

## ORIGINAL PAPER

# The value of the immunohistochemical exam in the diagnosis of the secondary malignant tumors to the thyroid gland

DELIA CIOBANU<sup>1)</sup>, CARMEN VULPOI<sup>2)</sup>, B. GĂLUȘCĂ<sup>2)</sup>, NICULINA FLOREA<sup>3)</sup>,  
SIMONA-ELIZA GIUȘCĂ<sup>4)</sup>, IRINA-DRAGA CĂRUNTU<sup>5)</sup>

<sup>1)</sup>Department of Pathology

<sup>2)</sup>Department of Endocrinology

"Gr. T. Popa" University of Medicine and Pharmacy, Iassy

<sup>3)</sup>Department of Pathology

<sup>4)</sup>S<sup>c</sup> Surgery Clinic

"Sf. Spiridon" University Hospital, Iassy

<sup>5)</sup>Department of Histology,

"Gr. T. Popa" University of Medicine and Pharmacy, Iassy

### Abstract

The aim of the study was to analyze several cases of secondary tumors to the thyroid, by focusing on the role of the immunohistochemical (IHC) exam in specifying the origin of the tumoral process. The studied group included 16 patients, investigated by fine-needle aspiration biopsy, frozen sections at the surgical moment, routine histopathological exam and immunohistochemical staining, using different antibodies, in accordance with the histological aspects. The final diagnosis was established as follows: metastases of squamocellular carcinoma with different degree of differentiation (seven cases), metastases of adenocarcinoma (four cases), metastases of renal cell carcinoma (two cases), metastases of Hodgkin (one case) and non-Hodgkin lymphoma (two cases). In four cases, the primary tumors were identified after the diagnosis of their metastases in thyroid. The immunohistochemical staining was useful in the diagnosis of squamocellular carcinoma metastases, poorly differentiated (CK19 positive), of renal cell carcinoma with clear cells (CK18, CK19 and CD10 positive) and in the establishing of the tumoral origin for adenocarcinomas (CK7 positive – respiratory tract, CK20 positive – digestive tract). Secondary tumors to the thyroid are rare tumors, with miscellaneous histological aspects, reason for which the diagnostic may be difficult. In these cases IHC is a useful method, allowing to the identification of the primary tumor.

**Keywords:** secondary tumors, thyroid, immunohistochemistry.

### Introduction

Metastases to the thyroid gland are rare, despite its rich vascular supply. Some authors even consider this a direct consequence of the rapid blood flow through the gland, estimated at around 560/100 grams of tissue/minute [1].

Secondary tumors to the thyroid gland are found at autopsy in up to 25% of patients, but in clinical series the frequency of secondary involvement of the gland is considerably lower [2].

The main pathways in which a tumor could develop metastasis to the thyroid are by contiguity, or via the lymph or the blood. Kidney, breast and lung are the most frequent primary sites from which thyroid metastases occur, due to the blood, whereas pharynx, trachea, esophagus, latero-cervical lymph nodes or soft tissue and mediastinum are the most common primary sites that can metastasize by direct extension [2].

In the series of cases reported by Lam KY *et al.* [3], the majority secondary tumors were carcinomas (81%), with primary sites in lung (43%), followed by the breast (9%) and stomach (8%). Rare sources of primary tumors, such as nasopharyngeal carcinoma,

choriocarcinoma, malignant phyllodes, and osteosarcoma, were also found [3].

Preoperative distinction between a primary *versus* secondary thyroid neoplasm is often impossible, because they may present non-specific symptoms or they may be asymptomatic and be found accidentally during a computer-tomography scan. Even so, it is very hard to make the difference between a malignant and a benign process such as multinodular goiter.

Starting from our personal experience in the thyroid pathology [4] and from other studies on metastases in thyroid gland, reported in the literature [5–12], the purpose of this study was the reevaluation of some cases of secondary tumors to the thyroid, by focusing on the role of the immunohistochemical (IHC) exam in specifying the origin of the tumoral process.

### Material and methods

The studied group included 16 cases of metastases to the thyroid gland diagnosed in the Department of Pathology of the "Sf. Spiridon" University Hospital Iassy, during a period of eight years (1999–2006).

