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Alcian blue and epithelial membrane antigen are useful markers in differentiating benign from malignant papillae in thyroid lesions

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Summary. Immunohistochemistry for epithelial membrane antigen (EMA) and histochemistry for alcianophilic substances were performed in 17 cases of papillary thyroid carcinoma (PTC) and 11 cases of benign thyroid lesions showing papillary changes (7 diffuse hyperplastic goitres-Graves'disease; 4 colloid cystic goitres). In all PTCs the glycocalix of the cells lining the papillary structures was stronly positive with anti-EMA antiserum. Alcian blue pH 2.5 stain (AB 2.5) was also positive in 15 of these cases. In contrast, no cases of benign thyroid lesions showed AB 2.5 positivity in the cells lining the papillary structures and the positivity with anti-EMA antiserum, present in only 5 out the 11 cases, was focal and very weak. These results indicate that the presence and distribution of EMA and alcianophilic substances may be useful in distinguishing benign from malignant thyroid lesions containing papillae.

Key words: Alcian blue – Epithelial membrane antigen – Thyroid – Papillary carcinoma – Hyperplastic goitre

Introduction

Papillary structures, the hallmark of papillary carcinoma of thyroid, are occasionally seen within benign lesions such as colloid and hyperplastic goitres. When present in these benign conditions they may cause diagnostic difficulties and lead to confusion with papillary carcinoma (Rosai and Carcangiu 1987). Several histological criteria have been proposed in order to distinguish papillary structures of papillary thyroid carcinoma (PTC) from the papillary fronds seen in benign conditions (Li-Volsi 1990; Rosai 1989). Alcian blue (AB) content and the epithelial membrane antigen (EMA) localization have been investigated in 11 benign lesions of the thyroid showing papillary structures and 17 PTCs, with the aim of identifying additional criteria in the differential diagnosis between the two types of papillary structures.

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Materials and methods

Eleven benign lesions of the thyroid (4 colloid cystic goitres with papillae and 7 diffuse hyperplastic goitres-Graves'disease) and 17 PTCs (11 of the classic and 6 of the mixed – follicular and papillary – variants) were selected from the files of the Institute of Pathology of the University of Bologna. Tissues were immediately fixed in 10% buffered formalin, embedded in paraffin and stained with haematoxylin- and eosin (H & E).

The following histochemical techniques for mucosubstances were performed: alcian blue pH 2.5 (AB 2.5); alcian blue pH 1 (AB 1); AB 2.5 combined with periodic acid-Schiff reaction with diastase pre-digestion (AB 2.5-PASd).

For immunohistochemistry all cases were stained with EMA monoclonal antibody diluted 1:6000 (kindly provided by J.P. Sloane, London), using the streptavidin-biotin-peroxidase complex technique (Hsu et al. 1981). The antibody used has been well characterized, as recently shown (Imrie et al. 1990).

Results

Data for the 11 benign lesions are shown in Table 1. In 7 of the 11 cases the thyroid tissue showed typical features of diffuse hyperplastic goitre microscopically and was almost entirely constituted by prominent papillary structures abutting in irregular follicular spaces, mostly devoid of colloid. All these cases underwent subtotal thyroidectomy because of a long-standing history of thyroid hyperfunction resistant to medical therapy. In the remaining 4 cases, most of the tissue was composed of large cystic follicles lined by flattened epithelium and focally showing papillary fronds (colloid cystic goitres with papillae). In all cases the papillary structures appeared to be composed of a central core of delicate fibrovascular tissue, sometimes myxomatous in appearance, lined by one layer of epithelial cells with basally located nuclei (Figs. 1, 2). Cells were cuboidal or colum-

