Evaluation of Sensitivity and Specificity of Napsin A and P40 in Non Small Cell Lung Cancer Patients

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Abstract :

The majority lung tumors are classified as Non-Small Cell Lung Carcinomas (NSCLCs) and Small Cell Carcinomas (SCCs). NSCLC needs to be further subclassifed into adenocarcinoma and squamous cell carcinoma for better treatment options. Napsin A and P40 are emerging as potential markers for the subclassification of adenocarcinoma and squamous cell carcinoma. So the present study evaluated expression of Napsin A and P40 in lung adenocarcinoma and SCC patients so as to evaluate its diagnostic utility, and correlate and check its sensitivity and specificity along with other diagnostic lung cancer markers. Expression of Napsin A and P40 was evaluated in 25 adenocarcinoma and 25 squamous cell carcinoma patients by Immunohisotochemistry. Napsin A expression was observed in 42% and P40 in 64% of the patients. The expression of Napsin A was significantly higher in adenocarcinoma patients with a positive correlation with adenocarcinoma markers Thyroid Transcription Factor 1(TTF-1) and Cytokeratin 7(CK7); and inverse correlation with squamous cell marker Cytokeratin 5/6(CK5/6) with a specificity of 76% which was highest and sensitivity of 60%, which was lower than that of CK7, Carcino Embryogenic antigen(CEA) and TTF-1 in adenocarcinoma patients. The expression of P40 was significantly higher in squamous cell carcinoma and patients with metastasis, along with higher incidence in patients with increased tumor size and advanced disease stage. P40 showed positive correlation with squamous cell marker p63 and CK 5/6 with a specificity of 52% and sensitivity of 80% in squamous cell carcinoma patients but was lower than CK5/6 and p63. Napsin A was found be most specific adenocarcinoma marker, but its sensitivity was low. Further, P40 failed to reach highest level of sensitivity and specificity in our set of patients which may be due to the less number of patients analyzed in the study and urges a need to be evaluated in a large cohort

Keywords : Napsin A, Non Small Cell Lung Cancer ,P40.

Introduction :

Lung cancer is one of the leading causes of cancer related mortality worldwide. Most lung cancers are usually detected in advanced stage with a low 5-years survival rate. [1, 2] Primarily, the lung tumors consist of different histological types, most of which can be defined as malignant epithelial tumors. The majority of these malignant tumors are classified as non-small cell lung carcinomas (NSCLCs) and small cell carcinomas (SCCs). [3] The majority of lung carcinomas are NSCLC that accounts approximately for 80%, with most of the remaining being SCC, which has neuroendocrine features and is treated with chemotherapy and radiotherapy. Previously, it was sufficient to diagnose primary lung carcinoma as either NSCLC or SCC for treatment purposes. But this previous distinction is now no longer sufficient due to the development of new, successful treatments and hence NSCLC is further sub classified into adenocarcinoma and squamous cell carcinoma. [3]

These new treatments include [1] therapies that target Epidermal Growth Factor Receptor(EGFR) mutations and Anaplastic Lymphoma Kinase(ALK) fusion genes that are found almost exclusively in adenocarcinomas; [2] bevacizumab (monoclonal antibody to Vascular Endothelial Growth Factor - VEGF), which is effective as a first-line agent in many adenocarcinomas but may cause severe, even life-threatening hemorrhage in patients with squamous cell carcinoma; and[3] pemetrexed (new antifolate agent) may be effective in adenocarcinomas but is not effective in squamous cell

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carcinomas. The lung is also a common site of metastases from other sites, including breast, gastrointestinal tract, and genitourinary tract and that of metastatic adenocarcinoma of an unknown primary. [3-7]

WHO defines with hematoxylin-eosin (H&E) staining method, by which lung cancers are classified; however, typing of NSCLC and the more poorly differentiated tumors is often hard to achieve by H&E stain alone. Immunohistochemistry (IHC) has emerged as a powerful, adjunctive tool for the differential diagnosis of lung carcinomas, whether primary or secondary to the lung. It may aid in the differential diagnosis of not only surgical specimens but also small biopsies. The currently used IHC markers for differential diagnosis NSCLC include p63, CK5/6, TTF-1, CK7 and CEA. [8]