Thirteen of the 16 patients had been initially investigated by fine-needle aspiration biopsy (FNAB). Preoperative FNAB were performed with a 22-gauge needle attached to a 30 ml plastic syringe. The aspirated fluid was expelled onto slides fixed on air and stained with a rapid method May–Grünwald Giemsa. At the surgical moment, for a preliminary diagnosis, frozen sections were performed on 10 of the 16 patients.

The corresponding surgical samples of all patients were formalin fixed and paraffin embedded for routine histopathological exam and immunohistochemical (IHC) staining, using the following antibodies (DAKO): thyroglobulin (Tg), thyroid transcription factor-1 (TTF-1), cytokeratin (CK7, CK18, CK19, CK20), galectin 3 (Gal-3), neuron specific enolase (NSE), CD10, CD20, CD45RA, calcitonin and chromogranin and the streptavidin–biotin method (LSAB Kit, DAKO).

## ✉ Results

The average age of the patients was of 56.3 years, and the sex ratio was F/M = 9/7. On clinical examination, for 10 of 16 patients we suspected a secondary tumor to the thyroid – in these 10 cases the thyroid function had not been affected and the patients showed known pulmonary or digestive tumoral lesions. The echographic exam indicated solitary nodular lesions in nine cases, multiple nodular lesions in three cases and diffuse lesions in four cases.

The FNAB represented a method of preliminary diagnosis in a ratio of 75% (12 cases), the smears being characterized as malign in 10 cases (83.3%). In two cases, the cellular material was made only of necrotic detritus and free nuclei in lysis, which did not allow the cytological interpretation and determined the recommendation of repeating the aspiration.

As for the other cases, the smears showed obvious features of malignancy in the cellular proliferation and were accompanied by abundant necrotic detritus, which represented the background of the smears. Hereby, we identified clusters or aggregates in which the nucleus/cytoplasm ratio was reversed, nuclei showed various forms and sizes, with a nuclear chromatin unequally distributed, frequent nucleoli and rare mitosis.

In seven cases (58.3%), we supposed a secondary determination at thyroid level, because of the absence of cytological elements that could indicate a thyroid primary tumor. The clear cases were the well-differentiated squamocellular carcinomas, which presented, beside the cellular tumoral clusters, frequent squamous cells. The accuracy ratio of frozen section diagnosis was 100%, but only for the squamocellular carcinoma could be indicated the primary tumor origin.

On the macroscopic exam of the surgical pieces, we identified single or multiple nodular lesions in 12 cases (corresponding to the results obtained from the echographic exam). In four cases we did not find any macroscopic lesion (corresponding to the diffuse lesions observed at the echographic exam), the presence of a secondary thyroid tumor was being revealed by the microscopic exam.

The final diagnosis was established accurately

through the standard pathological and immunohistochemical exams, as follows: metastases of squamocellular carcinoma with different degree of differentiation (seven cases – 43.75%), metastases of adenocarcinoma (four cases – 25%), metastases of renal cell carcinoma (two cases – 12.5%), metastases of Hodgkin (one case – 6.25%), and non-Hodgkin lymphoma (two cases – 12.5%).

### Metastases of squamocellular carcinoma

These metastases showed different degrees of differentiation, from well-differentiated, with keratin pearls, to poorly-differentiated, with large areas of necrosis and focal keratinization. A case was characterized by a proliferation of small cells, having the form of lymphocytes, associated with a reduced stromal reaction. This morphologic aspect suggested a malign non-Hodgkin lymphoma.

The Gordon Sweet stain was useful to demonstrate the epithelial origin of the tumoral process, the reticulin fibres marking the limits of the cellular clusters. For all seven cases, the IHC exam made by using CK19 (Figure 1) and CK7 revealed an intense positive reaction at the level of the tumoral islands and negative reaction for Tg (Figure 2), TTF-1 (Figure 3), NSE, calcitonin and chromogranin.

