

α -Smooth Muscle Actin Expression in Relation to Invasive Histopathological Features of Papillary Thyroid Carcinoma

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ABSTRACT

Papillary thyroid carcinoma (PTC) generally has a favourable prognosis, but some tumours show invasive histopathological features associated with more aggressive behaviour. Alpha-smooth muscle actin (α -SMA), a marker of activated cancer-associated fibroblasts, has been linked to postoperative recurrence in PTC in a companion analysis of this cohort (unpublished data), but its relationship with conventional invasive features is unclear. This retrospective study included 49 patients with PTC treated at a tertiary referral hospital in Surabaya, Indonesia. Stromal α -SMA expression was assessed immunohistochemically and quantified using the H-score. Tumour size category, multifocality, extrathyroidal extension, lymphovascular invasion, perineural invasion, lymph node metastasis and morphological subtype were extracted from archived histopathology reports. Groups were compared using the Mann-Whitney U and Kruskal-Wallis tests and Spearman correlation, using all patients with available data for each variable. Stromal α -SMA H-score did not differ significantly according to multifocality ($p=0.852$), extrathyroidal extension ($p=0.630$) or lymphovascular invasion ($p=0.854$), or across tumour size categories ($p=0.422$), and subtype was not significantly associated with H-score ($p=0.113$). Lymph node metastasis (2 node-negative patients) and perineural invasion (1 positive patient) were described only. Because subgroups were small and statistical power was low, a Type II error cannot be excluded. These findings suggest that stromal α -SMA expression is not simply a surrogate of conventional anatomical invasiveness and may reflect a distinct axis of tumour-stroma interaction that should be confirmed in larger multicentre cohorts.

Keywords: alpha-smooth muscle actin; cancer-associated fibroblast; invasive histopathological features; papillary thyroid carcinoma; tumour microenvironment

INTRODUCTION

Papillary thyroid carcinoma (PTC) generally carries an excellent prognosis, but a subset of tumours exhibits invasive histopathological features, such as lymphovascular invasion, extrathyroidal extension, multifocality and lymph node metastasis, that are incorporated into conventional staging systems and are associated with more aggressive clinical behaviour [1]. These anatomically defined features remain the cornerstone of postoperative risk assessment in PTC.

Beyond these conventional parameters, the tumour microenvironment, particularly cancer-associated fibroblasts (CAFs), has increasingly been recognised as an independent contributor to tumour behaviour. Among the immunohistochemical markers used to identify activated CAFs, including fibroblast activation protein (FAP), tenascin-C and platelet-derived growth factor receptor- β (PDGFR- β), α -SMA remains the most extensively applied across tumour

types and reflects the classical myofibroblastic CAF phenotype [2]. Stromal α -SMA expression has previously been shown, in a companion analysis of the same institutional cohort (unpublished data), to be significantly associated with postoperative locoregional recurrence in PTC. However, CAFs are biologically heterogeneous, with subpopulations that have divergent and sometimes opposing functions, so stromal activation does not necessarily track every dimension of tumour aggressiveness [3].

It therefore remains unclear whether stromal α -SMA expression simply mirrors conventional anatomical invasiveness, as captured by histopathological parameters such as the American Joint Committee on Cancer (AJCC) T category [1], or reflects a distinct biological process. This matters practically: if α -SMA closely tracks established invasive features, it would add little as a biomarker, whereas the absence of such a relationship would support its potential role as

a complementary axis of tumour biology. This study therefore analysed stromal α -SMA expression in relation to tumour size category, multifocality, extrathyroidal extension, lymphovascular invasion, perineural invasion, lymph node metastasis and morphological subtype in patients with PTC.

MATERIALS AND METHODS

Study design and patients

This retrospective study was conducted at the Department of Anatomical Pathology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, a tertiary referral hospital. It included 49 patients with histopathologically confirmed PTC who underwent thyroidectomy between 2016 and 2025, for whom the primary tumour paraffin block and the original histopathology report were available. The same cohort was used in a separate companion analysis of recurrence (unpublished data).

