ORIGINAL ARTICLE

Immunohistochemical Expression of CK19, AR, PHLDA1, CD10 and Ki67 in the Differentiation between Trichoepithelioma and Basal Cell Carcinoma: A Systematic Review

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ABSTRACT

Background. Basal cell carcinoma (BCC) and trichoepithelioma (TE) are follicular adnexal neoplasms that arise from the follicular germ but with divergent biological behavior. The gold standard in the differentiation is through histopathological examination using hematoxylin and eosin (H and E) stain. There are cases, however, when the distinction is not straightforward.

Objective. To assess the association and diagnostic accuracy of the immunohistochemical (IHC) expressions of CD10, Ki67, CK19, androgen receptor (AR), and PHLDA1 in distinguishing between basal cell carcinoma and trichoepithelioma.

Methods. We conducted a comprehensive search on cross-sectional studies on human tissue from 2000 to 2020 in MEDLINE (PubMed), CENTRAL and EMBASE for comparative studies and reference lists. The data were summarized and analyzed using Microsoft Excel and RevMan. We used Chi-square test for independence, summary receiver operator curves (sROC), and diagnostic odds ratio (OR).

Results. We included 15 articles containing 686 BCC and 367 TE in the systematic review. The pooled staining of biomarkers showed a significant difference in the staining of CK19 ($p<0.05$) and AR ($p<0.0001$), and PHLDA1 ($p<0.0001$). Diagnostic odds ratio was used to confirm these associations. AR was found to have the highest odds in the diagnosis of BCC (OR 27.92, 95% CI 10.69, 72.86). The pattern of staining of CD10 is significant ($p<0.001$) with staining of both tumor and stroma (OR 8.09, 95% CI 4.57, 13.53) and staining of tumor alone (OR 8.15, 95% CI 4.56, 14.35) ($p<0.001$) in the diagnosis of BCC. CD10 stromal staining, on the other hand, is significantly associated with the diagnosis of TE (OR 7.26, 95% CI 5.06, 10.44) ($p<0.0001$). There is no significant association between Ki67 staining (OR 1.22, 95% CI 0.48, 3.09) ($p=0.67$) and the diagnosis of BCC. The forest plot and sROC showed that AR had high specificity across all included studies in the diagnosis of basal cell carcinoma, while PHLDA1 demonstrated high specificity and high sensitivity in diagnosing trichoepithelioma.

Conclusion. The biomarkers AR and PHLDA1 are useful as an initial panel to distinguish between BCC and TE, given that both showed high sensitivity as well as significant association with BCC and TE respectively. CD10 and CK19 may also be used with AR and PHLDA1 for further confirmation.

Key Words: Basal cell carcinoma, trichoepithelioma, immunohistochemistry, diagnostic accuracy, diagnostic markers, CK19, Ki67, Androgen receptors (AR), CD10, PHLDA1

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INTRODUCTION

Trichoepithelioma (TE) and basal cell carcinoma (BCC) are tumors that differentiate from the follicular cell lines.[1] Being follicular in lineage and follicular germ in differentiation, both neoplasms are characterized by basaloid

islands with peripheral palisading. Classic histologic features seen using the standard hematoxylin and eosin (H and E) stain enable differentiation of one from the other. This is important as the management radically differs with TE being benign and BCC being a malignant neoplasm. TE is characterized by fibrous stroma surrounding the tumoral islands, while characteristic artefactual clefting separates the BCC nodules from its mucinous stroma. Moreover, both BCC and TE have histologic variants that may impose dilemmas in their histologic distinction. Desmoplastic TE can be difficult to differentiate from morpheaform BCC because of the sclerosing stroma that surrounds the neoplasm instead of the common characteristic mucinous stroma.[1] Furthermore, there are also BCC variants, such as BCC with thickened basement membrane, where the characteristic artefactual clefts are not seen.[2] The presence of circumscription, which is often a distinguishing feature of benign from a malignant neoplasm, may also not be possible for evaluation particularly in small and superficial biopsy specimens.

The advent of immunohistochemistry as a diagnostic tool has proven very useful in confirming the histologic diagnosis, particularly in difficult cases. Published literature is replete in immunohistochemical markers that differ in accuracy, but none of the markers applied appeared completely sensitive or specific in the distinction of BCC and TE. Androgen receptor (AR) expression in tumor cells of BCC was reported in the range of 52.4% to 78%.[3-5] CK 19 was expressed in BCC with the percentage of positive reactions ranging from 64% to 88%.[6-9] PHLDA1, which represents a follicular bulge stem cell marker,[10-11] was found to diffusely stain the tumor islands of TE[11] but may have limitations because it also highlights melanocytes which are found scattered within the BCC nodules. These staining cells may be falsely reported as positive expression. Being a malignant neoplasm, BCC was found to have higher expression of Ki67 which is a proliferation marker compared to TE.[12,13] Findings on cluster of differentiation 10 (CD10) expressions have been positive in both BCC and TE but with differences in patterns of staining.[14] This systematic review aims to determine which among the immunohistochemical biomarkers of AR, CK19, PHLDA1, CD10, and Ki67 are significantly associated with BCC or TE and therefore can be used in the initial immunohistochemical panel to assist in the distinction between these two follicular neoplasms.

METHODOLOGY

The gold standard in the differentiation of basal cell carcinoma and trichoepithelioma is histopathological examination using hematoxylin and eosin (H and E) stain and adequate clinical features. Presently, there is no gold standard of immunohistochemical (IHC) staining that can accurately differentiate BCC from TE. Hence, a panel of IHCs is recommended. For this study, the research questions are as follows:

1. Is there a difference between the positivity of immunohistochemical staining (AR, CK19, PHLDA1, CD10, and Ki67) between BCC and TE?
2. What is the diagnostic accuracy of the following immunohistochemical biomarkers (AR, CK19, PHLDA1, CD10, and Ki67) in distinguishing between BCC and TE?