The immunostaining had a diffuse character for CK19 and a focal one for CK7. As a result of this peculiarity and of the paraclinical investigations made afterwards, two cases were classified as metastases of laryngeal squamocellular carcinoma, the other five - as metastases of pulmonary squamocellular carcinoma.

### Metastases of adenocarcinoma

The routine microscopic exam revealed the presence of a tubular tumoral pattern or papillary isolated structures, with a marked cellular pleomorphism and an abundant stromal reaction.

The papillary architecture remained a primary papillary thyroid, fact that imposed special stains followed IHC. The alcian blue and the PAS stains proved the production of neutral and acid mucins, sustaining a digestive or a pulmonar origin of the tumoral process. In all the four cases, the IHC exam was made by using CK7 (specific to the respiratory tract) and CK20 (specific to the digestive tract).

The IHC reactions were strongly positive for CK20 (Figure 4) and negative for CK7 (Figure 5), Tg (Figure 6), TTF-1, calcitonin and chromogranin. Consecutively, the four cases were classified as adenocarcinoma metastases with a digestive origin.

### Metastases of renal cell carcinoma

On the background of multinodular goiters with secondary degenerative modifications, the two examined cases showed multiple focuses with cellular aggregates, characterized by clear cytoplasm and central hyperchromatic nuclei, associated with frequent intravascular tumoral emboli.

The IHC reactions were intensely positive for CK18 (Figure 7) and CK19 (Figure 8), moderate positive for CD10 (Figure 9) and negative for Tg,

TTF-1, CK7, CD15. In both cases, the thyroidian expression represented the first sign of a renal tumoral process, confirmed by supplementary echografic and radiologic exams.

### Metastases of Hodgkin and non-Hodgkin lymphoma

In the case diagnosed as Hodgkin lymphoma, the FNAB showed obviously malign characters and a surgical intervention was recommended. On the histological sections a double tumoral pathology was observed: a well differentiated papillary thyroid carcinoma and an abundant lymphocitar infiltrate with frequent eosinophilic polymorphonuclears, plasma cells, classical Sternberg-Reed cells or their mononucleate variant – Hodgkin cells.

The adjacent thyroidian tissue presented Hashimoto thyroiditis, and the lymph nodes revealed the Hodgkin disease, as well as pappillary thyroid metastases.

The IHC showed positive reaction for Gal-3 in papillary thyroid carcinoma areas (Figure 10) and the Sternberg-Reed cells was positive for CD45RA (Figure 11); the reactive lymphoid component was B-type, CD20 positive (Figure 12) and negative for Tg (Figure 13).

The cases diagnosed as non-Hodgkin lymphoma revealed a tumoral lymphocytic infiltrate with a diffuse disposition, made out of a monomorphic cellular population, characterized by small size, round nuclei with multiple nucleoli and a basophylic cytoplasm.

The IHC staining was strongly positive for CD20 (Figure 14). The lymph nodes with a modified architecture presented the same tumoral lymphocytic population.

### ☞ Discussions

Menegaux F [5] considered that only the tumors that occur in the thyroid gland because of lymph or vascular spread from distant sites are true metastases, not those appeared from direct tumoral invasion. Metastatic tumors were identified at a higher frequency in abnormal glands, with adenomatoid nodules, thyroiditis, and follicular neoplasms [5].

In our group, most of the studied cases (13 cases) appeared on a hipofonctional, colloid goiter background, two cases presented, in association, the Hashimoto thyroiditis and only one case preserved normal thyroidian tissue. Further, metastatic tumors may be found within primary thyroid lesions, such as renal cell carcinoma metastases localized in a thyroid papillary carcinoma [5].

In our group, one case alone showed a double tumoral pathology, namely a papillary thyroid carcinoma (primary tumor) and a Hogkin lymphoma (secondary tumor). Multifocal and bilateral disease is common, although some single masses are seen [2, 5]. Among the cases we investigated, five patients had a bilateral, and the others, an unilobular thyroidian involvement.

According to Baloch ZW and LiVolsi VA [6], although a thyroid gland mass may be the first clinical sign for a secondary malignancy, it is more often the underlying thyroid diseases (e.g., thyroiditis, adenomatoid nodules) that prompts clinical evaluation. The metastases at the thyroid level are the initial presentation of an occult primary tumor in as many as 40% of patient [6]. In our group, only in four cases (25%) the primary tumors were identifying after the diagnosis of their metastases in thyroid.

