Diagnostic reevaluation of 17 cases of pheochromocytoma – a retrospective study

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Abstract
A rare neuroendocrine tumor, the pheochromocytoma (PCC) raises problems due both the limited experience of the researchers in this field and its pathogenic mechanisms, still not fully elucidated. The malignant potential of this tumor cannot be predicted based on its macro- or microscopic aspects, but on the presence of metastases. The aims of this study were: (1) the reevaluation of data for a pertinent and complete tumor diagnostic and prognostic pattern; (2) the statistical correlation of all investigated parameters with the malignant form and the survival rate in order to obtain a possible predictor of malignancy; (3) the potential identification of initially diagnosed benign tumors that become malignant in time. The retrospective study was conducted on 17 patients diagnosed with pheochromocytoma. We investigated: the personal data, the associated neuroendocrine syndromes, the clinical, the laboratory, the macro- and microscopic data [location, size, Hematoxylin–Eosin (HE) pheochromocytoma of the adrenal gland scaled score (PASS score), and immunohistochemical aspects] and the survival rate (analyzed by Kaplan–Meier method and Log-Rank test). The influence of diagnostic parameters on malignancy was calculated taking into consideration the survival rate. By reevaluation of the 17 cases, we tried to emphasize the value of a complex diagnosis pattern for PCCs, based on the correlation between clinical data, laboratory findings and microscopic features. A significant statistical difference between benign and malignant forms was not registered, but there were parameters as age, association with neuroendocrine syndromes, PASS score and specifically Ki-67 mitotic index that had a powerful impact on the survival rate and could be consider as possible predictors of malignancy. The potential of PCC malignant transformation was revealed in our study, by two cases that have metastasized in time.

Keywords: benign/malignant pheochromocytoma, PASS score, neuroendocrine syndromes.

Introduction
As rare neuroendocrine tumors, with incidence ranging between 0.4–9.5/1 000 000 of individuals [1], pheochromocytomas (PCCs) raise diagnostic problems due to either the limited experience of the researchers in this field or the incompletely elucidated pathogenic mechanisms [1, 2].

Firstly described in 1886 by Fränkel and then in 1905 by Poll, PCCs develop from the adrenal gland [1]; the catecholamine-secreting cells stain black (“dusky colored”) when exposed to potassium dichromate, hence their name “chromaffin cells” [1, 3, 4]. According to World Health Organization (WHO) Classification (2004), the term “PCC” is only reserved for intra-adrenal tumors; the extra-adrenal locations (abdomen, pelvis, thorax and neck) are defined as “paragangliomas” [1–3, 5], but they are similar in origin, clinical manifestation, prognosis and management [3, 5].

PCCs are described as localized (present in one or both adrenal glands), regional (involving surrounding tissues or regional lymph nodes) and metastatic (malignant) tumors (containing cells from the primary tumor at distant non-chromaffin sites: bones, liver, lungs) [6, 7].

Traditionally, the PCCs malignancy has been cited as 10%, but lately it was reported as ranging from 2.4% to 50% [1–3, 8]. An accurate determination of malignant potential is difficult. A multitude of risk factors are involved: heredity (more than 10 genes hereditary and germline mutations or genotype–phenotype interactions contribute to the development of these neoplasms) [1, 9], age [1], race (African–American people) [7, 10], diet (either rich in manganese or tyramine [11, 12] or deficient in iron and copper [6, 7, 10, 13]), hypoxia, immunodeficiency [6, 10]; moreover, during the life-long follow-up of patients, which is mandatory, the development of metastatic or recurrent disease may occur [1, 3].

The traditional treatment possibilities – medication, chemotherapy and radiotherapy – have limited efficiency and poor prognosis for malignant tumors [6, 14–19]; thus, the only possible approach to cure both benign or malignant forms is the complete surgical resection [1–3, 19]. Ideally, the discrimination between malignant and benign neoplasms should be done preoperatively [1–3, 8].

Because of the low frequency of PCCs, there are only a few studies on the subject, generally performed on a limited number of patients [1–3, 7, 8]. Even though, these investigations have developed the knowledge regarding these tumors by describing the clinical characteristics, imaging aspects, histopathology, and genetics.

