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ORIGINAL PAPER

Immunohistochemical Profile of Polymorphous Adenocarcinoma of Minor Salivary Gland: A Systematic Review and Meta-Analysis

Taichiro Nonaka[1] · **Hidehiro Takei**[2]

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Abstract

Polymorphous adenocarcinoma (PAC) is a rare variant of minor salivary gland tumors. Because of its architectural diversity, histological diagnosis of PAC can be difficult especially for small biopsies, and immunohistochemistry is of great help in differentiating it from its histologic mimics. The aim of this study is to conduct a systematic literature review to identify reliable immunohistochemical markers for PAC. We conducted an electronic literature search of the MEDLINE, ScienceDirect, SpringerLink, and Wiley Online Library databases, covering the literature published in the period between 1988 and 2021. The eligibility criteria included case reports and retrospective studies of PAC cases with details of immunohistochemical markers. Following the search and selection process, 32 studies with 409 cases were included in this systematic review. Overall, > 90% positivity was observed for pan-cytokeratin (CK) (97.3%), CK7 (96.8%), CK7/8 (97.4%), E-cadherin (90.0%), Vimentin (92.5%), S100 (97.0%), p63 (91.7%), and SOX10 (100%), while little to no positivity was observed for CK20 (0.0%), p40 (0.0%), and GFAP (5.0%). The average MIB-1 labeling index was 3.78%. The results of this systematic review indicate that CK7+/CK20−, p63+/p40−, S100+, Vimentin+, and GFAP– immunophenotype have diagnostic value for PAC. In addition, the use of S100, MSA, p40, and c-Kit provide additional layers of information helpful to differentiate PAC from adenoid cystic carcinoma, one of challenging differential diagnoses.

Keywords Polymorphous adenocarcinoma · Adenoid cystic carcinoma · Salivary gland tumor · Immunohistochemistry · Diagnostic marker

Introduction

Polymorphous adenocarcinoma (PAC) is a malignant salivary gland tumor characterized by morphological diversity and infiltrative growth pattern but low metastatic potential [1]. This tumor was first recognized as a distinct entity by Evans and Batsakis in 1984 and named as polymorphous low-grade adenocarcinoma (PLGA: a classic terminology of PAC) [2]. In the 4th edition of the WHO classification of head and neck tumors, polymorphous low-grade adenocarcinoma (PLGA) was renamed as polymorphous adenocarcinoma (PAC) by removing the term "low-grade" to better reflect the wide spectrum of the tumor including high-grade variants [3]. Without a doubt, PAC has been the most contentious entity for the iteration of the WHO's reclassification. PAC represents a diagnostic challenge for pathologists because of its high morphological and histological heterogeneity. Some other salivary gland tumors, such as adenoid cystic carcinoma (ACC) and pleomorphic adenoma (PA), often show similar histological characteristics. Immunohistochemical analysis has been proposed to help distinguish PAC from other salivary gland tumors, but controversy still exists about relevant diagnostic markers. Due to the limited sample size and high heterogeneity of PAC, there are few systematic studies with quantitative assessment of candidate markers. Despite numerous reviews and case reports on PAC, no systematic review about immunohistochemical markers has been reported. In order to provide the most comprehensive immunohistochemical profiling of PAC, we conducted a literature review that included 409 PAC cases with immunohistochemical marker data reported

✉ Taichiro Nonaka
taichiro.nonaka@lsuhs.edu

[1] Department of Cellular Biology and Anatomy, Louisiana State University Health Sciences Center, Shreveport, LA 71103, USA

[2] Department of Pathology and Translational Pathobiology, Louisiana State University Health Sciences Center, Shreveport, LA 71103, USA

from 1988 to 2021. This study is the first systematic review of immunohistochemical markers for PAC to determine the best combination of diagnostic markers for PAC. In this manuscript, the term PAC is used for consistency with the latest WHO classification.

Materials and Methods

A systematic literature review was performed following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [4].

Database Search

We searched for PAC cases with immunohistochemical data in MEDLINE/PubMed, ScienceDirect, SpringerLink, and Wiley Online Library (Fig. 1). No limitations on the year of publication were placed on any database, but only studies published in English were included.

Eligibility

Original case reports and retrospective studies were included after full-text screening if they reported immunohistochemical marker data in patients with PAC. Articles were excluded if they did not meet the immunoreactivity scoring rule described in the *Data Collection and Outcomes* section.

Data Collection and Outcomes

Because different immunohistochemical staining scores were used across the studies, the following scoring rules were applied: Immunoreactivity was scored on a scale of 0 to 3+; 0 indicated negative or scattered spotty staining or 0–10% positive tumor cells; 1+ indicated 11–25% positive tumor cells; 2+ indicated 26–50% positive tumor cells; and 3+ indicated > 50% positive tumor cells. Weak immunoreactivity referred to tumors with an average score of 1+, moderate reactivity to tumors that scored 2+, and strong reactivity to tumors that scored 3+. These outcome measures were integrated into each immunohistochemistry assay and overall frequency was shown as percent positivity.