In order of frequency, the most common metastases to the thyroid [7–11] are carcinoma origin from kidney, lung, breast, and stomach; melanoma and lymphoma are less common. In the studied group, the most numerous metastases at the thyroid's level came from the respiratory tract's level (seven cases), followed by those from the intestinal tract's level (four cases), from lymphoid tissue (three cases) and from the kidney (two cases).

The metastatic lesions morphologically and architecturally resemble the primary site, yielding a distinctly different histologic appearance from primary thyroid tumor [2].

However, clear-cell carcinomas (e.g., renal cell carcinoma), small-cell carcinomas (e.g., neuroendocrine carcinoma) and tubular-papillary carcinoma (e.g. pulmonary and digestive carcinoma) may resemble a primary thyroid gland tumor [2].

In such circumstances, IHC analysis will help in the separation between primary and metastatic tumors: primary thyroid follicular tumors will usually be immunoreactive with thyroglobulin, CK7, and TTF-1, while C-cell – derived tumors will be reactive to calcitonin and chromogranin. With incredibly rare exception, metastatic tumors will not be thyroglobulin-reactive [2].

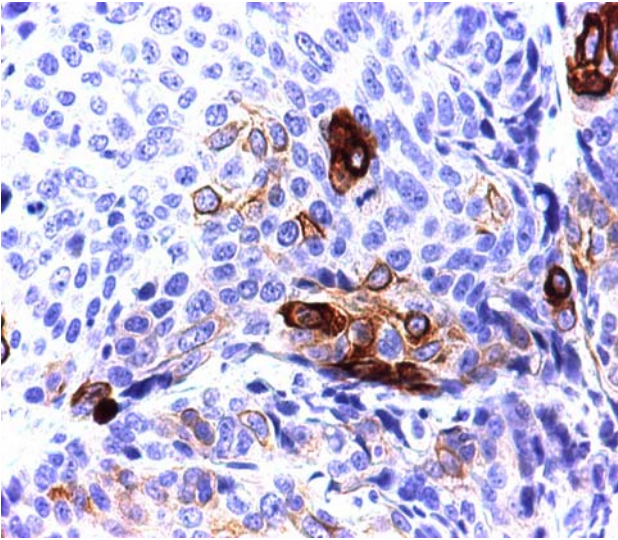
Nevertheless, the data reported in the literature focused on the IHC differentiation of the secondary malignancy to the thyroid are scarce. Recently, Booya F *et al.* [12] used the method in order to distinguish between the primary and secondary squamous cell carcinoma, respectively.

In our group, IHC staining was useful in the diagnosis of squamocellular carcinoma metastases, poorly differentiated (CK19 positive), of renal cell carcinoma with clear cells (CK18, CK19 and CD10 positive) and in the establishing of the tumoral origin for adenocarcinomas (CK7 positive – respiratory tract, CK20 positive – digestive tract).

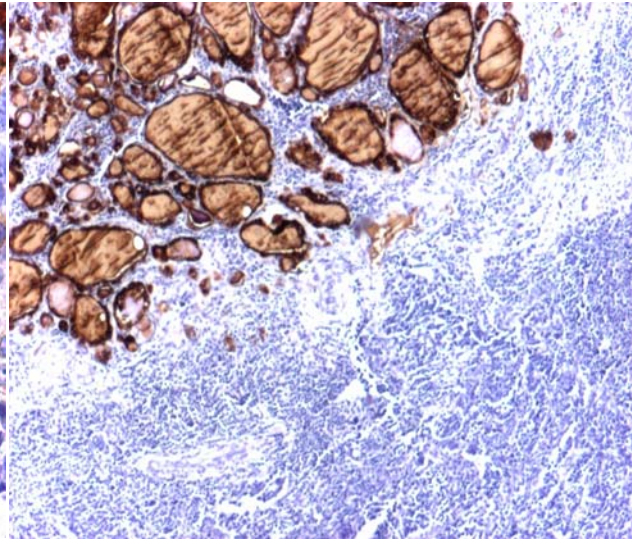
### ☞ Conclusions

Secondary tumors to the thyroid are rare tumors, with miscellaneous histological aspects, reason for which the diagnostic may be difficult. In these cases, IHC is a useful method, allowing to the identification of the primary tumor.