The reason for choosing the subject in this paper, besides PCC impact on the patient’s life and the scientific interest in this condition, was the fact that PCC is less investigated in our country; there are numerous cases

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left undocumented, because of the clinical and the screening difficulties.

The aims of this study were: (1) the reevaluation of data for a pertinent and complete tumor diagnostic and prognostic pattern; (2) the statistical correlation of all investigated parameters with the malignant form and the survival rate in order to obtain a possible predictor of malignancy; (3) the potential identification of initially diagnosed benign tumors that become malignant in time.

Materials and Methods

The retrospective study was initially conducted on 76 patients diagnosed with PCC in “Prof. Dr. Ion Chiricuță” Oncology Institute, Cluj-Napoca, Romania, between January 1, 2000–December 31, 2014. The procedures of the present study were in accordance with the ethical standards of the responsible Committee of “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca. For the statistical significance of the study, the patients were selected upon inclusion and exclusion criteria. Inclusion criteria were mainly represented by the positive diagnosis for PCC and the location of the tumor (right, left, bilateral or unspecified). Exclusion criteria included adrenal location of other tumor types (identified by imaging methods) and inaccessibility to slides or paraffin blocks. As a result, 17 patients were selected from the database and reevaluated.

To complete all the data, medical records of patients and operative protocols from the database of Department of Pathology, “Prof. Dr. Ion Chiricuță” Oncology Institute, Cluj-Napoca, were studied. We investigated the patients’ personal data: age (27–71 years old); gender; the associated neuroendocrine syndromes and the genetic tests (when possible); the clinical symptoms (hypertension) and laboratory data (urinary and serum metanephrine and vanillylmandelic acid values); the tumor pathological aspects: location, size, pheochromocytoma of the adrenal gland scaled score (PASS) score and survival rate.

Tissues from existing blocks (results of curative surgical procedures or biopsy samples) were reexamined for all patients by two independent examiners.

The tumoral samples were fixed for 72 hours in 10% neutral formalin solution, embedded in paraffin and the 4 μm thick sections cut from the blocks were stained with Hematoxylin and Eosin (HE).

Table 1 – PASS score

<table>
<thead>
<tr>
<th>Histological characteristic</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large nests/diffuse growth (&gt;10% of tumor volume)</td>
<td>2</td>
</tr>
<tr>
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<td>2</td>
</tr>
<tr>
<td>High cellularity</td>
<td>2</td>
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<td>Cellular monotonity</td>
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<td>Mitotic index &gt;3/10 HPFs (high-power fields)</td>
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<tr>
<td>Atypical mitosis</td>
<td>2</td>
</tr>
<tr>
<td>Periarenal adipose tissue invasion</td>
<td>2</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>1</td>
</tr>
<tr>
<td>Capsular invasion</td>
<td>1</td>
</tr>
<tr>
<td>Nuclear pleomorphism</td>
<td>1</td>
</tr>
<tr>
<td>Nuclear hyperchromasia</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
</tbody>
</table>

From the total score of 20 points, a lower numerical value (1) was assigned for features characteristic for both types of tumors, while a higher value (2) was attributed for features characteristic only for aggressive forms; the critical value that differentiates the malignant tumors from the benign ones was 4.

Immunohistochemical (IHC) stains were performed, according to protocol: 37°C/24 hours thermostat incubation, dewaxing, hydration, antigen unmasking, rinsing in tap water, 15 minutes washing in distilled water, blocking of the activity of endogenous peroxidase (30 minutes, 3% hydrogen peroxide at room temperature), 10 minutes washing in distilled water, 5 minutes washing in 1% phosphate-buffered saline (PBS), blocking of non-specific sites, incubation with primary antibodies at 4°C/18 hours, 30 minutes incubation with the biotinylated secondary antibody at room temperature, washing in 1% PBS for 5 minutes, three times, application of Streptavidin–HRP (Hors eradish peroxidase) 30 minutes, washing in 1% PBS three times, detection of signal using 3,3’-Diaminobenzidine (DAB) (DAKO) and contrasting with Mayer’s Hematoxylin.

