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ORIGINAL RESEARCH

A comparison of diagnostic panels in the immunohistochemical analysis of lung cancer

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Video abstract

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Purpose: Classification of non-small cell lung carcinoma (NSCLC), as adenocarcinoma or squamous cell carcinoma, is important both in the diagnosis and treatment of lung cancer. Use of appropriate markers for this identification is crucial in order to conserve patient tissue for further molecular testing that could guide treatment decisions and have prognostic implications.

Patients and methods: We constructed tissue microarrays from archival resections of 200 NSCLC that were previously subtyped based on morphology and immunohistochemistry (IHC) in some cases. We performed IHC with three TTF-1 clones (SP141, SPT24 and 8G7G3/1), Napsin A, p40, p63 and CK5/6 and panels of four or two markers that best help identify adenocarcinoma and squamous cell carcinoma were ascertained.

Results: Our results showed that the best four-marker panel utilized TTF-1 (clone SP141), Napsin A, p63 and CK5/6 with a sensitivity of 98.3% and high specificity of 91.7%. The best two-marker panel was TTF-1 (clone SP141) and p63 with 96.5% sensitivity and 85.71% specificity.

Conclusion: As there are variations in the performance of different clones of TTF-1 IHC antibodies, the clone chosen can increase the diagnostic value in differentiating adenocarcinoma from squamous cell carcinoma. In the panels analyzed, the survival of cases concordant with the diagnosis had longer survival compared to those that were discordant. The difference was however not statistically significant ($p>0.05$).

Keywords: non-small cell lung carcinoma, immunohistochemistry, squamous cell carcinoma, adenocarcinoma

Plain language summary

Lung cancer is the leading cause of cancer death. Non-small cell lung carcinomas are the most common type of lung cancers. They can further be divided into adenocarcinomas and squamous cell carcinomas. Accurately classifying these tumors is important to select the best treatment options for patients. Immunohistochemical tests help in improving the histopathological diagnosis of the cancer. However there are many antibodies used in the process and there is a lack of data on panels of antibodies that work best in diagnosing lung carcinomas. We have collected tissue from patients who were previously diagnosed and we performed immunohistochemistry with panels of antibodies. Our results show that the best four marker panel utilized the antibodies TTF-1 (clone SP141), Napsin A, p63 and CK5/6. The best two marker panel was TTF-1 (clone SP141) and p63.

Introduction

Lung cancer is a health problem worldwide with an estimated 1.8 million new cases being diagnosed in 2012, accounting for 12.9% of all new cancer cases. Amongst the 10 most commonly diagnosed cancers, lung cancer also has one of the lowest 5-year relative survival rates (14%).[1–3] Approximately 85% of lung cancers are non-small cell

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lung carcinomas (NSCLC) that include the subtypes adenocarcinomas (ADC) and squamous cell carcinomas (SCC). Tumors with histological features of ADC comprise approximately 70% of the NSCLC.[4,5] In a large proportion of cases (60–75%), the disease is metastatic or locally advanced at the time of diagnosis.[6]

Often the diagnostic sample is a small biopsy with minimal tumor tissue. It is thus imperative to conserve tissue for molecular testing that would optimize treatment options with prognostic implications for the patient,[7] as alterations of gene expression impact therapy selection. Testing for these alterations is important for identification of potentially effective targeted therapies, as well as avoidance of therapies unlikely to provide clinical benefit. These include EGFR, ALK, ROS1, BRAF V600E mutations and PD-L1 expression. According to the National Comprehensive Cancer Network (NCCN) guidelines, the outcomes from these molecular investigations determine the chemotherapeutic or targeted therapy that patients may receive.[8] SCC rarely have targetable mutations, and identifying them more precisely would avoid unnecessary further testing.[9]

Although a diagnosis of ADC or SCC can be readily apparent based on morphological features in lung cancer resections, smaller biopsies with limited tumor may be more problematic. Poorly differentiated tumors may lack clear morphologic criteria on histology and artifacts in specimens, scant tumor tissue in biopsies and tumor heterogeneity are factors contributing to the difficulty in providing an accurate diagnosis in small biopsies.[10,11] Another confounding factor is the co-localization of squamous and glandular markers that can occur in a significant number of NSCLC, making a precise diagnosis extremely difficult.[12] In order to preserve material for further studies, a limited panel of antibodies is recommended.[13]