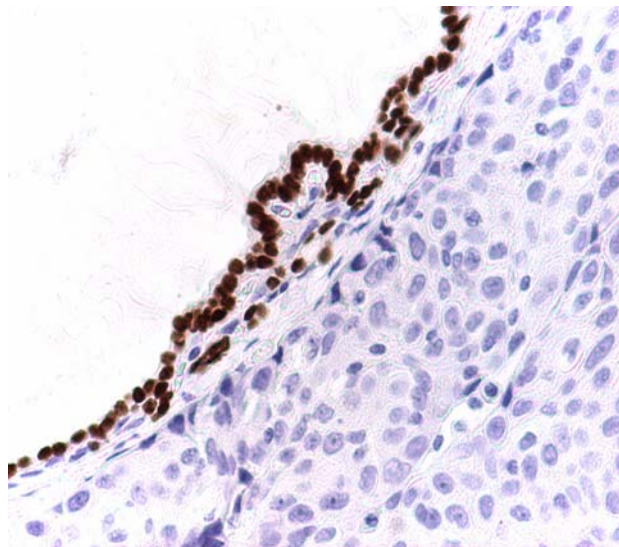
The prognosis of a patient with a tumor metastatic to the thyroid is determined by the underlying primary, but in most cases, it is poor. However, if metastatic disease is limited to the thyroid gland, surgery can prolong survival.



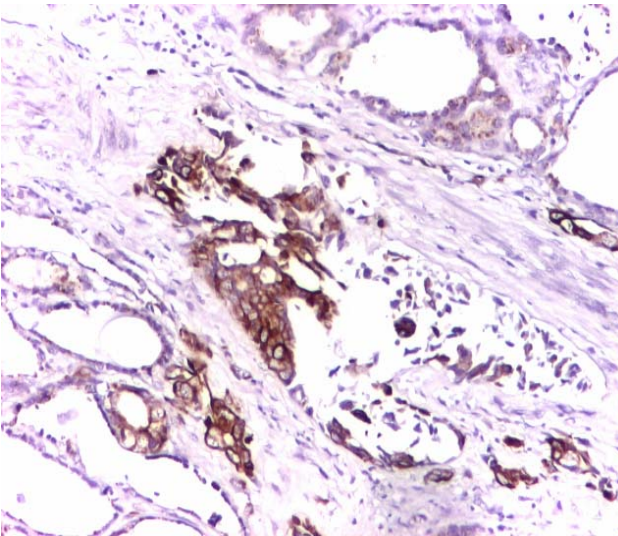
**Figure 1 – Metastasis of squamocellular carcinoma, positive reaction for CK19 in the tumoral islands (IHC, ×200)**



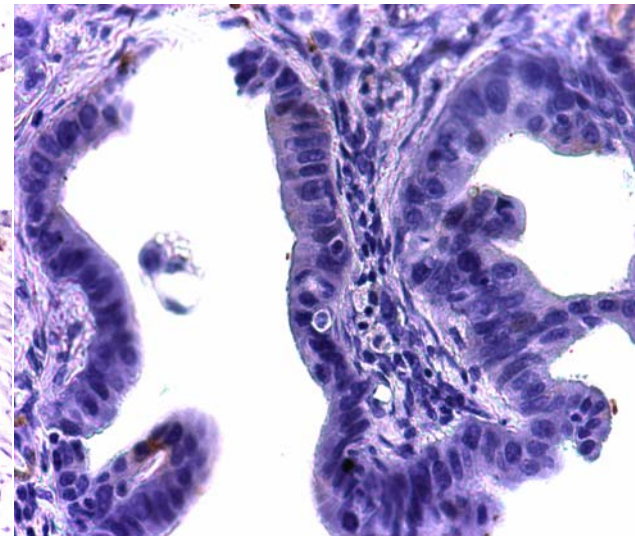
**Figure 2 – Metastasis of squamocellular carcinoma, negative reaction for Tg in the tumoral islands (IHC, ×40)**



