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RESEARCH ARTICLE

The Role of p40, p63, MAdL and TTF-1 in Differential Diagnosis of Primary Lung Adenocarcinoma, Squamous Cell Carcinoma and Metastatic Adenocarcinomas of The Lung

Primer Akciğer Adenokarsinomu ile Skuamöz Hücreli Karsinom Ayırımında ve Akciğerin Metastatik Adenokarsinomlarında p40, p63, MAdL ve TTF-1'in Rolü

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ABSTRACT

Objective: Lung cancer is the most common and fatal type of cancer in both women and men. Due to differences in treatment, it is important to accurately distinguish between lung cancer subtypes. Algorithms recommend using maximum of two markers (ie, a single adenocarcinoma marker and a single squamous marker) in each case to preserve tissue for molecular studies. This study aimed to determine the optimal combination of these markers.

Materials and Methods: This retrospective study included 62 cases diagnosed between 2010 and 2015, consisting of 29 primary lung adenocarcinoma (ADC), 19 squamous cell carcinoma (SCC), and 14 metastatic ADC to the lung. Immunohistochemical analyses for TTF-1, p40, p63, and MAdL were performed. Staining intensity and the percentage of positive tumor cells were recorded with sensitivity and specificity values also calculated.

Results: TTF-1 showed high intensity and positivity in 79.31% of primary lung ADCs, with a sensitivity of 96.55% and specificity of 100%. p40 showed high intensity staining in 84.21% of SCCs, with a sensitivity of 89.47% and specificity of 100%. p63 exhibited 100% sensitivity for SCC but low specificity (44.82%) due to focal positivity in ADCs. MAdL had 82.75% sensitivity and 100% specificity for ADC. No marker positively stained metastatic ADCs.

Conclusion: Of the tested markers, TTF-1 showed the highest diagnostic accuracy for ADC and p40 for SCC. MAdL could serve as a supportive marker in ADC. p63 should considered with caution due to its low specificity. Particularly in small biopsy samples, to preserve tissue for molecular studies, a limited panel combining p40 and TTF-1 immunohistochemical markers is recommended for routine diagnostic use.

Keywords: Lung cancer, p40, p63, MAdL, TTF-1

ÖZET

Amaç: Akciğer kanseri, hem kadınlarda hem de erkeklerde en sık görülen ve en ölümcül kanser türüdür. Tedavi farklılıkları nedeniyle, alt tipleri doğru bir şekilde ayırt etmek önemlidir. Algoritmalar, moleküler çalışmalar için dokuyu korumak amacıyla her vakada en fazla iki immunohistokimyasal belirteç (tek bir adenokarsinom belirteci ve tek bir skuamöz belirteç) kullanılmasını önermektedir. Bu çalışma, belirteçlerin optimal kombinasyonunu belirlemeyi amaçlamaktadır.

Gereç ve Yöntemler: Bu retrospektif çalışmaya, 2010 ile 2015 yılları arasında tanı konulan 62 vaka, 29 primer akciğer adenokarsinomu (ADK), 19 skuamöz hücreli karsinom (SHK) ve 14 akciğere metastatik ADK dahil edilmiştir. İmmünohistokimyasal olarak TTF-1, p40, p63 ve MAdL uygulanmıştır. Boyama yoğunluğu ve pozitif tümör hücrelerinin yüzdesi kaydedilmiştir. Duyarlılık (sensitivite) ve özgüllük (spesifite) değerleri hesaplanmıştır.

Bulgular: TTF-1, primer akciğer ADK'larının %79,31'inde yüksek yoğunlukta pozitiflik gösterdi, duyarlılığı %96,55 ve özgüllüğü %100 idi. p40, SHK'ların %84,21'inde yüksek yoğunluklu boyanma gösterdi, duyarlılığı %89,47 ve özgüllüğü %100 idi. p63, SHK için %100 duyarlılık gösterdi, ancak ADK'larda fokal pozitiflik nedeniyle düşük özgüllük (44,82%) sergiledi. MAdL, ADK için %82,75 duyarlılık ve %100 özgüllük gösterdi. Hiçbir marker metastatik ADK'ları boyamadı.