Several studies suggest p40 and TTF-1 are sufficient to discriminate between SCC and ADC of the lung, resulting in a reduction of cases in the NSCLC NOS (not otherwise specified) diagnostic group.[14–16] Although this two-marker analysis is recommended, there is still a "miss" rate in establishing the diagnosis with these markers, that can approximate 18%.[17]

p40 is an isoform of p63 (deltaNp63). The p63 antibody clone 4A4 used in this study recognizes p63 and its transactivation domain. On the other hand, the antibody clone of p40 used in this study (BC28) recognizes p40 but not the transactivating domain. This difference in antigenic structure has led to the conclusion that p40 is a more specific and sensitive marker for squamous cell lung carcinoma (SCC) than p63.[18] However, other studies revealed that p40 may have superior specificity but inferior sensitivity compared with p63 in the diagnosis of pulmonary SCC.[19] Expression of p63 has also been found in around 30% of lung ADC, reducing its specificity as a marker for SCC.[18,20,21]

CK5/6 is a high molecular weight cytokeratin expressed in several neoplasms of epithelial origin including SCC.[22] CK5/6 expression has been considered to be as good as p40 and even superior to p63 in the differentiation of ADC from SCC.[23] Nonetheless, others have found lower sensitivity of CK5/6 expression compared to p63 and p40.[24] There is therefore no consensus on the most ideal IHC (Immunohistochemistry) marker for SCC.

The most widely used IHC markers for pulmonary ADC include TTF-1 and Napsin A. TTF-1 is a homeodomain-containing transcription factor that is predominantly found in normal type II alveolar pneumocytes[25] that has shown high sensitivity and specificity in the immunohistochemical labeling of lung adenocarcinomas.[14,18,26,27] TTF-1 is a nuclear stain and has long been used to help confirm origin from a lung ADC at metastatic sites. The clone of antibody used matters, with different clones of antibodies exhibiting varying sensitivity and specificity.[28] The most widely used clones of TTF-1 are 8G7G3/1 and SPT24. Of these two, clone SPT24 has a stronger affinity for TTF-1.[28] Both clones 8G7G3/1 and SPT24 are mouse monoclonal primary antibodies while the more recent SP141 clone is a rabbit monoclonal primary antibody. This clone has been reported to be 98% specific for ADC.[29]

Napsin A is an aspartic proteinase and its expression is thought to be regulated by TTF-1 and is involved in the maturation of surfactant protein B. In some earlier studies, Napsin A has had a higher sensitivity and specificity for ADC than TTF-1.[30,31]

The addition of Napsin A to TTF-1 has been suggested by some to complement the diagnosis of lung adenocarcinomas. This may be especially relevant in cases where the lower sensitivity of TTF-1 is insufficient to render an exact diagnosis.[32,33] Interestingly, in other studies, the IHC performance of Napsin A has shown reduced sensitivity compared to TTF-1.[34,35] Although the nuclear stain of TTF-1 is easier to interpret compared to the cytoplasmic stain of Napsin A, mistyping of ADCs as SCCs can occur due to the labeling of trapped lung epithelial cells in the tumor.[33,36]

Comparing the results of IHC studies on a combination of 4 marker, 3 marker and 2 makers has been undertaken

by several others. Studies indicate that dual or triple marker combinations offer similar or higher sensitivities and specificities compared to labeling by individual IHC markers.[37]

The aim of our study was to compare the performance of IHC antibodies CK5/6, p40, p63, Napsin A, TTF-1 clones as well as to identify the best 4 and 2 IHC marker-panel to distinguish SCC and ADC among NSCLC. This study is unique in that the diagnostic utility of all three TTF-1 clones are compared to each other. We therefore assessed combinations of IHC antibodies in order to determine the most effective panel utilizing a minimum number of antibodies that can classify NSCLC into ADC and SCC subgroups more accurately.