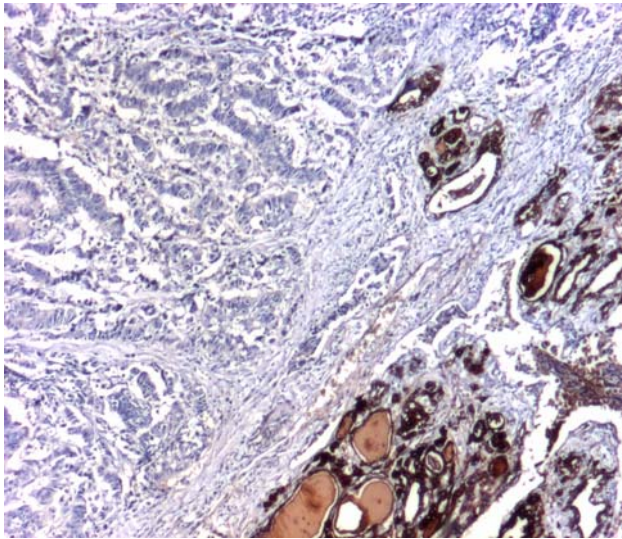
**Figure 3 – Metastasis of squamocellular carcinoma, negative reaction for TTF-1 in the tumoral islands (IHC, ×100)**



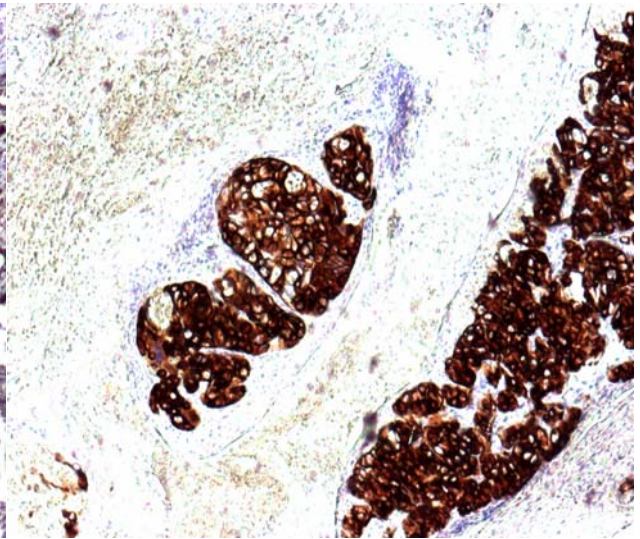
**Figure 4 – Metastasis of adenocarcinoma, positive reaction for CK20 in the tumoral glands (IHC, ×40)**



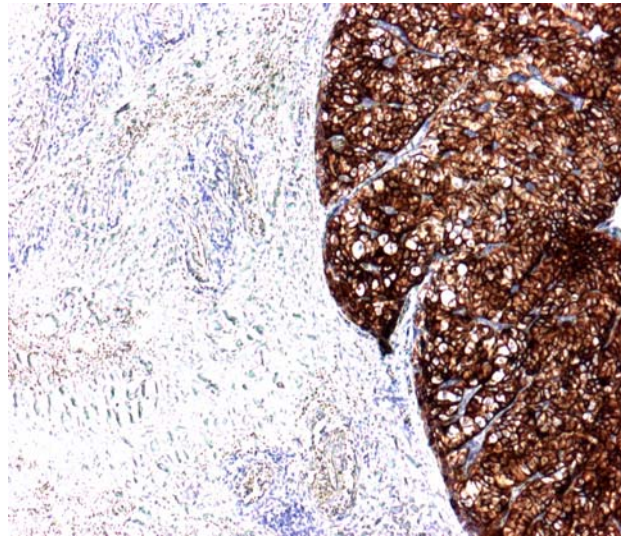
**Figure 5 – Metastasis of adenocarcinoma, negative reaction for CK7 in the tumoral glands (IHC, ×200)**