Sonuç: ADK için TTF-1 ve SHK için p40 en yüksek tanısal doğruluğu gösterdi. MAdL, ADK'da destekleyici bir marker olarak kullanılabilir. p63, düşük özgüllüğü nedeniyle dikkatle yorumlanmalıdır. Özellikle küçük biyopsi örneklerinde, moleküler çalışmalar için dokuyu korumak amacıyla, rutin kullanım için p40 ve TTF-1 immünohistokimyasal marker kombinasyonundan oluşan sınırlı bir panel önerilir.

Anahtar Kelimeler: Akciğer kanseri, p40, p63, MAdL, TTF-1

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INTRODUCTION

Lung cancer (LC) ranks first in incidence and mortality among men and second among women. The incidence and mortality of LC in men is approximately twice as high as in women. Globally, LC accounts for 18.7% of all cancer related deaths, with 2.5 million new cases reported and 1.8 million deaths annually. In 2022, LC was the most frequently diagnosed malignancy, representing 12.4% of all cancers worldwide (1). Nearly 99% of lung tumors are carcinomas (2). Following a steady increase in recent years, lung adenocarcinoma (ADC) has now become the most common histological subtype, comprising more than 50% of all LCs (3). Clinically, primary LCs are classified into two major categories of importance: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). The lung is also among the most frequent metastatic sites, with 15-25% of metastatic tumors presenting in the pulmonary parenchyma. The most common primary origins of LCs include breast, colorectal, gastric, pancreatic, renal, malignant melanoma, prostate, thyroid, and gynecologic malignancies (4). Although SCLC is highly sensitive to chemotherapy and while surgical intervention provides no major prognostic benefit except for early stage disease, the mainstay of early-stage NSCLC treatment is surgery, complemented with oncologic therapies in advanced disease (5). Therefore, accurate pathological evaluation and classification are crucial in guiding treatment decisions. Since the implementation of the 2015 WHO Classification, immunohistochemistry (IHC) has become indispensable in the diagnostic work-up of lung carcinomas (6). In addition to distinguishing SCLC from NSCLC, IHC is also essential for the subclassification of NSCLC to lung ADC or squamous cell carcinoma (SCC) (7). To preserve tissue for molecular studies, current diagnostic algorithms recommend the use of no more than two IHC markers, typically one ADC marker and one squamous marker (8). In most tumors, accurate classification can be made with this limited panel.

Thyroid transcription factor-1 (TTF-1) is highly expressed in lung and thyroid carcinomas and is widely used in routine practice for diagnosing lung ADC (9-13). Currently, TTF-1 is the most commonly used marker to distinguish lung ADC from SCC and from metastatic ADCs to the lung (7,14). p63, first described in 1998, is a gene located on chromosome 3q27-29, consisting of 15 exons and encoding at least six protein isoforms (15). In humans, p63 is expressed in head and neck SCC, pulmonary SCC, esophageal SCC, urothelial carcinoma of the bladder, and gastric carcinoma (16-18). p40 is an isoform of p63, specifically the ΔNp63 isoform. Several studies have evaluated the role of p40 in differentiating pulmonary SCC from ADC, reporting superior p40 specificity for SCC compared with p63 (19). In studies conducted by Bishop et al. (20) and Nonaka et al. (21), p40 showed 100% sensitivity and specificity for the diagnosis of pulmonary SCC. Similarly, Tacha et al. reported 85% sensitivity and 98% specificity for p40 (22). MAdL is a recently introduced, highly specific marker for lung ADC, as described by Schultz et al. Cytoplasmic immunoreactivity for MAdL shows strong positive signals in type II alveolar epithelial cells and intraalveolar macrophages following standard staining protocol optimization (23).

Given the therapeutic implications of NSCLC subtyping, establishing a definitive differential diagnosis is essential. Nevertheless, diagnostic challenges can still arise, even when using conventional IHC markers. Therefore, there remains a need for novel IHC markers that can support the pathologist in refining differential diagnosis, highlighting the necessity for further research in this field. In this context, this study aimed to evaluate the diagnostic significance of p40, p63, MAdL, and TTF-1 expression in the differential diagnosis of lung ADC, SCC, and metastatic ADCs to the lung.