Currently Napsin-A, an aspartic proteinase involved in the maturation of the surfactant protein B, recently has come to the attention of investigators as a potential marker of lung adenocarcinoma. The proteinase is expressed abundantly in the cytoplasm of normal lung cells (type II pneumocytes and Clara cells) and kidney cells (proximal and convoluted tubules) and in lung adenocarcinomas and renal cell carcinomas. It has been demonstrated that the expression of napsin-A is regulated by TTF-1, a member of the Nkx2 family of transcription factors that also regulate the expression of surfactant protein B. Previous studies using resected tumor tissues demonstrated that napsin-A was equal to or better than TTF-1 and surfactant protein A immunostains for determining lung origin in well to moderately differentiated adenocarcinomas. [9]

Another emerging marker for squamous cell carcinoma is P40 which is an isoform of p63 with alternative promoter site and splicing. The two best characterized p63 variants are long TAp63 isoform containing N – terminal transactivation domain and a truncated variant p40 (ΔNp63) lacking the N- terminal domain. These two variants have different functions; TAp63 activates p53 target gene, while p40 (DNp63) inhibits transcription activation of p53 gene. In contrast to the anti – p63 antibody (4A4) recognize both p63 variants, the antip40 (BC80 recognize only P40 (ΔNp63) isoform. [10] Although anti p63 (4A4) has high sensitivity in lung Squamous cell carcinoma, a limitation of this antibody is its low specificity due to its reactivity in substantial proportion of lung adenocarcinoma and other tumor types. So the present study was focused on the evaluation of expression of Napsin and p40 in lung adenocarcinoma and SCC patients so as to evaluate its diagnostic utility, and correlate and check its sensitivity and specificity in non small cell lung cancer patients along with other diagnostic markers.

Methods :

In this retrospective study, 50 lung cancer patients who had been diagnosed and treated at Gujarat Cancer & Research Institute (G.C.R.I) in the duration of 2016 to 2018 were included. The detailed clinical history such as patient's age, sex, habit (smoking), histopathological findings, and treatment offered and disease status was recorded in the division from the case file maintained at the Institutional Medical Record Department. Paraffin embedded block of these lung cancer patients were collected from Histopathology Department of G.C.R.I. The study was approved by Institutional Scientific Review Board and Ethics Committee.

Immunohistochemical localization of Napsin A and p40 along with CK5/6, CEA, TTF-1. P63 and CK7 were evaluated on formalin fixed paraffin embedded (FFPE) tissue blocks containing primary tumor evaluated by H&E staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). The commercially available antibodies used were Napsin A (clone MRQ 60, Cell marque. 1:100), p40 (clone ZR8, Master Diagnostic, RTU), CK7(clone OV-TL, Cell marque. 1:100), TTF-1 antibody (clone 8G7G3/1, Cell marque. 1:100), CEA(IL-7, Dako, 1:50), CK5/6 (Clone D5&16B4, Cell marquee,1:100) and p63 (clone 4A4, Ventana, RTU). 3-4 μm thin sections were cut on microtome (Leica, Germany) and taken on to 3-Aminopropyltriethoxysilane (APES) coated slides. Briefly, the protocol includes following steps of deparafinization using EZ prep solution, antigen retrieval for 30 minutes using retrieval solution CC1 and incubation with ultra view DAB inhibitor for 4 minutes, addition of 100μL of Napsin A, p40, TTF-1, CK7, p63 antibody at 37°C for 32 minutes and antigen retrieval for 60 minutes using retrieval solution CC1 and incubation with ultra view DAB inhibitor for 4 minutes antibody for CEA at 37°C for 32 minutes followed by ultra view HRP multimer for 8 minutes, ultra view DAB Detection kit for 8 minutes. The sections were counterstained with hematoxylin for 8

minutes and bluing reagent for 4 minutes and mounted with DPX.

