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THE EFFICACY OF THE THYROID PEROXIDASE MARKER FOR DISTINGUISHING FOLLICULAR THYROID CARCINOMA FROM FOLLICULAR ADENOMA

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Aim: Expression of thyroid peroxidase (TPO) in the thyroid gland tissue is well known as a sensitive marker of the thyroid malignancy. We have evaluated immunohistochemical assay of TPO for distinguishing follicular thyroid carcinoma from follicular adenoma. *Materials and Methods:* Sections of formalin-fixed tissues obtained from 92 patients with thyroid tumors (52 follicular carcinomas and 40 follicular adenomas including the Hürthle cell type) were analyzed using a monoclonal antibody (TPO mAb 47) and the avidin-biotin peroxidase complex immunohistochemical technique. Lesions with staining of more than 80% of the follicular cells/ specimen were considered benign, while less than 80% were considered malignant. *Results:* TPO immunostaining correlated with the histopathological diagnosis in 24/40 cases of follicular adenomas and 41/52 cases of follicular carcinomas, giving a specificity of 60% and a sensitivity of 79%. *Conclusion:* These results suggest that immunohistochemical assay of TPO expression has limited value for the differential diagnosis of follicular thyroid carcinoma from thyroid follicular adenoma.
Key Words: thyroid, follicular thyroid carcinoma, thyroid peroxidase, molecular marker, immunohistochemistry.

The pathology of the thyroid gland presents the pathologist with a set of diagnostic problems. A particular problem is distinguishing between follicular thyroid carcinoma, including Hürthle cell (oxyphilic or oncocytic) carcinoma (HCC), a variant of follicular carcinoma, and their benign counterparts, follicular thyroid adenoma and Hürthle cell adenoma (HCA). These tumors are encapsulated, and they can only be distinguished by assessing the vascular and/or capsular invasion on histological specimens. It is generally known that differential diagnosis is often difficult even with permanent sections. Therefore, specific molecular markers to discriminate between follicular thyroid carcinomas and adenomas in thyroid follicular lesions are needed.

One possibly suitable molecular marker to fulfill this role is thyroid peroxidase (TPO), a thyroid-specific enzyme essential for the biosynthesis of thyroid hormone. In the last few years, the diagnostic value of TPO immunodetection in thyroid neoplasms has been widely discussed, because several authors have demonstrated that TPO protein is expressed in benign tumors and normal tissue [1–3], but is absent or poorly expressed in a variety of thyroid follicular carcinomas [1, 4–9]. In addition, quantitative and qualitative changes in TPO activity and TPO messenger ribonu-

cleic acid (mRNA) expression have been reported in pathological thyroid tissues [10–16].

In general, immunohistochemical studies using mAb 47 have shown close correlation between negativity for TPO and thyroid carcinoma malignancy [1, 6, 8, 13, 17]. These findings have been confirmed in studies on FNA samples providing sensitivities between 97 and 100% for overall carcinomas [4–6, 8]. However, the recent studies of Lima et al. [3], Kholova et al. [18, 19] and Weber et al. [20] indicated that TPO has limited value in the diagnosis of malignant follicular thyroid tumors. Thus, the value of TPO immunohistochemistry using mAb 47 in thyroid carcinoma diagnostics is still the subject for discussion.

Disparate results in the literature prompted us to explore further the clinical usefulness of TPO staining for the differential diagnosis of follicular thyroid carcinoma from follicular adenoma of the thyroid.

MATERIALS AND METHODS

Clinical and morphological data. Formalin-fixed paraffin-embedded tissue sections from 92 patients with follicular thyroid tumors were stained immuno-histochemically. Tissue sections were obtained from the archival material of the Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia, Belgrade. Haematoxylin/eosin-stained sections from each sample were evaluated histologically by two pathologists to classify the tumors according to the World Health Organization criteria [21] with a consensus diagnosis being reached after discussion between the pathologists when a disagreement occurred. Consequently the histological diagnoses in these patients were: 28 cases of follicular adenoma (FTA), 12 cases of follicular adenoma — Hürthle cell variant