MATERIALS AND METHODS

Study Design and Case Selection

This retrospective study included 62 cases of lung ADC, SCC, and metastatic ADC to the lung. The cases were retrieved from the pathology archives of the Department of Medical Pathology, Faculty of Medicine, Necmettin Erbakan University, covering between March 2010 and May 2015. The sample set consisted of 29 cases of primary lung ADC, 19 cases of pulmonary SCC, and 14 cases of metastatic ADC diagnosed on transthoracic fine needle aspiration biopsies, bronchoscopy biopsies, or lobectomy specimens.

Ethics Approval

Ethical approval for the study was obtained from the Necmettin Erbakan University Meram Faculty of Medicine Drug and Non-Medical Device Research Ethics Committee (Approval No: 2013/543; Date: 06.12.2013).

Immunohistochemical Analysis

Formalin fixed, paraffin embedded tissue blocks that best represented the tumor and included adjacent nontumoral lung tissue were selected for IHC analysis. Sections of 4-5 µm thickness were cut and mounted on positively charged slides. Following deparaffinization, staining was performed with an automated IHC staining system (VENTANA BenchMark XT, Ventana Medical Systems, USA). The Ultraview Universal DAB Detection Kit (Cat. No: 760-500; Lot No: F00544) was used for secondary antibody detection.

The following primary antibodies were used:

- MAdL (1:100 dilution; Cat. No: MSK083-05, Zytomed, Germany)
- TTF-1 Ab-1 (1:50 dilution; Cat. No: MS-699-P0, P1; Thermo Scientific, USA)
- p63 Ab-1 (clone 4A4; 1:100 dilution; Cat. No: MS-1081-P0, P1; Thermo Scientific, USA)
- p40 (polyclonal; 1:100 dilution; Cat. No: PA5-28477; Thermo Scientific, USA)

Evaluation of Staining

Slides were stained with hematoxylin and eosin alongside IHC stained sections and were evaluated independently by two expert pathologists using a light microscope (Olympus BX51, Japan). For MAdL, cytoplasmic membranous staining was considered positive, while nuclear staining was accepted as positive for p40, p63, and TTF-1. Areas with the most abundant staining were selected, and 1000 tumor cells were counted at ×400 magnification to determine the percentage of positively

stained tumor cells.

The extent of staining for p40, p63, and TTF-1 was scored as:
(-) 0%
(+) 1-25%
(++) 26-50%
(+++) 51-75%
(++++): >75%
For MAdL, staining intensity was scored as:
(-) no staining
(+) weak
(++) moderate
(+++) strong

For statistical analyses, 0% was accepted as negative, (+) and (++) was low intensity staining, and (+++) or (++++) was high intensity staining. For MAdL, only (+++) was categorized as high intensity staining.

Statistical Analysis

All statistical analyses were performed with IBM SPSS version 21.0 (IBM Corp., Armonk, NY, USA). The sensitivity and specificity for each IHC marker was calculated as follows:
Sensitivity = True Positive / (True Positive + False Negative) × 100
Specificity = True Negative / (False Positive + True Negative) × 100

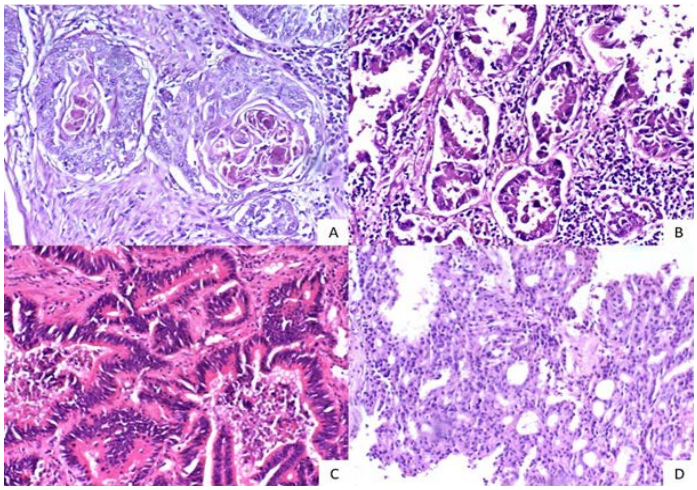

Figure 1. Hematoxylin and eosin stained slides of SCC, ADC, and metastatic ADC to lung

(A) SCC, hematoxylin and eosin, x100. (B) ADC, hematoxylin and eosin, x100. (C) Metastatic ADC (metastasis of colon ADC), hematoxylin and eosin, x100. (D) Metastatic ADC (metastasis of prostatic ADC), hematoxylin and eosin, x100.
ADC=adenocarcinoma. SCC=squamous cell carcinoma.