Clinicopathological data

Tumour size, multifocality, extrathyroidal extension, lymphovascular invasion, perineural invasion, lymph node metastasis and morphological subtype were extracted from the narrative conclusion of each archived report. A feature was recorded only when it was explicitly stated. Extrathyroidal extension was recorded as present when the report described tumour growth through the thyroid capsule or into perithyroidal tissue and as absent when the tumour was described as confined to the thyroid; statements such as tumour growing “to the edge of the thyroid” were treated as not available. Lymphovascular invasion included reported lymphatic or vascular (angio-) invasion. Multifocality was recorded as present when the report stated multifocal, multiple or bilateral tumours or listed two or more separately measured foci. Lymph node metastasis was recorded as present when nodal metastasis was documented in any nodal specimen cited in the report and as absent when nodes were examined and reported negative (pN0). Largest tumour dimension was grouped as ≤ 2 cm, >2 –4 cm and >4 cm, which correspond to T1, T2 and T3a of the AJCC eighth edition when the tumour is limited to the thyroid. T category was assigned from tumour size alone, and extrathyroidal extension was analysed as a separate variable; the pT category printed in the report was not used. Morphological subtype (classic, follicular variant, classic with tall cell features, or tall cell subtype) was taken from the study database. Because the reports were free text rather than structured synoptic reports, not every feature was documented for every patient. Missing information was left as missing and was not imputed, and each analysis used all patients with data for the variable concerned.

Immunohistochemistry

Four- to six-micrometre sections were cut from formalin-fixed, paraffin-embedded tumour blocks and mounted on polysine slides. Immunohistochemistry was performed manually according to the standard operating procedure of the Anatomical Pathology Laboratory with the Starr Trek Universal HRP Detection System (Biocare Medical, Pacheco, CA, USA).

Slides were heated on a hot plate at 60 °C for 1 h, deparaffinised in xylene and rehydrated through graded alcohol, and endogenous peroxidase was blocked for 15 min. Antigen retrieval used Diva Decloaker (Biocare Medical) in a decloaking chamber at 95 °C for 15 min, followed by background blocking with Background Sniper (Biocare Medical) for 15 min. Sections were incubated for 60 min with a mouse monoclonal anti- α -SMA antibody (clone IHC506, GeneAb, GenomeMe, Richmond, BC, Canada; dilution 1:100), followed by Trekkie Universal Link (20 min), Trekavidin-HRP label (10 min) and 3,3'-diaminobenzidine (DAB) chromogen (3 min), with phosphate-buffered saline washes between steps. Sections were counterstained with Mayer's haematoxylin, dehydrated, cleared and mounted. Normal uterine smooth muscle served as the positive control, and for the negative control the primary antibody was replaced with phosphate-buffered saline.

H-score assessment

Stromal α -SMA expression in fibroblasts of the tumour stroma was quantified using the Histochemical scoring (H-score) method, calculated as the sum of staining intensity (0–3) multiplied by the percentage of stromal cells at each intensity, giving a score from 0 to 300 [4]. Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate) or 3 (strong) (Figure 1). Two anatomical pathologists scored the slides, blinded to the recurrence status of the patients, and the final H-score was determined by consensus.

Statistical analysis

The distribution of the H-score within each comparison group was examined with the Shapiro-Wilk test. Because the comparison groups were small and unequal, a situation in which normality tests have limited power, non-parametric tests were used throughout, and H-scores are presented as median (interquartile range, IQR). Two groups were compared with the Mann-Whitney U test, three or more groups with the Kruskal-Wallis test, and the relationship between ordered tumour size category and H-score with Spearman correlation. Comparisons were not performed when a group contained fewer than five patients. A sensitivity analysis estimated the smallest standardised difference (Cohen's d) detectable with 80% power at a two-sided alpha of 0.05 for each comparison in G*Power 3.1 [5]. A two-tailed p-value <0.05 was considered statistically significant. All other analyses were performed using IBM SPSS Statistics version 31.0.1.0 (IBM Corp., Armonk, NY, USA).

Ethical approval

This study was approved by the Health Research Ethics Committee, Dr. Soetomo General Academic Hospital, Surabaya (Ethical Clearance No. 1602/KEPK/V/2026, valid 18 May 2026 – 18 May 2027). As a retrospective study using archived clinical and pathological records, the requirement for individual informed consent was waived.