Staining characteristics were reviewed and considered along with the intensity and distribution of staining patterns. A case was considered to be positive if greater than 5% of tumor cells with an appropriate staining pattern were identified; otherwise the case was considered to be negative. In terms of specific staining patterns, coarse granular cytoplasmic staining was considered positive for Napsin A. Nuclear staining was considered positive for TTF-1 and P63. Cytoplasmic staining was considered positive for CK7, CEA and CK5/6. Appropriate positive and negative controls were included in each assay. Care was taken not to interpret entrapped normal lung bronchial epithelium or pulmonary macrophages as a positive staining.

Statistical analysis:

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Pearson's chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between the two parameters.

Sensitivity, Specificity, Positive predictive value and Negative predictive value were calculated by following formulae:

True positive : A true positive test result is one that detects the condition when the condition is present.

Sensitivity =TP/TP + FN	**Specificity** =TN/TN+FP
Positive Predictive value (NPV) = FN/FN + FP	**Negative Predictive (PPV)** = TP/TP + FP

True negative : A true negative test result is one that does not detect the condition when the condition is absent.

False positive : A false positive test result is one that detects the condition when the condition is absent.

False negative : A false negative test result is one that does not detect the condition when the condition is present.

Results :

Expression of Napsin A and p40 in lung cancer patients:

Granular cytoplasmic expression of Napsin A was noted in 42 %(21/50) of the patients whereas, 58% (29/50) (Figure 1a) were negative for Napsin A expression (Figure 1b). Nuclear expression of P40 was observed in 64 % (32/50) (Figure 2) patients whereas, 36% (18/50) were negative for P40 expression.

Figure 1a: Granular Cytoplasmic Expression of Napsin A in Lung Cancer Patients.

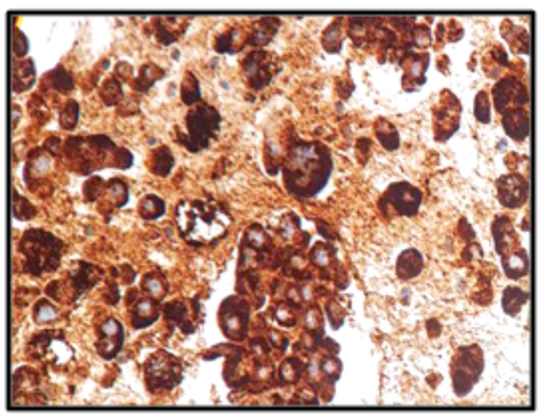

Figure 1b: Negative expression of Napsin A in Lung Cancer Patients.

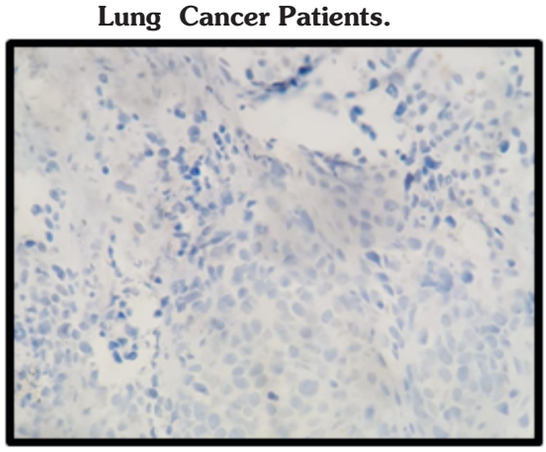

Figure 2: Nuclear expression of P40 in Lung Cancer Patients.

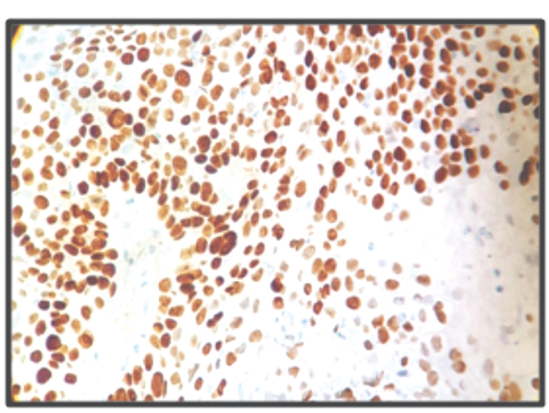

Table 1 : Correlation of Napsin - A Expression with Clinical and Pathological Parameters