Materials and methods

Patient recruitment

We selected a cohort of 200 patients, diagnosed with either ADC or SCC at the Department of Anatomical Pathology, Flinders Medical Centre between 1991 and 2011. All patients had histological diagnosis on resection specimens and cases were reclassified according to the 2015 WHO classification of tumors of the lung. These cases had initially been diagnosed based on morphology, IHC and electron microscopy. They included well and poorly differentiated tumors. The cohort is a mixed population including Caucasians and Asians. Definite ethnic status is not part of the clinical information kept on these cases. EGFR mutation status is unavailable as it was not performed in these historical cases. The work was approved by the Southern Adelaide Clinical Human Research Ethics Committee (OFR#136.16-HREC/16/SAC/). As this study was conducted retrospectively, all samples collected were historical archival FFPE tissue blocks with no direct contact with subjects. The ethics committee waived the need for individual consent due to the retrospective nature of the review. All data were anonymized (but re-identifiable with a study key) and maintained with confidentiality.

Tissuemicroarray (TMA) construction

Briefly, tissue cylinders with a diameter of 2.0 mm were punched from representative tumor regions of each donor tissue block and brought into recipient paraffin block using a Quick-Ray Manual Tissue Microarrayer. Control tissue was included in each TMA. There were 116 SCC and 84 ADC.

Immunohistochemical analysis

The immunohistochemical labeling for the different clones of TTF-1, Napsin A, CK5/6, p40 and p63 was performed on a Ventana BenchMark ULTRA immunostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) using QAP-compliant protocols which included in-house developed protocol for SPT24 and 8G7G3/1. The antibody clones used are summarized in Table 1. For Napsin A and CK5/6, membrane labeling was considered positive. For all other markers, nuclear labeling was indicative of a positive result. Focal labeling was regarded as a positive result for TTF-1 and >50% labeling was required for designation as positive for p40, in keeping with the IASLC recommendations.[38] Interestingly, all positive cases showed more than 5% positive labeling for TTF-1. All slides we considered as positive for p40 had more than 50% labeling. Similar to other studies, those cases expressing both TTF-1 and p63 were classified as ADC.[33] If a case was positive for TTF-1 but negative for Napsin A expression (or vice versa), this was considered sufficient to diagnose ADC. The results of combinations of IHC antibody labeling were analyzed to identify the number of correctly classified ADC and SCC with each panel. The combinations tested included 4 and 2 IHC marker panels shown in Table 2.

Statistical analysis

Sensitivity of the individual IHC antibody labeling was calculated as described.[39] Diagnostic reliability of the different antibodies for IHC classification was assessed by receiver-operating characteristic (ROC) curves and area under curve (AUC) calculations as done previously.[23] Sensitivity and specificity of panels of IHC antibodies was calculated using GraphPad Prism version 8.

Survival was calculated as the number of months between first diagnosis and death of the patient, or last follow-up in the case of patients who were still alive. Survival curves were generated using the Kaplan-Meier method, with significance evaluated using the Mantel-Cox log-rank test. Chi-square tests were used to examine the relationship between nominal variables. The limit of significance for all analyses was defined as a p-value of 0.05. This analysis was performed using SPSS software version 23.

Results

IHC expression in lung ADC and SCC

Among the antibodies that label SCC, expression in decreasing order was p63 labeling 112/116; p40 labeling 110/116

Table 1 Antibodies used for immunohistochemistry

Antibody	Clone	Source	Clonality	Species	Dilution
TTF-1	8G7G3/1	Ventana	Monoclonal	Mouse	Predilute
TTF-1	SPT24	Leica	Monoclonal	Mouse	1:50
TTF-1	SP141	Ventana	Monoclonal	Rabbit	Predilute
Napsin A	RQ-60	Ventana	Monoclonal	Mouse	Predilute
p40	BC28	Ventana	Monoclonal	Mouse	Predilute
p63	4A4	Ventana	Monoclonal	Mouse	Predilute
CK5/6	D5/16 B14	Ventana	Monoclonal	Mouse	Predilute

Abbreviations: TTF-1, thyroid transcription factor-1, p63, tumor protein p63; p40, deltaNp63; CK5/6, Cytokeratin 5/6.