Search strategies

An electronic literature search was performed starting from January 2000 to December 2020 in the following databases: the Cochrane Central Register of Controlled Trials, MEDLINE (PubMed), EMBASE, and Health Research and Development Information Network (HERDIN). Reference lists of articles were also searched. Search terms used were basal cell carcinoma or BCC, trichoepithelioma or TE, immunohistochemical markers, diagnostic markers, CD10, Ki67, CK19, AR, PHLDA1 (TDAG). Relevant journals were hand-searched. No language restrictions were imposed.

Eligibility criteria

Articles that examined the use of CK19, CD10, AR, Ki67, or PHLDA1 in distinguishing BCC from TE (published from 2000 to 2020) were evaluated.

Inclusion Criteria

- Types of Studies: Cross-sectional studies, retrospective studies
- Types of Participants: Patients with specimens or tissues with the diagnosis of basal cell carcinoma (and its subtypes) and trichoepithelioma (and its subtypes)
- Index tests: Studies that assessed one or more of the following diagnostic markers: CD10, Ki67, CK19, AR, PHLDA1 (TDAG)
- Reference test: Histopathologic examination using H and E
- Outcome Measures:
  - Primary: Percentage of specimens with positive and negative tests
  - Secondary: Sensitivity, specificity, accuracy, positive predictive values, negative predictive values

Exclusion Criteria

- Review or case series
- Duplication of previous publication
- No full-text available
- No relevant data/data that cannot be extrapolated

DATA COLLECTION AND ANALYSIS

Selection of studies

Two authors (EAC, JKG) independently performed the literature search, data extraction, and assessment. The titles

and abstracts were identified from the literature search, and assessment of the full text of all the articles was done for those that satisfied the inclusion criteria. Disagreements were resolved through consensus. The reasons for the exclusion of studies were listed.

Data extraction and management

Data extraction was done independently by the authors using a pre-tested form. The following details were extracted from each study: author's name, publication year, country, number of BCC and TE patients/tissues, number of BCC and TE tissues with positive IHCs of each marker, manufacturer of antibodies used.

Assessment of risk of bias in the included studies

The quality of the studies and the risk of bias were assessed independently by the authors using the Revised Tool for the Quality Assessment on Diagnostic Accuracy Studies (QUADAS-2).[15] Based on how the criteria were met, the methodological quality was classified into high (all criteria with low risk of bias), moderate (with one or more than one criteria with unclear risk of bias), or low (with one or more criteria with high risk of bias).[15]

Data analysis

Data was summarized using Microsoft Excel. Chi-square test for independence was initially performed to check the association of IHC with the tumors. Diagnostic odds ratio was used as the primary outcome for this study since data was pooled from different studies. The odds ratio and 95% confidence interval, test statistic, and p-value were computed using Microsoft Excel. Heterogeneity is presumed in a diagnostic accuracy systematic review; thus, a random-effects model was used in summarizing the data. Description of findings, especially those with patterns of staining, was also done.

Data synthesis for this study was done using a graphical representation. The values for sensitivity and specificity for all the included studies could not be pooled using one value, hence, paired forest plots was used to visualize these diagnostic accuracy measures side-by-side. Summary receiver operator curves (sROC) were also generated to visually represent the relationship of the sensitivity and specificity for each of the IHC stains. Meta-analysis using the univariate pooling method could not be performed with the data extracted in the study. This may be due to software limitations, or the inherent variability and different threshold that were set for each of the studies. Hence, a systematic review and visual summary of available data for this study were preferred. Qualitative data synthesis was done using RevMan 5.

Ethical consideration

No human participants were involved in this study. Given that secondary data analysis was performed, ethics approval was not necessary.

RESULTS

Study Selection

A total of 1379 articles were retrieved from the electronic databases and reference lists. After excluding irrelevant studies and duplicate records, 128 abstracts were evaluated for assessment of eligibility. A total of 18 articles were eligible for review, however, 2 articles did not have full texts, and 1 article did not have data that could be extrapolated. A total of 15 studies were included and analyzed in this systematic review (Figure 1).

Assessment of risk of bias in the included studies

Twelve studies[3,7,14,16-17,19,21-25] out of the 15 included studies were low risk in the risk of bias assessment and applicability concerns (Figure 2). Three studies[11,18,20] had unclear risk of bias in the index test component since it was not specified whether the slides were assessed independently. The tabular summary using QUADAS-2 assessment tool is in Appendix A.

Characteristics of the studies

The characteristics of the studies are summarized in Table 1. All studies that were included compared the IHC staining for BCC and TE. The 15 studies included and analyzed in this systematic review were published between 2000–2020. Seven of the studies were from the USA,[6,11,20-23,25] two each from Iran[18,19] and Turkey,[3,7] one each from Brazil,[16] Germany,[24] Netherlands,[17] and Egypt.[14] A total of 686 BCC and 367 TE were analyzed. Six studies examined CD10 expression on 494 BCC and 221 TE; five studies on AR assessing 165 BCC and 72 TE; five studied expressions of PHLDA1 on 112 BCC and 81 TE; four trials conducted on Ki67 on 73 BCC and 64 TE, and lastly, CK19 examined by three studies on 61 BCC and 48 TE (Table 1).

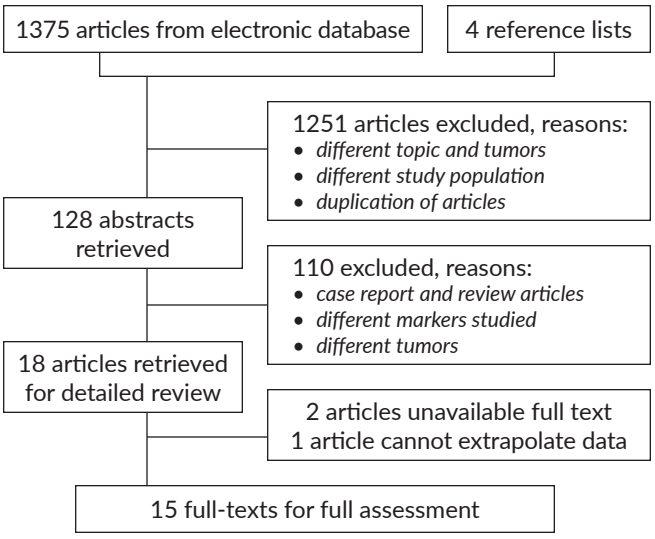


Figure 1. Study Flow Diagram (PRISMA).