Quality Assessment

Quality assessment was performed using NIH/NHLBI Study Quality Assessment Tools developed by the National Heart, Lung, and Blood Institute at the National Institutes of Health [5]. This assessment comprises of 9 categories that assess the study objective, population, case, subjects, intervention, outcome, follow-up, statistical methods, and results described in the studies. Points are awarded for adequacy of reporting in the study. Total score ranges from 0 to 9, where 9 is the highest score. High quality is defined by scores between 7 and 9, moderate quality is defined between 4 and 6, and poor quality is less than 4. We have included the

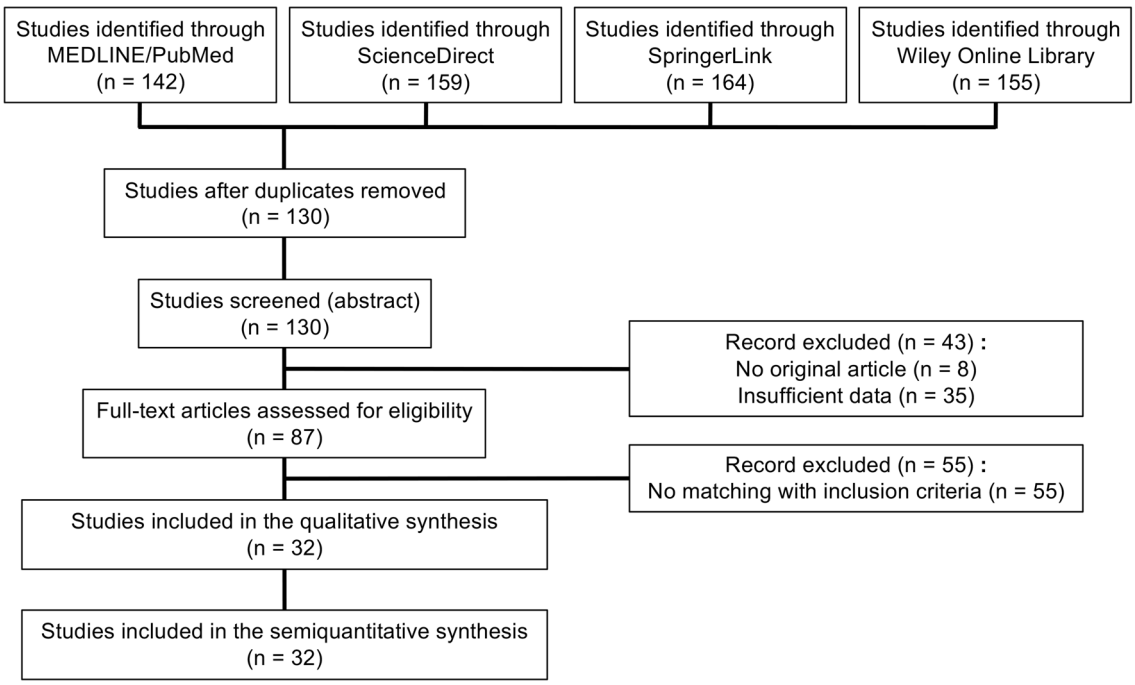

Fig. 1 Flow chart of the literature search and selection process

intervention category in the quality assessment since Ki-67 expression is significantly decreased after chemotherapy in patients with breast cancer and any intervention could affect immunohistochemical profiles in PAC [6].

Meta-analysis

Statistical heterogeneity among studies was assessed using the inconsistency index I^2. Heterogeneity was considered insignificant for $I^2 < 30\%$, moderate for $I^2 < 50\%$, substantial for $I^2 < 75\%$, and high for $I^2 > 75\%$. In case of $I^2 < 30\%$, the fixed-effect model of Mantel-Haenzel was used. Otherwise, the random-effect model was used. The association between marker expression and PAC was assessed using odds ratio (OR): p value < 0.05 indicated a significant association. OR was calculated for each study and displayed on forest plot with 95% confidence interval (CI).

Results

Systematic Review

A summary of the main study characteristics is presented in Table 1. Consequently, 409 PAC cases from 32 articles were finally included in the analysis after eliminating cases that did not satisfy the scoring rules. The mean age of patients was 45.5–91.0 years (mean, 63.8 years). In total, 50 males, 78 females, and 281 patients whose gender details were not reported were included. The locations of PAC were as follow: 122 palates, 19 buccal mucosa, 6 lips, 4 maxilla, 2 tongues, 4 retromolar triangles, 4 parotid glands, 2 floor of the mouth, 1 gingiva, 1 tonsil, 1 oropharynx, 1 submandibular gland, 1 nasal mucosa, and 241 unspecified locations. A flow chart of the literature search and selection process are shown in Fig. 1.

Quality Assessment and Risk of Bias

The quality assessment revealed that the quality of included studies was mostly from moderate to high (Table 2). In all studies, objective and population were clearly stated, cases were consecutive, and subjects were comparable. Area of weakness included lack of details on intervention and outcome. Although 20 of 32 studies did not describe statistical methods, statistical analysis is usually not applicable to case report or qualitative study.

