56 expression

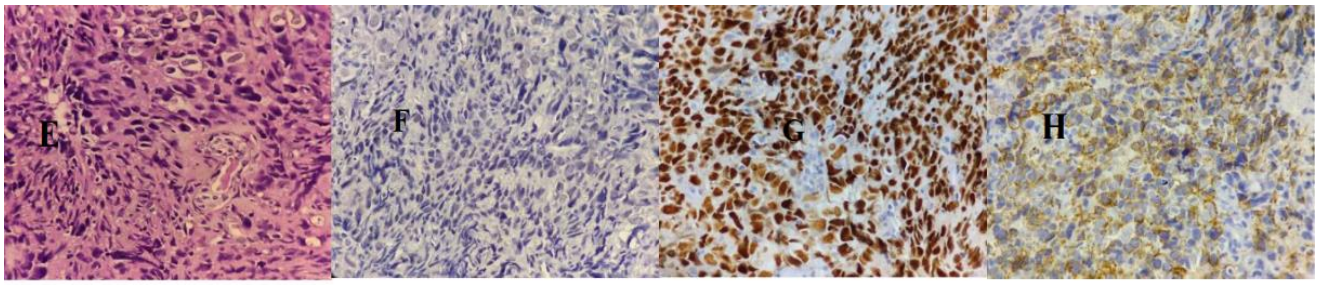

Figure 2. E. Squamosa cell carcinoma, F. Negative staining of TTF1 expression, G. Positive staining of P40 expression showed as yellow or brown particle expressed in nucleus, H. Positive staining of CK5/6 expression showed as yellow or brown particle expressed in membrane cell and cytoplasm.

Table 5 showed significant relationship between PA diagnosis and TTF-1, p40 and CK5/6 expression. The adenocarcinoma group showed a higher positive proportion of TTF-1 compared to squamous cell carcinoma (inversely related with adenocarcinoma, the negative proportion of TTF1 in SCC was higher than the positive result). **Table 6** showed similar result negative proportion of p40 and CK5/6 were higher on adenocarcinoma than in squamous cell carcinoma.

Age distribution as a numerical variable was analyzed based on mean-median, standard deviation, skewness, and kurtosis. Normal distribution was fulfilled. Hence, a T-test was used to analyzed age and TTF-1, p40, and CK5/6 expression. There was no statistically significant difference in age median between the three groups (Table 5).

Table 6. Result of TTF-1, P40 and CK5/6 expression between adenocarcinoma and squamous cell carcinoma.

Cancer Cell Type	TTF-1				P40				CK5/6			
	Positive		Negative		Positive		Negative		Positive		Negative	
	N	%	N	%	N	%	N	%	N	%	N	%
Adenocarcinoma	25	71,4	10	28,6	6	17,1	29	82,9	4	11,4	31	88,6
Squamous Cell Carcinoma	4	33,3	8	66,7	8	66,7	4	33,3	8	66,7	4	33,3

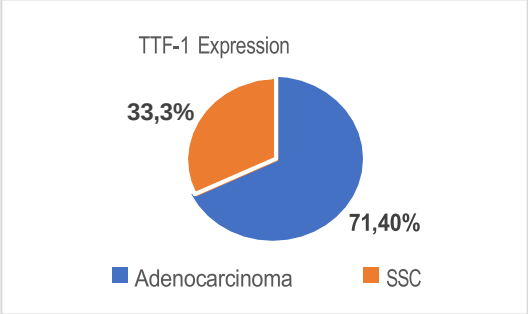

Figure 3. Positive expression of TTF-1 on adenocarcinoma and squamous cell carcinoma

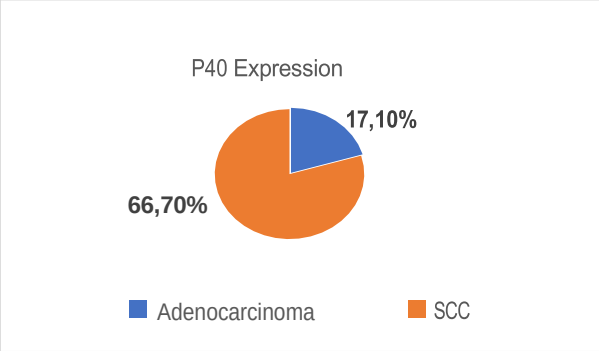

Figure 4. Positive expression of P40 on adenocarcinoma and squamous cell carcinoma