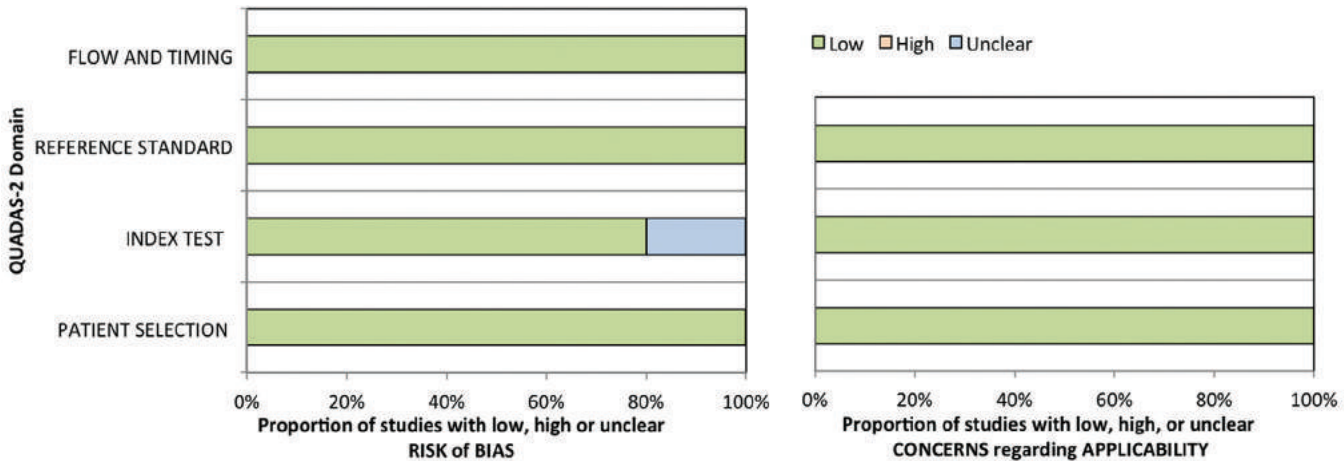


Figure 2. Summary of QUADAS-2 assessment of included studies.

Table 1. Summary of positivity of markers in the selected studies

Study Number	First Author (Year)	Country	Biomarkers Examined	BCC Number of Positive Tissue (Total Tissues Examined)	TE Number of Positive Tissues (Total Tissues Examined	Antibody Manufacturer
1	Mostafa (2018)	Egypt	CD10	17 (19)	8 (10)	Thermo Fisher Scientific, Fremont, CA, USA
			AR	12 (19)	0 (10)	DAKO, Glostrup, Denmark
			CK19	11 (19)	4 (10)	Thermo Fisher Scientific, USA
			Ki67 Mean (SD)	33.79 (10.2)	14.9 (3.1)	DAKO, Glostrup, Denmark
			PHLDA1	0 (19)	10 (10)	Santa Cruz Biotechnology, Heidelberg, Germany
2	Astarci (2015)	Turkey	CD10	32 (39)	12 (15)	Thermo Fisher Scientific, Fremont, CA, USA
			AR	23 (39)	0 (15)	DAKO North America, Inc., Carpinteria, CA, USA
3	Tebcherani (2012)	Brazil	CD10	307 (310)	137 (144)	Thermo Fisher Scientific, USA
4	Sellheyer (2011)	USA	PHLDA1	0 (11)	19 (19)	Santa Cruz Biotechnology, Santa Cruz, CA, USA
5	Arits (2011)	Netherlands	AR	32 (38)	5 (18)	Dako, Carpinteria, CA
6	Bedir (2015)	Turkey	CK19	22 (25)	7 (17)	ScyTek, USA
7	Heidarpour (2011)	Iran	CD10	28 (30)	11 (12)	manufacturer not indicated
8	Aslani (2013)	Iran	CD10	54 (55)	13 (13)	DAKO, Denmark
9	Sellheyer (2013)	USA	CK19	7 (17)	12 (21)	Santa Cruz Biotechnology, Santa Cruz, CA, USA
			PHLDA1	0 (17)	21 (21)	Santa Cruz Biotechnology, Santa Cruz, CA, USA
10	Evangelista (2015)	USA	AR	40 (51)	0 (15)	DAKO
			PHLDA1	51 (51)	15 (15)	Santa Cruz Biotechnology, Dallas, TX, USA
11	Pham (2006)	USA	CD10	20 (23)	12 (13)	DAKO
12	Sellheyer (2010)	USA	PHLDA1	0 (14)	15 (16)	Santa Cruz Biotechnology, Heidelberg, Germany
13	Abdelsayed (2000)	USA	Ki67 Mean (SD)	51.25 (6.06)	30.50 (6.46)	DAKO
14	Costache (2008)	Germany	AR	18 (18)	0 (14)	DAKO
			Ki67	12 (18)	12 (12)	DAKO
15	Lum (2004)	USA	Ki67 Proliferative index %	50%	13%	Dako, Carpinteria, CA, USA

Table 2. Comparison of biomarkers (CD10, CK19, AR, PHLDA1) staining in BCC and TE