The following markers: anti-chromogranin A, anti-synaptophysin, anti-CE56, anti-S100, anti-vimentin, were used for positive diagnosis of PCCs, while, anti-inhibin, anti-cytokeratin (CK) AE1/AE3 were used for differential diagnosis with other adrenal tumors or epithelial origin neoplasms. The vascular invasion and cellular proliferation score were analyzed by anti-CD34 and by Ki-67 index (Table 2).

Table 2 – List of antibodies used for immunohistochemistry and their characteristics

<table>
<thead>
<tr>
<th>Antigen or antibody</th>
<th>Primary antibody / clone</th>
<th>Manufacturer</th>
<th>Dilution</th>
<th>Antigen unmasking solution, pH and time</th>
<th>Cellular localization</th>
<th>Role in PCCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogranin A</td>
<td>Monoclonal / DAK-A3</td>
<td>DAKO</td>
<td>1:100</td>
<td>Sodium citrate 0.01 M, pH 6, 20 minutes pressure cooker</td>
<td>Cytoplasm</td>
<td>Positive diagnosis highlights the neuroendocrine origin</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Monoclonal rabbit / DAK-SYNAP</td>
<td>DAKO</td>
<td>1:50</td>
<td>Citrate buffer 0.01 M, pH 6, 20 minutes pressure cooker</td>
<td>Membrane Cytoplasm</td>
<td>Positive diagnosis highlights the neuroendocrine origin</td>
</tr>
<tr>
<td>CD56</td>
<td>Monoclonal / 1B6</td>
<td>LEICA</td>
<td>1:100</td>
<td>Citrate buffer 0.01 M, pH 6, 20 minutes pressure cooker</td>
<td>Membrane Cytoplasm</td>
<td>Neoplasmic chromaffin cells</td>
</tr>
<tr>
<td>S100</td>
<td>Polyclonal</td>
<td>DAKO</td>
<td>1:400</td>
<td>Citrate buffer 0.01 M, pH 6, 20 minutes pressure cooker</td>
<td>Cytoplasm of sustentacular cells</td>
<td>Presence of sustentacular cells</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Monoclonal / V9</td>
<td>DAKO</td>
<td>1:100</td>
<td>Citrate buffer 0.01 M, pH 6, 20 minutes pressure cooker</td>
<td>Cytoplasm</td>
<td>Connective–vascular stroma</td>
</tr>
</tbody>
</table>
Diagnostic reevaluation of 17 cases of pheochromocytoma – a retrospective study

<table>
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<tr>
<th>Antigen or antibody</th>
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<th>Manufacturer</th>
<th>Dilution</th>
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<th>Cellular localization</th>
<th>Role in PCCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibin</td>
<td>Monoclonal / R1</td>
<td>DAKO</td>
<td>1:40</td>
<td>Epitope retrieval solution (&gt;10 concentrate), pH 9, 30 minutes steamer</td>
<td>Cytoplasm</td>
<td>Differential diagnosis with adrenal tumors</td>
</tr>
<tr>
<td>Cytokeratin AE1/AE3</td>
<td>Monoclonal / AE1/AE3</td>
<td>LEICA</td>
<td>1:400</td>
<td>Citrate buffer 0.01 M, pH 6, 20 minutes pressure cooker</td>
<td>Cytoplasm</td>
<td>Differential diagnosis with epithelial tumors</td>
</tr>
<tr>
<td>CD34</td>
<td>Monoclonal / 1A10</td>
<td>LEICA</td>
<td>1:60</td>
<td>Epitope retrieval solution (&gt;10 concentrate), pH 9, 30 minutes steamer</td>
<td>Membrane</td>
<td>Angiogenesis, vascular embolus</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Monoclonal / MM1</td>
<td>LEICA</td>
<td>1:200</td>
<td>Citrate buffer 0.01 M, pH 6, 20 minutes pressure cooker</td>
<td>Nucleus</td>
<td>Mitotic index</td>
</tr>
</tbody>
</table>

PCCs: Pheochromocytomas.