Table 2 Combinations of 4 and 2 antibody panels used for immunohistochemistry

1	TTF-1 (SP141), NapsinA, p63, CK5/6
2	TTF-1 (SP141), NapsinA, p40, CK5/6
3	TTF-1 (SPT24), NapsinA, p63, CK5/6
4	TTF-1 (SPT24), NapsinA, p40, CK5/6
5	TTF-1 (8G7G3/1), NapsinA, p63, CK5/6
6	TTF-1 (8G7G3/1), NapsinA, p40, CK5/6
7	TTF-1 (SP141), p63
8	TTF-1 (SP141), p40
9	TTF-1 (SP141), CK5/6
10	TTF-1 (SPT24), p63
11	TTF-1 (SPT24), p40
12	TTF-1 (SPT24), CK5/6
13	TTF-1 (8G7G3/1), p63
14	TTF-1 (8G7G3/1), p40
15	TTF-1 (8G7G3/1), CK5/6
16	NapsinA, p63
17	NapsinA, p40
18	NapsinA, CK5/6

Abbreviations: TTF-1, thyroid transcription factor-1, p63, tumor protein p63; p40, deltaNp63; CK5/6, Cytokeratin 5/6.

and CK5/6 labeling 108/116 SCC. Labeling of ADC with these antibodies was seen in 29/84, 5/84 and 3/84 cases, respectively. CK5/6 had the highest specificity (96.4%) and p63 has shown the lowest specificity (65.4%). Sensitivities and specificities of these IHC markers are shown in Table 3.

Within the ADC cases, the individual TTF-1 clones varied slightly in sensitivity with SP141 labeling 72/84, SPT24 labeling 71/84 while both 8G7G3/1 and Napsin A labeled 69/84 ADC. These three TTF-1 clones also labeled 13/116, 14/116 and 10/116 of the SCC, respectively. The difference in sensitivities between the TTF-1 clones SPT 24 and 8G7G3/1 (84.5% and 82.1%, respectively) in our study is comparable to that found by others, who found sensitivities of 84.1% and 93% for SPT24 and 89% and 79.3% for 8G7G3/1.[40,41] The IHC platform and detection system used has been implicated as one of the reasons for the differences in sensitivities.[42] The SP141 clone had the highest sensitivity (85.7%) compared to the other TTF-1 antibody clones. The 8G7G3/1 clone had the highest specificity (91.3%). Napsin A labeled only 4/116 SCC. Sensitivities and specificities of individual IHC markers are also shown in Table 3.

ROC analysis (not shown) revealed that the values of the area under the curve were high and relevant for each marker. This demonstrates the high performance of individual IHC antibodies to correctly classify NSCLC into ADC and SCC. CK5/6 had the highest AUC of 0.947 for SCC markers and Napsin had the highest AUC of 0.893 of all of the ADC makers. Among the three TTF-1 clones, SP141 and 8G7G3/1 had the highest AUC of 0.868, slightly higher than that seen with clone SPT24 (AUC 0.862).

Table 3 Sensitivity and specificity of individual IHC(immunohistochemistry) markers and AUC (area under the curve)

Antibody	n	Sensitivity %	Specificity %	AUC	Std error
CK5/6	108/116	93.9	96.4	0.947	0.018
p40	110/116	94.8	94	0.944	0.019
p63	112/116	96.5	65.4	0.804	0.034
Napsin A	69/84	82.1	96.5	0.893	0.027
TTF-1(8G7G3/1)	69/84	82.1	91.3	0.868	0.029
TTF-1(SP141)	72/84	85.7	88.7	0.868	0.028
TTF-1(SPT24)	71/84	84.5	87.9	0.862	0.029

Abbreviations: TTF-1, thyroid transcription factor-1, p63, tumor protein p63; p40, deltaNp63; CK5/6, Cytokeratin 5/6.