Marker	Staining Pattern in BCC	BCC Number of patients or tissues		Staining Pattern in TE	TE Number of patients or tissues		P value
		n	%		n	%	
CD10[3,14,16,18,19,21]							0.12
Positive	(see Table 4)	457	96.0	(see Table 4)	193	93.2	
Negative	<25% stain	19	4.0	<25% stain	14	6.8	
CK19[7,14,20]							<0.05
Positive	Diffuse staining of cells	41	67.2	Focal staining of cells	23	47.9	
Negative	<25% stain	20	32.7	<25% stain	25	52.1	
AR[3,6,14,17,24]							<0.001
Positive	Clusters / scattered	125	75.8	Focal	5	6.9	
Negative	<25% stain	40	24.2	<25% stain	59	93.1	
PHLDA1[6,11,14,20,22]							<0.001
Positive	> 75% of cells	6	5.4	> 75% of cells	81	100	
Negative	<25% stain	106	94.6	<25% stain	0	0	
Ki67[14,23-25]							0.98
Positive	Strong nuclear staining confluent	42	71.2	Strong nuclear staining peripheral	27	71.1	
Negative	<25% stain	17	28.8	<25% stain	11	28.9	

Proportions of Positivity of Biomarkers between BCC and TE

Using chi-square test of association, there is a statistically significant difference between the positivity of BCC and TE in the following biomarkers AR (p<0.001), CK19 (p<0.05), and PHLDA1 (p<0.001) (Table 2).

CD10 expression was not significant given that it showed high positive staining in both BCC (96%) and TE (93.2%). However, there was a statistically significant difference when the pattern of staining was examined (p<0.001). A trend towards a predominantly stromal pattern was seen among tumor islands of TE (Table 3). For the studies that showed proportions with the proliferative index Ki67,[14,23,25] there was no significant difference between the positivity seen in BCC and TE. Mean indices for all studies under Ki67 are summarized in Table 4.

Immunohistochemical staining

Comparing the biomarkers CK19 and AR staining in BCC and TE, both showed significant positive staining in BCC tissues more than in TE specimens (p<0.05 and p<0.0001, respectively). PHLDA1 expression, on the other hand, was more significantly positive in TE (p<0.001) than in BCC (Table 2). These markers, therefore, are useful in differentiating BCC from TE.

CD10 expressions to distinguish BCC from TE was not significant as it showed high positive staining in both BCC (96%) and TE (93.2%) (Table 2). The pattern of staining for CD10 though was found to be significant (p<0.001). Larger studies, however, would be needed to confirm which among the three patterns are significantly associated with BCC. A trend towards a predominantly stromal pattern was seen among tumor islands of TE (Table 3).

Four studies examined the biomarker Ki67.[14,23-25] Ki67 proliferation index (PI) was used to summarize data in three out of the four studies.[14,23,25] Among these, two studies showed a Ki67 greater than 50% for BCC compared with

Table 3. Comparison of staining patterns in the CD10 positive specimens of BCC and TE

Marker***	BCC Number of positive patients or tissues		TE Number of positive patients or tissues		P value
	n	%	n	%	
CD10[3,14,16,18,19,21]					
Tumor	176	37.0	14	6.7	<0.001
Tumor + stroma	196	41.2	45	21.7	
Stroma	96	20.2	134	64.7	
Total (+) specimens examined	**476**		**207**		

Note: there are overlaps of pattern hence higher value of n in CD10 than previous +/- table

Table 4. Summary of studies that compared Ki67 proliferative indices

First Author (Year)	BCC Number of patients or tissues	TE Number of patients or tissues	Proliferative Index Mean (SD)
Mostafa (2018)	19	10	BCC: 33.79 (10.2) TE: 14.9 (3.1)
Abdelsayed (2000)	20	20	BCC: 51.25 (6.06) TE: 30.50 (6.46)
Lum (2004)	16	20	BCC: 50 TE: 13
			Number of (+) patients/tissue
Costache (2008)	18	14	BCC: 18 TE: 12

<31% Ki67 PI in TE tissues. Abdelsayed et al.[23] reported the mean of 51.25 ± 6.06 for BCC and mean of 30.5 ± 6.46 for TE, while Lum[25] demonstrated a statistically different proliferative index for BCC and TE (50.0% vs 13.0%, p<0.001). Mostafa et al.[14] described the mean for BCC at 33.79 ± 10.2 and the mean for TE at 14.9 ± 3.1. Positivity of Ki67 expression was used as outcome measure in the study of Costache et al.[24] and they found that both BCC and TE showed positivity of the cells for the marker. In BCC, 67.7% stained many cells, 33.3% stained few cells, while in trichoepithelioma, only a few cells stained in all of the samples (Table 4).

Measures of Diagnostic Accuracy

Sensitivity and Specificity

The values for sensitivity and specificity for each biomarker are summarized in the paired forest plot seen in Figure 3. Other measures of diagnostic accuracy are summarized in Appendix B.

For the diagnosis of basal cell carcinoma, AR had high specificity (72%–100%) in all studies, as well as moderate sensitivity (59%–100%). Thus, a positive AR rules in the diagnosis of BCC. The biomarker Ki67 also showed high specificity (94%–100%) and moderate sensitivity (63%–95%). There is low to moderate sensitivity for CD10 tumor (38%–77%) and CK19 (41%–88%). The specificity CD10 tumor is moderate to high (73%–100%). There is moderate specificity for CK19 (43%–60%). CD10 with staining of tumor and stroma demonstrated low to moderate sensitivity (10%–51%) and moderate to high specificity (74%–100%).

For the diagnosis of trichoepithelioma, PHLDA1 showed high specificity (88%–100%) and high sensitivity (100%). CD10 stromal only showed moderate to high sensitivity (60%–100%) and specificity (72%–100%). A test with moderate to low sensitivity could miss most of the disease (more false negative), while a test with moderate to low specificity could have many false positives.[26]

The summary receiver operator curves (sROC) represented the trade-off of sensitivity and specificity better (Figure 4). The closer the curves come to the 45-degree diagonal of the ROC space, the less accurate the test. When the area under the curve (AUC) equals 0.5, it corresponds to random chance, while an AUC of 1.0 corresponds to perfect accuracy.[26,27] The sROC curve for AR and CD10 with tumor staining showed values close to 1 and thus, were more accurate markers in diagnosing basal cell carcinoma than the markers of CK19 and stromal and tumor staining in CD10. The use of the biomarker PHLDA1 was more accurate in diagnosing trichoepithelioma compared to CD10 stromal staining.