Parameters		Napsin - A		χ^2	P- value
		Negative (N=29)	Positive (N=21)		
Age (years)	≤60	16 (55%)	11(52%)	0.038	0.845
	>60	13 (44%)	10 (47%)		
Gender	Male	28 (96%)	19 (90%)	0.797	0.372
	Female	1 (4%)	2 (10%)		
Habit	None	9 (31%)	11 (52%)	2.784	0.249
	Smokers	19 (65%)	10 (47%)		
	Chewers	1 (03%)	0 (00%)		
Tumor size	N=45	25	20	2.675	0.617
	T1 + T2	8 (32%)	10 (50%)		
	T3 + T4	17 (68%)	10 (50%)		
Nodal status	N=46	26	20	2.819	0.420
	Negative (N0)	11 (42%)	5 (25%)		
	Positive (N1, N2, N3)	15 (58%)	15 (75%)		
Stage	N=47	27	20	4.450	0.217
	I + II	4 (15%)	5 (25%)		
	III + IV	23 (85%)	15 (75%)		
Histological subtypes	N=50	29	21	6.665	0.01
	Adenocarcinoma	10 (40%)	15 (60%)		
	Squamous cell carcinoma	19 (76%)	6 (24%)		
Metastasis	N=47	27	20	1.719	0.423
	No metastasis (M0)	22 (81%)	14 (70%)		
	Metastasis	05 (19%)	06 (30%)		

Correlation of Napsin A and P40 with Clinical and Pathological parameters :

In relation to clinical parameters no significant difference in expression of Napsin A was noted with age, gender and smoking habits. In relation to pathological parameters a significant higher expression of Napsin A was noted in patients with adenocarcinoma (60%, 15/21; p=0.01) as compared to squamous cell carcinoma patients (24%, 6/21). Also higher expression of Napsin A was noted in patients with no metastasis (70%, 14/20), lymph node positivity (75%, 15/20), advanced stage patients {(Stage III + Stage IV) (75%, 15/20)} as compared to their respective counterparts (Table 1).

In relation to clinical parameters, no significant difference in expression of P40 was noted with age, gender and smoking habits. With pathological parameters correlating P40 expression with histological subtypes, a significant higher expression was observed in patients with squamous cell carcinoma (80%, 20/25; p=0.01) as compared to Adenocarcinoma patients (37%, 12/32). Also expression of P40 was found to be significantly higher in patients with metastasis (66%, 19/29, p = 0.004), as compared to patients without metastasis. Further, trend of higher P40 incidence was found in patients advanced disease stage {(Stage III + Stage IV), (86%, 25/29; p=0.06)} T3 + T4 tumor size (64%, 18/28) and lymph node positivity (57%, 16/28) than patients with early disease stage (stage I + stage II), T1 + T2 tumor size and lymph node negativity (Table 2).

Correlation of Napsin A and P40 with other lung cancer diagnostic markers

When intermarker correlation of Napsin A was performed with Adenocarcinoma markers, a significant positive correlation was noted between the

Table 2: Correlation of p40 Expression with Clinical and Pathological Parameters

Parameters (N=50)		P40		χ^2	P - value
		Negative (N=18)	Positive (N=32)		
Age (years)	≤60	10 (55%)	17 (53%)	0.027	0.869
	>60	8 (45%)	15 (47%)		
Gender	Male	17 (94%)	30 (94%)	0.010	0.921
	Female	1 (06%)	2 (06%)		
Habit	None	7 (39%)	13 (41%)	0.618	0.734
	Smokers	11 (61%)	18 (56%)		
	Chewers	0 (00%)	1 (03%)		
Tumor size (N=45)	N=45	17	28	4.237	0.375
	T1 + T2	8 (47%)	10 (36%)		
	T3 + T4	9 (53%)	18 (64%)		
Nodal status	N=46	18	28	2.05	0.152
	Negative (N0)	4 (22%)	12 (43%)		
	Positive (N1, N2, N3)	14 (78%)	16 (57%)		
Stage	N=47	18	29	7.319	0.062
	I + II	5 (28%)	4 (14%)		
	III + IV	13 (72%)	25 (86%)		
Histological subtype	N=50	18	32	5.55	0.01
	Adenocarcinoma	13 (72%)	12 (37%)		
	Squamous cell carcinoma	5 (28%)	20 (63%)		
Metastasis	N=47	18	29	8.33	0.004
	No metastasis (M0)	14 (78%)	10 (34%)		
	Metastasis	4 (22%)	19 (66%)		

