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Abstract

The available data on the natural history of pheochromocytomas and paragangliomas after radical surgery are heterogeneous and discordant. The aim of our retrospective multicenter study was to find predictors of recurrence in patients with pheochromocytomas and sympathetic paragangliomas submitted to radical surgery in Piedmont (a region in northwest Italy). We collected data from 242 patients diagnosed between 1990 and 2016. Forty-two patients (17.4%) had disease recurrence. Multivariate analysis showed that genetic mutation (HR = 3.62; 95% CI 1.44−9.13; $p = 0.006$), younger age (HR = 0.97; 95% CI 0.95−0.99; $p = 0.031$) and larger tumor size (HR = 1.01; 95% CI 1.00−1.02; $p = 0.015$) were independently associated with a higher recurrence risk of pheochromocytoma and paraganglioma; in pheochromocytomas, genetic mutation (HR = 3.4; 95% CI 1.00−11.48; $p = 0.049$), younger age (HR = 0.97; 95% CI 0.94−0.99; $p = 0.02$), higher tumor size (HR = 1.01; 95% CI 1.00−1.03; $p = 0.043$) and PASS value (HR = 1.16; 95% CI 1.03−1.3; $p = 0.011$) were associated with recurrence. Moreover, tumor size was the only predictor of metastatic pheochromocytoma and paraganglioma (HR = 4.6; 95% CI 1.4−15.0; $p = 0.012$); tumor size (HR = 3.93; 95% CI 1.2−16.4; $p = 0.026$) and PASS value (HR = 1.27; 95% CI 1.06−1.53; $p = 0.007$) were predictors of metastatic pheochromocytoma. In conclusion, our findings suggest that the recurrence of pheochromocytoma and sympathetic paraganglioma develops more frequently in younger subjects, patients with a family history of chromaffin tissue neoplasms, mutations in susceptibility genes, larger tumors and higher values of PASS. We recommend genetic testing in all patients with PPGL and strict follow-up at least on an annual basis.

Introduction

Pheochromocytoma (PCC) and paraganglioma (PGL) are rare tumors arising from adrenomedullary cells and from sympathetic or parasympathetic ganglia, respectively. Approximately 80−85% of chromaffin-cell tumors are PCCs, whereas 15−20% are PGLs. The prevalence of pheochromocytoma and paraganglioma (PPGL) in hypertensive patients varies between 0.2 and 0.6%, while PCC is observed in 5% of patients with adrenal

incidentaloma. PCCs and sympathetic PGLs commonly produce catecholamines: epinephrine, norepinephrine and dopamine, while parasympathetic PGLs are often silent [1]. Metanephrines and CT attenuation values are useful parameters to distinguish PPGL from other tumors [2]. It is important to recognize these tumors early to reduce related cardiovascular morbidity/mortality, prevent growth and extension into adjacent tissues, development of metastases and address syndromic forms.
The rule that 10% of chromaffin tumors are paragangliomas, malignant, associated with genetic mutations, affect patients without arterial hypertension, have bilateral adrenal involvement and pediatric onset [3] is no longer epidemiologically correct. In fact, PPGL have a higher risk of recurrence, not only for the development of distant metastases but also for the incidence of local relapse or new tumor localizations. Although two multiparametric scoring systems have been proposed, namely, the Pheochromocytoma of the Adrenal gland Scaled Score (PASS) [4] and the Grading system for Adrenal Pheochromocytoma and Paraganglioma (GAPP) [5] or the modified-GAPP [6], the prediction of recurrence is still a clinical challenge, even after pathological examination. The Endocrine Society [1] and the European Society of Endocrinology Guidelines [7] recommend considering genetic testing in all patients with PPGL. Many mutations in susceptibility genes for the development of PPGLs have been identified in recent years, and research in this field has advanced our understanding of cancer biology [8]. Genetic mutations often confer a low rate of malignancy but a high risk of relapse (local recurrence or metachronous tumors). Studies on this issue, performed in surgical or medical settings, often explore only the risk of malignant disease, include patients not radically cured and have a high risk of referral bias. A recent meta-analysis [9] of 42 studies suggests that the risk of recurrence following complete resection of PPGLs is lower than previously estimated, but the heterogeneity of methodological designs and the clinical results of the included articles preclude any firm conclusion. Therefore, the aim of our study was to analyze clinical and pathological predictors of recurrence of radically excised PPGLs over 27 years of experience.