(HCA), 19 cases of follicular thyroid carcinoma (FTC) and 33 cases of follicular thyroid carcinoma — Hürthle cell variant (HCC). FTC and HCC were diagnosed by the presence of complete capsular and/or vascular invasion, and the absence of nuclear features of papillary thyroid carcinoma. Hürthle cell carcinomas were composed of greater than 75% oncocytic cells with moderate to abundant eosinophilic granular cytoplasm [22]. Fifteen out of the total of 52 follicular carcinomas were defined as minimally invasive (10 Hürthle cell carcinomas) due to the histological finding of a single focus of vascular invasion, a single focus of complete capsular invasion, or both.

Immunohistochemistry. Mouse monoclonal antibody against thyroid peroxidase (TPO mAb 47, Biocytex, Switzerland) was employed for immunostaining on 4–6 μm thick sections using the avidin-biotin peroxidase complex (ABC) technique with reagents supplied by Vector Laboratories (Burlingame, CA).

Following deparaffination and rehydration, endogenous peroxidase activity was blocked with 0.3% H_2O_2/methanol followed by non-immune horse serum for 20 min to block non-specific binding. The sections were then incubated with primary antibody against TPO at 4 °C overnight at a dilution of 1 : 50. This was followed by incubation with biotinylated horse anti-mouse IgG for 30 min and thereafter with the avidin-biotin-peroxidase complex (ABC reagents) for 30 min. Between each step, sections were washed three times in phosphate buffered saline (PBS). The reaction was visualized using 3, 3′-diaminobenzidine tetra hydrochloride (DAB) solution.

After counterstaining with haematoxylin, slides were dehydrated, coverslipped and examined using a Reichart-Jung microscope supplied with a Photostar automatic camera system. Controls were incubated with PBS in place of the primary antibody and no positive staining was observed.

Scoring of staining and statistical data. The percentage of positively stained cells was assessed in a semi quantitative fashion, i.e. the following system was employed to score the percentage of positive thyrocytes: (0) no staining, (1) less than 20% positive cells, (2) 21 to 50% positive, (3) 51 to 80% positive and (4) more than 80% positive. The cut-off values proposed by De Micco et al. [4] were applied. Thus, lesions were considered positive and hence allegedly benign, if more than 80% of the thyroid epithelial cells stained for TPO. Tissue staining of less than 80% of the follicular cells/specimen was considered malignant.

The sensitivity was defined as the number of malignant lesions with a negative TPO staining score as a fraction of the total number of true malignancies.

The specificity was defined as the number of true benign lesions with a positive TPO staining score as a fraction of the total number of true benign lesions.

Data were analyzed with the Statgraphics 4.2 statistical software. The nonparametric Mann-Whitney U-test was used to determine the significance of differences between groups. A value $P < 0.05$ was considered to be statistically significant.

RESULTS

The results of immunohistochemical staining for TPO protein expression in tissue sections of 92 follicular tumors are summarized in the Table. The 19 FTC showed great variability in the degree and intensity of staining. Thus, virtual absence of TPO positivity was observed in two specimens, while ten showed weak to moderate cytoplasmic positivity with more than 20% and less than 80% of stained cells, but seven showed positivity across the entire section (score 4). Three of these last seven cases, definitely positive for TPO staining, were minimally invasive carcinomas, but the remaining four were classified as widely invasive carcinomas.

Table. Immunohistochemical detection of TPO expression in follicular thyroid tumors

Expression grade	0	1	2	3	4	Total
			Cut off	⇓		
Malignant tumors						
FTC	2	1	4	5	7	19
HCC	9	9	8	3	4	33
Total	11	10	12	8	11	52
Dg confirmed by TPO immunohistochemistry: 41/52 (79%)						
Benign tumors						
FTA			2	7	19	28
HCA	1	1	3	2	5	12
Total	1	1	5	9	24	40
Dg confirmed by TPO immunohistochemistry: 24/40 (60%)						

Notes: FTC — follicular carcinoma; HCC — follicular carcinoma — Hürthle cell variant; FTA — follicular adenoma; HCA — follicular adenoma — Hürthle cell variant; Dg — diagnosis. The following system was employed to score the percentage of positive thyrocytes: (0) no staining, (1) 1 to 20% positive cells, (2) 21 to 50% positive, (3) 51 to 80% positive and (4) more than 80% positive. Tissue staining of more than 80% of the follicular cells/specimen was considered benign, less than 80% was considered malignant. FTC *vs* FTA $P = 0.01$; HCC *vs* HCA $P = 0.012$; total benign *vs* total malignant $P < 0.0001$.

