CASE REPORT

The histopathology analysis of the diffuse sclerosing variant of the papillary carcinoma of the thyroid: a distinctive and rare form

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Abstract
Diffuse sclerosing variant (DSV) is a rare variant of papillary thyroid carcinoma (PTC) and its features have not yet been fully characterized. The aim of this case report is to analyze the immunohistochemistry profile of this disease and to highlight this rare entity. We analyzed the histology and immunohistochemistry of a female patient admitted in the Surgery Department of the "Sf. Ioan" Emergency Hospital, Bucharest, in April 2008. We especially used a wide panel of antibodies (Thyroglobulin mono, Thyroglobulin poly, Ki-67, TTF 1, Cytokeratin 19, Cytokeratin 34betaE12, and p63) in order to point out the follicular origin of the cells and to investigate the extensive squamous metaplasia lesions. The immunohistochemistry (IHC) was performed on 3 µm thick sections from 10% formalin-fixed paraffin-embedded tissues, according to the indirect tristadial Avidin–Biotin–Complex method. Our case report reveals that the DSV of PTC has distinctive pathologic features and its diagnosis importance is suggested by the high incidence of recurrence after operation.

Keywords: thyroid, papillary carcinoma, diffuse sclerosing variant, immunohistochemistry.

Introduction
Papillary carcinoma is the most common well-differentiated cancer of the thyroid. Papillary/follicular carcinoma must be considered a variant of the papillary thyroid carcinoma [1]. Despite its well-differentiated characteristics, papillary carcinoma may be overtly or minimally invasive [2].

Thyroid cancers are quite rare, accounting for only 1.5% of all cancers in adults and 3% of all cancers in children. During the last few years, the frequency of papillary cancer has increased, but this growth is related to an improvement in the diagnostic techniques and the information campaign about this carcinoma [3]. Of all thyroid cancers, 74–80% are papillary. Thyroid cancer is usually curable. Most thyroid cancers grow slowly and the mean survival rate after 10 years is higher than 90% [3].

Considering its history, it is very important to state that numerous cases of papillary thyroid cancer are subclinical and the most common presentation is an asymptomatic thyroid mass or nodule that can be felt in the neck. At the same time, it is necessary to obtain a history regarding prior exposure to ionizing radiation and/or a family history of thyroid cancer [4].

At the time of diagnosis, 10–15% of patients have distant metastases to the bones and lungs [5].

The lab studies include the investigation of the thyroid function and the TSH suppression test.

The study of echography imaging must be the first performed on any patient with possible thyroid malignancy. It is non-invasive and inexpensive and represents the most sensitive procedure for identifying thyroid lesions and determining the diameter of a nodule.

Before the fine needle aspiration biopsy (FNAB), the initial diagnostic procedure of choice for a thyroid evaluation is thyroid scintigraphy (or thyroid scanning), performed with technetium 99mTc-pertechnetate (99mTc) or radioactive iodine (iodine I 131 or iodine I 123) [5]. The procedure is not as sensitive or specific as FNAB for distinguishing benign nodules from malignant ones.

FNAB is considered the best first-line diagnostic procedure for a thyroid nodule, being a safe and minimally invasive procedure [6, 7].

Surgery is the definitive management of thyroid cancer, and various types of operations may be performed: lobectomy with isthmectomy, subtotal and total thyroidectomy [4].

The diffuse sclerosing variant (DSV) is a rare morphologic variant of papillary carcinoma, characterized by diffuse involvement of one or both thyroid lobes, dense sclerosis, abundant psammoma bodies, extensive solid foci, squamous metaplasia, and heavy lymphocytic infiltration [3]. Clinically, it may be misdiagnosed as Hashimoto’s thyroiditis. Nodal metastases are nearly always present, lung metastases are common, and the disease-free survival rate is lower than for conventional papillary carcinoma [8].
Patient and Methods

We present the case of a 57-year-old female, which was admitted in our hospital with the clinical diagnosis of left lobe thyroid nodule (suspicion of papillary thyroid carcinoma).

Her personal history revealed diabetes mellitus type II, autoimmune primary myxedema, level II hypertension and anemia.

The patient had thermophobia, bradykinesia and was feeling fatigue. The serum level of TSH (thyroid stimulating hormone) was 65.14 mIU/dL (reference range is 0.2–4.7 mIU/dL).