Methods

Design

We conducted a retrospective multicenter study to evaluate the role of several predictors of the recurrence of PPGL, diagnosed in our centers between 1990 and 2016. The inclusion criteria were radical surgery with an apparent cure (R0 resection confirmed at pathological examination, absence of other disease localization and hormonal normalization at 6 weeks after surgery) and an appropriate follow-up at least annually with proper diagnostic methodologies (annual determination of metanephrines and chromogranin A for secretive tumors or the annual control of CT/MRI for nonsecretive PPGLs). We excluded only patients with metastatic disease at diagnosis and patients not submitted to radical surgery. Nine centers in Piedmont (a region in northwest Italy) with recognized expertise in adrenal gland disorders were involved. The data were collected from prospective registries and analyzed retrospectively. Approval from local ethics committees was obtained for the analysis of patient data from all centers. Written informed consent was obtained from the patients in all centers.

Study population

A total of 242 patients met the inclusion criteria. Personal data, age at diagnosis, familial history of PPGL or syndromic forms, plasma and/or 24 hour urinary catecholamine/metanephrine levels, plasma chromogranin A values, imaging test data (magnetic resonance and/or computed tomography, tumor size and side), functional imaging test results, tumor type (PCC or PGL), date of surgery, genetic testing and mutation type, pathological features of PCCs by a single pathologist with the re-evaluation of the PASS, date of the last follow-up visit, development and type of recurrence, disease-free survival time, and the cause and date of death were collected from all patients.
Three levels of signs/symptoms were identified: (1) asymptomatic; (2) mild symptomatic (arterial hypertension, hypertensive crisis associated or not associated with adrenergic symptoms or resistant hypertension); (3) severe symptomatic (hypertensive emergencies, arrhythmias, cardio- and cerebrovascular events).
Genetic testing was performed only on clinical indication (in cases of positive familial history, young age, multiple tumor localizations, large tumors, bilateral adrenal gland involvement, extra-adrenal localization and high PASS values) in 46.3% of the series (112 patients). In fact, it is only after the publication of Endocrine

Society (2014) and European Society of Endocrinology guidelines (2016) that it became clinical practice to engage patients with PPGL in shared decision making for genetic testing [1, 8]. The samples collected for genetic testing were periodically revaluated to search for new mutations discovered in more recent years. The patients were informed on the new tests and asked to give written informed consent when required by law. We considered clinically appropriate to search for mutations in the following genes: *RET*, *VHL*, *NF1*, *SDHAF2*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *TMEM127*, *MAX*, *EPAS1* and *FH*.
All patients were followed-up annually with hormonal values in case of secreting tumors and with CT/MRI if the neoplasm was biologically silent. Recurrence was defined as local relapse, new chromaffin tumor or metachronous metastases, detected with CT/MRI and/or functional imaging. According to the 2017 World Health Organization criteria, we considered metastatic those PPGL whose recurrence was in sites where normally there is not chromaffin tissue [10].

Statistical analysis

The baseline characteristics of all patients included in the analysis were summarized using median and interquartile range (IQR) for continuous data (or mean and standard deviation when specified) and rate and percent values (*n*, %) for binary and categorical data. Between-group differences in personal and clinical features at diagnosis were evaluated by the Mann–Whitney *U* test for continuous variables and the chi-square test or Fisher's exact test for categorical variables.
To describe the timing of recurrence, the cumulative incidence was estimated using the Kaplan−Meier method. Statistical significance ($p < 0.05$) of differences in the cumulative incidence of recurrence between groups (i.e., by genetic testing results, familial history for PPGL, lesion size, age, localization and several clinical measures) was tested using the log-rank test for homogeneity.
The observation period for time to recurrence (TTR) started on the day of surgery until death for any cause or the date of recurrence diagnosis (failures) or until the last follow-up visit (censoring). A Cox proportional hazard model was employed to estimate the crude and the multivariable-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) and to evaluate possible predictors of recurrence. The proportional hazard assumption was also verified by graphical checks and formal tests based on Schoenfeld residuals. The effect of the following selected factors, potentially associated with recurrence, was considered in the univariate models: age, gender, familial history for syndromic form, level of symptoms, 24 h urinary fractionated metanephrines and chromogranin A levels, secretive phenotype, tumor type (PCC or PGL) and size (categorized above and below the median value), lesion side (left, right, bilateral) in cases of PCC, genetic testing (positive, negative, not performed) and functional imaging result and PASS value (only for 177 cases of PCC). Prognostic factors and clinically relevant variables were included in the multivariable models. As a sensitivity analysis, the factors considered as possible predictors of relapse were separately evaluated in benign ($n = 25$) and metastatic recurrence ($n = 17$) to highlight different effects in the two groups. Due to the small number of cases and the substantially similar effect of predictors in the two groups, the main analyses were reported for the overall recurrences.
Statistical analyses were performed using Stata 13.1 software (StataCorp LP, College Station, TX, USA).