Napsin A and TTF - 1 expression (62%, 13/21; p=0.001). Also, a higher incidence of Napsin A was noted in CK7 positive patients (77%, 16/21) and CEA positive patients (57%, 09/19). With relation to squamous cell markers a significant higher expression of Napsin A was noted in CK5/6 negative tumor (70%, 12/17; p=0.03) as compared to CK 5/6 positive tumors. When intermarker correlation of P40 expression was performed with squamous cell carcinoma markers, a significant positive correlation was noted between the P40 and P63 expression (83%, 24/29; p=0.001) and P40 and CK5/6 positive patients (61%, 17/28; p=0.02). P40 when compared with Adenocarcinoma markers, 55%, 32% and 58% patients showed positive expression of CEA, TTF – 1 and CK7, respectively (Table 3).

Sensitivity, Specificity, PPV and NPV of Adenocarcinoma and squamous cell carcinoma

In accordance with the expression of Adenocarcinoma in lung cancer, CK7 showed 96% of sensitivity, 69% specificity, 75% PPV and 94% NPV. CEA showed 89% sensitivity, 69% specificity, 69% PPV and 89% NPV , while TTF – 1 showed 71% sensitivity, 00% specificity, 41% PPV and 00% NPV, whereas Napsin A showed 60% sensitivity, 76% specificity, 71% PPV and 65% NPV (Table 4).

In accordance with the expression of squamous cell carcinoma in lung cancer, P63 showed sensitivity of 100%, specificity of 76%, PPV of 83% and NPV of 95% while CK 5/6 showed sensitivity of 80%, specificity of 76%, PPV 95% and NPV of 95%, while

Table 3 : Intermarker correlation of and Napsin A and p40 with Diagnostic Lung Cancer Panel Markers

Markers		Napsin A expression		P -40 expression		
					Negative	Positive
P63	N=46	27	19	N=46	13	29
	Negative	06 (22%)	10 (52%)	Negative	11 (85%)	05 (17%)
	Positive	21 (78%)	09 (48%)	Positive	06 (15%)	24 (83%)
	χ^2= 4.5464, r – value=-0.326, P – value = 0.03			χ^2= 10.64, r – value=0.48, P – value = 0.001		
CK5/6	N=41	24	17	N=41	13	28
	Negative	09 (37%)	12 (70%)	Negative	10 (77%)	11 (39%)
	Positive	15 (63%)	05(30%)	Positive	3 (23%)	17 (61%)
	χ^2= 4.36, r – value=0.350, P – value = 0.02			χ^2= 5.03, r – value=0.350, P – value = 0.02		
CEA	N=41	25	16	N=41	12	29
	Negative	11 (44%)	7 (43%)	Negative	5 (42%)	13 (45%)
	Positive	14 (56%)	9(57%)	Positive	7 (58%)	16 (55%)
	χ^2= 0.02, r – value=-0.002, P – value = 0.987			χ^2= 0.03, r – value=-0.029, P – value = 0.85		
TTF 1	N=48	27	21	N=48	17	31
	Negative	23 (85%)	08 (38%)	Negative	10 (59%)	21 (68%)
	Positive	4 (15%)	13 (62%)	Positive	7 (41%)	10 (32%)
	χ^2= 11.54, r – value=-0.488, P – value = 0.001			χ^2= 0.38, r – value=-0.089, P – value = 0.53		
CK7	N=46	25	21	N=46	15	31
	Negative	12 (48%)	5 (23%)	Negative	4 (27%)	13 (42%)
	Positive	13 (52%)	16 (77%)	Positive	11 (73%)	18 (58%)
	χ^2= 1.06, r – value= 0.152, P – value = 0.30			χ^2= 1.01, r – value=-0.148, P – value = 0.31		

Table 4 : Sensitivity, Specificity, PPV and NPV of Adenocarcinoma and Squamous cell carcinoma

Markers	Sensitivity	Specificity	PPV*	NPV#
Adenocarcinoma markers				
Napsin A	60%	76%	71%	65%
CK7	96%	69%	75%	94%
CEA	89%	69%	69%	89%
TTF – 1	71%	00%	41%	00%
Squamous cell carcinoma markers				
P40	80%	52%	62%	72%
CK5/6	95%	95%	95%	95%
P63	100%	76%	83%	100%

*PPV: Positive Predictive Value; # NPV: Negative Predictive Value

P40 showed sensitivity of 80%, specificity of 52%, PPV of 62% and NPV of 72% (Table 4).