An echography and a scintigraphy were performed and these procedures showed the presence of a suspect nodule of 27/27 cm and a cyst of 7 cm.

A cytology exam by fine-needle aspiration was performed, revealing an epithelial proliferation, with compacting trabecular pattern, of large dimensions, abnormal nucleus and few mitoses.

Surgery is the definitive management of thyroid cancer, and a total thyroidectomy was performed (removal of all thyroid tissue, but preserving the contralateral parathyroid glands). It was not necessary to practice lymph nodes dissection because the patient had no detectable lymph nodes during surgery. The macroscopic examination of the tumor showed the presence of a whitish pseudo-encapsulated nodule, firm, and without colloid on one lobe. The other lobe had a normal macroscopic aspect.

Written informed consent was obtained from the patient before processing and analyzing the specimens, using consent forms and protocols approved by the Hospital Committee of Ethics in Medical Research.

The specimen was fixed in 10% buffer formalin and paraffin-embedded, using the standard histological procedure. Five-μm thick section steps were stained using routine morphologic methods (Hematoxylin–Eosin). Afterwards, microscopic examination immunohistochemistry (IHC) was performed using several antibodies.

The antibodies applied and their working dilutions are listed in Table 1. Reactions were revealed using working system LSAB; DAB was used as chromogen.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Dilution</th>
<th>Clone</th>
<th>Provider</th>
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<tbody>
<tr>
<td>Thyro mono</td>
<td>Follicular cells</td>
<td>1/100</td>
<td>DAK-Tg6</td>
<td>DAKO</td>
</tr>
<tr>
<td>Thyro poly</td>
<td>Follicular cells</td>
<td>1/250</td>
<td>Polyclonal Neomarker</td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>Nuclear proliferating factor</td>
<td>1/50</td>
<td>Mib-1</td>
<td>DAKO</td>
</tr>
<tr>
<td>TTF 1</td>
<td>Follicular cells</td>
<td>1/100</td>
<td>SPT24</td>
<td>Novocastra</td>
</tr>
<tr>
<td>CK 19</td>
<td>Papillary carcinoma</td>
<td>1/50</td>
<td>RCK 108</td>
<td>DAKO</td>
</tr>
<tr>
<td>CK 34betaE12</td>
<td>Squamous metaplasia</td>
<td>1/50</td>
<td>34betaE12</td>
<td>DAKO</td>
</tr>
<tr>
<td>P63</td>
<td>Squamous metaplasia</td>
<td>1/20</td>
<td>4A4</td>
<td>Biogenex</td>
</tr>
</tbody>
</table>

Result

Surgical treatment of the patient included total thyroidectomy. Modified radical lymph node dissection was not performed because the patient comprised any conditions as (1) clinically detectable lymph nodes, (2) suspicious lymph nodes noted on preoperative ultrasonography, and (3) lymph nodes found during active intraoperative sampling.

The thyroid gland tumoral lobe was enlarged, with ill-defined nodular masses replacing much of the thyroid parenchyma.

The predominant histological pattern was characterized of the cytomorphologic features of thyroid papillary carcinoma (Figure 1) which included nuclear enlargement, irregularities in nuclear size and shape, dispersed to optically clear appearing nuclear chromatin, crowding or overlapping nuclei, nuclear grooves, and intranuclear cytoplasmic inclusions.

The microscopic examination showed a diffuse proliferation of the tumor cells, with papillary distribution (Figure 1), without colloid, vascular metastasis, abundant lymphocyte and plasma cells infiltration, multiple small islands of papillary thyroid carcinoma (Figure 1), diffuse sclerosis, psammoma bodies (Figure 2) and multiple foci of squamous metaplasia with keratinisation (Figure 3).

Light microscope examination showed diffuse stromal sclerosis and diffuse infiltration of the thyroid by lymphocytes forming secondary follicles with islands of squamous cells in vascular and lymphatic spaces, but the striking feature was the presence of numerous psammoma bodies.

The second lobe, which had a normal macroscopic examination, showed an aspect of diffuse colloid goiter and an infiltration of islands compounded of PTC and rich squamous metaplasia, lymphoid infiltration and many psammoma bodies. Tumor necrosis was absent.