Results

The data from 242 patients, including 121 females (50%) and 121 males (50%) diagnosed between 1990 and 2016 (220 cases of PCC—90.9%, 22 cases of sympathetic PGL—9.1%), were collected. The median follow-up was 73.9 (IQR 41.2−121) months. The personal and clinical characteristics of the patients at diagnosis are shown in Table 1. The mean age at diagnosis was 49.2 ± 16.2 years. Thirty-three patients (13.6%) had a positive familial history of PPGLs or associated syndromes. A total of 77 (31.8%) subjects were defined as asymptomatic, 138 (57%) as mild symptomatic and 27 (11.2%) as severe symptomatic.
Given the distribution of the diagnosis over 27 years, 24 h urinary metanephrine levels were available in 142 patients (58.7%), with median values of 1824 (850−4038) and 553.5 (195−2130) μg/day for normetanephrines and metanephrines, respectively. The median chromogranin A level, measured in 149 patients (61.6%), was 290 (110−568) ng/mL.

To identify the localization of tumors, CT was used in 168 subjects and MRI was used in 74 patients, either with or without contrast media. A total of 155 patients (64%) underwent functional imaging: ^{123}I-MIBG scintigraphy, ^{18}F-FDG PET, ^{18}F-FDOPA PET and ^{111}In-pentetreotide scintigraphy were positive in 110/132 (83.3%), 4/8 (50%), 17/19 (89.5%) and 8/8 (100%) patients at diagnosis, respectively (data not shown). Notably, 97/220 (44.1%) PCCs were localized only in the left adrenal gland, while 108 (49.1%) were only localized in right adrenal gland and 15 (6.8%) were bilateral; 4/22 (18.2%) sympathetic paragangliomas were localized in the organ of Zuckerkandl, 14 (63.6%) in the infradiaphragmatic para-aortic ganglia, 1 (4.5%) in the mediastinum and 3 (13.6%) in other sites. The median tumor size at diagnosis was 50 (34−60) mm.

A single pathologist performed a blind re-evaluation of the histology of 177/220 (80.5%) PCCs: 94 patients (42.7%) had PASS ≥ 4, and 83 subjects (37.7%) had PASS < 4. The mean PASS was 3.97 ± 2.9.

Out of the 112 cases (46.3%) submitted to genetic testing, 58 patients (24%) had a negative result, and 54 (22.3%) presented a mutation in one of the susceptibility genes, identifying 15 *VHL*, 11 *MEN2a*, 5 *MEN2b*, 13 *NF-1*, 7 *SDHB*, 2 *SDHD* and 1 *MAX* gene mutations.

Recurrence occurred in 42 patients (R group, 17.4%): 8 (19%) were local relapses, 16 (38.1%) were pheochromocytomas of the contralateral gland, 1 (2.4%) was new evidence of pheochromocytoma (in a patient with *SDHD* mutated PGL), 6 (14.3%) paragangliomas and 11 (26.2%) metastases. Two hundred subjects did not develop recurrence (NR group, 82.6%). Moreover, six patients developed metastatic disease after a benign recurrence. Of the 17 subjects with metastatic PPGLs, 1 patient with *NF-1*, 1 with *VHL*, and 1 with *SDHB* mutations, 3 with sporadic PPGLs and 5 subjects that did not perform genetic testing had a primary metastatic recurrence. One patient with *MAX*, 2 with *SDHB*, and 1 with *SDHD* mutations, 1 sporadic PCC and 1 subject that did not perform genetic testing had metastatic diseases after a benign recurrence.

Metastases were located in the liver (four cases), lungs (two cases), lungs and liver (five cases), abdominal lymph nodes (three cases), bone (one case) and multiple sites (two cases). At the end of the observation period, 17 deaths were observed, 8 of which were observed after disease recurrence. In the R group, the deaths were due to five metastatic PCCs, one metastatic PGL, one metastatic medullary thyroid carcinoma and one acute myocardial infarction. The causes of death in the NR group were one metastatic GIST, one metastatic colorectal cancer, one metastatic lung cancer, two acute myocardial infarctions (one case during the recovery in the reanimation department a few days after adrenalectomy), one sudden cardiac arrest, one lymphoma and two acute heart failures.

The comparison between the R group and the NR group (Table 1) showed no difference in gender, symptoms, levels of normetanephrine, levels of chromogranin A and rate of positive ^{123}I-MIBG scintigraphy. Patients in the R group tended to have larger tumors (50.5, 40−70 vs. 48, 32−60 mm; $p = 0.063$) and a higher rate of PGL (16.7% vs. 7.5%, $p = 0.060$), but these results did not reach statistical significance. Age, familial history and genetic testing were significantly associated with recurrence: the R group patients were younger (36.4 ± 16.1 vs. 51.9 ± 14.9, $p < 0.001$), showed a higher rate of familial history of PPGLs (35.7% vs. 9%, $p < 0.001$) and a higher rate of positive genetic testing (64.3% vs. 13.5%, $p < 0.001$) compared with the NR group. Bilateral adrenal involvement was significantly associated with recurrence (17.1% vs. 4.8%, $p = 0.033$).