RESULTS

With the advent of molecular testing and targeted therapies in LC, the distinction between ADC and SCC and accurate histological subtyping have become increasingly crucial for delivering patient-specific treatments. ADC and SCC can be diagnosed based on morphology alone in 50-70% of cases; however, IHC is generally required for poorly differentiated tumors that lack a definitive morphology (8) (Figure 1). In this study, TTF-1 was accepted as the reference marker for primary lung ADC and p40 for SCC. The expression profiles of TTF-1, p63, p40, and MAdL were evaluated in primary lung ADC, pulmonary SCC, and metastatic ADC to the lung (Table 1). TTF-1 Expression: Among the 29 primary lung ADC cases, 16 had (++++) staining in the tumor area, seven showed (+++), one showed (++), and four showed (+), while one case was negative. Of the 28 positive cases, 23 displayed high intensity and five had low intensity staining (Figure 2). No staining was detected in SCC (n=19) or in metastatic ADC (n=14). TTF-1 positivity with high intensity was observed in 79.31% (23/29) of primary ADC cases, and low intensity staining was seen in 17.24% (5/29) of cases. The sensitivity and specificity of TTF-1 for primary ADC were 96.55% and 100%, respectively.

p63 Expression: All SCC cases stained positively for p63 (15 [++++] , two [+++], two [+], and none [++]), with 17 showing high intensity and two with low intensity staining. In primary lung ADC, 16 cases showed (+) and 13 were negative; all positive cells stained with low intensity (Figure 3). No staining was observed in metastatic ADC. Overall, p63 had 100% sensitivity for SCC, but specificity was 44.82% due to low intensity staining in 55.17% (16/29) of ADC cases. p40 Expression: In SCC, 13 cases showed (++++) staining, three (+++), and one (++), while two

Table 1. Evaluation of immunohistochemistry markers in all cases

Tumor type	**Squamous cell carcinoma (n=19)**				**Primary lung adenocarcinoma (n=29)**				**Metastatic adenocarcinoma to lung (n=14)**			
Staining Intensity	Negative	Positive (low)		Positive (high)		Negative	Positive (low)		Positive (high)		Negative	Positive (low)		Positive (high)	
	0	(+)	2 (+)	3 (+)	4 (+)	0	1 (+)	2 (+)	3 (+)	4 (+)	0	1 (+)	2 (+)	3 (+)	4 (+)
p63	0	2	0	2	15	13	16	0	0	0	14	0	0	0	0
p40	2	0	1	3	13	29	0	0	0	0	14	0	0	0	0
TTF-1	19	0	0	0	0	1	4	1	7	16	14	0	0	0	0
MAdL	19	0	0	0	*	5	8	5	11	*	14	0	0	0	*

*The intensity of staining for MAdL was scored at most as 3 (+), so no result value was written for these areas.

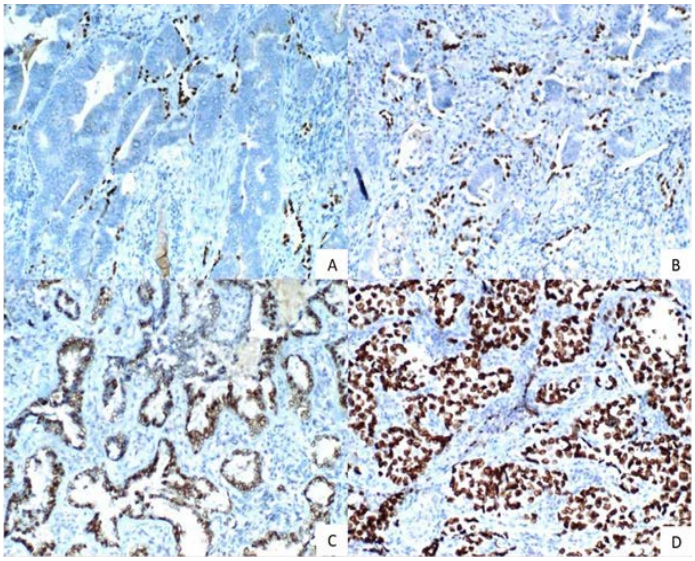

Figure 2. TTF-1 staining in primary lung ADC
(A) (+), x100. (B) (++), x100. (C) (+++), x100. (D) (++++), x100.
ADC=adenocarcinoma.