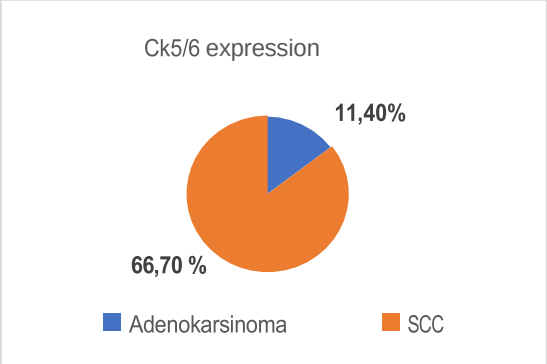

Figure 5. Positive expression of CK5/6 on adenocarcinoma and squamous cell carcinoma

Table 7 showed multiple regression analysis on 3 gene expression. The regression coefficient (beta) was not consistently indicated from P (z) higher than the expected significant result (0.05), Hence three modalities cannot be used simultaneously. This statement was strongly supported by simple regression analysis on table 8 to table 10.

All coefficient marks on each modality were significantly different from 0 (P (z) < 0.05). Only TTF-1 showed a positive correlation (positive coefficient mark) with pathology anatomy result od adenocarcinoma. P40 and CK5/6 expression negatively correlated with Adeno Ca, which had more association with SCC. Table 11 showed TTF1 had the highest accuracy (ROC area 70%, 95% CI 50;80%) compared to P40 and CK 5/6.

Table 7. Multiple Regression Analysis

PA Diagnosis	Coefficient	P (Z)	P Chi	Pseudo RSq
TTF1	0.6	0.5	0.00	0.3
P40	14.6	0.9		
CK 5/6	-16.9	0.9		
Constanta	1.5	0.1		

Table 8. TTF 1 Regression Analysis

PA Diagnosis	Coefficient	P (Z)	P Chi	Pseudo RSq
TTF1	1.6	0.02	0.02	0.1
Constanta	0.2	0.6		

Table 9. P 40 Regression Analysis

PA Diagnosis	Coefficient	P (Z)	P Chi	Pseudo RSq
P40	-2.3	0.003	0.00	0.2
Constanta	1.9			

Table 10. PA Regression Analysis

PA Diagnosis	Coefficient	P (Z)	P Chi	Pseudo RSq
CK 5/6	-2.7	0.0	0.0	0.2 37
Constanta	2	0.0		ch

Table 11. Accuracy

PA Diagnosis	ROC area	95% CI	
TTF1	0.7	0.5	0.8
P40	0.2	0.1	0.4
CK 5/6	0.2	0.1	0.4

Discussion

TTF-1 is a tissue-specific homeodomain with a transcription factor mainly found on alveolar pneumocyte type II. TTF-1 can also be found on thyroid tissue since embryogenesis in human lungs and brain. TTF-1 have primarily functions on epithelial morphogenesis and stimulates pneumocyte surfactant protein regulating transcription gene secretion on Klara's cell.[8-10] TTF-1 is a 38 kDa nuclear protein member of

the NKx2 family of homeodomain transcription factors. Human TTF-1 is a single polypeptide of 371 amino acids. With gene located on chromosome 14q13.[11-13]

TTF-1 specific to lung carcinoma and carcinoma origin from the thyroid.[14] Nowadays, TTF-1 is mainly used as marker for adenocarcinoma. Recent studies have found that TTF-1 has high sensitivity and specificity for adenocarcinoma.[12] In lung non-small cell carcinoma, TTF-1 was found in 76% adenocarcinoma but rarely on squamous cell carcinoma.[8] Moldvay et al[7] reported in primary bronchial adenocarcinomas found immunopositivity in 46/50 cases among them 30 cases showed strong nuclear immunostaining and four primary adenocarcinoma cases observed immunopositivity was localized to the cytoplasm. Stenhouse et al[8] reported of the pulmonary adenocarcinomas, 75% were strong positive for TTF-1.