Lower levels of urinary 24 h metanephrines were associated with disease recurrence (209, 88−400 vs. 694.5, 204.5−2386.5 µg/day, $p = 0.001$). Moreover, among the different secretive phenotypes, nonsecretive PPGLs had a higher risk of recurrence ($p = 0.015$). Histological re-evaluation revealed that a high PASS value predicted recurrence (6.2 ± 4.4 vs. 3.68 ± 2.7, $p < 0.001$; PASS ≥ 4: 75.0% vs. 49.0%; $p = 0.013$).

The comparison between the demographic and clinical characteristics of PGLs and PCCs (Table 2) showed that extra-adrenal tumors are more often silent ($p < 0.001$) and larger than adrenal tumors (55.5, 48−80 vs. 48.5, 32−60 mm; $p = 0.003$). PGLs tended to produce higher levels of normetanephrines (3995, 2854−8575 vs. 1785, 826−3997 µg/day; $p = 0.063$) and lower levels of metanephrines than PCCs (157, 82−373 vs. 574, 198−2200 µg/day; $p = 0.065$), but these differences did not reach statistical significance.

The results on the association between several clinical/pathological data and recurrence are reported in Tables 3 and 4. Younger age (HR = 0.95; 95% CI = 0.92−0.96; $p < 0.001$), positive genetic testing (HR = 4.59; 95% CI = 1.98−10.6; $p < 0.001$), the strictly related familial history of syndromic forms (HR = 3.34; 95% CI = 1.73−6.46; $p < 0.001$), nonsecretive phenotype (HR = 3.80; 95% CI = 1.42−10.1; $p = 0.008$), bilateral adrenal involvement (HR = 3.16; 95% CI = 1.14−8.76; $p = 0.027$), lower levels of 24 h urinary metanephrines (HR = 0.58; 95% CI = 0.40−0.85; $p = 0.005$), a higher PASS value (HR = 1.24; 95% CI = 1.13−1.36; $p < 0.001$)

and a PASS value ≥ 4 (HR = 3.01; 95% CI = 1.29−7.02; $p = 0.011$) were strong predictors of recurrence in the univariate analysis (Table 3).

The absence of genetic testing, larger tumor size, tumor type (PGL vs. PCC), normetanephrine values, symptoms, and a positive ^{123}I-MIBG scintigraphy were not associated with recurrence.
In the multivariate analysis, age, tumor size, tumor type and the results of genetic testing were considered as covariates. We did not consider the secretive phenotype because of the lack of data in 39 patients.
In the first model (Table 4a), genetic mutation was the strongest independent predictor of recurrence (HR = 3.62; 95% CI = 1.44−9.13; $p = 0.006$); also young age (HR = 0.97; 95% CI = 0.95−0.99; $p = 0.031$) and large tumor size (HR = 1.01; 95% CI = 1.00−1.02; $p = 0.015$) but not extra-adrenal localization (HR = 1.17; 95% CI = 0.48−2.82; $p = 0.735$) were independently associated with higher recurrence risk.
In the second model (Table 4b), we analyzed all patients with a diagnosis of pheochromocytoma (220 cases) to enable the introduction of PASS values into the analysis. Genetic mutation was still proven to be the strongest predictive factor (HR = 3.4; 95% CI = 1.00−11.48; $p = 0.049$). Additionally, young age (HR = 0.97; 95% CI = 0.94−0.99; $p = 0.02$), large tumor size (HR = 1.1; 95% CI = 1.00−1.03; $p = 0.043$) and high PASS value (HR = 1.16; 95% CI = 1.03−1.30; $p = 0.011$) were significantly associated with recurrence.
The cumulative incidence of recurrence with the Kaplan−Meier curve showed that at the end of the follow-up period (12 years), 21.5% (95% CI 14.9−30.4) of patients developed disease recurrence (Fig. 1a). The median recurrence time in our population was 2.91 years (95% CI 1.5−3.49), i.e., 50% of recurrent diseases occurred within the first 3 years from surgery. The recurrence risk was 2.49% (95% CI 1.13−5.46) at 1 year and 12.3% (95% CI 8.66−17.8) at 5 years after surgery.
Figure 1b shows the comparison between the Kaplan−Meier curves of the subjects with positive, negative and not performed genetic testing: the presence of mutation was an excellent predictor of recurrence (log-rank test, $p < 0.001$). In 9 years, 49.3% (95% CI 34.2−66.8) of cases with germline mutations developed recurrence compared to 13.2% (95% CI 6.5−25.8) of subjects without detected mutations and 5.9% (95% CI 2.7−12.7) of subjects without genetic evaluation. We found no difference between patients with negative genetic testing and subjects not submitted to genetic testing: the selection of cases to refer for genetic testing based on clinical indication, a normal practice until 10 years ago, appears to be appropriate.
Possible predictors of relapse were also separately evaluated in benign ($n = 25$) and metastatic recurrence ($n = 17$, 11 patients with metastases at first recurrence and 6 subjects with metastases after a first benign recurrence). In this analysis, tumor size was the only predictor of metastatic PPGL (HR = 4.6; 95% CI = 1.4−15.0; $p = 0.012$), while tumor size (HR = 3.93; 95% CI = 1.2−16.4; $p = 0.026$) and PASS value (HR = 1.27; 95% CI = 1.06−1.53; $p = 0.007$) were predictors of metastatic PCC. In our sample, genetic mutation was not a predictor of metastatic disease for PPGL (HR = 1.25; 95% CI = 0.29−5.21; $p = 0.764$) and for PCC (HR = 0.78; 95% CI = 0.12−4.89; $p = 0.790$), probably due to the low malignancy rate of most identified mutations (data not shown).