Statistical analysis

Statistical analysis employed the Microsoft Office Excel (ver. 2013) for statistical processing; the File Maker software (ver. 6) for data processing and study included patients database; the Kaplan–Meier (KM) method and Log-Rank test for survival curves. Due to the limited number of cases, mainly malignant PCCs, the correlations indices could not be used in this study, lacking accuracy. The influence of diagnostic parameters on malignancy was calculated taking into consideration the survival rate.

Results

Personal data

The age of the patients ranged between 27–71 years old; the average age was 46.4 years old (Figure 1).

The distribution of patients by gender included nine (53%) women and eight (47%) men. None of these two parameters were statistically significant.

Regarding both the distribution of patients by age and gender, the peak incidence of PCCs in women was between 40–49 years old and in men, around 50–59 years old (Figure 1).

![Figure 1 – Distribution of PCCs by age and gender. M: Men; W: Women.](image)

These parameters were not statistically significant for survival ($P_{KM}=0.45$ for age and $P_{KM}=0.39$ for gender). However, the value of 63% on the survival curve for “age” indirectly indicated a positive correlation between the two parameters.

Associated neuroendocrine syndromes and genetic tests

In our study, 12 (71%) patients had sporadic PCC, while five (29%) patients had PCCs associated with neuroendocrine syndromes (MEN2A).

Three of the five patients with neuroendocrine MEN2A syndromes presented malignant forms (they suffered bilateral adrenalectomy and thereafter, in a timeframe of between two months and one year underwent total thyroidectomy due to medullar thyroid carcinoma).

Simultaneous occurrence of adrenal medullar hyperplasia and PCC (i.e., diffuse medullar hyperplasia, nodular medullar hyperplasia) was found in two patients with MEN2A syndrome. A hyperplastic nodule (1 cm in diameter) with discrete capsule and compression of surrounding adrenal tissue was considered to be a small PCC. Mutations of the RET gene were analyzed for all five cases. In three cases, a correlation between malignancy and hereditary forms presenting mutations of the RET gene, was found.

The association of PCCs with neuroendocrine syndromes was not statistically significant for survival ($P_{KM}=0.5$), though its value of 64% indirectly indicated a positive correlation.

Clinical data

Hypertension was present in 15 (88%) patients, prior to the diagnosis of PCC and absent in two (12%) patients.

Laboratory data

Generally linked to clinical symptoms, a tumor biochemical-screening test is compulsory. In our study, 15 patients had significantly elevated values of free plasma metanephrine ($355±18$ pg/mL) and normetanephrine ($231±15$ pg/mL), as well as measurements of fractionated urinary metanephrines ($2.5±0.03$ mg/24 h) ($p<0.001$).

Tumor pathological aspects

Location within the study group: PCC was located on the right in 11 cases, on the left in four cases and bilateral in two cases. Bilateral PCCs occurred in two of five cases associated with neuroendocrine syndromes (Figure 2).

![Figure 2 – Location of PCC.](image)
The histological diagnosis is relatively straightforward. Macroscopically, the tumors appeared as solid, encapsulated, yellow-gray masses. In most cases (12 patients), PCCs were well demarked from the adjacent normal tissue, with areas of hemorrhage, necrosis and calcification. Five cases presented invasion of adjacent structures.

PASS score. In our study, of the 17 cases, 13 (76.4%) patients had metastatic tumors associated with the PASS score >4; of the four (23.6%) patients diagnosed with benign PCC, two (11.8%) patients had the PASS score >4 and the other two (11.8%) had the PASS score <4.

Histological analysis revealed, in 12 cases, the typical cell arrangement for the PCC: small alveoli surrounded by fibro-vascular stroma (“zellballen”) (Figure 3, A and B).

Cellular and nuclear pleomorphism was present in most patients (16 of 17 cases). Pheochromocytes were polygonal in shape, whereas in five cases, sheaths of fusiform cells were evident (Figure 4).

Binuclear and giant cells were evident in six cases. Nuclei exhibiting hyperchromasia, giant nucleoles, or atypical mitotic figures could also be identified (Figures 5 and 6).