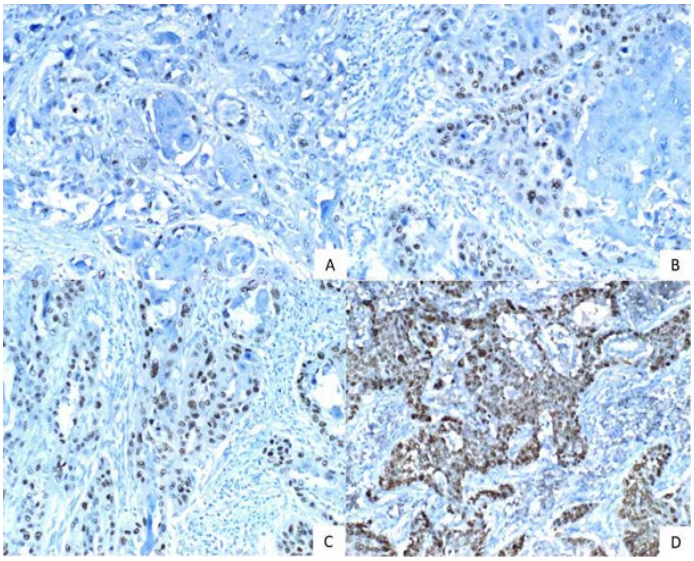

Figure 4. p40 staining in SCC
(A) (+), x100. (B) (++), x100. (C) (+++), x100. (D) (++++), x100.
SCC=squamous cell carcinoma.

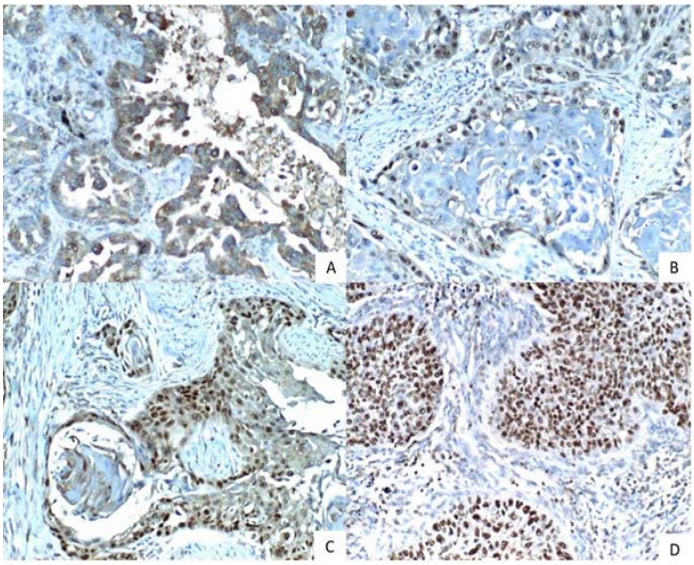

Figure 3. p63 staining in SCC and ADC
(A) p63 staining in ADC (+), x100. (B) p63 staining in SCC (+), x100. (C) p63 staining in SCC (+++), x100. (D) p63 staining in SCC (++++), x100.
ADC=adenocarcinoma. SCC=squamous cell carcinoma.

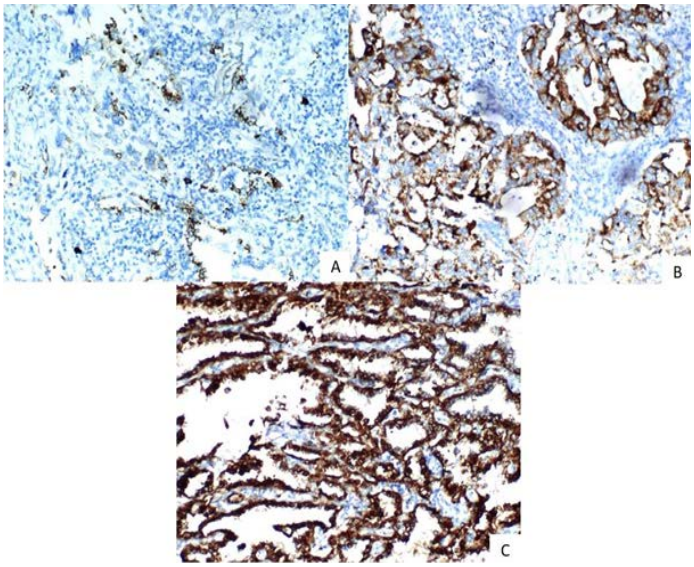

Figure 5. MAdL staining in primary lung ADC
(A) (+), x100. (B) (++), x100. (C) (+++), x100.
ADC=adenocarcinoma.