Discussion

The population of our multicenter study showed a prevalence of recurrent PPGLs of 17.36%, displaying a lower risk of recurrence if compared with previous reports [9]. Our rate is justified since we collected patients submitted to radical surgery and defined recurrence as local relapse or the development of metachronous chromaffin tumors or metastases. A recent systematic review and meta-analysis by Amar et al. [9] revealed a variable percentage of recurrent disease from 1 to 34% with a 5-year cumulative incidence of 4.7%. These authors considered studies published from 1980 to 2012, but often the aim of these papers was not the analysis of predictors of recurrence. In these studies, the description of the follow-up was only an ancillary part, especially in the surgical series. The quality of the included studies was low, and Amar et al. could not derive firm conclusions.
Our study confirmed the importance of the secretory phenotype for the incidence of recurrence; we found that recurrent PPGL is associated with higher levels of norepinephrine and lower levels of adrenaline. The production of adrenaline is dependent on the cytosolic expression of the enzyme phenylethanolamine *N*-methyltransferase (PNMT), which is downregulated in metastatic or *VHL*-related chromaffin tumors [11]. On this topic, Ayala-Ramirez et al. [12] discovered that after the normalization of urinary excretion of

catecholamines per unit of tumor volume, the adrenaline secretion in metastatic PPGL was lower than that of the other metabolites. Unfortunately, we did not have the results for 3-methoxytyramine, a dopamine metabolite for which, in the literature, there is growing consideration as a predictor of recurrence [13,14,15,16]. Recently, Eisenhofer et al. [17] reported a series of 365 patients with PPGL compared to 846 healthy patients, showing that subjects with metastatic disease had higher norepinephrine, normetanephrine and methoxytyramine levels. Higher values of chromogranin A (CgA) in our study were not associated with an increased recurrence risk. This finding is in contrast with some previous reports: in fact, some authors observed that the progressive increase in CgA correlates with the probability of recurrence. In particular, Rao et al. [18] showed a progressive increase in chromogranin A levels from healthy subjects compared to those with benign and metastatic PCCs. We believe that this discrepancy can be explained by the features of the patients: chromogranin A seems to reflect a large tumor load [19], which was not frequent in our patients.

The association between tumor size and recurrence was described by Amar et al. [20], Park et al. [21], Ayala-Ramirez et al. [12] and Press et al. [22], who observed that the lesion size was correlated with both metastatic disease and patient survival. The incidence of recurrence did not show significant differences depending on the right or left localization of the primary tumor, whereas bilateral localization was associated with the relapse in the association study, losing significance in the multivariate analysis. The literature concerning the importance of this variable is incomplete and discordant: Park et al. [21] and John et al. [23] described this association as not significant, while Feng et al. [24] demonstrated a significant correlation. In our sample, 14/15 (93%) tumors with bilateral localization were associated with genetic mutations, features that can exert a considerable confounding effect in the evaluation of the importance of this variable as a predictor of recurrence.

The extra-adrenal localization (PGL) in our study was not associated with recurrence in either univariate or multivariate models. This point is debated in the literature. John et al. [23] discovered an association between PGL and a higher rate of metastatic disease, which can reach up to 36%. Additionally, Ayala-Ramirez et al. [12] showed that the incidence of metastasis was 4.5 times higher in PGL than in PCC, while Cho et al. [25] did not find an effect of the localization on the outcome of PPGL, and Goffredo et al. [26] showed a high rate of recurrence in metastatic PCCs compared with PGLs.