Extensive necrosis was present in 14 of 17 cases (Figure 7).

The immunohistological studies indicated that all the PCCs were immunopositive for chromogranin A, synaptophysin, CD56 and vimentin (Figures 8–11).

S100 protein positive sustentacular cells surrounding the cell islets were found in three cases related to family syndromes and in two sporadic PCCs (Figure 12).

All PCCs were immunonegative for inhibin (Figure 13). Most PCCs were immunonegative for cytokeratin A1/A3; however, slightly positive immunostaining was seen in three patients in our study (Figure 14).

Five patients in our study had multiple foci of angiogenesis immunopositive for CD34 (Figure 15).

Ki-67 >15% immunopositive nuclei were present in nine patients (53%), whereas Ki-67 <15% immunopositive nuclei were found in eight patients (47%) (Figure 16). The two patients found with metastases after the initially benign diagnosis had a Ki-67 value <15, but >7.

Survival rate

The follow-up of the patients in our study ranged from 5.1 to 132.6 months, with a mean of 50.4 months. Given the limited group of patients, we calculated the median to be 43.3 months. At the end of the study, nine patients were deceased and eight patients were alive (Figure 17).

Survival of patients at four years was 47%. The value of Ki-67 strongly correlated with patients’ survival: Ki-67 <15, correlated with 66% survival at four years, while Ki-67 >15% correlated with 33% survival (Figure 18).

![Figure 3 – Typical arrangement of cells in form of “zellballen”. HE staining: (A) ×100; (B) ×400.](image1)

![Figure 4 – Sheaths of fusiform cells. HE staining, ×400.](image2)

![Figure 5 – Binuclear cells and nuclear pleomorphism. HE staining, ×400.](image3)
Figure 6 – Giant cells and nuclear pleomorphism. HE staining, ×400.

Figure 7 – Area of necrosis. HE staining, ×200.

Figure 8 – Chromogranin A: diffuse granular cytoplasmic pattern of staining. IHC, ×200.

Figure 9 – Synaptophysin: diffuse granular cytoplasmic pattern of staining. IHC, ×200.

Figure 10 – CD56: membrane pattern of staining. IHC, ×200.

Figure 11 – Vimentin: diffuse stromal pattern of staining. IHC, ×200.
**Figure 12** – S100 staining. IHC, ×400.

**Figure 13** – Inhibin was undetectable on the PCC’s histological sections, except for the normal adrenal tissue. IHC, ×200.

**Figure 14** – Cytokeratin AE1/AE3. IHC, ×200.

**Figure 15** – CD34: foci of angiogenesis and a vascular embolus. IHC, ×200.

**Figure 16** – Ki-67: brown granular nuclear pattern. IHC, ×200.
the different statistical methods were used to demonstrate for the elaboration of a pertinent diagnostic pattern; information [4].

A tumor may undergo, after excision, malignant transformation excepting the emergence of metastases and that a benign tumor is not a firm predictor of malignancy in PCCs, orientating towards this evolution. However, the time of diagnosis there were parameters that could be tumors that metastasized over time and if at the time of the diagnosis there were parameters that could distinguish between benign and malignant tumors their influence concerning PCC malignancy if they could estimate a future aggressive behavior. Moreover, we investigated the cases of benign PCCs, to see if there were tumors that metastasized over time and if at the time of diagnosis there were parameters that could orientate towards this evolution.

Our study indicated that PCCs occurred at any age and had relatively equal gender distribution; these results were consistent with the published data [1, 2, 6, 7, 14]. However, the PCCs incidence peak was between 40–60 years old, which was also reported by Eisenhofer et al. [20]. The positive (yet not statistically significant) correlation between age and the survival curve integrates age as a possible risk factor, as it is cited in the literature [1].