Table 4 Sensitivity and Specificity of 4 IHC antibody panels

Panels of 4 IHC antibodies	n	Sensitivity %	n	Specificity %	*p* value	Std error
TTF-1(SP141), NapsinA,p63,CK5/6	77/84	98.2	114/116	91.6	<0.0001	0.019
TTF-1(SP141), NapsinA,p40,CK5/6	77/84	96.5	112/116	91.6	<0.0001	0.01957
TTF-1(SPT24), NapsinA,p63,CK5/6	76/84	98.2	114/116	90.4	<0.0001	0.02007
TTF-1(SPT24), NapsinA,p40,CK5/6	76/84	97.4	113/116	90.4	<0.0001	0.0206
TTF-1(8G7G3/1), NapsinA,p63,CK5/6	76/84	98.2	114/116	90.4	<0.0001	0.02007
TTF-1(8G7G3/1), NapsinA,p40,CK5/6	75/84	97.4	113/116	89.2	<0.0001	0.02157

Abbreviations: TTF-1, thyroid transcription factor-1, p63, tumor protein p63; p40, deltaNp63; CK5/6, Cytokeratin 5/6.

Table 5 Sensitivity and Specificity of 2 IHC antibody panels

Panels of 2 IHC antibodies	n	Sensitivity %	n	Specificity %	*p* value	Std error
TTF-1(SP141),p63	72/84	96.5	112/116	85.7	<0.0001	0.02459
TTF-1(SP141),p40	72/84	94.8	110/116	85.7	<0.0001	0.02538
TTF-1(SP141),CK5/6	72/84	93.1	108/116	85.7	<0.0001	0.02613
TTF-1(SPT24),p63	71/84	96.5	112/116	84.5	<0.0001	0.02535
TTF-1(SPT24),p40	71/84	94.8	110/116	84.5	<0.0001	0.0261
TTF-1(SPT24),CK5/6	71/84	93.1	108/116	84.5	<0.0001	0.02683
TTF-1(8G7G3/1),p63	69/84	96.5	112/116	82.1	<0.0001	0.02677
TTF-1(8G7G3/1),p40	69/84	94.8	110/116	82.1	<0.0001	0.02746
TTF-1(8G7G3/1),CK5/6	69/84	86.2	100/116	82.1	<0.0001	0.03051
NapsinA,p63	69/84	96.5	112/116	82.1	<0.0001	0.02677
NapsinA,p40	69/84	92.2	107/116	82.1	<0.0001	0.02844
NapsinA,CK5/6	69/84	93.1	108/116	82.1	<0.0001	0.02812

Abbreviations: TTF-1, thyroid transcription factor-1, p63, tumor protein p63; p40, deltaNp63; CK5/6, Cytokeratin 5/6.

The four-marker IHC panels that had the highest sensitivity and specificity was TTF-1 (clones SP141, NapsinA, p63 and CK5/6 with sensitivity of 98.3% and specificity of 91.7%). This panel accurately identified 77/84 ADC and 114/116 SCC. Four-marker panels with both clones SPT24 and 8G7G3/1 closely followed with sensitivities of 98.28% and specificities of 90.48%. They accurately identified 76/84 ADC and 114/116 SCC. These panels were highly accurate in poorly differentiated tumors in this study. The Chi square test for association was however not significant (p=0.7) (data not shown). Sensitivities and specificities of the 4 antibody panels are shown in Table 4.

We then analyzed panels of 2 IHC antibody markers with 1 ADC and 1SCC marker. The two-marker IHC panel with the highest sensitivities consisted of TTF-1 (either of the clones SP141, SPT24 or 8G7G3/1) and p63 correctly identifying 72/84, 71/84 and 69/84 ADC, respectively. They also identified 112/116 SCC. Although the sensitivities were the same in these panels (96.55%), the specificities varied (85.71%, 84.52% and 82.14%, respectively). The 2 IHC marker panel with the highest sensitivity and specificity was TTF-1 (SP141) and p63 with sensitivity of 96.55% and specificity of 85.71%. The results of labeling with these panels were not significantly associated with either stage or differentiation status. Sensitivities and specificities of the two-antibody panels are shown in Table 5.