Discussion :

Lung cancer is the 2nd most common cancer leading to high rate of mortality worldwide. Of all histological subtypes of lung cancer, NSCLC accounts for approximately 85%. Differentiation of NSCLC into histologic types is very important because of new successful therapies that target lung adenocarcinoma and squamous cell carcinoma. Although, the majority of NSCLC, including FNA cases, can be sub-classified based on morphologic examination using H&E stained slides, in day to day practice, an accurate diagnosis might be challenging in some of the small biopsy specimens due to: paucity of tumor cells in a given specimen, loss of characteristic architecture, preparation-related artifacts and factors related to differentiation and heterogeneity of tumor cells. In these situations, IHC markers come to play a crucial role in the sub-classification of NSCLC.[11] So, it is crucial to develop highly sensitive and specific immunohistochemistry (IHC) panel to differentiate subtypes of NSCLC.

Napsin A and P40 are emerging markers for differentiating adenocarcinoma and squamous cell carcinoma along with CK5/6, P63, CK7, TTF–1 and CEA markers are used for differential diagnosis of NSCLC that have shown different sensitivity and specificity in different studies to sub-classify NSCLC.[8] In the present study along with other diagnostic markers, Napsin A and P40 expressions were evaluated in 25 adenocarcinoma and 25 squamous cell carcinoma patients. The overall expression of Napsin A was 42% with 60 % expressed in adenocarcinoma and 24% of squamous cell carcinoma cases. Napsin A expression in lung is regulated by TTF–1, has also shown promise in helping to differentiate primary lung from metastatic adenocarcinoma. Ye J et al, observed that Napsin A and TTF – 1 is a strong indication that an adenocarcinoma originated from lung. [12]

P40 expression was observed in 62% of patients with expression in 80 % of squamous cell carcinoma cases and 37% of adenocarcinoma cases. P40 expression can also be seen in Urothelial carcinoma, Skin carcinoma, Esophageal carcinoma, Head and Neck carcinoma and Cervical carcinoma.[13] P40 is a relatively unknown antibody that recognizes ΔNp63-a p63 isoform suggested to be highly specific to confirm squamous cell carcinoma.[14]

In the present study, similar expression of Napsin A was noted with respect to age and habit. Similar to our study, another observed that there was no significance obtained with clinical parameters like age and gender. Similar expression was observed among different tumor size which was in discordance with study of Ueno et al analyzed that Napsin A expression was significantly higher in patients with primary tumor size less than 3 cm, when compared with their counterparts. [15] Further, an increased expression of Napsin A was observed in patients with nodal positivity and advanced stage. The study reported that univariate analysis of Napsin A showed positive expression associated with pathological parameters like nodal metastasis, primary tumor size and was also positively associated with tumor differentiation. Study by Ao et al failed to show any statistical significance with reference to clinical outcome. [16] In the present study, when Napsin A was correlated with histologic subtype of NSCLC, its expression was found to be highly significant in adenocarcinoma (p=0.01). The studies of Gruda et al also showed higher Napsin A expression in Adenocarcinoma patients. [8]

In the present study, while considering clinical parameters similar expression of P40 was noted with respect to age and habit. However, another study reported that expression of P40 was found higher in males and in younger group of patients in Urothelial carcinoma.[16] With pathological parameters, higher expression of P40 was observed in (T3 + T4) tumor size. In the present study, while considering lymph node status a similar expression of P40 was noted in patients with positive and negative lymph node. An increasing trend was observed when expression of P40 was correlated with disease stage, suggesting its expression to be associated with the development of the disease.