Integrated Immunohistochemical Profile of PAC

Immunoreactivity scores of each case were integrated into a comparable framework based on the scoring rule described in the *Data Collection and Outcomes* section. Overall summary of immunohistochemical profiles of previously reported PAC cases is shown in Table 3. With regard to staining for epithelial markers, > 90% positivity was observed for in pan-cytokeratin (CK) (36/37), CK5/6 (1/1), CK7 (60/62), CK7/8 (37/38), and E-cadherin (18/20). In addition, 33.3% and 77.3% positivity was observed for CK19 (10/30) and epithelial membrane antigen (EMA) (17/22), respectively. Both pan-CK and CK7 staining often showed strong (3+) immunoreactivity. CK20 staining was negative in all cases. With regard to myoepithelial markers, Vimentin (49/53), S100 (32/33), and p63 (60/65) showed > 90% positivity; 5.0–33.3% positivity was observed for muscle markers such as glial fibrillary acidic protein (GFAP) (6/119), muscle-specific actin (MSA) (9/70), smooth muscle actin (SMA) (12/43), and calponin (1/3). Transcription factor SOX10 was found to be positive in 100% (25/25) whereas GATA3 was positive in 17.2% (5/29). Overall, 90–100% sensitivity was observed for pan-CK (97.3%), CK5/6 (100%), CK7 (96.8%), CK7/8 (97.4%), E-cadherin (90.0%), Vimentin (92.5%), S100 (97.0%), p63 (92.3%), and SOX10 (100%), whereas CK5/6 staining was reported only in a single case. Conversely, no positive staining was observed for CK20 (0.0%) or CD10 (0.0%). The results of CK7 and CK20 staining were concordant with the CK7+/CK20− immunophenotype of malignant salivary gland tumors, as reported previously [20]. The mean MIB-1 labeling index was 3.78%.

Meta-analysis of Differential Marker Expression Between PAC and ACC

Meta-analysis was performed when there were multiple studies addressing the same immunohistochemical markers in PAC versus ACC. We constructed Forest plots to show ORs of the proportions of marker expression in PAC and ACC. Among myoepithelial markers analyzed, S100 was more associated with PAC than ACC with overall OR of 13.56 (95% CI 1.48–124.16, p = 0.02), whereas MSA and p40 were more associated with ACC with overall ORs of 0.03 (95% CI 0.00–1.00, p = 0.05) and 0.01 (95% CI 0.00–0.04, p < 0.00001), respectively (Fig. 2). In the subgroup of cases stained for both p63 and p40, p63+/p40− immunophenotype was significantly associated with PAC but not ACC (OR 801.32, 95% CI 99.09–6480.09, p < 0.00001) (Fig. 2). In the subgroup of cases stained for c-Kit, ACC exhibited a greater tendency to express c-Kit than PAC (OR 0.11, 95% CI 0.03–0.43, p = 0.002) (Fig. 3).

Additionally, performance of individual markers to differentiate PAC from ACC was evaluated across the independent studies, and sensitivity and specificity were extracted from each study (Table 4). p63+/p40− immunoprofile is the top performer across the different studies, and the pooled sensitivity was 97.44% (95% CI 86.52–99.94) and the pooled specificity was 99.35% (95% CI 96.46–99.98).

Table 1 Characteristics of the studies included

Author	M	F	Age (mean)	Location	Staining
Gnepp et al. [7]	2	2	65.3	4 palate	EMA, pan CK, S100, MSA, CEA
Norberg et al. [8]	1	2	72.7	1 palate, 1 retro, 1 nasal	EMA, pan CK[a], Vimentin, S100, MSA, CEA
Regezi et al. [9]	n = 15		NA	NA	GFAP, MSA
Simpson et al. [10]	3	3	45.5	5 palate, 1 floor	pan CK[b], CK7/8, Vimentin, S100, CEA
Skalova et al. [11]	7	14	59.3	16 palate, 2 buccal, 1 gingiva, 1 floor, 1 tonsil	Ki-67
Perez-Ordonez et al. [12]	6	11	58	12 palate, 2 tongue, 1 lip, 1 buccal, 1 retro	EMA (12)[c], S100, GFAP (16)[c], MSA (15)[c], SMA (16)[c], CEA (14)[c], Ki-67
Araujo et al. [13]	n = 30		55.2	20 palate, 2 maxilla, 2 lip, 1 retro, 5 unknown	CK7, CK8, CK19, Vimentin, MSA
Curran et al. [14]	n = 42		61.5	28 palate, 3 buccal, 1 lip, 1 maxilla, 9 unknown	GFAP
Penner et al. [15]	7	7	61.1	9 palate, 3 buccal, 1 retro, 1 maxilla	c-Kit
Simpson et al. [16]	2	0	64.5	2 palate	EMA, pan CK, CK7, CK7/8, CK20, Vimentin, S100, GFAP, SMA, Calponin, p63, CEA, Ki-67
Edwards et al. [17]	4	13	67	8 palate, 4 buccal, 1 parotid, 4 unknown	c-Kit
Edwards et al. [18]	4	13	67	8 palate, 4 buccal, 1 parotid, 4 unknown	p63
Nagao et al. [19]	2	1	65.0	2 parotid, 1 submandibular	CK 20, GFAP, MSA, SMA, Ki-67
Nikitakis et al. [20]	n = 11		NA	NA	CK7, CK20
Andreadis et al. [21]	n = 10		NA	NA	E-Cadherin
Beltran et al. [22]	n = 10		56	NA	Ki-67
Furuse et al. [23]	n = 4		NA	NA	E-Cadherin
Curran et al. [24]	n = 30		NA	NA	GFAP
Epivatianos et al. [25]	n = 12		NA	NA	Vimentin, SMA, c-Kit
Meer et al. [26]	n = 21		NA	NA	pan CK, CK7, CK20
Vargas et al. [27]	4	6	71.6	10 oral mucosa	Ki-67
Schwarz et al. [28]	5	3	61.0	4 palate, 2 buccal, 1 lip, 1 oropharynx	CK7, c-Kit, Ki-67
Dultra et al. [29]	n = 6		NA	NA	E-Cadherin
Schwartz et al. [30]	n = 4		NA	NA	GATA3
Argyris et al. [31]	0	1	91	1 lip	EMA, pan CK, CK5/6, CK7, S100, GFAP, Calponin, p63, CD10, CEA
Zaib et al. [32]	n = 10		NA	NA	GFAP, SMA, c-Kit
Projetti et al. [33]	3	2	61	5 minor salivary gland	p63
Rooper et al. [34]	n = 11		NA	NA	p63, p40
Argyris et al. [35]	n = 5		65.6	5 palate	p63, p40
Xu et al. [36]	n = 12		NA	NA	p63 (1)[c], p40 (11)[c]
Adkins et al. [37]	n = 25		NA	NA	SOX10, GATA3
Atiq et al. [38]	n = 23		NA	NA	p63, p40