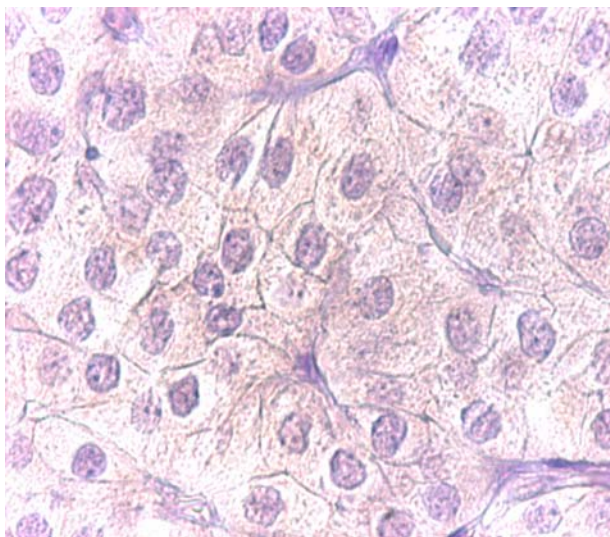
**Figure 6 – Metastasis of adenocarcinoma, negative reaction for Tg in the tumoral glands (IHC, ×40)**



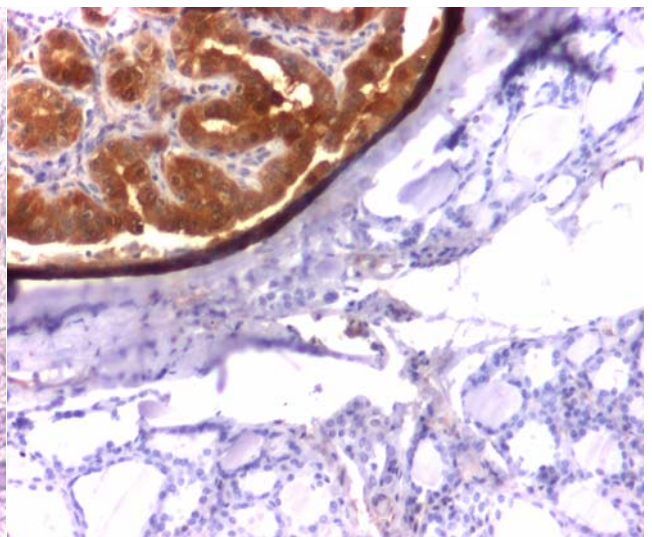
**Figure 7 – Metastasis of renal cell carcinoma, strongly positive reaction for CK18 in the tumoral islands (IHC, ×40)**



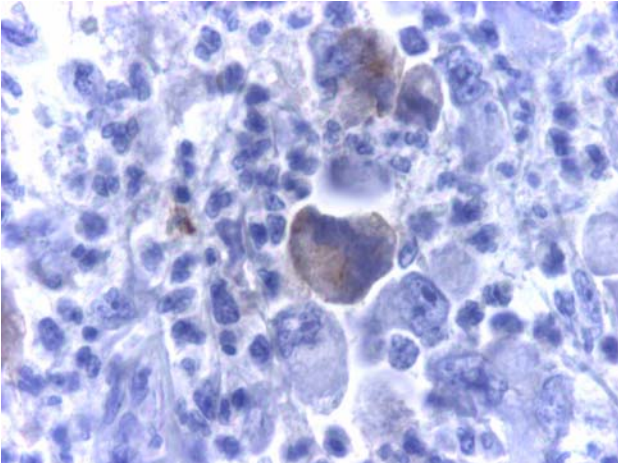
**Figure 8 – Metastasis of renal cell carcinoma, strongly positive reaction for CK19 in the tumoral islands (IHC, ×40)**