Interestingly, two-marker panels of TTF-1 clone SP141 utilizing either p40 or CK5/6 also had very high specificities (85.71%) but definitely lower sensitivities compared to the two-marker panel of TTF-1 (SP141) and p63. Kaplan-Meier survival curves were constructed to determine if there were differences in the survival distribution between cases that had concordant immunohistochemical results and the final diagnosis, versus those cases that had discordant results. The cases whose expression was concordant with the diagnostic subtype had higher survival compared to cases discordant with the diagnosis. The differences were not statistically significant. (Log Rank p>0.05. Figure 1)

Discussion

The purpose of histopathological examination of tumor tissue is to establish a correct diagnosis with the aim of directing the most effective therapy based on the type of

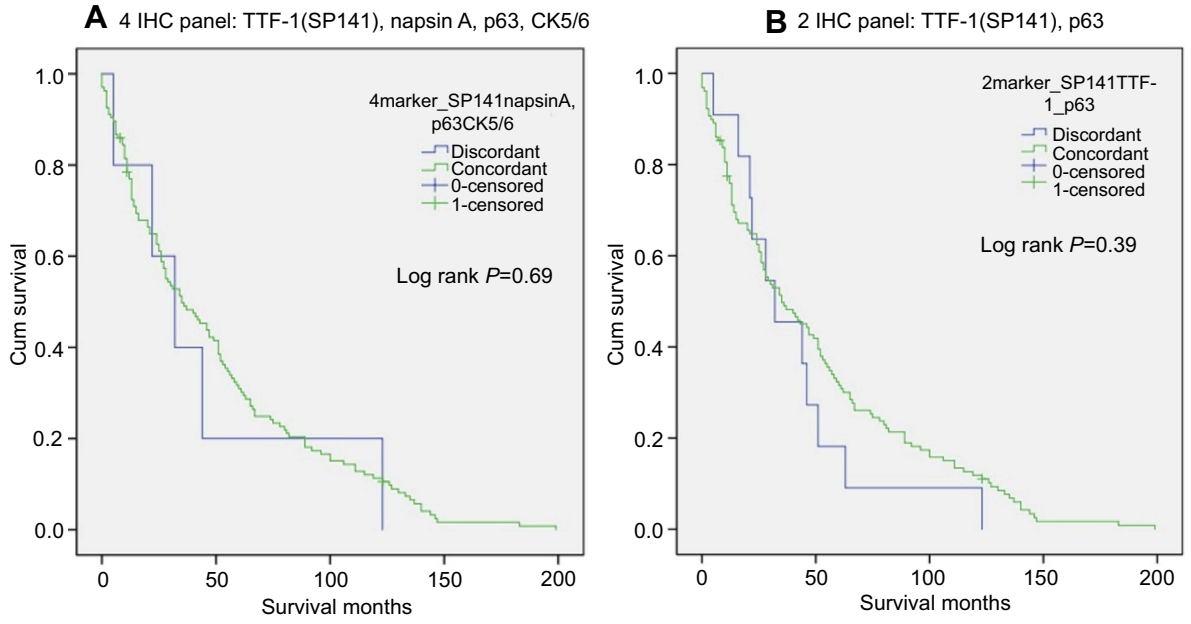

Figure 1 Kaplan-Meier survival curves of cases classified correctly with the panel of 4 IHC markers: TTF-1 (SP141), NapsinA, p63 and CK5/6, and the panel of 2 IHC markers: TTF-1 (SP141) and p63. (**A**) Kaplan-Meier survival curves for cases classified correctly with the panel of 4 IHC markers: TTF-1 (SP141), NapsinA, p63 and CK5/6 in the cohort of 200 NSCLC patients. The green line represents cases in whom the results of immunohistochemistry and final diagnosis were concordant (utilizing the specified panel of antibodies); the blue line represents cases in whom the results of immunohistochemistry and final diagnosis were discordant. The difference was not statistically significant with a log-rank *p*-value of 0.69. (**B**) Kaplan-Meier survival curves for cases classified correctly with the panel of 2 IHC markers: TTF-1 (SP141), and p63 in the cohort of 200 NSCLC patients. The green line represents cases in whom the results of immunohistochemistry and final diagnosis were concordant (utilizing the specified panel of antibodies); the blue line represents cases in whom the results of immunohistochemistry and final diagnosis were discordant. The difference was not statistically significant with a log-rank *p*-value of 0.39.