Table 2. Expression of p40 + p63 combined in cases of squamous cell carcinoma and primary lung adenocarcinoma

p40 + p63	Squamous cell carcinoma	Primary lung adenocarcinoma
Positive	19	16
Negative	0	13

Table 3. Expression of MAdL + TTF-1 combination in cases diagnosed with primary lung adenocarcinoma and squamous cell carcinoma

MAdL + TTF-1	Primary lung adenocarcinoma	Squamous cell carcinoma
Positive	28	0
Negative	1	19

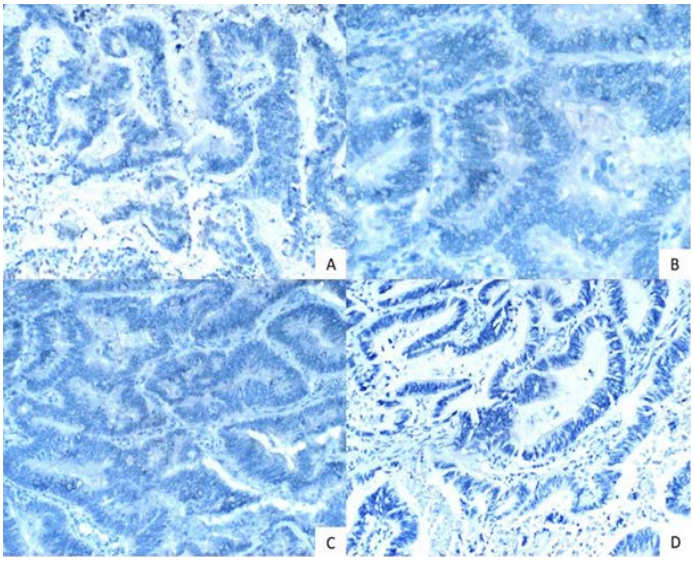

Figure 6. Metastatic ADC to lung (metastasis of colon ADC)
(A) TTF-1, x100. (B) p63, x100. (C) p40, x100. (D) MAdL, x100.
ADC=adenocarcinoma.

Table 4. Diagnostic values of p40, p63, MAdL, and TTF-1 in differential diagnosis

Marker	Tumor type	Sensitivity (%)	Specificity (%)
p40	Squamous cell carcinoma	89.47	100
p63	Squamous cell carcinoma	100	44.82
MAdL	Primary lung adenocarcinoma	82.75	100
TTF-1	Primary lung adenocarcinoma	96.55	100

were negative and none for (+). Of the 17 positive SCC cases, 16 showed high intensity and one had low intensity staining (Figure 4). No staining was observed in any primary lung ADC (n=29) or metastatic ADC (n=14). For SCC, p40 showed high intensity staining in 84.21% (16/19) and low intensity in 5.26% (1/19) of cases. The sensitivity and specificity of p40 for SCC were 89.47% and 100%, respectively.

MAdL Expression: In primary lung ADC, 11 cases showed (+++), five were (++), eight were (+), and five were negative. Among the 24 positive cases, 11 were high intensity and 13 were low intensity (Figure 5). No staining was detected in SCC (n=19) or metastatic ADC (n=14). For positive MAdL staining of ADC, 37.93% (11/29) showed was high intensity and 44.82% (13/29) showed low intensity. The sensitivity and specificity of MAdL for primary lung ADC were 82.75% and 100%, respectively. Combined p40 and p63: Cases were considered positive if either marker showed positive expression. All SCC cases were positive. In primary ADC, 16 cases were positive and 13 were negative, while all metastatic ADC cases were negative (Table 2). Combined sensitivity and specificity for SCC were 100% and 44.82%, respectively. Given the 100% specificity and 89.47% sensitivity, p40 alone was more meaningful for SCC diagnosis. Combined MAdL and TTF-1: Cases were considered positive if either marker showed positive expression. No SCC cases showed staining. In primary ADC, 28 were positive and one was negative; all metastatic ADCs were negative (Table 3). Combined sensitivity and specificity for ADC were 96.55% and 100%, identical to TTF-1 alone; thus, TTF-1 was more informative than MAdL for ADC diagnosis.