Diagnostic Odds Ratio (DOR)

Diagnostic odds ratio was used to compare each biomarker and is seen in Tables 5 and 6. All the biomarkers showed diagnostic odds ratio greater than 1. The odds of having BCC with positive staining was highest with the biomarkers AR, followed by CD10 staining pattern for tumor and both stromal and tumor, and lastly, with CK19. There was a significant association seen in the use of AR (p<0.001), the tumor staining pattern seen in CD10 (p<0.001), and the tumor and stromal staining pattern in CD10 (p<0.001). These markers increased the odds of a diagnosis of BCC 27.92 times higher for AR, 8.15 times higher for CD10 staining of tumor and stromal staining, and 8.09 times higher for CD10 tumor staining, compared to their negative staining counterparts.

The computed odds ratio for Ki67 was 1.22, which was not significant since the 95% confidence interval estimate was between 0.48–3.09. There was no significant association between Ki67 and the diagnosis of BCC (p=0.67).

There was a significant association of stromal staining with CD10 (p<0.0001) and PHLDA1 (p< 0.0001) with the diagnosis of trichoepithelioma. The odds of TE was 7.26 times higher for CD10 stromal staining compared to CD10 negative staining. For PHLDA1, the diagnostic odds ratio was significantly higher. However, caution should be observed in the use of this output because of the zero-cell value, which leads to a very high odds ratio. Looking at the studies that reviewed PHLDA1, it may be noted that both sensitivity and specificity were high for this biomarker, with minimal to zero rates of false positive and false negatives (Table 6).

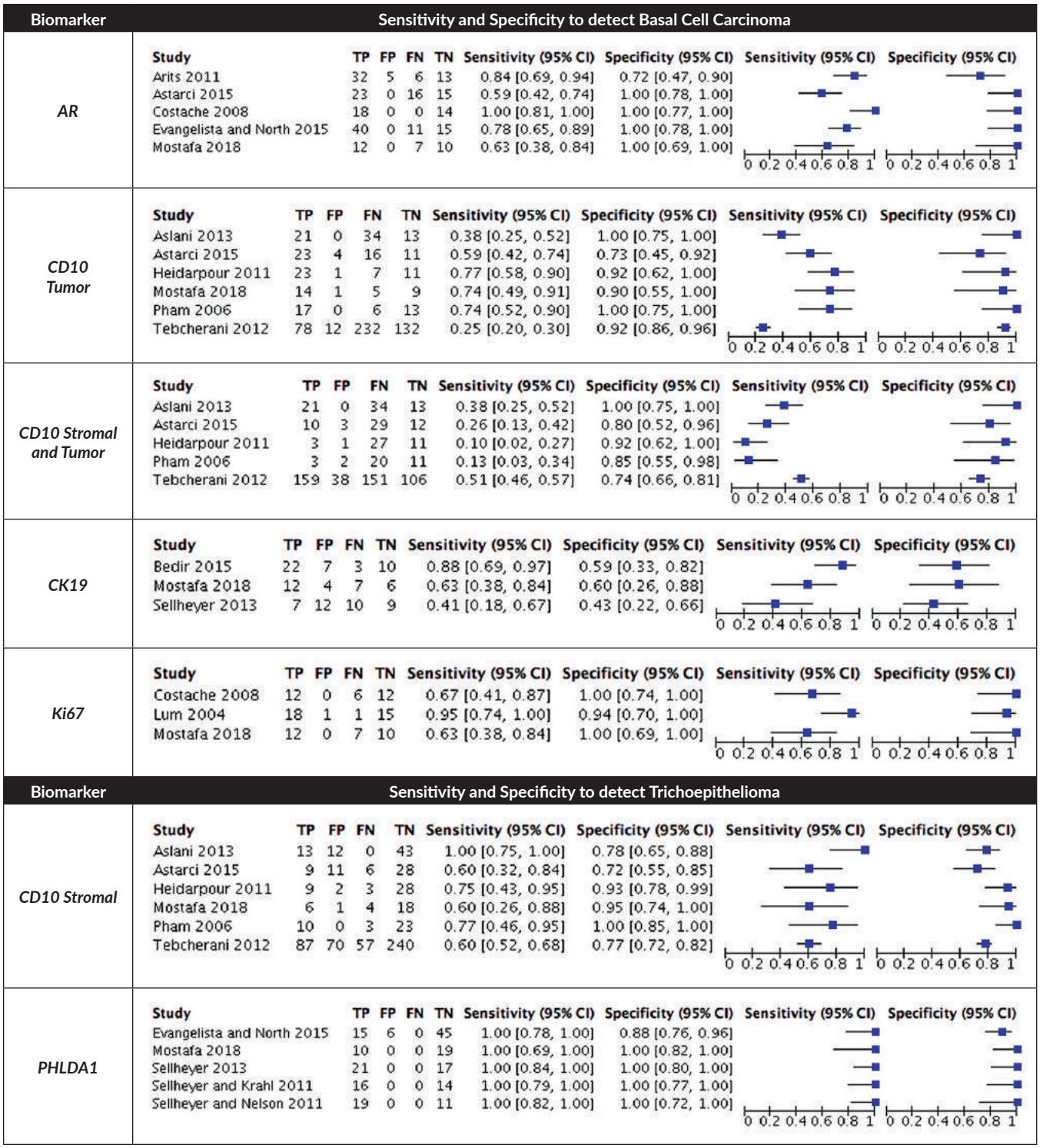
Table 5. Association of use of markers in favoring diagnosis of BCC than TE

Marker	BCC	TE	Diagnostic Odds Ratio (95% CI)	P value
AR +	125	5	**27.92 (10.69-72.86)**	**<0.0001**
AR -	60	67		
CD10 both +	196	14	**8.15 (4.57-14.53)**	**<0.0001**
CD10 both -	261	152		
CD10 tumor +	176	14	**8.09 (4.56-14.35)**	**<0.0001**
CD10 tumor -	300	193		
CK19 +	41	23	**2.23 (1.0-4.90)**	**(0.04)**
CK19 -	20	25		
Ki67 +	42	27	**1.23 (0.48-3.09)**	**0.67**
Ki67 -	14	11		