Retro, retromolar triangle; nasal, nasal mucosa; floor, mouth floor; buccal, buccal mucosa; parotid, parotid gland; submandibular, submandibular gland

NA not applicable

[a]40–65 kD

[b]AE1

[c]Number of samples included in this systematic review

Discussion

In this study, we conducted a systematic review and meta-analysis of immunohistochemical markers for PAC. The result of our meta-analysis of individual patient data provides a more robust insight into the overall effectiveness of combined immunoprofiles versus individual marker in cases presenting diagnostic challenge. To our knowledge, this is the first systematic review of immunohistochemical markers for PAC, including the most comprehensive panel of

Table 2 Quality assessment of studies using National Institutes of Health Quality Assessment Tools

Author	Study design	Objective/population clearly stated	Case consecutive/subjects comparable	Intervention/outcome clearly defined	Length of follow-up adequate	Statistical methods/results well-described	Total score
Gnepp et al. [7]	Case series	+/+	+/+	+/+	+	NA/+	8
Norberg et al. [8]	Case series	+/+	+/+	+/+	+	NA/+	8
Regezi et al. [9]	Case series	+/+	+/+	NA/NA	NA	NA/+	5
Simpson et al. [10]	Case series	+/+	+/+	+/+	+	NA/+	8
Skalova et al. [11]	Case series	+/+	+/+	+/+	+	+/+	9
Perez-Ordonez et al. [12]	Case series	+/+	+/+	+/+	+	NA/+	8
Araujo et al. [13]	Case series	+/+	+/+	NA/NA	NA	NA/+	5
Curran et al. [14]	Case series	+/+	+/+	NA/NA	NA	+/+	6
Penner et al. [15]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Simpson et al. [16]	Case series	+/+	+/+	+/+	+	−/+	8
Edwards et al. [17]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Edwards et al. [18]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Nagao et al. [19]	Case series	+/+	+/+	+/+	+	NA/+	8
Nikitakis et al. [20]	Case series	+/+	+/+	NA/NA	NA	+/+	6
Andreadis et al. [21]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Beltran et al. [22]	Case series	+/+	+/+	+/+	+	+/+	9
Furuse et al. [23]	Case series	+/+	+/+	NA/NA	NA	NA/+	5
Curran et al. [24]	Case series	+/+	+/+	NA/NA	NA	+/+	6
Epivatianos et al. [25]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Meer et al. [26]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Vargas et al. [27]	Case series	+/+	+/+	NA/NA	NA	+/+	6
Schwarz et al. [28]	Case series	+/+	+/+	+/+	+	+/+	9
Dultra et al. [29]	Case series	+/+	+/+	NA/NA	NA	+/+	6
Schwartz et al. [30]	Case series	+/+	+/+	NA/NA	NA	NA/+	5
Argyris et al. [31]	Case series	+/+	+/+	+/+	+	NA/+	8
Zaib et al. [32]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Projetti et al. [33]	Case series	+/+	+/+	NA/NA	NA	+/+	6
Rooper et al. [34]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Argyris et al. [35]	Case series	+/+	+/+	NA/NA	NA	−/+	5
Xu et al. [36]	Case series	+/+	+/+	+/+	+	+/+	9
Adkins et al. [37]	Case series	+/+	+/+	NA/+	+	+/+	8
Atiq et al. [38]	Case series	+/+	+/+	NA/NA	NA	+/+	6