Similar variability in TPO expression was also noted for oncocytic epithelial cells in HCC. Namely, virtual absence of TPO staining was observed in 9/33 specimens, while variable positivity occurred in the remaining cases. Two of four HCC cases definitely positive for TPO were widely invasive carcinomas. Thus, the number of TPO positive cases in the whole carcinoma group was 11/52, which means that TPO immunohistochemistry gave 21% false negative results in confirming the diagnosis of follicular cancer (Table, Figure, *c*, *d*). The sensitivity of TPO immunostaining for FTC and for HCC was 63 and 88%, respectively, with an average sensitivity of 79% for both types of carcinomas. In addition, no significant difference in TPO underexpression could be established between minimally invasive and widely invasive carcinomas ($p > 0.05$).

Among the 40 adenomas 24 cases were positive for TPO (including five cases of HCA) showing labeling across the entire section (Table, Figure, *e*, *f*). The remaining nine cases of FTA and seven cases of HCA expressed TPO under the threshold of 80% positive thyrocytes and would therefore be considered malignant from the immunostaining result (see Table). The specificity of TPO immunostaining for FTA and HCA was 68 and 42%, respectively, with an average specificity of 60% for the whole series of follicular adenomas.
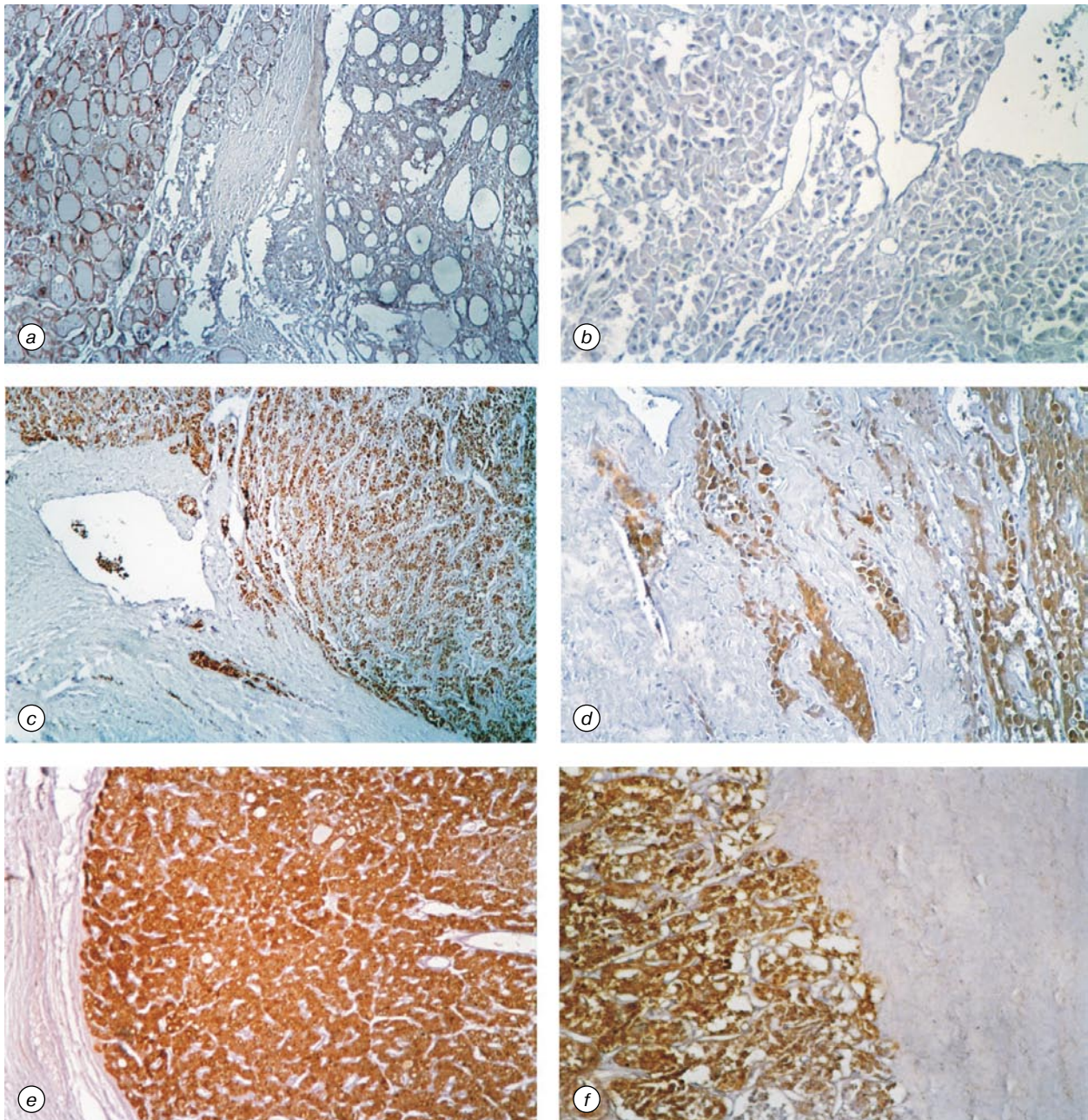
Figure. Immunohistochemical staining for thyroid peroxidase (TPO) in follicular thyroid carcinoma and follicular thyroid adenoma (indirect immunoperoxidase technique; hematoxylin diaminobenzidine counterstaining). (*a*): FTC case showing negative immunostaining for TPO (right) which confirms the malignant nature of the lesion and TPO positivity in nonmalignant surraunding tissue (left). *(b):* Complete TPO immunonegativity in one case of HCC. (*c*): Positive TPO staining in a case of widely invasive FTC failed to confirm the diagnosis. (*d*): Widely invasive HCC expressing TPO in more than 80% of thyrocytes. (*e*): Characteristic positive TPO immunostaining in FTA indicating a benign thyroid tumor. (*f*): HCA showing strong cytoplasmic immunopositivity for TPO present throughout the whole section which confirms the benign nature of the lesion (Original magnification *a, c, e* x 10 and *b, d, f* x 20)