Table 6. Association of use of markers in favoring diagnosis of TE than BCC

Marker	TE	BCC	Diagnostic Odds Ratio (p value)	95% CI
CD10 stroma +	134	96	**7.26 (5.06-10.44)**	**<0.0001**
CD10 stroma -	73	380		
PHLDA1 +	81	6	**2670.69* (148.29-48098.86)**	**<0.0001**
PHLDA1 -	0	106		

**High value is due to the zero value in one of the cells*



Sensitivity and specificity are reported with a mean (95% confidence limits). Forest plot shows the estimated sensitivity and specificity (blue squares) and its 95% confidence limits (horizontal black line).
Abbreviations: DOR, Diagnostic Odds Ratio; TP, True positive; FP, False positive; FN, False negative; TN, True negative

Figure 3. Paired forest plot of sensitivity and specificity for the biomarkers.

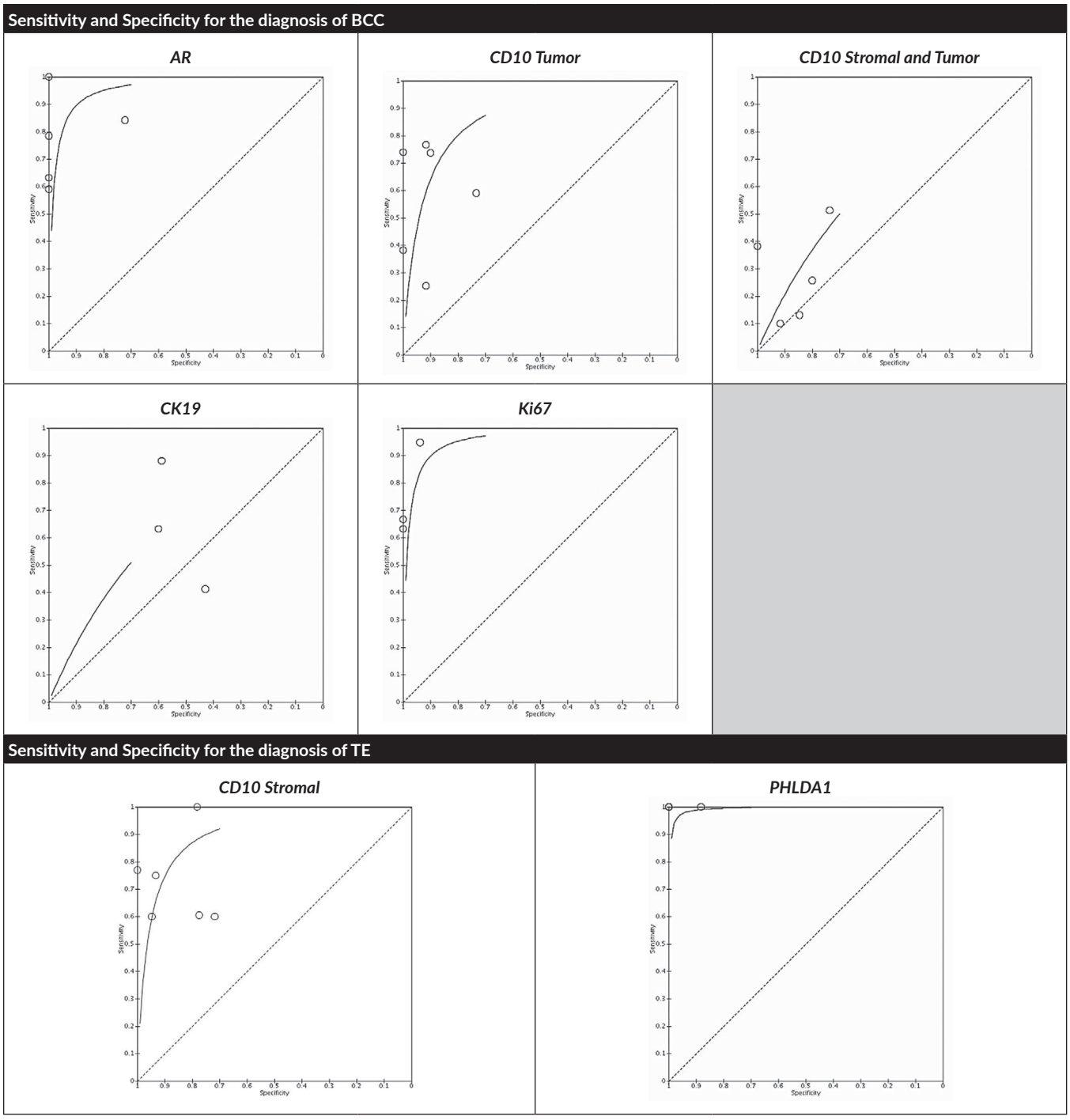


Figure 4. Summary Receiver Operator Curves for the Biomarkers for BCC and TE. This figure shows the summary receiver operator curves (sROC) for each of the biomarker. Sensitivity is on the y-axis, and the x-axis shows inverted specificity. The circles represent the included studies for each of the biomarker. The closer the curve to the value of 1 (top left), the more accurate the marker is for the disease. The closer the value to the diagonal line, the less accurate is the test for the disease. In this figure, AR and Ki67 are more accurate markers than CD10 with tumor staining, CD10 with stromal and tumor staining, and CK19 in diagnosing basal cell carcinoma. PHLDA1 is more accurate in diagnosing trichoepithelioma than CD10 stromal straining.

DISCUSSION

The histologic features of trichoepithelioma and basal cell carcinoma are often distinctive and straightforward but can be inconclusive in small and superficial biopsy specimens and can be difficult to distinguish in some histologic variants of both neoplasms. Immunohistochemistry has proven useful in these difficult cases. There have been numerous studies that evaluated the diagnostic accuracy of the expression of some markers to distinguish BCC from TE but with conflicting results against the reliability of the stains. In the systematic review, we aimed to assess the expression of AR, CK19, PHLDA1, CD10 and Ki67 in BCC and TE specimens.