In our study, 29% of patients had MEN2A syndrome, while 71% of patients showed sporadic PCCs, results comparable to those from the literature. Previous studies have demonstrated that PCCs occur sporadically in 70% of cases or in neuroendocrine syndromes, in 30% of cases [9, 13, 21, 22]. Family syndromes associated with PCC are classified into three categories: (1) Von Hippel–Lindau syndrome, (2) multiple endocrine neoplasia MEN (MEN1 and MEN2) and (3) neurofibromatosis type 1 [7, 10, 13, 21, 22, 24–26]. Multiple endocrine neoplasia type MEN2A is characterized by thyroid carcinoma, parathyroid adenoma, Hirschsprung’s disease and PCC [13, 21, 27–30]. The clinical diagnosis of MEN2A syndrome implies the existence of at least two endocrine tumors, with 50% risk of PCC [31, 32].

Regarding the involvement of heredity in the development of PCCs, our study confirms these data, since three cases of the total five were correlated with mutations of the RET gene. Several cases report that about 10% of tumors associated with neuroendocrine syndromes are related to genetic mutations [1, 9, 33], so family history is an important feature for the diagnosis of this neoplasm. For that matter, increasing evidence demonstrated that genetic diversity is typical for PCCs, about 35% of these tumors having a genetic origin [34–36]; yet, no gene signature is known to accurately distinguish between benign and malignant PCCs. The most common gene mutations identified until now are: RET, VHL, NF1, SDHB, SDHC, SDHD and SDHAF2 [1, 4–6, 9]. In MEN2 syndrome, the mutation of RET gene (mainly codon 634) is highly suggestive for hereditary tumors and the position of RET mutation is strongly correlated with the clinical phenotype [33, 37]; in sporadic PCCs, the heterozygous germine mutations in SDHA are the most frequent; the affected subunit dictates the clinical features of the tumor. On the other hand, the SDHB gene mutations are suggestive for malignant PCCs in 50% of cases [27, 38, 39].

In our study, the association of PCCs with the neuroendocrine syndromes was not statistically significant, though there was an indirect positive correlation with the survival rate.

Hypertension was present in most patients in the study group, in accordance with literature data [15, 23, 40, 41]. We interpreted the absence of hypertension in 12% of cases as being a possible adaptation of the cardiovascular system to the increased levels of circulating catecholamines. Intermittent or continuous release of excessive catecholamines may cause various clinical manifestations [20, 28, 29], similar to common hypertension [14]. There is a characteristic clinical tetrad (palpitations, headache, profuse sweating, elevated blood pressure values [6, 20, 32, 36, 39], which facilitates the diagnosis of PCCs. The symptoms may be paroxysmal (when patients are agitated, pale, with hypertension values [12, 14, 26, 27]). We interpreted the absence of hypertension in 12% of cases as being a possible adaptation of the cardiovascular system to the increased levels of circulating catecholamines. Intermittent or continuous release of excessive catecholamines may cause various clinical manifestations [20, 28, 29], similar to common hypertension [14]. There is a characteristic clinical tetrad (palpitations, headache, profuse sweating, elevated blood pressure values [6, 20, 32, 36, 39], which facilitates the diagnosis of PCCs. The symptoms may be paroxysmal (when patients are agitated, pale, with hypertension values [12, 14, 26, 27]). In metastatic tumors, catecholamine production and the degree of hypertension may be more elevated [7, 12, 14, 26]. Correct diagnosis and treatment of PCCs enable the normalization of hypertension [14]. However, the symptoms may be inconspicuous due to the underlying condition and some cases of PCCs are diagnosed only upon autopsy [35].
In our study, the elevated values of free plasma metanephrine (normal >90 pg/mL) [37] and normetanephrine (normal <180 pg/mL) [37], as well as measurements of fractionated urinary metanephrines (normal range 0–1 mg/24 h) [37], correlated well with PCC presence.

Biochemical measurement of the excessive catecholamines in serum, urine or both is regarded as a diagnostic cornerstone [7, 9, 14, 30] and used as a screening test. The optimal collection of biological samples is recommended to be done during or immediately after the hypertensive crisis [1]. There are several methods for catecholamine determination: high performance liquid chromatography, radioenzymatic tests and liquid chromatography associated with spectrophotometry [32, 38].