The literature attributed a potential role for functional imaging techniques in the discrimination of metastatic tumors, which are less differentiated and have a lower capacity for collecting the tracer. Nevertheless, the clinical impact of functional imaging in all PPGL patients remains unknown. Historically, ^{123}I-MIBG scintigraphy was the most commonly used diagnostic technique because patients with MIBG-avid lesions may benefit from treatment with therapeutic doses of ^{131}I-MIBG [27]. In our study, most cases (132 patients, 54.5%) underwent ^{123}I-MIBG scintigraphy, showing a comparable positive rate in patients with and without recurrence. Approximately 40% of the series did not undergo functional imaging tests because the biochemical and morphological data were considered sufficient to confirm the diagnosis. In the last 10 years, several radionuclides (^{18}F-FDOPA and ^{68}Ga-conjugated peptide) were introduced for PET studies [28], but only a few patients in our series underwent these techniques.

PPGL associated with genetic mutations are more frequently bilateral or extra-adrenal, associated with multiple synchronous or metachronous tumors and therefore implying a higher risk of recurrence. The rate of metastatic disease greatly varies from one syndrome to another, being low in carriers of *RET* and *SDHD* mutations and in *VHL* disease, 12% in NF-*1* and reaching 30−70% in carriers of *SDHB* mutations [29, 30]. In our multicenter study, the presence of genetic mutations was also confirmed to be the strongest predictor of recurrence, although genetic testing was not performed in all patients. The good prognosis of patients not selected for genetic testing on a clinical basis indicated that the selection of patients for genetic screening was correct. However, the growing number of genetic-related PPGLs (approximately 40% of cases) [29] and the clinical implications of positive genetic testing for family members suggest screening for germline mutations in candidate genes in all patients with PPGL.

We also found a correlation between high values of PASS and recurrence risk. The histological score, introduced by Thompson in 2002 [4], was tested in many studies [31] to verify the reliability as a predictor of recurrence of PCC. Strong et al. [32] showed, on a series of 51 pheochromocytomas, a significant difference between the PASS value of malignant tumors (score > 6) and that of benign tumors. In our population, both PASS ≥ 4 (binary variable) and high values of PASS (considered as continuous variable) were correlated with disease recurrence. A retrospective study on 93 PCCs [33] showed that although all PASS parameters were

more frequently found in tumors with a diameter greater than 6 cm, the difference with smaller pheochromocytomas was not significant, except for cellular monotony. This finding could be explained by the high inter- and intraobserver variation in the assignment of PASS observed, even between expert pathologists [34]. In our study, PASS was evaluated by a single pathologist, limiting this type of discrepancy. We believe that if the use of PASS can be validated in future studies, this value could be an important parameter for the prediction of recurrence.

Recently, a study by Hamidi et al. [35], including 272 patients with metastatic disease (21−22% in stage IV at diagnosis), observed a markedly variable course and an overall survival longer than previously reported. In a meta-analysis, Hamidi et al. [36] also tried to clarify the outcomes of metastatic PPGL. Due to the low-quality evidence, these authors could not derive precise conclusions, even if the data seem to suggest low mortality rates and poor prognoses in male patients with synchronous metastases.

The strength of our study is the high number of patients included in the analysis, the exclusive inclusion of patients submitted to radical surgery, the careful re-evaluation of all cases, including medical records and the description of the surgical intervention, the selection of patients with a continuous follow-up and the reanalysis of the histological samples by a single expert pathologist. Moreover, our patients were managed in centers with recognized expertise in adrenal diseases belonging to a regional network, ensuring a relatively homogeneous management of these tumors.

There are some limitations that should be taken into account when interpreting these results. Only approximately 50% of the patients were submitted to genetic testing. We currently screen all patients with PPGL for germline mutations, but this practice has become routine only a few years ago, after the publication of important studies confirmed by international guidelines. Unfortunately, at this time, our cohort was already initiated. Among the apparently sporadic PPGL, approximately 25% of tumors are associated with a germline mutation that has incomplete penetrance and/or variable expression [37]. Another weakness of our study is the lack of dopamine and its plasma metabolite values, a measurement performed in our laboratories only recently on plasma and urine samples.