The scale score ranges from 0 to 9, where 9 is the highest score

NA not applicable

22 immunohistochemical markers. PAC is one of infiltrating basaloid salivary gland tumors primarily at low power, characterized by cytologic uniformity and architectural diversity with occasional production of myxohyaline matrix and has some histological overlap with adenoid cystic carcinoma (ACC), pleomorphic adenoma (PA), myoepithelial carcinoma, and metastatic carcinoma. Given its characteristic histologic features and common anatomical site of origin (i.e., palate), the pathologic diagnosis of PAC is often straightforward; however, it is occasionally challenging especially for small biopsies or tumors occurring in uncommon locations such as oropharynx, sinonasal tract, and nasopharynx. In these settings, immunohistochemistry is of great help in addition to detailed histocytological examination. However, PAC immunoreactivity can vary significantly among cases, and no consistent immunohistochemical profile has yet been

Table 3 Overall summary of immunohistochemical profiles of previously reported polymorphous adenocarcinoma (PAC) cases in Table 1

Marker	Staining	No. of positive cases/total no. of cases (3+/2+/1+/0)	% of positivity
Epithelial	pan CK	36/37 (31/5/0/1)	97.3
	CK5/6	1/1 (0/1/0/0)	100.0
	CK7	60/62 (43/15/2/2)	96.8
	CK7/8	37/38 (17/14/6/1)	97.4
	CK19	10/30 (0/3/7/20)	33.3
	CK20	0/37 (0/0/0/37)	0.0
	EMA	17/22 (4/1/12/5)	77.3
	E-Cadherin	18/20 (10/4/4/2)	90.0
Myoepithelial	Vimentin	49/53 (44/3/2/4)	92.5
	S100	32/33 (23/9/0/1)	97.0
	GFAP	6/119 (2/1/3/113)	5.0
	MSA	9/70 (3/2/4/61)	12.9
	SMA	12/43 (3/5/4/31)	27.9
	Calponin	1/3 (0/0/1/2)	33.3
	p63	60/65 (52/6/2/5)	92.3
	p40	0/50 (0/0/0/50)	0.0
	CD10	0/1 (0/0/0/1)	0.0
Transcription factor	SOX10	25/25 (25/0/0/0)	100.0
	GATA3	5/29 (0/0/5/24)	17.2
Tyrosine kinase	c-Kit	35/61 (11/11/13/26)	57.4
Cancer	CEA	4/30 (1/0/3/26)	13.3
MIB-1 index	Ki-67	66/70 (NA)	3.78[a]

Immunoreactivity was scored as 0 for negative, or scattered spotty staining, or 0–10% of tumor cell positive; 1+ for 11–25% of tumor cells positive; 2+ for 26–50% positive; and 3+ for > 50% positive

No number, *NA* not applicable

[a]MIB-1 labelling index

identified. Several epithelial and myoepithelial markers are considered valuable diagnostic markers for PAC, but individual studies have often included a small number of patients and results have varied. We conducted a systematic literature review of 409 reported PAC cases with immunohistochemical marker data to evaluate an immunohistochemical profile for PAC.

The results of the present review demonstrate that PAC shows high epithelial and relatively low myoepithelial characteristics. The summary of immunohistochemical profiles indicated nearly 100% expression status for pan-CK, CK7, and CK7/8. In the present systematic review, PAC is completely negative for CK20. For other types of cancers, CK7–/CK20+ phenotype is often associated with carcinomas of colorectal origin, whereas CK7+/CK20− phenotype is seen in a wide variety of carcinomas, including carcinomas of the lung, breast, thyroid, pancreas, salivary gland, and female genital tract [39]. The loss of CK20 expression is associated with poorly differentiated colorectal carcinomas, and the presence of CK7 along with the absence of CK20 may have prognostic value [40]. However, practical utility of CK20 in salivary gland cancers remains uncertain and requires further investigation. In addition, nearly 100% positivity for Vimentin and S100 staining associated with negative GFAP expression may help differentiate PAC from PA, as PA has been reported to strongly express GFAP [14, 24].

Previous studies reported a poor prognosis for myoepithelial carcinoma [41, 42]. Considering the high metastatic and mortality rate of myoepithelial carcinoma, PAC with predominant myoepithelial features may provide an explanation for the risk of recurrence. Myoepithelial marker p63 is a transcriptional factor expressed in salivary glands and participating in morphogenesis, myoepithelial cell maturation, and tumorigenesis of human salivary glands [43–45]. Nuclear staining for p63 has been used to discern neoplastic myoepithelial processes as well as basal cell differentiation [44, 45]. In the present study, intense nuclear immunostaining for p63 was noted in 92.3% PAC cases, but its pathological role in PAC is uncertain. Evans et al. reported a series of 40 cases of PAC with long follow-up with recurrences in 13 cases (32.5%) [46]. Of the recurrences, 6 cervical lymph node metastases (15%) occurred in 11 years and 3 distant metastases (7.5%) occurred in 24 years after initial diagnosis. Given the metastatic potential and p63 expression status