The immunohistochemical profile excluded a metastasis from the breast (Figures 4 and 5), and pointed out the histogenesis of the tumor (Figures 6 and 7), the follicular cells (Figures 4 and 5).

Sections were intensely stained for all antibodies, diffuse or focal; the reactivity usually was confined to follicular cells (Thyro, TTF 1) and to the squamous metaplastic foci (Figures 8–10).

All of the foci of papillary carcinoma and the areas of squamous metaplasia were immunoreactive with keratin (Figure 8).

Furthermore, these same areas were all immunoreactive with both CK 19 (Figures 6 and 7) and TTF 1 (Figure 5), that support to the notion that the squamous metaplasia is arising from thyroid follicular epithelial cells.

Thyroglobulin is a capricious stain antibody with difficulties in interpretation related to its presence in the serum and therefore, the tendency for a high background. In spite of this difficulty, thyroglobulin was immunoreactive in nearly all of the foci of papillary carcinoma and in a few of the foci of squamous metaplasia (Figure 5).

Ki-67 was positive in a few nuclei in both the papillary carcinoma and areas of squamous metaplasia in the evaluated case. None of the areas of squamous differentiation were highlighted with the Ki-67.
Although axiomatic, immunohistochemistry was not requisite for the diagnosis of diffuse sclerosing variant of papillary thyroid carcinoma, but only for differentiated and support diagnosis.

Figure 1 – Typical features of papillary thyroid carcinoma, and benign follicles with colloid, abundant inflammatory infiltrate (HE stain, ob. 4×).

Figure 2 – A psammoma body between two solid squamoid aggregates (HE stain, ob. 10×).

Figure 3 – An obvious squamoid metaplasia island (HE stain, ob. 10×).

Figure 4 – Positivity for TTF 1 antibody in follicular cells (ob. 10×).

Figure 5 – The immunostaining for Thyro mono (ob. 10×).

Figure 6 – Perimembrane immunostaining for CK 19, in carcinoma area (ob. 20×).

Figure 7 – Perimembrane strong immunostaining for CK 19, in carcinoma area (ob. 4×).

Figure 8 – Immunostaining for CK 34betaE12 (ob. 4×).
Discussion

The current accepted diagnosis of PTC is based on nuclear features that include optical clearing, elongation, overlapping, micronucleoli, and irregular contours with grooves and pseudo-inclusions [9]. However, the identification of these features remains controversial and some markers have been proposed as useful means of diagnosis of PC [10].

The key morphological feature, wide spread lymphatic permeation of the thyroid, is clearly related to the incidence of nodal spread and post-therapy disease persistence. The diffuse lymphatic permeation also causes the diffuse and sometimes painful enlargement of the thyroid mimicking a thyroiditis clinically. The presence of elevated levels of antibodies in some cases complicates the issue. The lymphocytic infiltration and the raised antibody titers could be the result of an antigenic response to the tumor. As for the view that DSPC develops in a Hashimoto’s thyroiditis, Hurthle’s cells, which are a feature of Hashimoto’s thyroiditis, are absent in DSPC.

Few prognostic scoring systems have been elaborated in order to determine the process of choosing the appropriate treatment [11–13].

The most important scoring systems are: AGES [14] (Table 2), AMES [8] (Table 3), and MACIS [15] (Table 4).

In 1985, Vickery AL Jr et al. described a new variant of papillary thyroid carcinoma (PTC), for which they proposed the term diffuse sclerosis [20]. Diffuse sclerosing variant (DSV) of PTC is an uncommon variant of PTC that shows diffuse involvement of the thyroid, dense sclerosis, extensive squamous metaplasia, patchy to dense lymphocytic infiltrate, numerous psammoma bodies, and marked lymphatic permeation during histological examination [21, 22]. Survival data from the literature for patients with DSV is derived mainly from case reports and small reported series [23]. The clinical behavior of this variant of PTC remains uncertain [1].

Concerning the immunohistochemical markers used for the PTC diagnosis [16], some studies have proposed HBME-1, specific cytokeratins (CK) [17], such as CK 19 and ret; the latter two markers each identify a subpopulation of PC [18, 19].