Androgen Receptor

Androgen receptors (AR) belong to a nuclear receptor family of transcription that are normally found in sebaceous glands, pilosebaceous duct keratinocytes, epidermal keratinocytes, eccrine glands, and dermal fibroblasts.[28] They are present in cutaneous neoplasms including BCC but are not expressed in mature hair follicles and have been found not expressed by hair follicle tumors such as TE.[29] Any focal nuclear staining is considered positive as confirmed by Astarci et al.[3] and Evangeline and North.[6] In previous studies, AR has consistently demonstrated high specificity (72.2-100%).[3,6,14,17,24] In the systematic review, AR was significantly expressed in BCC compared to TE. Furthermore, AR was found to have the highest odds in the diagnosis of BCC. With the high specificity of this test as well as the high odds ratio demonstrated in this study, a positive AR can therefore be used to confirm BCC.

Cytokeratin 19

Cytokeratin 19 (CK19) is expressed in germinative basaloid cells, upregulated in the outermost layer of the outer root sheath in the bulge region of the hair follicles as well as the outer root sheath proximal and distal to the bulge.[14] Cytoplasmic staining is considered positive and was found to favor the diagnosis of BCC.[14] CK19 only showed moderate sensitivity and moderate specificity across all studies. In this review, CK19 staining was significantly positive in BCC tissues compared to TE and is found to be significantly associated with the diagnosis of BCC. There is a high association of CK19 with the diagnosis of BCC, however caution may be exercised in using this marker alone due to the moderate sensitivity and specificity seen also in the study. This marker may still be used as one of the immuno-histochemical markers for confirmation in the context of an IHC panel in diagnosing BCC.

Pleckstrin Homology Like Domain Family A Member 1

Pleckstrin Homology Like Domain family A member 1 (PHLDA1), also known as T-cell death-Associated Gene 51 (TDAG51), is involved in the regulation of apoptosis and represents a follicular bulge stem cell marker.[6,10] Uniform cytoplasmic immunoreactivity is seen in TE which could refer to a hamartomatous recapitulation of the hair follicle bulge.[14] There are limitations though to PHLDA1 staining. Melanocytes express PHLDA1 which can be found scattered in BCC tumor islands thereby resulting in false positive reporting in BCC tissues.[14] Melanocytes are also present in TE but are usually difficult to discriminate amidst the diffuse staining of PHLDA1. Ulcerated BCC can also pose inaccuracy since tumor islands near the surface become PHLDA1-positive whereas the deeper portions of the neoplasm are negative. Mostafa et al.[14] postulated that the inflammatory response upon ulceration of a BCC could stimulate PHLDA1 expression in the tumor islands close to the ulcerated surface. For this study, PHLDA1 showed high sensitivity and high specificity in diagnosing trichoepithelioma, with minimal to zero rates of false positives and false negatives. PHLDA1 staining significantly distinguished TE from BCC with a significantly high association. A strong and diffuse PHLDA1 staining, therefore, favors a diagnosis of TE over BCC, and thus can be used as one of the immunohistochemical protocols for workup.

Cluster of Differentiation 10

Cluster of differentiation 10 (CD10) is a 100 kDa transmembrane glycoprotein which is expressed in the inner sheath of hair follicles, hair matrix, and perifollicular fibrous sheath.[3] In the systematic review, both BCC and TE stained positive for CD10. It is in the staining pattern that distinction may be made favoring a stromal pattern of expression in TE tissues as seen in this systematic review. This may reflect the difference in stromal morphology, tumor growth, or tumor host response in TE compared to BCC.[3] On the other hand, CD10 expression in BCC samples showed staining of tumor alone and staining of both tumor and stroma to be significantly associated in the diagnosis of BCC. For this biomarker, there is moderate sensitivity and moderate specificity for both staining patterns- stromal staining for the diagnosis of TE, and tumor staining for the diagnosis of BCC. CD10 may be useful as part of the IHC panel, but the staining pattern should be carefully assessed.

Marker of Proliferation Ki67

Ki67 is a high molecular weight non-histone protein expressed in the nucleus during active phases of the cell cycle.[14] It is considered to be a marker of proliferating cells. BCC, being a malignant neoplasm exhibits a high Ki67 proliferation index, particularly with the adenoid and morpheaform subtypes.[30] TE on the other hand, was seen to have lower mean Ki67 compared to BCC which reflects its lower proliferative ability.[12,13] Aside from providing a quantitative measure, there may also be a difference in the pattern of Ki67 staining. Diffuse nuclear staining throughout the tumor islands was reported in BCC, while positive staining cells mainly found at the periphery of tumor islands

are more evident in tissues of TE.[14] Although there was high sensitivity and specificity seen for Ki67 in the study, no significant association was seen between Ki67 staining and the diagnosis of BCC; hence, it may not be useful in distinguishing between BCC and TE.

Among the immunohistochemical markers included in the systematic review, AR was significantly associated with BCC, while PHLDA1 was significantly associated with TE. These two biomarkers may be useful as an initial panel in distinguishing BCC from TE.

Furthermore, the biomarkers CK19 and CD10 (tumor staining and tumoral with stromal patterns) were found to be significantly associated with BCC, and CD10 stromal pattern were significantly associated with TE. These stains may be useful in the context of an immunohistochemical panel, if the initial panel shows inconclusive findings. Ki67 marker, on the other hand, is not useful in making the distinction between the two neoplasms.

CONCLUSION

The biomarkers AR and PHLDA1 were found to have a significant association with BCC and TE, respectively. These markers also demonstrated high sensitivity across all included studies. Hence, these are useful as an initial panel in distinguishing BCC and TE. CK19 and CD10 also showed significant associations: with CK19 more associated with BCC, CD10 stromal pattern more associated in TE and CD10 tumor staining and tumoral with stromal staining pattern more associated with BCC. However, the summary of their diagnostic accuracy showed only moderate sensitivity and specificity. Thus, CK19 and CD10 biomarkers may be performed with AR and PHLDA1 for further confirmation.