Table 1. Benign lesions

Cases	EMA		PASd		AB 2.5	
	Pap.	Foll.	Pap.	Foll.	Pap.	Foll.
Colloid Goitres						
1	gly +F	gly +F	N	co +D	N	N
2	gly +F	gly +F	N	co +D	N	N
3	N	gly +F	N	co +D	N	N
4	N	N	N	co +F	N	N
Hyperplastic Goitres						
5	N	gly +F	N	N	N	N
6	N	N	N	co +F	N	N
7	N	co +F	N	co +F	N	N
8	gly +F	gly +D	N	co +F	N	co +F
9	gly +F	N	N	co +F	N	N
10	N	gly +D	N	co +F	N	N
11	gly +F	N	N	co +F	N	N

AB, Alcian blue; EMA, epithelial membrane antigen; PASd, periodic acid Schiff (diastase); Pap, papillae; Foll, follicles; gly, glycocalix; co, colloid positivity: +, weak; ++, medium; +++, strong; N, negative F, focal; D, diffuse

Table 2. Classic papillary carcinomas

Cases	EMA		PASd		AB 2.5	
	Pap.	Foll.	Pap.	Foll.	Pap.	Foll.
12	gly +++D	N	N	N	gly +F	N
13	gly +++D	N	N	N	gly ++D	N
14	gly ++D	N	N	N	gly +D	N
15	gly +++D	N	N	N	gly +D	N
16	gly +++D	N	co +D	N	gly +++D co +D	N
17	gly +++F	N	co +F	N	gly +F	N
18	gly +++D	gly +++F co +F	N	co +F	gly ++F	N
19	gly ++D	N	N	N	gly ++F	N
20	gly +++D	gly +++D	N	co +F	gly +D	gly +D
21	gly ++F	gly +F co +F	N	co +F	gly +F	gly +F
22	gly +D	gly +F	N	co +F	gly +F	N

Pap, papillae; Foll, neoplastic follicles; gly, glycocalix; co, colloid positivity: +, weak; ++, medium; +++, strong; N, negative; F, focal; D, diffuse

nar in hyperplastic lesions. In 3 of these cases the nuclei were large and irregular.

Histochemically the epithelial cells lining the fibrovascular core of the papillae were not stained by the AB methods. A PAS-positive basal lamina was evident in all cases.

The colloid within the follicles appeared PASd-positive in 10 of the 11 cases of benign lesions studied (4 colloid-cystic goitres with papillae and 6 hyperplastic goitres). PASd positivity was diffuse in 3 cases and focal in 7 cases. In 1 case of hyperplastic goitre a focal colloid AB 2.5 positivity was seen.

Immunohistochemically the outer surface (glycocalix) of most of epithelial cells was negative with anti-EMA antiserum (Fig. 3). A weak positivity was seen in the follicular component of 6 and in the papillary component of 5 cases. In 1 case colloid was also immunoreactive with anti-EMA antiserum. This positivity was present in a very limited number of follicles. No cytoplasmic positivity with anti-EMA antiserum was found.

The 17 PTCs examined were considered to be of classic type when most of the tumour appeared composed of papillary structures (11 cases), and as mixed (follicular and papillary) type when the presence of neoplastic follicle-like structure was prominent (6 cases). The neoplastic

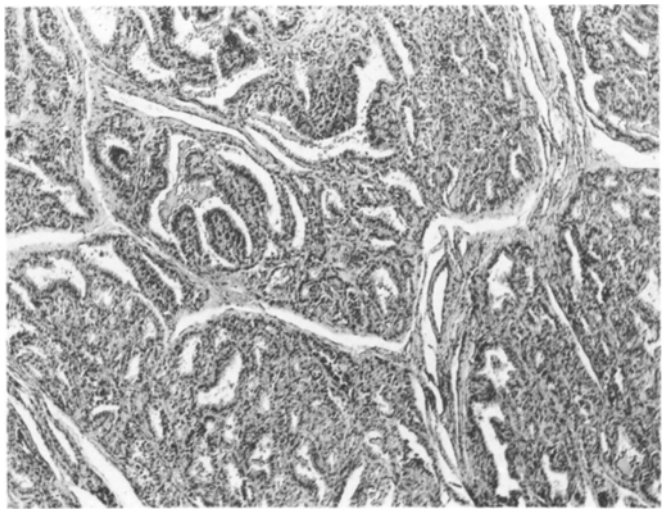

Fig. 1. Case 6: in this case of diffuse hyperplastic goitre there are several papillary projections abutting into irregular follicular spaces. H & E, ×100