tumor and biomarker profile. The selection of molecular tests performed depends on the histological subtype – for example, EGFR testing performed on ADC. The aim of our study was to compare the performance of IHC antibodies CK5/6, p40, p63, Napsin A, three TTF-1 clones as well as to identify the best four- and two-marker panel to accurately distinguish SCC and ADC. As each of these groups are associated with unique molecular alterations, their treatment options differ. Several factors influence the ability to distinctly identify and segregate NSCLC samples into either the SCC or ADC groups. Co-localization of markers of the two subtypes within a tumor can add to the conundrum, often delaying a diagnosis. The WHO suggests a minimal panel of TTF-1 and p40 or p63 (one ADC marker and one SCC marker, respectively) to differentiate between the two tumors especially in small biopsies where tumor tissue can be sparse.[26] In such cases, the presence of TTF-1 labeling with absence of p40 labeling should be classified as NSCC favor adenocarcinoma.[43]

The most commonly used TTF-1 antibody clones are 8G7G3/1, SPT24 and SP141. Several studies have demonstrated increased sensitivity of the SPT24 and SP141 clones in lung carcinomas. A recent NordiQC assessment on TTF-1 deemed clones SP141 and SPT24 as being more sensitive than clone 8G7G3/1 in detecting primary lung adenocarcinomas.[42] However, this comes at the cost of decreased specificity as evidenced by SP141 labeling in sarcomatoid mesotheliomas, sarcomatoid carcinoma of the lung and in atypical squamous lesions of the lung.[44] The present study is unique in that all 3 TTF-1 clones are compared to each other individually and also in combination with commonly used IHC antibodies. The difference in sensitivities between the TTF-1 clones SPT24 and 8G7G3/1 were probably due to the difference in IHC platforms and detection systems used.[42]

Others have combined TTF-1 with a range of antibodies to diagnose Adenocarcinoma.[10,33,45,46] One of the four-marker combinations we examined (p63, TTF-1 (SP141), Napsin A and CK5/6) accurately classified more cases (91.6%) of Adenocarcinomas, compared to an identical panel by Mukhopadhyay et al where just over three-fourths of cases were classified correctly.[33] The best four-marker IHC panel in our study that had the highest sensitivity and specificity was TTF-1 (clones SP141, NapsinA, p63 and CK5/6 with sensitivity of 98.3% and specificity of 91.7%). When the other clones of TTF-1 were substituted in this panel, the sensitivity was lower. As the expression of TTF-1 overrides that of the squamous markers, the choice of the TTF-1 antibody clone

determines the sensitivity in ADC diagnosis. This could mean that using these four-marker panels identify more ADC cases who would then have further molecular testing and help in optimizing treatments.

In situations where tissue is sparse and the need to preserve material for molecular and immune marker studies is vital, the use of a two-marker panel of antibodies would help conserve residual tumor tissue. Rekhtman et al suggested that a combination of TTF-1 and p63 was adequate to classify both ADC and SCC with CK5/6 used only if this combination required additional support.[27] We found that the two-marker combinations of TTF-1 and p63 had similar sensitivity (96.55%) but higher specificity only when the clone SP141 of TTF-1 was used emphasizing the advantage of utilizing these markers. When we finally compared all the IHC antibody combination results comprehensively, the panel with the highest sensitivity and specificity was the four-marker IHC antibody panel TTF-1 (SP141), Napsin A, p63 and CK5/6. We analyzed the survival time of patients whose IHC expression was either concordant or discordant with the final diagnosis but did not find a statistically significant difference between the two groups.

Napsin A has been shown to have higher specificity compared to TTF-1 by others as well as Agackiran et al.[28,32,47] Napsin A expression is valuable when the TTF-1 expression results are inconclusive in ADCs. Our results showed that the combination of Napsin A and p63 had similar sensitivity of 96.55% and could be utilized if have ambiguous results are obtained with TTF-1.[42]

Our results provide a rationale for using TTF-1 or p63 in combinations of IHC antibody panels. Such panels would be particularly useful in diagnosing poorly differentiated tumors and small biopsies. In instances where the differential diagnosis would include mesothelioma, pathologists should be aware of the increased likelihood of labeling with the SP141 clone. Metastatic lung tumors sometimes rely on the expression of TTF-1 in the tumor to help identify their origin or confirm recurrence. While it is important to take measures to conserve as much tissue as possible for molecular testing, it is good to be aware of choosing antibody clones that are optimally sensitive.

Abbreviations

ADC; adenocarcinoma; SCC squamous cell carcinoma.

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Disclosure

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