RESULTS

The 49 patients comprised 33 women and 16 men, with a median age of 53 years (range 25–77). The median stromal α -SMA H-score of the whole cohort was 180.0 (IQR 120.0–240.0). The subtype was classic in 38 patients, follicular variant in 8, and classic with tall cell features or tall cell subtype in 3.

Largest tumour dimension was recorded numerically in 45 patients (median 4.0 cm, IQR 2.95–6.0 cm, range 0.3–10.8 cm), and one further tumour was reported as <1 cm. Stromal α -SMA staining ranged from negative to strong, and representative examples of each intensity are shown in Figure 1.

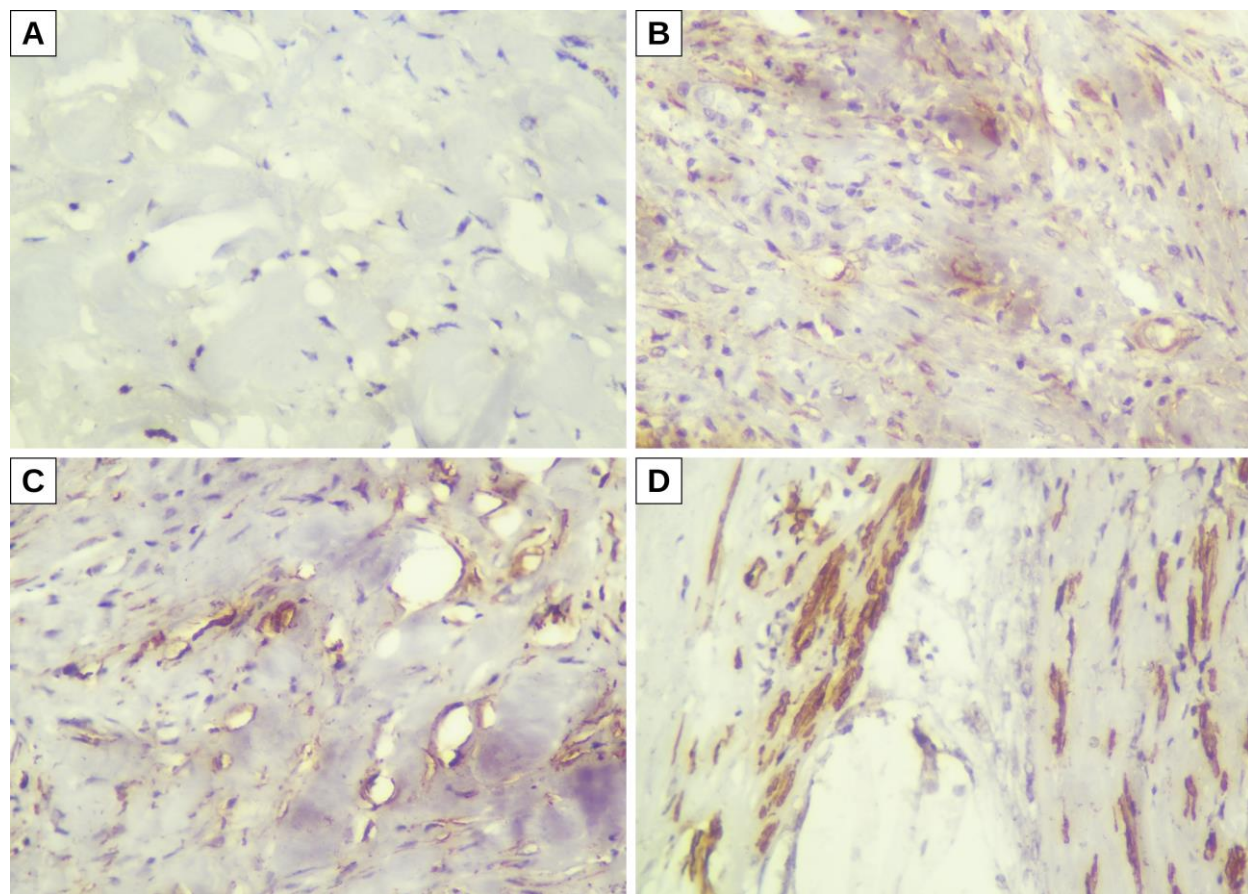


FIGURE 1: Representative Stromal α -SMA Immunostaining Intensities Used For H-Score Assessment in Papillary Thyroid Carcinoma. (A) Negative, intensity 0. (B) Weak, intensity 1. (C) Moderate, intensity 2. (D) Strong, intensity 3. Positive staining appears as brown cytoplasmic staining of stromal spindle cells (immunohistochemical staining; original magnification $\times 400$).