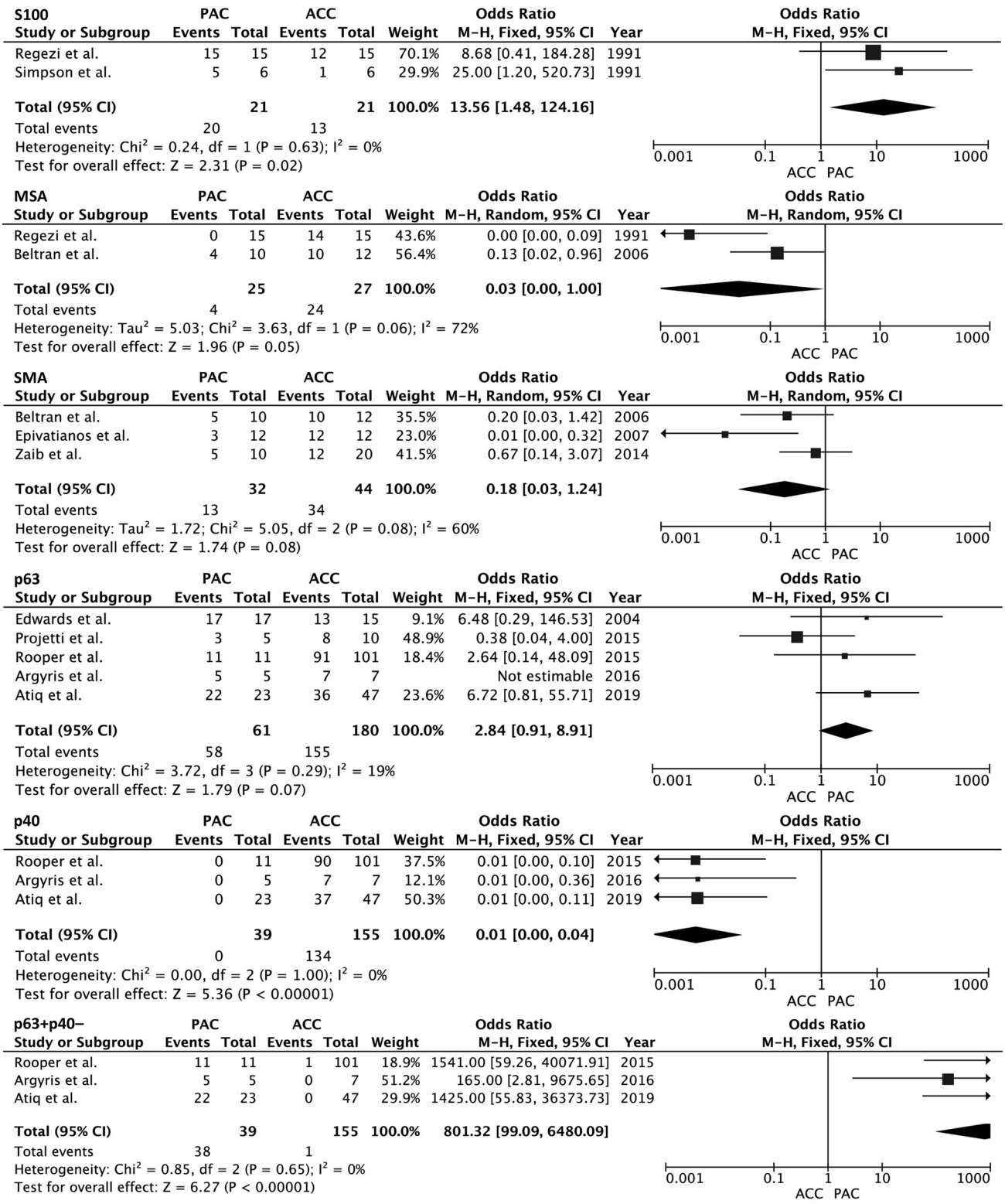

Fig. 2 Forest plot of individuals studies and pooled odds ratio of myoepithelial marker expressions associated with PAC versus ACC