In this study, when P40 was correlated with histological subtype of NSCLC squamous cell carcinoma was found to be highly significant (p= 0.01). While correlating

P40 expressions with metastasis a significant higher incidence was observed in patients with metastasis (p= 0.004) as compared to patients without metastasis, suggesting its presence to be associated with persistent disease and metastasis.

When correlated with other markers of lung cancer, a significant positive correlation of Napsin A was noted with TTF-1 inverse correlation with squamous cell marker CK5/6 which signifies its role as adenoocarcinoma marker. Regarding P40, a positive correlation was observed with squamous cell marker p63 and CK 5/6 that further strengthens its value in squamous cell carcinoma. Among the markers for Adenocarcinoma, highest sensitivity of 96% was observed for CK7 followed by CEA (89%), TTF – 1 (71%) and Napsin A (60%). Further, in the study, highest specificity was observed of Napsin A (76%) whereas, CK7 and CEA showed same specificities of 69% each.

Another study observed that the overall sensitivity of TTF–1 was 81% and that of Napsin A was 65%. Bishop et al found that the Napsin -A positivity decreased with more poorly differentiated lung tumors.[1] Another study found that Napsin A shows the higher specificity as compared to CK7 and CK20, but when Napsin A was assessed with TTF – 1, both revealed the same sensitivity. It was also observed that monoclonal Napsin A (76%) is less sensitive than TTF – 1 (82%) for metastatic adenocarcinoma of lung. [17] In another study, although the performance characteristics of Napsin A has been observed generally equivalent to those of TTF-1, some comparative studies found increased sensitivity and specificity of Napsin A. [18] TTF-1 showed 84.5% and 86.9% positivity in primary and metastatic ADCs, respectively. Although TTF-1 had a higher sensitivity, Napsin-A was useful as a surrogate marker when encountering a poorly differentiated lung adenocarcinoma or an unknown primary tumor. [19]

In contrast to adenocarcinoma, other histologic types of lung neoplasms, including squamous cell carcinoma and neuroendocrine tumors (carcinoid, atypical carcinoid, small cell carcinoma), have been shown to generally lack Napsin A. Studies of Stoll LM et al stated that besides pulmonary adenocarcinomas, the only other type of tumor frequently demonstrating Napsin A positivity in renal cell carcinoma. [19] In our study, 24% of squamous cell carcinomas were found positive for Napsin A. Recent immunohistochemical studies, however, have reported similar expression of Napsin A in squamous cell carcinomas of the lung; that is 26%, a finding that indicates that this marker may not be as specific for lung adenocarcinomas as it is generally believed.

Regarding squamous cell markers, highest sensitivity of 100% was observed for P63 followed by 95% of CK5/6 and 80% of P40. However, specificity of CK5/6 was higher than p63 and P40 and were 95%, 76% and 52%, respectively. But considering specificity, P63 showed highest specificity of 89.6% while that of CK5/6 and P40 was 80% each. P40 provides a nuclear staining pattern which is similar in morphologic appearance to p63, in contradiction to the cytoplasmic decoration seen with CK5/6. [13] Unlike our findings, a study by Collins et al observed sensitivity and specificity of P40 was 89.4% and 100% respectively, while of P63 was 86.8% and 96.7%. [20] P40 specificity and sensitivity for SCC was 100% in FNA cell blocks and FNA specimens in a study. In another study the overall sensitivity of P40 and P63 in SCC FNA cases were 76.6% and 93.3% and specificity were 90.02% and 50.7%, respectively. The key findings of these studies showed P40 was markedly superior to p63 in specificity. [14] The low specificity in the present study could be due to less number of the patients in the study group. CK5/6 is typically a specific marker for SCC. The high specificity and sensitivity of CK5/6 for SCC in our study is similar to earlier studies. [21]

Briefly, in the present study Napsin A was found to be most specific adenocarcinoma marker, but its sensitivity was low. Further P40 failed to reach highest level of sensitivity and specificity in our set of patients which may be due to the less number of patients analyzed in the study and urges a need to be evaluated in a large cohort. But when included in Lung Cancer Panel they may be an important marker in the differential diagnosis of NSCLC to further sub-classify the disease for the best treatment modality.

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