Given the tumor variations of secretory level, dosing serum free metanephrines remains the method that offers the highest sensitivity [9, 14, 30, 34–36, 38]. Determination of vanillylmandelic acid, although with high specificity, should be used with caution because of reduced sensitivity [9, 14, 30, 38]. Significantly increased values of catecholamines (i.e., four times above the normal range) in suggestive clinical context, plead for PCC [7, 9, 14, 30].

In our study, the tumor location was mainly on the right (65% of patients) and two patients from the total five with MEN2A syndrome, presented a bilateral PCC. Concerning location, Thompson et al., found the same tumor position, without clear explanation [14]. Besides, the PCCs “rule of 10” claims that 10% of PCCs are bilateral [25], but recently higher percentages are taken into consideration [14, 26]. Bauters et al. mentioned the frequent association of MEN syndromes with bilateral PCCs [33].

Regarding the size, in most cases, the tumors were larger than 6 cm. Several studies demonstrated that a tumor over 6 cm may be malignant and associated with a poor prognosis [1, 8]. However, malignancy has been also demonstrated in smaller tumors, so the tumor size alone is not a sensitive predictor of malignancy [28, 47].

In our study, none of these parameters was statistically significant. There is a debate in the literature, about the association of PCCs location and size with malignancy. Some authors consider location and tumor size as possible risk factors for malignancy, therefore useful in predicting the biological behavior of PCCs [5, 7, 14, 22].

We used imaging methods [computed tomography, magnetic resonance imaging, positron emission tomography (PET) scan] necessary for tumor localization and size assessment, as exclusion criteria of PCCs. Nevertheless, these parameters are similar in terms of sensitivity [10, 14]: 65% of PCCs are correctly diagnosed, while 35% are incorrectly interpreted as adenomas or other malignancies [43].

Since two patients with the PASS score >4 and initially diagnosed with benign tumors developed metastases in time, we interpreted that the PASS score >4 could predict an aggressive behavior of the tumor and possibly its trend towards malignancy. In our study, none of the parameters involved in PASS score were statistically significant though there was an indirect positive correlation with the survival rate (Table 3).

<table>
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The PASS score, conceived by Thompson in 2002 [8], is still widely used to guide pathologists in the diagnosis of PCCs malignancy [1, 3–5, 13, 22, 28]. It allows the histological evaluation of each tumor, by identifying the microscopic features. Several histological parameters are assessed: necrosis, vascular/capsular invasion, mitotic index, tumor cell spindling, large cell nests (Table 1); these parameters try to define the benign and the malignant PCCs [1, 44–47]. It is assumed that a score value >4 may indicate a possible but not mandatory aggressive nature of the tumor; a score value <4 does not necessarily indicate a non-malignant tumor, but a slower evolution. Metastases may occur many years after a primary benign considered tumor, as they were reported by several studies [44–47]. The PASS score reliability is under debate, its relativity being proven by several authors [47–52]. They found significant inter-/intra-observer variation in the interpretation of PASS score or reported low values PASS score associated with metastases [47–52]. Thompson et al. described different cases: patients with PASS score <4 and lack of metastases for 14 years; patients with PASS score >4, that did not present metastases in five years, or, one patient with a PASS score >15, who has not showed metastases for 28 years after the PCC diagnosis [8].

Microscopically, the cells characteristically demonstrated a nested “zellballen” pattern, surrounded by sustentacular cells [1].

In our study, the histological appearance of PCCs presented significant variety regarding cell morphology. Ten cases showed the typical cell-nesting pattern with abundant basophilic cytoplasm. Histological features suggesting an aggressive behavior have included, besides capsular and vascular invasion, or local extension and necrosis, fusiform cells, high cellularity, marked nuclear pleomorphism, macronucleoli, high mitotic index, and atypical mitoses.

Standard histopathological techniques are not always reliable for PCCs diagnosis, since there is a risk of recurrence or metastatic spread [1, 4]; ancillary tests, such as immunohistochemistry are necessary.