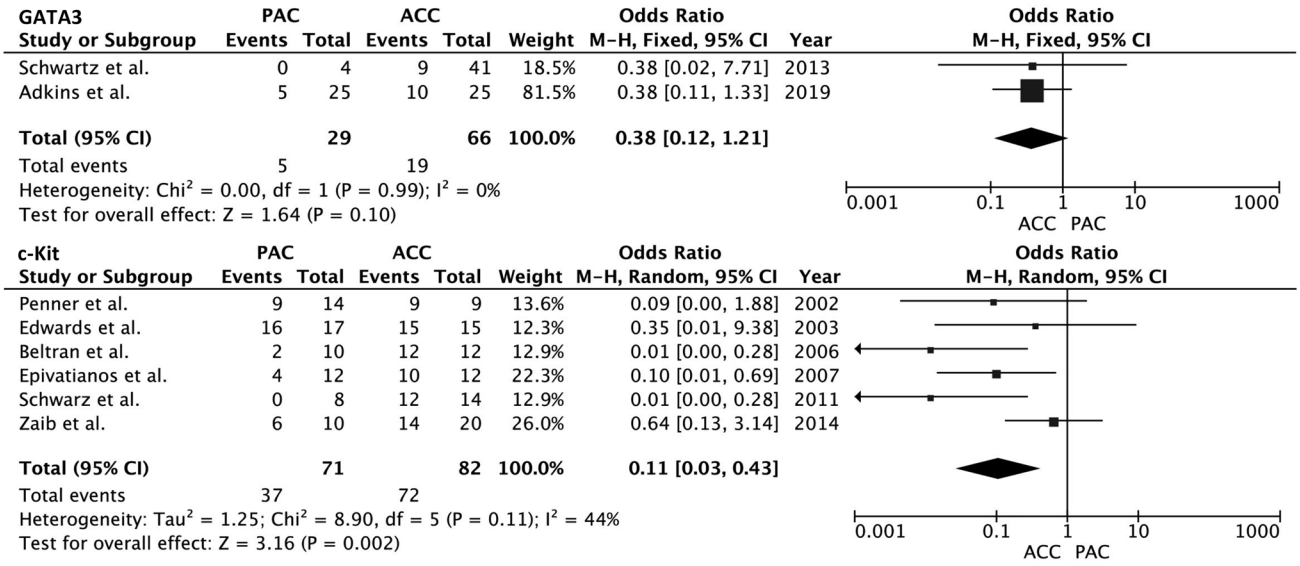

Fig. 3 Forest plot of individuals studies and pooled odds ratio of GATA3 and c-Kit expressions associated with PAC versus ACC

in PAC, association between p63-positive immunoprofile and clinical outcomes warrants further investigation.

The cell proliferation-associated nuclear marker Ki-67 and adhesion molecule E-cadherin are used to assess the malignant potential of a tumor. The present study showed a low MIB-1 index (3.78%) and a high frequency of E-cadherin immunoreactivity (90.0%), indicating low-grade malignancy. The expression status of c-Kit, a transmembrane receptor tyrosine kinase, was intermediate (57.4%) and its significance remains unclear. The number of cases with CK5/6, calponin, and CD10 staining was too small to discuss the utility of these markers, and the significance of these markers awaits further study.

ACC resembles PAC from an architectural standpoint and is the most important differential diagnosis of PAC histologically as well as clinically. Both tumors can have cribriform, tubular, and solid patterns with infiltrative borders and perineural invasion [1]. Distinction between PAC and ACC remains a diagnostic challenge especially in small biopsy samples. We conducted meta-analysis to assess differences and similarities in immunohistochemical marker expression between PAC and ACC (Figs. 2, 3). We found significant differences in the expression of S100, MSA, p40, and c-Kit. Among these markers, p40 and c-Kit clearly showed distinct expression profiles (OR 0.01 and 0.11, respectively). c-Kit is particularly interesting since it appears to have diagnostic value especially in differentiating PAC from ACC, despite its intermediate expression status (57.4%) in PAC. c-Kit has a diagnostic value only if the staining is negative (i.e. high negative predictable value). If c-Kit staining is positive, ACC is more likely than PAC, but given its intermediate expression status in PAC, c-Kit-positive status should not be taken into account when making a final diagnosis. In contrast, SMA, p63, and GATA3 showed no statistical difference but had large 95% CIs, suggesting insufficient sample sizes to draw definitive conclusions. Future studies with larger numbers of cases will be needed to address whether these markers are specifically involved in PAC or ACC.

Recent studies have suggested the utility of a combined p63/p40 immunophenotype in differentiating PAC (p63+/p40−) from ACC (p63+/p40+) [34, 35, 38]. p40 is an N-terminal truncated form of p63 protein (ΔNp63) and shown to have higher specificity for squamous cells than full-length p63 [47, 48]. In our meta-analysis, 97.4% of PAC cases (38/39) were p63+/p40−, while only 0.006% ACC cases (1/155) were p63+/p40−(OR 801.32, $p < 0.00001$) (Fig. 2). p63+/p40− immunophenotype is a promising tool for making a distinction between PAC and ACC.

As a literature-based systematic review, it is vital to note the limitations of our study. First, all included studies were retrospective, potentially leading to a case-selection bias. Second, it was not possible to perform meta-analysis for the full staining panel listed in the present review as only limited immunohistochemical data were available for comparison between PAC and ACC in case series. Finally, most of the studies did not provide longitudinal information about recurrence or overall survival corresponding to each immunohistochemistry result, therefore, we could not calculate hazard ratios. Nevertheless, this is the largest and most comprehensive systematic review to date that has semiquantitatively assessed the diagnostic value of 22 immunohistochemical markers in patients with PAC. An important direction for future research will be to undertake a meta-analysis that includes more

Table 4 Pooled sensitivity and specificity of markers for differentiating PAC from ACC