None of the four markers stained positively in metastatic ADCs (Figure 6). MAdL, athough 100% specific similar to TTF-1, had lower sensitivity and was insufficient alone. p63 showed excellent sensitivity but low specificity. The joint use of p40 for SCC and TTF-1 for ADC is recommended as the most reliable combination for differentiating between primary ADC and SCC in the lung (Table 4).

DISCUSSION

In our study and consistent with previous literature, TTF-1 and p40 were the most viable markers for distinguishing between primary ADC and SCC. Both of these markers are nuclear and prevent background staining caused by cytoplasmic antibodies (6). Moldvay et al. highlight the importance and reliability of TTF-1 for distinguishing between primary and metastatic lung ADCs, reporting TTF-1 expression as a reliable marker for identifying lung derived cancers. TTF-1 showed very high specificity (100%) and sensitivity (70%) in the differential diagnosis of ADCs originating in the lung and extrapulmonary metastatic ADCs (excluding thyroid tumors). Thus, TTF-1 is considered a very valuable marker that can be used routinely in IHC (14). In our study, TTF-1 had a sensitivity of 96.55% and a specificity of 100% for primary lung ADCs. No staining was detected in SCC or metastatic ADC. Thus, consistent with the literature, our study also highlights the diagnostic importance of TTF-1 in ADC. As a marker for SCC, p63 was more commonly used before the introduction of the p40 antibody. TTF-1 and p63 have been shown to be useful in distinguishing primary ADC from SCC. However, p40, a variant of p63, is reportedly more specific and sensitive for detecting a squamous histology (6). Uramoto et al. reported that p40 to be more specific than high molecular weight cytokeratin for identifying the SCC component (24). Bishop et al. showed that p40 and p63 have equal sensitivity (96%), but p40 provides higher specificity because it does not show false positives in ADCs (20). Butnor et al. reported that p40 and keratin 34βE12 as being more reliable than p63 for distinguishing SCLC from poorly differentiated NSCLC (25). Tatsumori et al. report p40 as the most sensitive marker for SCC (96.8%) and it also being useful for distinguishing between ADC, neuroendocrine carcinoma, and malignant mesothelioma (26). Furthermore,

Collins et al. report p40 being 89% sensitive and 100% specific for NSCLC (27).

In our study, p63 showed 89.47% sensitivity and 100% specificity in cases of SCC, demonstrating high intensity staining in 84.21% of these cases and low intensity staining in 5.26%. p63 showed 100% sensitivity, providing considerable benefit in diagnosis, but the 44.82% specificity caused some difficulties in differential diagnosis. p63 showed high intensity staining in 89.47% of SCC cases and low intensity staining in 10.52%. However, low intensity staining for p63 was also detected in 55.17% of ADC cases, and our statistical studies revealed that p63 had false positives for ADC. As supported by the literature, low intensity staining with p63 creates difficulties in differentiating SCCs from primary ADCs; therefore, p40 is evaluated as a more useful marker than p63 in the diagnosis and differential diagnosis of SCCs. There is currently only one published study related to MAdL, which included 362 cases of primary lung carcinoma and 111 cases of extrapulmonary carcinoma. 154 of the primary lung carcinomas were SCCs, 167 were ADCs, two were adenosquamous carcinomas, 19 were SCLCs, 17 were large cell carcinomas, and three were diagnosed as carcinoids. Of the extrapulmonary carcinomas, 28 were colon, 19 were breast, 11 were prostate, six were pancreatic, ten were gastric, 21 were renal, one was biliary tract, one was liver, three cases were endometrial, two cases urothelial, and eight cases were mesothelial in origin. Staining revealed positive expression of TTF-1 in 92.2% of ADCs and in 74.2% of cases when stained with MAdL. While TTF-1 expression was not observed in metastatic tumors originating from tissues other than the lung, MAdL positivity was detected in 4.7% (1/21) of cases with renal cell carcinoma metastasis (23). Due to the lack of sufficient studies on MAdL, our study is an important investigation into the feasibility of using MAdL alone or in combination with other IHC markers for the diagnosis and differential diagnosis of ADC.