<table>
<thead>
<tr>
<th>Table 2 – Scoring system AGES</th>
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<tbody>
<tr>
<td>Prognostic score $= 0.05 \times \text{age (if age } \geq 40)$</td>
</tr>
<tr>
<td>$+1$ (if grade 2)</td>
</tr>
<tr>
<td>$+3$ (if grade 3 or 4)</td>
</tr>
<tr>
<td>$+1$ (if extrathyroidal)</td>
</tr>
<tr>
<td>$+3$ (if distant spread)</td>
</tr>
<tr>
<td>$+0.2 \times \text{tumor size (cm maximum diameter)}$</td>
</tr>
<tr>
<td>Survival by AGES score (20- yrs.):</td>
</tr>
<tr>
<td>$\leq 3.99 = 99%$</td>
</tr>
<tr>
<td>$4-4.99 = 80%$</td>
</tr>
<tr>
<td>$5-5.99 = 67%$</td>
</tr>
<tr>
<td>$\geq 6 = 13%$</td>
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</table>

AMES – patient age, histological grade of the tumor, tumor extent (extrathyroidal invasion or distant metastases) and size of the primary tumor.

<table>
<thead>
<tr>
<th>Table 3 – Scoring system AMES</th>
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<tbody>
<tr>
<td>Younger patients (men $=\leq 40$, women $=\leq 50$) with no metastases</td>
</tr>
<tr>
<td>Older patients (intrathyroid papillary, minor capsular invasion for follicular lesions)</td>
</tr>
<tr>
<td>Primary cancers $&lt;5 \text{ cm}$</td>
</tr>
<tr>
<td>No distant metastases</td>
</tr>
<tr>
<td>All patients with distant metastases</td>
</tr>
<tr>
<td>Extrapapillary papillary, major capsular invasion follicular</td>
</tr>
<tr>
<td>Primary cancers $\geq 5 \text{ cm}$ in older patients (men $&gt;40$, women $&gt;50$)</td>
</tr>
<tr>
<td>Survival by AMES risk-groups (20- yrs.):</td>
</tr>
<tr>
<td>Low risk = 99%</td>
</tr>
<tr>
<td>High risk = 61%</td>
</tr>
</tbody>
</table>

AMES – patient age, presence of distant metastases, extent and size of primary tumor.

<table>
<thead>
<tr>
<th>Table 4 – Scoring system MACIS</th>
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<tbody>
<tr>
<td>Score $= 3.1$ (if age $&lt;40$ years) or $0.08 \times \text{age (if age } \geq 40$ years)</td>
</tr>
<tr>
<td>$+0.3 \times \text{tumor size (cm maximum diameter)}$</td>
</tr>
<tr>
<td>$+1$ (if incompletely resected)</td>
</tr>
<tr>
<td>$+1$ (if locally invasive)</td>
</tr>
<tr>
<td>$+3$ (if distant spread)</td>
</tr>
<tr>
<td>Survival by MACIS score (20- yrs.):</td>
</tr>
<tr>
<td>$&lt; 6 = 99%$</td>
</tr>
<tr>
<td>$6-6.99 = 89%$</td>
</tr>
<tr>
<td>$7-7.99 = 56%$</td>
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<tr>
<td>$\geq 8 = 24%$</td>
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MACIS – metastases, patient age, completeness of resection, local invasion and tumor size (Mayo Clinic).
The immunoreactivity for CK 19 is not specific for PTC, but it is a useful ancillary technique for diagnosis of PTC. Focal CK 19 staining may be found in benign lesions, but diffuse positivity is characteristic of PC.

HBME-1 is a marker of mesothelial cells. Several studies have demonstrated its utility as a marker of malignant thyroid tumors of follicular epithelial derivation [24]. HBME-1 positivity in thyroid follicular epithelial tumors is, therefore, indicative of malignancy, but it does not necessarily indicate papillary differentiation [24].

Papillary carcinomas have been shown to express CK 19 with strong diffuse cytoplasmic reactivity, which is characteristic in PC [25, 26]. This marker is expressed focally in reactive thyroid follicular epithelium; however, most authors have not identified diffuse CK 19 positivity in follicular adenomas or carcinomas [27, 28].