Data availability differed by variable, as expected for narrative reports: tumour size category was available for 46 patients, multifocality for 46, extrathyroidal extension for 37, lymphovascular invasion for 44, lymph node status for 28 and perineural invasion for 27. The H-score did not clearly depart from normality in any group (Shapiro-Wilk $p > 0.05$ in all groups, lowest $p = 0.055$).

Stromal α -SMA H-score did not differ significantly according to multifocality (present, $n = 10$: median 185.0, IQR 142.5–217.5; absent, $n = 36$: 170.0, IQR 97.5–240.0; $U = 173.0$, $p = 0.852$), extrathyroidal extension (present, $n = 15$: 190.0, IQR 120.0–240.0; absent, $n = 22$: 170.0, IQR 120.0–240.0; $U = 149.5$,

$p = 0.630$) or lymphovascular invasion (present, $n = 16$: 180.0, IQR 120.0–237.5; absent, $n = 28$: 170.0, IQR 90.0–240.0; $U = 216.5$, $p = 0.854$) (Table 1).

Lymph node metastasis was documented in 28 patients, of whom 26 had metastasis (median H-score 180.0, IQR 112.5–240.0) and only 2 were node-negative (H-score 120.0 in both); no inferential comparison was made because of the very small node-negative group. Perineural invasion was reported in 27 patients and was present in only one (H-score 210.0); the other 26 had a median H-score of 185.0 (IQR 110.0–242.5), and no test was performed. Representative histological appearances of the invasive features are shown in Figure 2.