papillae in all cases were composed of a central core of fibrovascular tissue lined by one or several layers of cells showing crowded nuclei with a ground-glass appearance and occasional intranuclear cytoplasmic invaginations and groovings (Fig. 4). The follicular component in the mixed PTCs showed cytological features superimposable on those of the papillary structures.

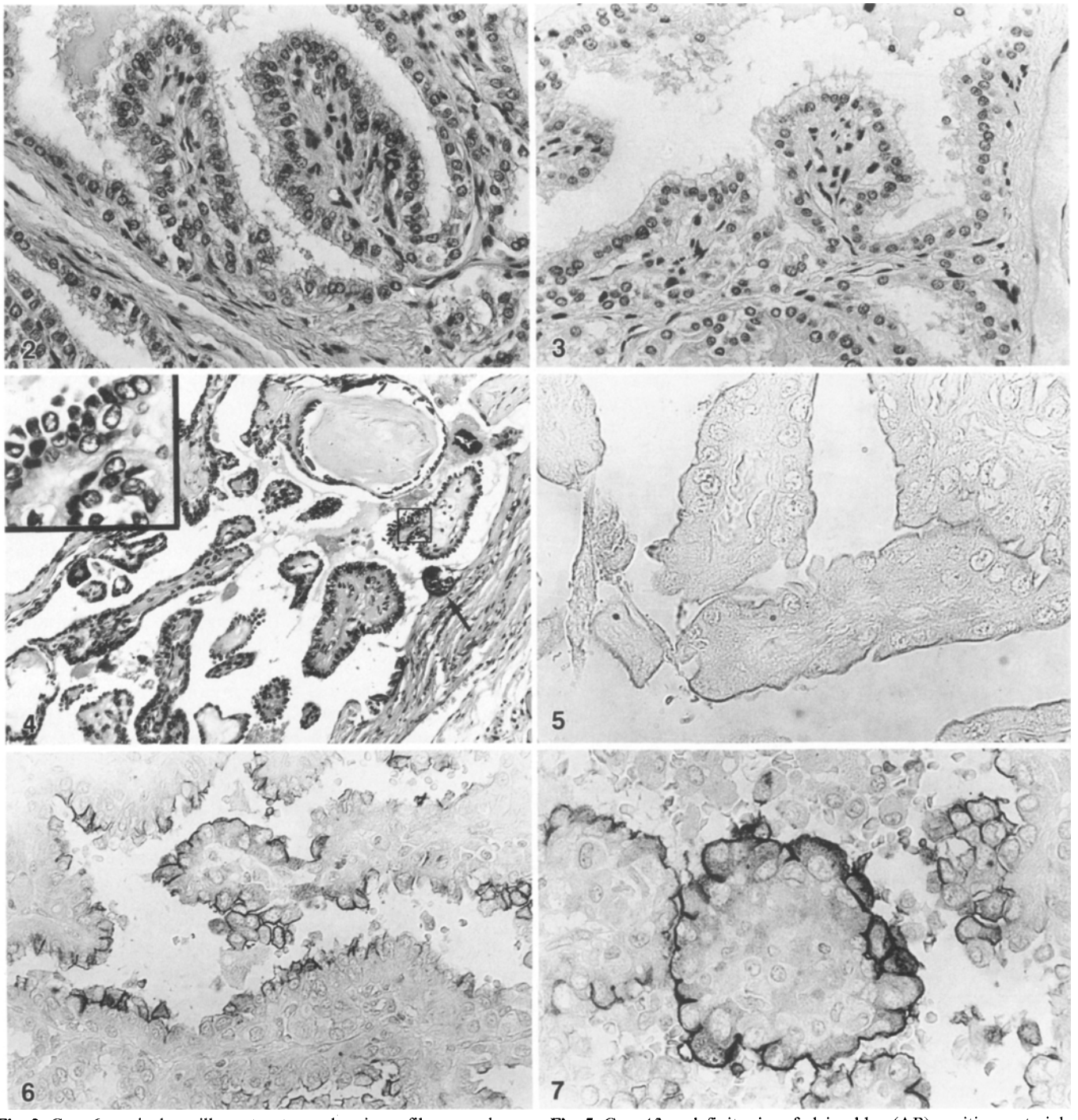

Fig. 2. Case 6: typical papillary structures showing a fibrovascular core. The cuboidal lining cells have round nuclei, H & E, ×250

Fig. 3. Case 6: deeper level of sectioning. The fluffy glycocalix appears unstained by anti-epithelial membrane antigen (EMA) antiserum. Streptavidin-biotin-peroxidase, ×250

Fig. 4. Case 12: in this case of papillary carcinoma the fibrovascular core is sclero-hyaline. A psammoma body is present (*arrow*). *Inset*: Cells with clear nuclei are visible. H & E, ×100; *inset* ×350

Fig. 5. Case 13: a definite rim of alcian blue (AB)-positive material outlines the neoplastic cells. AB 2.5, ×350

Fig. 6. Case 14: EMA is present on the glycocalix of cells lining papillae. Neoplastic follicles are devoid of EMA. Streptavidin-biotin-peroxidase, ×250

Fig. 7. Case 15: the neoplastic cells are strongly outlined by anti-EMA antiserum. Streptavidin-biotin-peroxidase ×350

Table 3. Mixed papillary carcinomas

Cases	EMA		PASd		AB 2.5	
	Pap.	Foll.	Pap.	Foll.	Pap.	Foll.
23	gly ++F	gly ++F	N	co +F	N	N
24	gly +D	gly +D	N	co +D	N	gly ++D
25	gly ++D	gly ++D	N	co +F	gly ++D	gly ++D
26	gly ++D	gly ++D	N	co +F	N	N
27	gly ++D	gly ++D	N	co +F	gly +F	gly +F
28	gly ++D	gly ++D	N	co +F	gly +D	gly +D