Lau et al[10] demonstrated TTF-1 positivity in the majority of tumours (75-80% in reported series). Unlike adenocarcinomas, squamous carcinomas in consistently express TTF-1, with reported positivity ranging from 0 to 37,5% of cases studied.

TTF-1 appears to be a reasonably sensitive and highly specific marker for pulmonary adenocarcinomas, with potential for diagnostic use in distinguishing primary pulmonary adenocarcinomas from metastatistic extrapulmonary adenocarcinomas metastatic to the lung, or establishing a lung origin for metastatic adenocarcinomas of unknown primary site.

Kawai et al[15] found TTF-1 expression in 53 out of 82 adenocarcinoma case (65%) with moderate and weak differentiation. Expression P40 was 2% and CK5/6 0% in adenocarcinoma. TTF-1 was observed in only 9% of squamous cell carcinomas, while P40 was 85% and CK5/6 81%. Yaman et al[14] reported TTF-1 was 84,8% in adenocarcinoma but being negative in SCC. TTF- 1 it was for AC with 84,4% sensitivity and 100% specifity.

Yatabe et al[16] reported that expression of TTF-1 in adenocarcinoma was 72% and had statistically significant prevalence of female and nonsmoker. In this research, we reported 25 positive results of TTF-1 expression on adenocarcinoma (71.4%) and 4 positive results on squamous cell carcinoma (33.3%). And same with studied TTF-1 in adenocarcinoma was positive 70-85% and SCC was 0-37,5%.

P40 is one of the most specific markers for basal cells and squamous cells. It has a significant function compared to p63 in diagnosing squamous cell carcinoma.[17] P40 antibody that has been identified as Np63 has been widely available. Nowadays, it has known to differentiate squamous cell carcinoma and adenocarcinoma.[17] P40 is a fraction isoform from N-terminal p63, dominantly expresses on squamous cell carcinoma, and has great specificity in diagnosing squamous cell carcinoma.[18,19] Bishop et al[18] showed that p40 has a sensitivity and specificity of 100% for squamous cell carcinoma. Thus p40 is highly recommended in cases of poorly differentiated carcinomas.[17]

While Tacha et al[19] reported that p40 sensitivity was 85% and specificity was 98% on squamosa cell carcinoma. Affandi et al[17] found that p40 was expressed in 27 of squamous cell carcinoma cases (77.1%) and all adenocarcinoma cases showed negative expression of P40. Reactivities P40 on carcinoma cell squamosa were diffuse and strong. p40 isan excellent marker for distinguishing lung squamous cell carcinoma from adenocarcinoma with specificity 100% sensitivity 77,1%. Wang et al[20] TTF-1 was positive in adenocarcinoma 84,2% and SCC 7,2% with specificity 93,44% and sensitivity 79,82%. While P40 was positive in KSS 100% and adenocarcinoma 8,7% with specificity 98,8% and sensitivity 24,9%. CK5/6 was positive in KSS 94% and adenocarcinoma 6,67% with specificity 96,5% and sensitivity 77,05% P40 more specificity CK5/6 in SCC.

This study found p40 positive expression in 66.7% SSC and 17.1% on adenocarcinoma. P40 is a nucleus marker that is easier to evaluate. CK5/6 is essential keratin with moderate size and molecular weight of 58 kDa.[21] CK5/6 is cytokeratin with high molecular weight and found on epithelial breast muscle and basal epithelial on lung bronchiole and spinous cell in skin which however is rarely expressed in glandular ephitelium.[20,22]

CK5/6 showed as a stain on cytoplasm, useful in diagnosis of SCC especially in the poorly differentiative cell type.[5] Sensitivity on squamous cell carcinoma reported 73-100%.[6] Xu et al[22] reported CK5/6 was positive in biopsies from 91 of 99 SCC, and 5 of 111 adenocarcinoma. TTF-1 was present in 105 of 111 adenocarcinoma and none of SCC than TTF- 1 specificity 100% , sensitivity 94,59%.

CK5/6 has relatively high specificity of 91.92% in carcinoma cell squamous. Although CK5/6 positivity

has been reported in a small percentage (2-8%) of primary pulmonary adenocarcinoma. Kriegsmann et al.[24] found two markers (CK5/6, P40) are similar to identifying squamous cell carcinoma. On lung cancer, sensitivity to identify squamous cell carcinoma from CK5/6, p40, and is 93.94%, and specificity CK5/6 is 98% and p40 is 97%. P40 showed the highest sensitivity and specificity combination for squamous differentiation compared CK5/6. These findings align with a previous study that reported range 80-99% for CK5/6, 85-100% for P40.