In our study, the use of the immunohistochemical markers enabled the accurate positive and differential diagnosis. Immunopositivity for synaptophysin, and chromogranin A found in all cases correlated with literature data [53]. Positive S100 sustentacular cells are suggestive for good prognosis; these cells are fewer or
absent in malignant PCCs [1, 4]. Vimentin is part of PCC immunohistochemical diagnostic battery [54]. Immunonegativity for inhibin and cytokeratin A1/A3 excluded another type of tumor: adrenal cortical tumors are positive for inhibin-α, melan A, calretinin, weakly positive for keratin, but negative for chromogranin A [1, 4]. CD34 was used to reveal angiogenesis and vascular emboli, which are signs of aggressive behavior [1, 4].

Recently, immunohistochemical studies have brought new and valuable information in the diagnosis of PCC evolution potential. Proteins found in neurosecretory granules (for example, chromogranin) are antigens that resist degradation [55]. Chromogranin A is considered a marker of PCC: its levels are directly related to tumor volume and indicate the risk of malignant transformation [55]. It was demonstrated that chromogranin A expression in malignant tumors was more pronounced as compared to benign types [56]. Similar to all neuroendocrine tumors, PCC is positive for synaptophysin [1, 4].

In our study, Ki-67, a marker strictly associated with cell proliferation, had the most powerful impact on the survival rate and was indirectly correlated with malignancy. Due to the fact that this protein is present in all active phases of the cell cycle (G1, G2), but absent in resting cells (G0), it allows an excellent evaluation of cell population proliferation [57]. According to literature, Ki-67 labeling might be helpful in differentiating benign from malignant PCCs [13]. A Ki-67 index >3% could predict the malignant potential, since benign PCCs have never been shown to have scores >3%. However, despite a high specificity for malignancy, Ki-67 index lacks sensitivity; indices <3% have been also shown in patients with malignant PCCs [4]. By the absence of a standardized method of evaluation, its reliability is greatly reduced [1, 4, 58].

Recently, Kimura et al. proposed a new score for adrenal tumors [1, 4]. This system combines histological criteria with the tumor Ki-67 scores (a maximum of 10) and the type of catecholamine produced by the tumor. The higher the score achieved by individual tumors, the greater the correlation with metastatic potential and patient survival. All patients with a score of 7–10 points were found to have malignant tumors [4]. Yet, this model needs further validation in order to establish its applicability in clinical setting [4].

Following the reevaluation of the cases, we observed that two patients, initially diagnosed with benign tumors developed metastases in time. These patients aged of 44 and 47 years old, with large tumors (7.5 and 9 cm) had the PASS score >7, a Ki-67 >15 and were both associated with neuroendocrine syndromes. The correlation of these parameters at diagnosis could have direct to an aggressive tumor behavior and alert the physicians to the possibility of malignant transformation.

In our study, the four-year patient survival was consistent with the literature: for patients with malignant PCC, a five-year survival rate less than 50% was reported; metastatic disease is the main factor associated with low survival rate. The overall five-year survival for patients with PCCs is approximately 89%; five-year survival for patients with benign PCC is more than 95% [18–20, 29–31, 57, 59–61]. Since PCCs prognosis may be poor, an accurate distinguishing between benign and malignant tumors at the time of diagnosis may enable appropriate treatment and adequate follow-up. All patients should be evaluated at least 10 years after diagnosis; however, tumors with high risk of malignant transformation (e.g., those over 5 cm in size or associated with endocrine syndromes) need to be assessed indefinitely [18–20, 57].

### Conclusions

By reevaluation of the 17 cases, we tried to emphasize the value of a complex diagnosis pattern for PCCs, based on the correlation between clinical data, laboratory findings and microscopic features. These elements must be taken into account when the tumor is diagnosed, in order to make the best decision for treatment. A significant statistical difference between benign and malignant forms was not registered, but there were parameters as age, association with neuroendocrine syndromes, PASS score and specifically Ki-67 mitotic index that had a powerful impact on the survival rate and could be consider as possible predictors of malignancy. The potential of PCC malignant transformation was revealed in our study, by two cases that have metastasized in time.

### Conflict of interests

The authors declare that they have no conflict of interests.

### References


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