Thus, TPO underexpression was seen in a significantly higher number of FTC cases than in its benign counterpart (FTA) (see Table; P = 0.01). Concerning Hürthle cell thyroid tumors, TPO underexpression was observed in 88% of HCC and in many (58%) HCA (P = 0.01). Thus, the proportion of immunoreactive cells for TPO in follicular carcinomas as a whole was significantly lower than that observed for the adenomas (P < 0.0001).

DISCUSSION

Immunohistochemical investigations of follicular thyroid neoplasms have detected many molecular markers for follicular carcinomas, but with varying de-grees of sensitivity and specificity for these neoplasms [23]. Among the molecular markers that appeared to yield high sensitivity and specificity values for thyroid tumors differential diagnostics was TPO. Thus, several studies using mAb 47 for the immunohistochemical determination of TPO indicated that it may be a potentially important diagnostic tool for thyroid cancer [1, 4, 6, 8, 17, 24, 25]. However, some later results showed disagreement between mAb 47 findings and the histological diagnosis of follicular tumors [3, 18–19, 20].

In this study, we focused on the utility of TPO immunostaining using mAb 47 as a tool for distinguishing between follicular thyroid adenoma and carcinoma,

including the Hürthle cell type. The results showed that TPO immunostaining correlated with the histopathological diagnosis in 24/40 cases of follicular adenomas giving a specificity of 60%. On the other hand, malignancy was correctly identified in 41 of 52 patients with follicular carcinomas, giving sensitivity of 79%. This is a higher incidence of TPO false-positivity for follicular cancer than previously observed in some studies [1, 4–6], but lower than reported by other authors [3, 20] with the same antibody and methodology. Namely, in Lima's series of follicular neoplasms with the same threshold for positivity applied (80%), it was noted that 46.2 % of the follicular thyroid carcinomas were of dismissed diagnosis [3]. Recently, Weber et al. [20] reported TPO staining sensitivity of only 11% for follicular thyroid carcinoma with a different threshold (less than 5% of follicular cells staining positively for TPO considered malignant). Moreover, variable expression of TPO was described in benign follicular adenomas, with specificity ranging from 68 to 96% [4, 18]. De Micco et al. [4] found that the specificity of TPO staining was greater in microfollicular adenomas (83.3%) than in oncocytic nodules (47.1%). In our series of adenomas, specificity for TPO protein in FTA was also higher (68%) than in HCA (42%), indicating a difference between these two types of benign tumors. Thus, in confirmation of the findings of De Micco et al. [4] and Faroux et al. [6], the practical role of TPO as a marker in Hürthle cell tumors may be questioned by its low specificity.

Evidence from earlier reports indicated that TPO protein is frequently underexpressed in human differentiated cancers and is particularly poorly expressed in more malignant and dedifferentiated tumor lesions [6, 11, 17]. Thus, no TPO expression was found in anaplastic thyroid carcinoma, the most aggressive thyroid cancer representing the last stage of thyroid epithelium dedifferentiation [3, 6, 26]. In addition, Weber et al. [20] found some evidence that the lack of expression of TPO in papillary cancer might predict a worse clinical outcome.

In our study, no relationship was found between TPO staining and the type of invasion in follicular carcinomas, because the widely invasive type, a highly aggressive malignancy, was not necessarily negative (see Figure c, d). We therefore failed to establish a significant decrease in TPO protein expression in follicular carcinoma with higher aggressiveness.

In summary, from our evaluation of the utility of TPO immunostaining, we conclude that the clinical use of this marker has limited diagnostic value and that the distinction between follicular thyroid adenoma and follicular thyroid carcinoma should be primarily based on classical histological criteria.

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ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ ПЕРОКСИДАЗЫ В КАЧЕСТВЕ МАРКЕРА ДЛЯ ОПРЕДЕЛЕНИЯ РАЗЛИЧИЙ МЕЖДУ ФОЛЛИКУЛЯРНОЙ КАРЦИНОМОЙ И ФОЛЛИКУЛЯРНОЙ АДЕНОМОЙ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

Цель: уровень экспрессии тироидной пероксидазы (ТПО) в ткани щитовидной железы является чувствительным маркером малигнизации этого органа. В работе представлено попытку оценки метода иммуногистохимический детекции ТПО для дифференциальной диагностики фолликулярной карциномы и фолликулярной аденомы щитовидной железы (ФКЩЖ и ФАЩЖ соответственно). *Материалы и методы:* срезы ткани, зафиксированные в формалине, были получены у 92 пациентов с опухолями щитовидной железы (52 случая — ФКЩЖ и 40 — ФАЩЖ, в том числе тип с клетками Хюртля). Для иммуногистохимического анализа этих срезов использовали моноклональные антитела против ТПО (TPOmAb47) и авидин-биотиновый комплекс. Препараты опухолей, содержащих более 80% позитивно окрашенных фолликулярных клеток, признавали доброкачественными, а те, что содержали менее 80% таких клеток, — злокачественными. *Результаты:* интенсивность иммуноокрашивания препаратов коррелировала с гистопатологическим диагнозом в 24 из 40 случаев ФАЩЖ и в 41 из 52 случаев ФКЩЖ. При этом чувствительность метода составляла 79%, специфичность — 60%. *Выводы:* иммуногистохимический анализ ТПО имеет недостаточную специфичность для дифференциальной диагностики фолликулярной карциномы и фолликулярной аденомы.

Ключевые слова: щитовидная железа, фолликулярная карцинома щитовидной железы, тироидная пероксидаза, молекулярный маркер, иммуногистохимия.