Statement of Authorship

All authors participated in the data collection and analysis and approved the final version submitted.

Author Disclosure

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APPENDICES

Appendix A. Summary of QUADAS-2 assessment for included studies

Study	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Mostafa (2018)	☺	☺	☺	☺	☺	☺	☺
Astarci (2015)	☺	☺	☺	☺	☺	☺	☺
Tebcherani (2012)	☺	☺	☺	☺	☺	☺	☺
Sellheyer (2011)	☺	?	☺	☺	☺	☺	☺
Arits (2011)	☺	☺	☺	☺	☺	☺	☺
Bedir (2015)	☺	☺	☺	☺	☺	☺	☺
Heidarpour (2011)	☺	?	☺	☺	☺	☺	☺
Aslani (2013)	☺	☺	☺	☺	☺	☺	☺
Sellheyer (2013)	☺	?	☺	☺	☺	☺	☺
Evangelista (2015)	☺	☺	☺	☺	☺	☺	☺
Pham (2006)	☺	☺	☺	☺	☺	☺	☺
Sellheyer (2010)	☺	☺	☺	☺	☺	☺	☺
Abdelsayed (2000)	☺	☺	☺	☺	☺	☺	☺
Costache (2008)	☺	☺	☺	☺	☺	☺	☺
Lum (2004)	☺	☺	☺	☺	☺	☺	☺

☺ *Low Risk* ☹ *High Risk* ? *Unclear Risk*

Appendix B. Summary of measures of diagnostic accuracy from the included studies

	BCC Positive for IHC (Total Tissue)	TE Positive for IHC (Total Tissue)	Specificity (%)	Sensitivity (%)	Accuracy (%)	PPV (%)	NPV (%)
AR							
Arits 2011	32 (38)	5 (18)	72.2	84.2	80.4	86.5	68.4
Astarci 2015	23 (39)	0 (15)	100.0	59.0	70.4	100.0	48.4
Costache 2008	18 (18)	0 (14)	100.0	100.0	100.0	100.0	100.0
Evangelista and North 2015	40 (51)	0 (15)	100.0	78.4	83.3	100.0	57.7
Mostafa 2018	12 (19)	0 (10)	63.1	100.0	75.8	100.0	58.8
	125 (165)	**5 (72)**					
CK19							
Bedir 2015	22 (25)	7 (17)	58.8	88.0	76.2	75.9	76.9
Mostafa 2018	12 (19)	4 (10)	63.1	60.0	62.0	75.0	46.2
Sellheyer 2013	7 (17)	12 (21)	42.9	41.2	42.1	36.8	47.4
	41 (61)	**23 (48)**					
PHLDA1							
Evangelista and North 2015	6 (51)	15 (15)	88.2	100.0	90.9	71.4	100.0
Mostafa 2018	0 (19)	10 (10)	100.0	100.0	100.0	100.0	100.0
Sellheyer 2013	0 (17)	21 (21)	100.0	100.0	100.0	100.0	100.0
Sellheyer and Krahl 2011	0 (14)	16 (16)	100.0	100.0	100.0	100.0	100.0
Sellheyer and Nelson 2011	0 (11)	19 (19)	100.0	100.0	100.0	100.0	100.0
	6 (112)	**81 (81)**					
CD10							
Astarci 2015	32 (39)	12 (15)					
Stromal	11 (39)	9 (15)	71.79	60.0	68.52	45.0	82.4
Peripheral	23 (39)	4 (15)	73.33	58.97	62.96	85.2	40.8
Both	10 (39)	3 (15)	80.0	25.7	40.74	76.9	28.3
Aslani 2013	54 (55)	13 (13)					
Stromal	12 (55)	13 (13)	78.18	100.0	82.35	52.0	100.0
Tumor	21 (55)	0 (13)	100.0	38.18	50.0	100.0	27.7
Both	21 (55)	0 (13)	100.0	38.18	50.0	100.0	27.7
Heidarpour 2013	28 (30)	11 (12)					
Stromal	2 (30)	9 (12)	75.0	93.33	88.1	81.8	90.3
Tumor	23 (30)	1 (12)	76.67	91.67	80.95	95.8	61.1
Both	3 (30)	1 (12)	10.0	91.7	33.3	75.0	28.95
Mostafa 2018	16 (19)	8 (10)					
Stromal	1 (19)	6 (10)	60.0	94.7	82.8	85.7	81.82
Tumor	14 (19)	1 (1)	90.0	73.6	79.3	93.3	45.2
Tebcherani 2012	307 (310)	137 (144)					
Stromal	70 (310)	87 (144)	77.42	60.42	72.03	55.4	80.1
Tumor	78 (310)	12 (144)	91.67	25.15	46.26	86.7	36.3
Both	159 (310)	38 (144)	73.61	51.29	58.37	80.7	41.3
Pham 2006	20 (23)	12 (13)					
Stromal	0 (23)	10 (13)	100.0	76.9	91.7	100.0	88.5
Tumor	17 (23)	0 (13)	100.0	73.9	83.3	100.0	68.4
Both	3 (23)	2 (13)	84.6	13.0	38.9	60.0	35
	457 (476)	**193 (207)**					
Stromal	**96 (476)**	**134 (207)**					
Tumor	**176 (476)**	**14 (207)**					
Both	**196 (476)**	**45 (207)**					
Ki67							
Abdelsayed 2000	51.25 (6.06)	30.5 (6.46)	P = 0.02				
Costache 2008	12 (18)	12 (12)	66.67	100.0	80.0	66.7	100.0
Lum 2004	18 (20)	15 (16)	94.7	93.8	94.29	93.8	94.7
Mostafa 2018	12 (19)	0 (10)	63.13	100.0	75.8	100.0	58.82

BCC, Basal cell carcinoma; TE, Trichoepithelioma; IHC, Immunohistochemistry