Pap, papillae; Foll, neoplastic follicles; gly, glycocalix; co, colloid positivity: +, weak; ++, medium; +++, strong; N, negative; F, focal; D, diffuse

Histochemically, in the 11 classic PTCs (Table 2) the epithelial cells lining the papillary fronds showed a definite AB 2.5 positivity confined exclusively to the glycocalix in all of the 11 cases studied (Fig. 5). Positivity was seen in most of the cells in 5 and limited to some areas in 6 cases. Only 4 of the 11 cases showed a focal PASd positivity in the colloid within follicular structures.

In mixed (papillary and follicular) PTCs (6 cases) (Table 3) the glycocalix of the neoplastic cells was stained by AB 2.5 in the follicular component in 4 cases (diffuse in 3 cases and focal in 1) and in the papillary component in 3 cases (diffuse in 2 cases and focal in 1). In 2 cases no staining was visible in the epithelium lining the papillae.

Colloid appeared diffusely positive with PASd in all cases of mixed PTCs. No AB 2.5 – positive substances were present within the cytoplasm of the neoplastic cells of the classic, or the mixed PTCs. A PAS-positive basal lamina was always observed in all cases of classic and mixed PTCs.

In all cases of classic and mixed PTCs, the glycocalix of the neoplastic cells lining the fibrovascular core of the papillary structures was immunoreactive with anti-EMA antiserum (Figs. 6, 7). Positivity was diffuse in the papillary component of most of the cases of both variants, while the follicular component appeared negative (7 cases) or only weakly positive (10 cases). No positivity was observed within the cytoplasm of the neoplastic cells in any of the cases.