In our study, which yielded results supporting the literature, MAdL showed 82.75% sensitivity and 100% specificity for ADC. Furthermore, the rate of ADC diagnosed cases showing positive expression with MAdL was 37.93% at high intensity and 44.82% at low intensity. TTF-1, on the other hand, showed rates of 79.31% for high intensity staining and 17.24% for low intensity staining. Since TTF-1 showed both 96.5% sensitivity and 100% specificity along with high intensity staining in 79.31% of ADC cases, it emerged as a more viable marker than MAdL for the diagnosis of ADC. When we evaluated the statistics regarding binary combinations, combined p40 and p63 staining evaluation revealed 100% sensitivity and 62.79% specificity for SCC cases. Since p40 alone showed 89% sensitivity and 100% specificity in the same cases, p40 was a more appropriate marker for SCC diagnosis. When MAdL and TTF-1 staining were evaluated together, they showed 96.55% sensitivity and 100% specificity for cases of ADC. These rates were the same when TTF-1 was used alone. Therefore, TTF-1 was more viable than MAdL for diagnosing lung ADC. For metastatic ADC, if thyroid cancers are suspected in the differential diagnosis or if there is a histopathological suspicion, the use of MAdL staining might be useful to exclude TTF-1 positive cases. The difficulties in diagnosis and differential diagnosis in LC necessitate the use of a panel of multiple IHC markers rather than focusing on a single marker. Therefore, similar studies exist that include specific markers for SCC and ADC, as in our study. Montezuna et al. used a panel consisting of four IHC markers—CK 7, CK 20, TTF-1, and p63—to distinguish primary and metastatic lung carcinomas in biopsies. TTF-1 is reportedly the best marker for ADC, while p40 (an isoform of p63), was found to be specific for SCC (28).

Pelosi et al. aimed to create a panel using the fewest markers to distinguish ADCs from SCCs, stating that a combination of TTF-1 and p40 IHC markers was the best approach for identifying ADCs and SCCs (29). Whithaus et al. used a panel containing Napsin A, CK 5/6, p63, and TTF-1 to distinguish ADCs from SCCs in their study, demonstrating that the Napsin A and p63 had 94% specificity and 96% sensitivity when distinguishing ADCs from SCCs (30).

Limitations

Since the subtypes included in the current lung classification exhibit morphologically distinct patterns (eg, lepidic and acinar), our study mainly included cases with a solid pattern that were difficult to distinguish between primary and metastatic LCs, and where additional tests were required for the differential diagnosis of primary ADC and SCC. The sample size was limited and based on data from a single center; therefore, the generalizability of the findings might be limited. Additionally, retrospective design could lead to missing data or recording errors. Another limitation was that power analysis was not performed at the beginning of the study due to the insufficient number of included metastatic ADC cases. However, the systematic investigation of the role of the combinations of markers used in the study for distinguishing ADC and SCC in the lung and the potential contribution of MAdL in thyroid derived tumors supports the consistency of the findings with the literature and the value of the study. Future multicenter, large sample, prospective studies could contribute to validating the findings by reducing the impact of these limitations.

CONCLUSION

The results of this study show that among the four evaluated IHC markers, TTF-1 and p40 were the most reliable and diagnostically valuable markers for distinguishing primary lung ADC and SCC. TTF-1 exhibited high sensitivity and specificity for primary lung ADC, whereas p40 showed excellent specificity and superior diagnostic performance for SCC compared with p63. Although MAdL showed high specificity for ADC, the lower sensitivity limits its use as a standalone marker; however, it could serve as a useful complementary marker in complex cases. Our findings suggest that TTF-1 could be useful for the differential diagnosis of metastatic ADCs other than thyroid carcinoma metastases, while MAdL might be useful in cases with a suspected diagnosis of thyroid carcinoma or a primary tumor of thyroid origin. The combined use of p40 and TTF-1 is recommended in an optimal limited

IHC panel for the differential diagnosis of NSCLC subtypes, particularly in small biopsy samples where tissue preservation is crucial for subsequent molecular testing.

DECLARATIONS

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