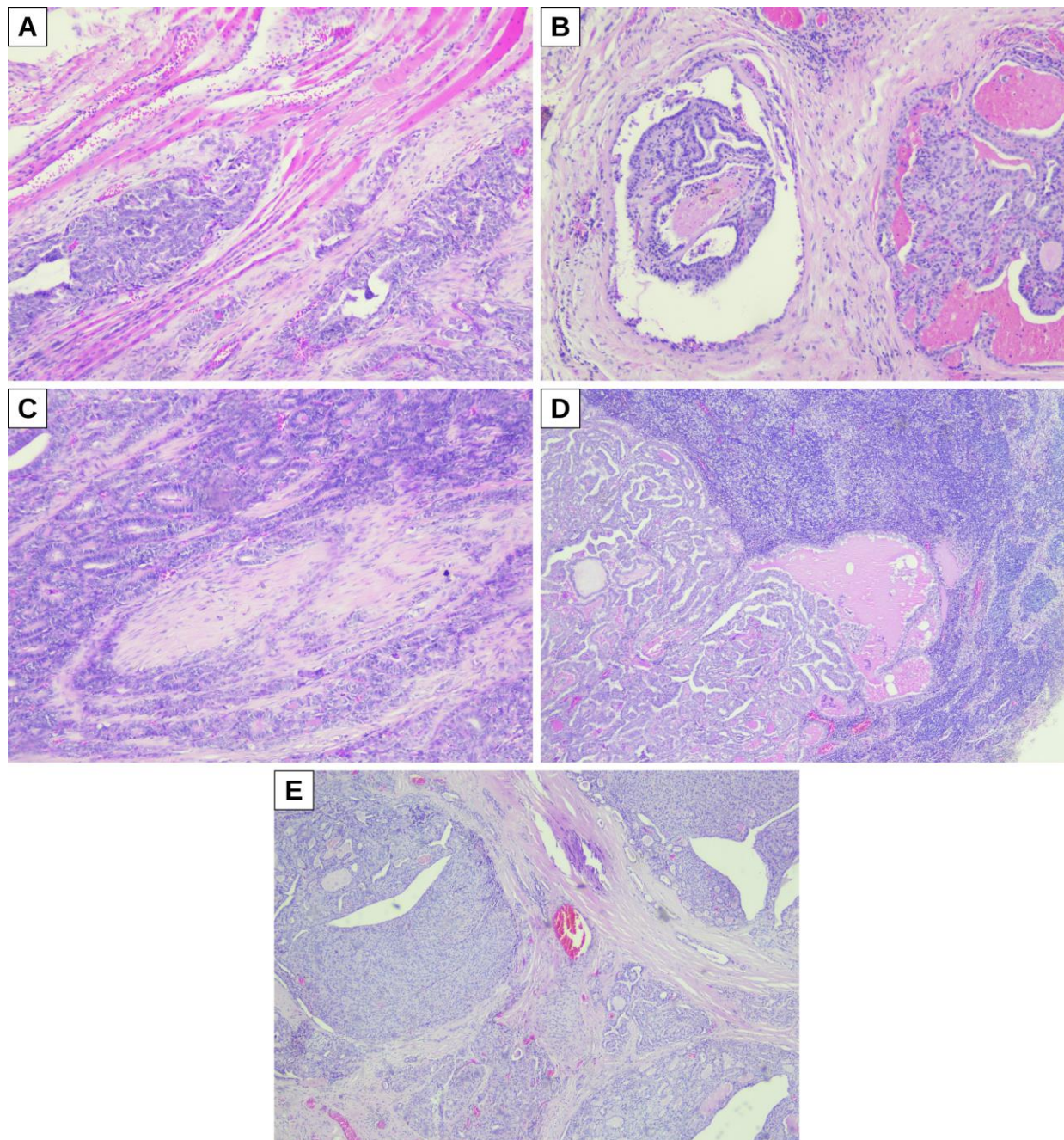


FIGURE 2: Representative Invasive Histopathological Features of Papillary Thyroid Carcinoma. Haematoxylin and eosin staining. (A) Extrathyroidal extension, with tumour nests infiltrating perithyroidal skeletal muscle ($\times 100$). (B) Lymphovascular invasion, with a tumour cluster within a vascular space ($\times 200$). (C) Perineural invasion, with tumour cells surrounding a nerve bundle ($\times 100$). (D) Lymph node metastasis, with papillary carcinoma deposits within lymphoid tissue ($\times 40$). (E) Multifocality, with separate tumour foci divided by fibrous stroma ($\times 40$). Magnifications are original magnifications.

The H-score tended to be higher with increasing tumour size category, but this was not statistically significant (Kruskal-Wallis $H=1.73$, $df=2$, $p=0.422$; Spearman $\rho=0.134$, $p=0.374$, $n=46$) (Table 2). Subtype was not significantly associated with H-score (Kruskal-Wallis $H=4.37$, $df=2$, $p=0.113$; classic versus follicular variant $U=120.5$, $p=0.360$) (Table 3). The group with tall cell features or tall cell subtype ($n=3$) had the highest median H-score (270.0, range 190.0–300.0), but with three patients this is a descriptive observation only.

The sensitivity analysis showed that the comparisons for multifocality, extrathyroidal extension, and lymphovascular invasion had 80% power only for large, standardised differences (Cohen's d of about 1.02, 0.96, and 0.90, respectively), whereas the observed differences were very small ($d=0.08$ to 0.10), and the post-hoc power for the observed differences was below 7%.

TABLE 1: Stromal α -SMA H-Score According to Invasive Histopathological Features of Papillary Thyroid Carcinoma.

Feature (n assessed)	Category	n	H-score, median (IQR)	U	p
Multifocality (n=46)	Absent	36	170.0 (97.5–240.0)	173.0	0.852
	Present	10	185.0 (142.5–217.5)		
Extrathyroidal extension (n=37)	Absent	22	170.0 (120.0–240.0)	149.5	0.630
	Present	15	190.0 (120.0–240.0)		
Lymphovascular invasion (n=44)	Absent	28	170.0 (90.0–240.0)	216.5	0.854
	Present	16	180.0 (120.0–237.5)		
Lymph node metastasis (n=28)	Absent	2	120.0 (120.0–120.0)	not tested	
	Present	26	180.0 (112.5–240.0)		
Perineural invasion (n=27)	Absent	26	185.0 (110.0–242.5)	not tested	
	Present	1	210.0		

Mann-Whitney U test. Patients without data for a feature were excluded from that comparison only. The Shapiro-Wilk test did not reject normality in any group; non-parametric tests were chosen because of small, unequal groups. U is the smaller of the two U values.