Residual non-neoplastic thyroid tissue was observable in 14 of 17 cases of PTC. Twelve of these showed PASd positivity in the colloid, while in 6 cases colloid also appeared positive with AB 2.5. Benign thyroid follicular cells were devoid of AB 2.5-positive substances in all cases.

The glycocalix of normal thyroid cells appeared focally positive with anti-EMA antiserum in 7 cases. In 1 case (case 7) EMA positivity was also found in the cytoplasm of occasional thyroid cells. Colloid was weakly EMA-immunoreactive in 10 cases.

The stromal tissue was weakly AB 2.5 positive in all cases of benign and malignant lesions, as well as in the residual normal thyroid tissue.

No positivity for AB 1 was observed in normal thyroid tissue, in benign lesions, or in papillary carcinomas.

Discussion

It is generally accepted that the observation of papillary structures is not a reliable criterion to establish the diagnosis of papillary carcinoma of the thyroid, as in the follicular variant of PTC papillae are not present (Rosai 1989). In addition, typical papillary structures or papilloid projections are seen in benign conditions such as colloid and hyperplastic goitres (Rosai and Carcangiu 1987). Therefore it appears that it is important to distinguish papillary structures of PTC from those appearing in benign conditions. Ground-glass nuclei and intranuclear invaginations of the cytoplasm are very useful cytological criteria characteristic of PTCs (LiVolsi 1990). However, not all papillary carcinomas show optically clear nuclei (Rosai 1989), and the presence of nuclear pseudoinclusions has been recently described in hyalinizing trabecular adenomas (Carney et al. 1987). Papillary fronds in PTC are short and have an irregular structure. In contrast, the papillae present in benign conditions are elongated, have a more organoid disposition and tend to converge towards the centre of the lesion (Rosai and Carcangiu 1987). Nevertheless, there are cases in which confusion might arise, especially when atypical nuclei, capsular and/or vascular and skeletal muscle invasion are encountered in diffuse or nodular hyperplasia (Rosai 1989).

In the present study, the different distribution of EMA and AB 2.5 – positive substances was apparent in benign and malignant lesions bearing papillary structures. Anti-EMA antiserum stained diffusely the glycocalix of the cells lining the papillary structures in all cases of classic and mixed PTC. Similarly, all classic PTCs and more than 50% of mixed PTCs displayed AB 2.5-positive glycocalix of the epithelial cells constituting the papillae (Tables 2, 3). In contrast, the epithelium lining the papillary fronds in the benign lesions did not show any AB 2.5-posistive substance. In addition, the expression of EMA in the same cases was only focal and weak (Table 1). It appears therefore that the study of the localization and distribution of these two markers can be of additional help in the histological distinction of benign from malignant papillary lesions of the thyroid.

EMA is usually expressed by most of the glandular epithelia (Heyderman et al. 1979) and its presence in normal and neoplastic thyroid tissue has been previously reported by Wilson et al. (1986), who studied 10 cases of PTC. Their results differed somewhat from those reported here since they found only a weak positivity for EMA in their cases. Nevertheless this statement appears contradicted by one illustration in the same paper where

a strong and diffuse staining of the glycocalix is clearly evident. Therefore this discrepancy may be more apparent than real and it is due to different evaluation of the same results.