In conclusion, our study demonstrated that among patients who undergo radical resection of PPGL, genetic mutation in susceptibility genes, positive family history of PPGL or associated syndromes, large tumor size and high PASS values are major predictors of recurrence. These observations have clinical relevance for the possibility to plan the follow-up to identify recurrence early and to give correct and prompt therapy to patients at greater recurrence risk. Unfortunately, the stronghold of cure is currently surgical removal (medical treatment has only palliative intent), but the future will provide new therapeutic strategies. Genetic testing should be considered in all patients with PPGL, giving peculiar clinical features of syndromic forms and important prognostic significance. Additionally, histology could also help to identify aggressive cases. Our study also suggests that clinical and biochemical follow-up in patients with PPGL should continue life-long, probably on a yearly basis.

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Compliance with ethical standards

Conflict of interest: the authors declare that they have no conflict of interest.

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Table 1 Overall data and association study on predictors of recurrence

Overall data (*N* = 242)		**Recurrence**		
		No (*N* = 200)	**Yes (*N* = 42)**	***p* value**
Age (years)[a]	49.2 ± 16.2	51.9 ± 14.9	36.4 ± 16.1	<0.001
Male gender	121 (50%)	100 (50.0%)	21 (50.0%)	1.000
Positive familial history	33 (13.6%)	18 (9%)	15 (35.7%)	<0.001
Levels of symptoms				
Asymptomatic	77 (31.8%)	65 (32.5%)	12 (28.5%)	0.778
Mild	138 (57.0%)	112 (56%)	26 (62.0%)	
Severe	27 (11.2%)	23 (11.5%)	4 (9.5%)	
u24h-normetanephrines (µg/day) (*N* = 142)[b]	1824 (850–4038)	1843.5 (888.5–4017.5)	1613 (722–4219)	0.879
u24h-metanephrines (µg/day) (*N* = 142)[b]	553.5 (195–2130)	694.5 (204.5–2386.5)	209 (88–400)	0.001
Secretory phenotype (*N* = 203)		*N* = 173	*N* = 30	0.015
Noradrenaline and adrenaline	111 (54.7%)	101 (58.4%)	10 (33.3%)	
Only noradrenaline	61 (30.0%)	50 (28.9%)	11 (36.7%)	
Only adrenaline	11 (5.4%)	9 (5.2%)	2 (6.7%)	
Not secretive	20 (9.9%)	13 (7.5%)	7 (23.3%)	
Positive chromogranin A (*N* = 149)	118 (79.2%)	98 (83.1%)	20 (19.9%)	0.387
Chromogranin A values (*N* = 149, ng/mL)[b]	290 (110–568)	284.4 (109–568)	423 (157–600)	0.420
Tumor type				0.060
Pheochromocytoma (PCC)	220 (90.9%)	185 (92.5%)	35 (83.3%)	
Paraganglioma (PGL)	22 (9.1%)	15 (7.5%)	7 (16.7%)	
Lesion side (only PCC, *N* = 220)		*N* = 185	*N* = 35	0.033
Left	97 (44.1%)	85 (46.0%)	12 (34.3%)	
Right	108 (49.1%)	91 (49.2%)	17 (48.6%)	
Bilateral	15 (6.8%)	9 (4.8%)	6 (17.1%)	
Tumor size (mm)[b]	50 (34-60)	48 (32–60)	50.5 (40–70)	0.063
^{123}I-MIBG Scintigraphy (*N* = 132)		*N* = 106	*N* = 26	0.328

Overall data (*N* = 242)		**Recurrence**		
		No (*N* = 200)	**Yes (*N* = 42)**	***p* value**
Positive	22 (16.7%)	16 (15.0%)	6 (23.1%)	
Negative	110 (83.3%)	90 (85.0%)	20 (76.9%)	
PASS (*N* = 177)[a]	3.97 ± 2.9	3.68 ± 2.7	6.2 ± 4.4	<0.001
PASS (*N* = 177)		*N* = 149	*N* = 28	0.013
<4	83 (46.9%)	76 (51.0%)	7 (25.0%)	
≥4	94 (53.1%)	73 (49.0%)	21 (75.0%)	
Genetic testing				<0.001
Negative	58 (24.0%)	51 (25.5%)	7 (16.7%)	
Positive	54 (22.3%)	27 (13.5%)	27 (64.3%)	
Not performed	130 (53.7%)	122 (61.0%)	8 (19.0%)	

[a]Data represent the mean ± standard deviation
[b]Data represent the median and interquartile range (IQR)

Table 2 Comparison between PCCs and PGLs

	Pheochromocytoma (*N* = 220)	**Paraganglioma (*N* = 22)**	***p* value**
Age (years)[a]	**49.8 ± 16.5**	**43.3 ± 11.7**	**0.072**
Gender			**0.371**
Male	**112 (50.9%)**	**9 (40.9%)**	
Female	**108 (49.1%)**	**13 (59.1%)**	
Genetic testing			**0.415**
Positive	**51 (23.2%)**	**7 (31.8%)**	
Negative	**48 (21.8%)**	**6 (27.3%)**	
Not performed	**121 (55.0%)**	**9 (40.9%)**	
u24h-normetanephrines (µg/day) (*N* = 142)[b]	**1785 (826–3997)**	**3995 (2854–8575)**	**0.063**