TABLE 2: Stromal α -SMA H-Score by Tumour Size Category (T Category by Size).

T category (largest dimension)	H-score, median (IQR)	n	Test
T1 (≤ 2 cm)	120.0 (50.0–300.0)	7	Kruskal-Wallis H=1.73, df=2, p=0.422
T2 (>2 –4 cm)	155.0 (67.5–217.5)	18	Spearman rho=0.134, p=0.374 (n=46)
T3a (>4 cm)	190.0 (145.0–240.0)	21	

T category was assigned from tumour size alone. T3b and T4 categories are not represented (see Limitations).

TABLE 3: Stromal α -SMA H-Score by Morphological Subtype.

Subtype	H-score, median (IQR)	n	Test
Classic subtype	170.0 (120.0–240.0)	38	Kruskal-Wallis H=4.37, df=2, p=0.113
Follicular variant	140.0 (22.5–232.5)	8	Classic vs follicular U=120.5, p=0.360
Classic with tall cell features, or tall cell subtype	270.0 (range 190.0–300.0)	3	

DISCUSSION

This study found that stromal α -SMA expression, a marker of activated cancer-associated fibroblasts, was not significantly associated with any of the conventional invasive histopathological features examined, namely tumour size category, multifocality, extrathyroidal extension and lymphovascular invasion, nor with morphological subtype. This is notable because stromal α -SMA expression has been significantly associated with postoperative locoregional recurrence in the same institutional cohort (unpublished data).

This apparent dissociation is biologically plausible given growing evidence that CAFs are functionally heterogeneous. Single-cell studies across tumour

types have identified distinct CAF subpopulations, from matrix-remodelling myofibroblastic to inflammatory phenotypes, that contribute differently to tumour behaviour [2,3]. As a single marker, α -SMA cannot separate these subpopulations, and it may capture a dimension of stromal activation relevant to recurrence, such as paracrine signalling or field-level microenvironmental remodelling, without necessarily reflecting gross anatomical invasion.

The earlier PTC-specific study of stromal α -SMA found higher expression in tumour stroma than in normal thyroid tissue, but only a non-significant trend towards higher expression in metastatic small PTCs [6]. Two other recent studies reported positive findings: a 2026 study of 85 mixed thyroid carcinomas

found higher α -SMA immunoreactivity in larger tumours, in tumours with aggressive features and in men [7], and an earlier PTC study found stromal CAFs to predict cervical lymph node metastasis [8]. Our null results may reflect differences in cohort composition (PTC only), scoring method, the very small node-negative group, or limited power rather than a true absence of association.

Clinically, these findings suggest that stromal α -SMA is unlikely to substitute for conventional staging in PTC. Its association with recurrence in the same cohort raises the possibility that it could provide complementary rather than redundant prognostic information.

This study has several limitations. With these sample sizes the comparisons could detect only large differences (Cohen's *d* of about 0.9 or more), so a Type II error cannot be excluded and a subtle association between α -SMA expression and anatomical invasion might become evident in a larger cohort; these null findings should be regarded as hypothesis-generating. Some subgroups were too small to test, namely node-negative patients (*n*=2), perineural invasion (1 positive case) and the tall cell group (*n*=3). Data were missing for several features, most often extrathyroidal extension (12 patients) and lymph node status (21 patients), because they were extracted from narrative rather than synoptic reports. Missing data are probably not random; for example lymph node status is often absent when no nodes were submitted, so results for these variables may be biased. T category was assigned from tumour size alone, so T3b and T4 tumours are not represented and six tumours reported as pT4a by the pathologist were classified as T3a. Block storage time varied widely (16 to 116 months) and was not analysed, although antigen loss in older blocks could affect staining. Only stromal staining was scored, and the study was retrospective and single centre. Larger multicentre studies using structured synoptic reporting are needed to confirm whether stromal α -SMA is an anatomically independent axis of tumour biology in PTC.

CONCLUSION

In conclusion, within the limits of this study cohort, stromal α -SMA expression was not significantly associated with conventional invasive histopathological features of PTC. These findings suggest that α -SMA expression may represent a biologically distinct axis of tumour-stroma interaction rather than a surrogate marker of anatomical invasiveness. Larger prospective multicentre studies are required to confirm these observations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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