Argon et al[6] reported CK5/6 of the 72 SCC cases 56 had 3+ strong, 4 had 2+ and 10 had 1+ and no staining was seen in any of adenocarcinoma. CK5/6 had specificity 100% and sensitivity 97,8%. TTF-1 was see 100% in all adenocarcinoma cases but not seen in any of SCC. Nishino[25] TTF-1 demonstrated specificity 99% for adenocarcinoma and sensitivity 94% for TTF-1.

P40 demonstrated superior specificity to CK5/6 (94% vs 59%, respectively) for SCC and sensitivity P40 and CK5/6 100%. Marson et al[26] reported TTF-1 was 3% in SCC and 98% in adenocarcinoma. While CK5/6 was not found in adenocarcinoma and was detectable in 100% of primary lung SCC.

With specificity and sensitivity 100% This report found a positivity rate 66.7% on SCC and 11.4% for adenocarcinoma. And we found P40 and CK5/6 was positive 66,7% in KSS. But P40 more positivity compared CK5/6 in adenocarcinoma (17,1% PS 11,4%). Expression of CK5/6 in some pulmonary adenocarcinomas is not surprising because CK5/6 is present in normal basal cells of the respiratory epithelium.[27]

There was a significant difference between the expression of TF-1, P40, and CK 5/6 in differentiating adenocarcinoma and squamous cell carcinoma. However, these three expressions cannot be used simultaneously. Because the coefficient values were significantly different. TTF-1 expression has the highest accuracy compared to p40 and CK5/6.

Conclusion

The positive expression of TTF-1 is 71.4% in adenocarcinoma and 33,3% in SCC. The positive expression of p40 and CK5/6 are 66.7% in squamous cell carcinoma, but p40 expression positivity was 17,1% in adenocarcinoma and CK5/6 expression 11,4% in adenocarcinoma. There was a significant difference between the expression of TTF-1, P40, and CK 5/6 in differentiating adenocarcinoma and squamous cell carcinoma. However, these three expressions cannot be used simultaneously. TTF-1 expression has the highest accuracy compared to p40 and CK5/6.

References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview [Internet]. Vol. 149, International Journal of Cancer. 2021. Available from: http://globocan.iarc.fr/Pages/
2. Kemkes. Pedoman Nasional Pelayanan Kedokteran Kanker Paru Komite Penanggulangan Kanker Nasional [Internet]. [cited 2017 Apr 5]. Available from: Kemkes.go.id
3. Jusuf A, Haryanto A, Syahruddin E, Endart S, Midjiantoro S, Sutantio N. Kanker Paru Jenis Karsinoma Bukan Sel Kecil. Pedoman Diagnosis dan Penatalaksanaan Kanker Paru Jenis Karsinoma Bukan Sel Kecil di Indonesia. Jakarta: PDPI, PO; 2015.
4. Herbst RS, Heymach JV, Scott, Lippman M. Molecular origins of cancer lung cancer. Onco Targets and therapy. 2008;359:1367-80.
5. Rahman MAA, Nada NAM, Zalata KR, Badrawy MKE, Salkh IME, Hamid AA . Role of minimal panelimmuno-staining in accurate diagnosis of lung cancer using small biopsies. Eqypt J Chest Dis Tuberc. 2016;10.011:1- 6.
6. Argon A, Nart D, Veral A. The value of cytokeratin 5/6, p63 and thyroid transcription factor-1 in adenocarcinoma, squamous cell carcinoma and non-small-cell lung cancer of the lung. Turk Patologi Derg.2015;31:81-8.
7. Moldvay J, Jackel M, Bogos K, Soltesz I, Agocs L, Kovacs G, Schaff Z. The role of TTF-1 in differentiating primary and metastatic lung adeno-carcinomas. Path Oncol Res.2004;10 (2):85-8.
8. Stenhouse G, Fyfe N, King G, Chapman A, Kerr KM. Thyroid transcription factor 1 in pulmonary adenocarcinoma. J Clin Pathol.2004;57:383-7.
9. Boggaram V. Thyroid transcription factor-1 (TTF-I/Nkx2.1/TITFI) gene regulation in the lung. Clin Sci.2009;116:27-35.
10. Lau SK, Luthringer DJ, Eisen RN. Thyroid transcription factor-1: Review. Appl Immunohistochem Mol Morphol.2002;10(2):97-102.
11. Bingle CD. Thyroid transcription factor-1. Int J Biochem. Cell Biol.1997;29(12):1471-3.
12. Ordonez NG. Thyroid transcription factor-1 is a marker of lung and thyroid carcinomas. Adv Anatomic Pathol.2000;7(2):123-7.
13. Ordonez NG. Value of thyroid transcription factor-1 immunostaining in tumor diagnosis: A review and update. Appl Immunohistochem Mol Morphol.2012;20:429-44.