The Ret /PTC family of chimeric oncogenes results from several rearrangements involving the ret gene of chromosome 10q; they are specific to papillary carcinomas and are present in up to 77% of these tumors [29]. All protein products of the ret/PTC oncogenes contain the intracellular tyrosine kinase domain of the normal ret proto-oncogene product and can therefore be immunohistochemically detected with an antibody to the carboxyl terminus of Ret [29].

The expression of the CD44 molecule has been correlated to carcinogenesis and aggressive biological behavior of several malignant tumors. CD44 is a polymorphic family of cell surface proteoglycans and glycoproteins implicated in cell–cell and cell–matrix adhesion interactions, lymphocyte activation and homing, cell migration and tumor metastasis [30]. CD44 protein is encoded by a gene located on 11p13 chromosome and consists of at least 20 exons. Interest has recently been focused on the abnormal transcription of CD44 variants by cancer cells, and on their role in local invasion and metastatic dissemination. Alternations in the composition of CD44 protein and of its isoforms are associated with neoplastic transformation and metastasis in a number of different tissues. So, increased expression of CD44 has been implicated in melanoma metastasis, pancreatic adenocarcinomas, and colorectal carcinomas. On the other side, the decreased expression of CD44 is related to tumor recurrence and unfavorable outcome in poorly differentiated squamous cell and laryngeal carcinomas, as well as in superficial bladder and prostate carcinomas [31]. Some studies indicate that most papillary carcinomas of the thyroid express the cell-adhesion molecule CD44, in contrast to other neoplastic and non-neoplastic thyroid lesions and normal follicular cells [32]. Therefore, it seems that the deregulated expression of CD44 plays a role in the carcinogenesis of the thyroid gland and possibly contributes to the mild biological behavior of differentiated papillary carcinomas [32].

P63 proteins are p53 homologues that are postulated to regulate squamous stem cell commitment [33]. An immunohistochemical survey of p63 expression in normal thyroid and in reactive, neoplastic and inflammatory thyroid disorders was performed. The results showed that the p63 expression was negative in normal thyroid tissue, nodular goiters and oncocytic follicular adenomas. Positivity was rare and weak in follicular adenomas. Twenty-seven of 33 papillary thyroid carcinomas (81.8%) displayed p63-positive foci. All squamoid structures were p63-positive. The finding of p63 in benign squamoid nests supports a possible inter-relationship between these structures and Hashimoto’s thyroiditis and papillary carcinoma [34]. P63 is detected in squamoid aggregates and solid cell nests, and the high percentage of papillary carcinomas with p63-positive foci appears to distinguish papillary carcinoma from other neoplasms originating in the thyroid.

The positivity of p63 in SCNs (solid cell nests) indicates the commitment of p63-positive undifferentiated cells toward thyroid follicular epithelial differentiation, and also the presence of a subset of PCs which is closely homologous to stem cell-associated p63 staining pattern (described in squamous and bronchial epithelia). The p63-positive cells are pluripotent and may stay undifferentiated or may undergo benign squamoid or glandular maturation, thyroid follicular epithelial differentiation, oncogenic change leading to PC, or may trigger an immune reaction (as in Hashimoto’s thyroiditis) [34].

Conclusions

The case report presents the diffuse sclerosing variant of papillary thyroid carcinoma.

DSPC is an unusual type of papillary carcinoma that occurs especially in young patients. Clinically it can be mistaken for Hashimoto’s thyroiditis as it often presents as a diffuse firm thyroid swelling. It is important to recognize this rare variant as the patients invariably have lymph node metastasis at the time of diagnosis. Contrary to the previous reports of a poor prognosis of this variant when compared to the classical papillary carcinoma, recent reports indicate a good prognosis. DSV–PTC is more biologically aggressive than conventional PTC, although the patients’ survival is not significantly different.

The prognostic of the disease seems to be as good as that of classic PTC after complete surgical treatment and postoperative radiiodine ablation. Close follow-up is necessary because of the frequent tumor recurrence and the association with unfavorable pathologic features. This diagnosis should lead the clinician to aggressively manage these patients (thyroidectomy and lymph node dissection) in an effort to achieve an excellent long-term clinical outcome.

In conclusion, the presented case report reveals the importance of this histopathological entity first, albeit of the “biologically” aggressive presentation, these patients have an excellent long-term clinical outcome, and the secondly, the immunohistochemical useful tool for the analysis of difficult thyroid nodules.

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References


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