Marker	Study	PAC		ACC		Sensitivity (%) [95% CI]	Specificity (%) [95% CI]
		P	N	P	N		
S100	Regezi et al. (1991)	15	0	12	3	100.00 [78.20, 100.00]	20.00 [4.33, 48.09]
	Simpson et al. (2019)	5	1	1	5	83.33 [35.88, 99.58]	83.33 [35.88, 99.58]
	Pooled	20	1	13	8	95.24 [76.18, 99.88]	38.10 [18.11, 61.56]
MSA	Regezi et al. (1991)	0	15	14	1	0.00 [0.00, 21.80]	6.67 [0.17, 31.95]
	Beltran et al. (2006)	4	6	10	2	40.00 [12.16, 73.76]	16.67 [2.09, 48.41]
	Pooled	4	21	24	3	16.00 [4.54, 36.08]	11.11 [2.35, 29.16]
SMA	Beltran et al. (2006)	5	5	10	2	50.00 [18.71, 81.29]	16.67 [2.09, 48.41]
	Epivatianos et al. (2007)	3	9	12	0	25.00 [5.49, 57.19]	0.00 [0.00, 26.46]
	Zaib et al. (2014)	5	5	12	8	50.00 [18.71, 81.29]	40.00 [19.12, 63.95]
	Pooled	13	19	34	10	40.62 [23.70, 59.36]	22.73 [11.47, 37.84]
p63	Edwards et al. (2004)	17	0	13	2	100.00 [80.49, 100.00]	13.33 [1.66, 40.46]
	Projetti et al. (2015)	3	2	8	2	60.00 [14.66, 94.73]	20.00 [2.52, 55.61]
	Rooper et al. (2015)	11	0	91	10	100.00 [71.51, 100.00]	9.90 [4.85, 17.46]
	Argyris et al. (2016)	5	0	7	0	100.00 [47.82, 100.00]	0.00 [0.00, 40.96]
	Atiq et al. (2019)	22	1	36	11	95.65 [78.05, 99.89]	23.40 [12.30, 38.03]
	Pooled	58	3	155	25	95.08 [86.29, 98.97]	13.89 [9.19, 19.82]
p40	Rooper et al. (2015)	0	11	90	11	0.00 [0.00, 28.49]	10.89 [5.56, 18.65]
	Argyris et al. (2016)	0	5	7	0	0.00 [0.00, 52.18]	0.00 [0.00, 40.96]
	Atiq et al. (2019)	0	23	37	10	0.00 [0.00, 14.82]	21.28 [10.70, 35.66]
	Pooled	0	39	134	21	0.00 [0.00, 9.03]	13.55 [8.59, 19.96]
p63+/p40−	Rooper et al. (2015)	11	0	1	100	100.00 [71.51, 100.00]	99.01 [94.61, 99.97]
	Argyris et al. (2016)	5	0	0	7	100.00 [47.82, 100.00]	100.00 [59.04, 100.00]
	Atiq et al. (2019)	22	1	0	47	95.65 [78.05, 99.89]	100.00 [92.45, 100.00]
	Pooled	38	1	1	154	97.44 [86.52, 99.94]	99.35 [96.46, 99.98]
GATA3	Schwartz et al. (2013)	0	4	9	32	0.00 [0.00, 60.24]	78.05 [62.39, 89.44]
	Adkins et al. (2019)	5	20	10	15	20.00 [6.83, 40.70]	60.00 [38.67, 78.87]
	Pooled	5	24	19	47	17.24 [5.85, 35.77]	71.21 [58.75, 81.70]
c-Kit	Penner et al. (2002)	9	5	9	0	64.29 [35.14, 87.24]	0.00 [0.00, 33.63]
	Edwards et al. (2003)	16	1	15	0	94.12 [71.31, 99.85]	0.00 [0.00, 21.80]
	Beltran et al. (2006)	2	8	12	0	20.00 [2.52, 55.61]	0.00 [0.00, 26.46]
	Epivatianos et al. (2007)	4	8	10	2	33.33 [9.92, 65.11]	16.67 [2.09, 48.41]
	Schwarz et al. (2011)	0	8	12	2	0.00 [0.00, 36.94]	14.29 [1.78, 42.81]
	Zaib et al. (2014)	6	4	14	6	60.00 [26.24, 87.84]	30.00 [11.89, 54.28]
	Pooled	37	34	72	10	52.11 [39.92, 64.12]	12.20 [6.01, 21.29]

P positive, *N* negative

consistent panel and extensive assessment of potential markers for survival in PAC, ACC, and other salivary gland tumors. The precise diagnosis is vital for management of PAC since PAC is capable of regional and distant metastases that may become uncontrollable. Combination of markers may be useful for differential diagnosis of PAC, especially for small biopsies or tumors occurring in uncommon locations.

Conclusion

We conducted a systematic review of immunohistochemical markers for PAC, indicating the diagnostic value of CK7+/CK20−, p63+/p40−, S100+, Vimentin+, and GFAP− immunophenotype. In addition, meta-analysis demonstrated that the use of S100, MSA, p40, and c-Kit provide additional layers of information helpful to

differentiate PAC from ACC. However, the utility of these markers requires further investigations due to the current paucity of studies in this area. Key future tasks will be validating markers and demonstrating the practical utility of markers to differentiate PAC from ACC and other differential diagnoses.

Author Contributions TN conceived and carried out study design, data collection, data analysis, data interpretation, literature search, generation of figures, writing of the manuscript. HT carried out data interpretation and writing of the manuscript.

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Declarations

Conflict of interest Authors declared that they have no conflict of interest.

Ethical Approval This meta-analysis study is exempt from ethics approval.

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