14. Yaman B, Nart D, Ekren PK, Cok G, Veral A. Expression of p63, TTF-1 and maspin in non-small cell lung carcinoma and their effect of the prognosis and differential diagnosis. Turk Patologi Derg.2015;31:163-74.
15. Kawai T, Tominaga S, Hiroi S, Kameda K, Ogata S, Nakashima H, et al. Expressions of thyroid transcription factor-1, napsin A, p40, p63, CK5/6 and desmocollin-3 in non small cell lung cancer, as revealed by imprint cytology using a malinol-based cell-transfer technique. Acta Cytologica.2015;59:457-64.
16. Yatabe Y, Mitsudomi T, Takahashi T. TTF-1 Expression in pulmonary adenocarcinomas. Am J Surg Pathol. 2002;26(6):767-73.
17. Affandi KA, Tizen NMS, Mustangin, Rahayu R. pP40 immunohistocemistry is an excellent marker in primary lung squamous cell carcinoma. *J Pathol Transl Med*. 2018;52:283-9.
18. Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. P40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol.* 2012;25:405-15.
19. Tacha D, Bremer R, Haas T, Qi W. An immunohistochemical analysis of a newly developed, mouse monoclonal P40 (BC28) antibody in lung, bladder, skin, breast, prostate, and head and neck cancers. *Arch Pathol Lab Med*. 2014;138:1358-64.
20. Wang JY, Wang XM, Xu XY, Li SR, Liu XL. Expression and significance of CK5/6, P63, P40, CK7, TFF-1, NapsinA, CD56, Syn and CgA in biopsy specimen of squamous cell carcinoma, adenocarcinoma and small cell lung carcinoma. *Int J Morphol*. 2020;38(2):247-51.
21. Chu PG, Weiss LM. Expression of cytokeratin 5/6 in epithelial neoplasms: An immunohistochemical study of 509 cases. *Mod Pathol*. 2002;15(1):6-10.
22. Xu XY, Yang GY, Yang JH, Li J. Analysis of clinical characteristics and differential diagnosis of the lung biopsy specimens in 99 adenocarcinoma cases and 111 squamous cell carcinoma cases: Utility of an immunohistochemical panel containing CK5/6, CK34βE12, p63, CK7 and TTF-1. *Pathol Res Pract*. 2014;210:680-5.
23. Zhao W, Wang H, Peng Y, Tian B, Peng L, Zhang DC. Δnp63, 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.
24. Kriegsmann K, Cremer M, Zgorzelski C, Harms A, Muley T, Winter H, et all. Agreement of CK5/6, p40, and p63 immunoreactivity in non-small cell lung cancer. *Pathol*. 2019;51(3):240-5.
25. Nishino M, Hoang MP, Pelle PD, Oyarvide VM, 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.
26. Marson VJ, Mazieres J, Groussard O, Garcia O, Berjaud J, Dahan M, et al. Expression of TTF-1 and cytokeratins in primary and secondary epithelial lung tumours: Correlation with histological type and grade. *Histopathol.* 2004;45(2):125-34.
27. Camilo R, Capelozzi VL, Siqueira SAC, Bernardi FDC. Expression of p63, keratin 5/6, keratin 7, and surfactant-A in non-small cell lung carcinomas. *Hum Pathol*. 2006;37(5):542-6.