There are contrasting data in the literature concerning the presence of mucosubstances in normal and neoplastic thyroid tissue. In current textbooks of pathology, the production of mucins by primary thyroid tumours is considered an extremely rare event, with the exception of medullary carcinoma (LiVolsi 1990; Rosai 1989). Nevertheless, recent studies revealed the presence of mucosubstances in up to 50% of thyroid carcinomas (Mlynek et al. 1985; Rigaud and Bogolometz 1987) and in up to 57% of metastatic PTCs (Chan and Tse 1988). In addition, acid mucins have also been found in 18% of normal thyroid glands (Harach 1985). Whether alcianophilic substances in thyroid have to be considered as mucins or as products of degradation of thyroglobulin is still a matter of discussion (Carcangiu et al. 1985; Mendelsohn 1984; Rigaud and Bogolometz 1987). In addition, the presence of AB 2.5-positive substances is difficult to evaluate in thyroid tissue, as these are often obscured by the presence of colloid (Rigaud and Bogolometz 1987). In conclusion, the true nature of the AB 2.5-positive substances in PTCs is difficult to ascertain and for this purpose the standardization of the histochemical techniques for detection of mucins in the thyroid seems to be necessary. A novel approach was recently attempted by Vierbuchen et al. (1989), who employed two different monoclonal antibodies directed against carbohydrate antigens. These same authors found results similar to our own; immunoreactivity was seen in malignant conditions only, while no staining was present in the several benign lesions studied. It is pertinent to quote their words: as "in well-differentiated papillary carcinomas the reaction product was concentrated in the apical portion of the papillary structures".

At the present time, the reason why EMA and alcianophilic substances are not consistently seen to outline the glycocalix of cells constituting the papillary fronds in benign lesions as opposed to those in PTCs is not clear. This might be the consequence of different functional stages, the benign cells having different functional activity from the malignant counterpart.

In conclusion, the purpose of this paper was to show different patterns of distribution of AB 2.5- and EMA-positive substances in benign and malignant lesions of the thyroid containing papillary structures and to suggest that their evaluation might be useful in the differential diagnosis of these lesions.

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References

Carcangiu ML, Sibley RK, Rosai J (1985) Clear cell change in primary thyroid tumors. A study of 38 cases. Am J Surg Pathol 9:705–722

Carney JA, Ryan J, Goellner JR (1987) Hyalinizing trabecular adenoma of the thyroid gland. Am J Surg Pathol 11:583–591

Chan JKC, Tse CCH (1988) Mucin production in metastatic papillary carcinoma of the thyroid. Hum Pathol 19:195–200

Harach HR (1985) Thyroid follicles with acid mucins in man: a second kind of follicles? Cell Tissue Res 242:211–215

Heyderman E, Steele K, Ormerod MG (1979) A new antigen on the epithelial membrane: its immunoperoxidase localization in normal and neoplastic tissues. J Clin Pathol 32:35–39

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Imrie SF, Sloane JP, Ormerod MG, Styles J, Dean CJ (1990) Detailed investigation of the diagnostic value in tumour histopathology of ICR 2, a new monoclonal antibody to epithelial membrane antigen. Histopathology 16:573–581

LiVolsi VA (1990) Surgical pathology of the thyroid. Saunders, Philadelphia

Mendelsohn G (1984) Signet-ring cell-simulating microfollicular adenoma of the thyroid. Am J Surg Pathol 8:705–708

Mlynek ML, Richter HJ, Leder LD (1985) Mucins in carcinomas of the thyroid. Cancer 56:2647–2650

Rigaud C, Bogolometz WV (1987) "Mucin secreting" and "mucinous" primary thyroid carcinomas: pitfalls in mucin histochemistry applied to thyroid tumours. J Clin Pathol 40:890–895

Rosai J (1989) Thyroid gland. In: Ackerman's surgical pathology, 7th edn. Mosby, St. Louis pp 391–447

Rosai J, Carcangiu ML (1987) Pitfalls in the diagnosis of thyroid neoplasms. Pathol Res Pract 182:169–179

Vierbuchen M, Schroder S, Uhlenbruck G, Ortmann M, Fischer R (1989) CA 50 and CA 19-9 antigen expression in normal, hyperplastic and neoplastic thyroid tissue. Lab Invest 60:726–732

Wilson NW, Pambakian H, Richardson TC, Stokoe MR, Makin CA, Heyderman E (1986) Epithelial markers in thyroid carcinoma: an immunoperoxidase study. Histopathology 10:815–829