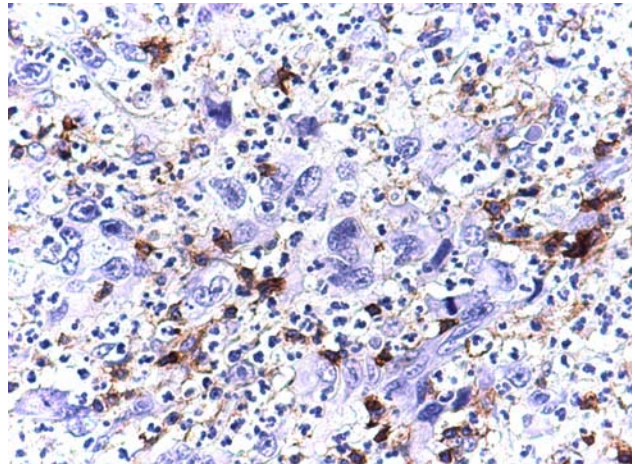
**Figure 9 – Metastasis of renal cell carcinoma, weakly positive reaction for CD10 in the tumoral islands (IHC, ×400)**



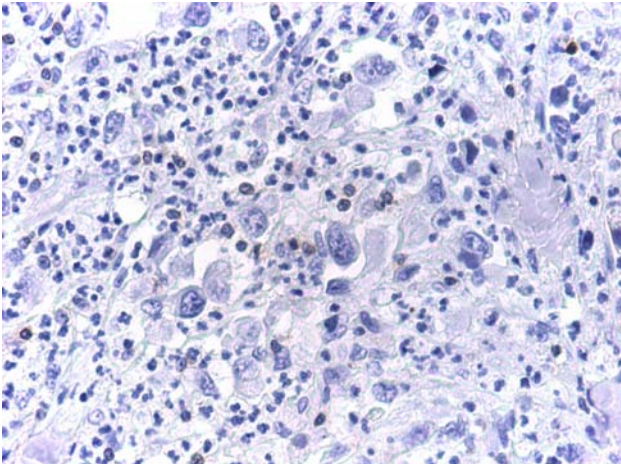
**Figure 10 – Primary papillary thyroid carcinoma associated with metastasis of Hodgkin lymphoma, positive reaction for Gal-3 in the tumoral thyroid (IHC, ×100)**



**Figure 11** – *Primary papillary thyroid carcinoma associated with metastasis of Hodgkin lymphoma, Sternberg-Reed cells positive for CD45RA (IHC, ×400)*

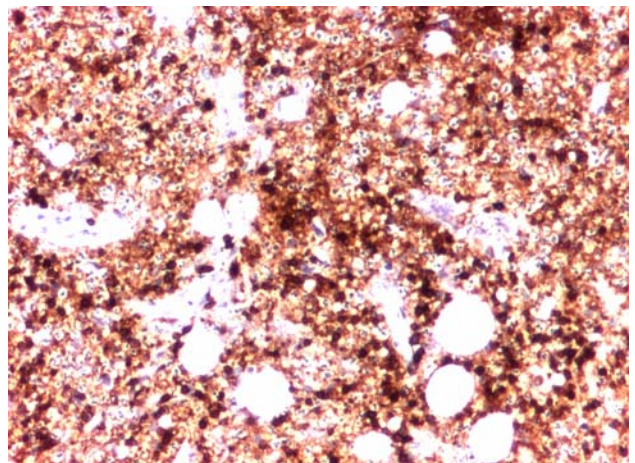


**Figure 12** – *Primary papillary thyroid carcinoma associated with metastasis of Hodgkin lymphoma, reactive lymphoid component positive for CD20 (IHC, ×200)*



**Figure 13** – *Primary papillary thyroid carcinoma associated with metastasis of Hodgkin lymphoma, tumoral lymphoid infiltrate negative for Tg (IHC, ×200)*

**Figure 14** – *Metastasis of non-Hodgkin lymphoma, tumoral lymphoid infiltrate strongly positive for CD20 (IHC, ×200)*



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**Corresponding author**

Delia Ciobanu, Assistant, MD, PhD, Department of Pathology, Faculty of Medicine, “Gr. T. Popa” University of Medicine and Pharmacy, 16 University Street, 700 115 Iassy, Romania; Phone +40232–215 350, Fax +40232–215 288, E-mail: deliaku@yahoo.com

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