	Pheochromocytoma (*N* = 220)	Paraganglioma (*N* = 22)	*p* value
u24h-metanephrines (µg/day) (*N* = 142)[b]	574 (198−2200)	157 (82−373)	0.065
Secretory phenotype (*N* = 203)	*N* = 185	*N* = 18	<0.001
Noradrenaline and adrenaline	108 (58.5%)	3 (16.7%)	
Only noradrenaline	55 (29.7%)	6 (33.3%)	
Only Adrenaline	11 (5.9%)	0 (0%)	
Not secretive	11 (5.9%)	9 (50.0%)	
Tumor size (mm)[b]	48.5 (32−60)	55.5 (48−80)	0.003
^{123}I-MIBG Scintigraphy (*N* = 132)	*N* = 121	*N* = 11	0.376
Negative	22 (18.2%)	0 (0%)	
Positive	99 (81.8%)	11 100%)	

[a]Data represent the mean ± standard deviation
[b]Data represent the median and interquartile range (IQR)

Table 3 Univariate analysis of predictors of recurrence with the Cox model

Pheochromocytoma/paraganglioma (*n* = 242)	Univariate model	
	HR (95% CI)	*p* value
Age	0.95 (0.92−0.96)	<0.001
Genetic testing		
Negative	1.00	
Positive	4.59 (1.98−10.6)	< 0.001
Not performed	0.50 (0.18−1.38)	0.183
Tumor size	1.01 (1.00−1.02)	0.072
Tumor type		
Pheochromocytoma	1.00	

Pheochromocytoma/paraganglioma (n = 242)	Univariate model	
	HR (95% CI)	p value
Paraganglioma	1.83 (0.81−4.16)	0.146
u24h-normetanephrines (Log) (N = 142)	1.07 (0.68−1.67)	0.779
u24h-metanephrines (Log) (N = 142)	0.58 (0.40−0.85)	0.005
Secretive phenotype		
Noradrenaline and adrenaline	1.00	
Noradrenaline only	2.14 (0.91−5.05)	0.082
Adrenaline only	1.78 (0.39−8.13)	0.458
Not secretive	3.80 (1.42−10.1)	0.008
^{123}I-MIBG Scintigraphy (N = 132)		
Negative	1.00	
Positive	0.82 (0.30−2.22)	0.693
Familial history		
No	1.00	
Yes	3.34 (1.73−6.46)	<0.001
Levels of symptoms		
Asymptomatic	1.00	
Mild	1.10 (0.55-2.20)	0.777
Severe	1.04 (0.34-3.24)	0.942
Lesion side (only PCC, N = 220)		
Left	1.00	
Right	1.41 (0.67−2.96)	0.364
Bilateral	3.16 (1.14−8.76)	0.027
PASS (only PCC, N = 177)	1.24 (1.13−1.36)	<0.001
PASS (only PCC, N = 177)		
< 4	1.00	
≥4	3.01 (1.29−7.02)	0.011

Table 4 Multivariate analysis of predictors of recurrence of (a) PPGL (LR $X^2 = 43.55$, Prob > $X^2 = 0.0000$) and (b) PCC (LR $X^2 = 38.59$, Prob > $X^2 = 0.0000$)

Pheochromocytoma/paraganglioma ($N = 242$)	Multivariable model	
	HR (95% CI)	p value
Age	0.97 (0.95–0.99)	0.031
Tumor type		
Pheochromocytoma	1.00	
Paraganglioma	1.17 (0.48–2.82)	0.735
Tumor size	1.01 (1.00–1.02)	0.015
Genetic testing		
Negative	1.00	
Positive	3.62 (1.44–9.13)	0.006
Not performed	0.59 (0.21–1.63)	0.309
Pheochromocytoma ($N = 177$)	Multivariable model	
	HR (95% CI)	p value
Age	0.97 (0.94–0.99)	0.02
Lesion side		
Left adrenal	1.00	
Right adrenal	1.49 (0.62–3.63)	0.371
Left and right	1.33 (0.35–5.04)	0.679
Tumor size	1.01 (1.00–1.03)	0.043
Genetic testing		
Negative	1.00	
Positive	3.4 (1.00–11.48)	0.049
Not performed	0.6 (0.17–2.04)	0.41
PASS	1.16 (1.03–1.3)	0.011

Figure 1 Kaplan−Meier curves. a Cumulative incidence of recurrence. b Cumulative incidence of recurrence in patients with genetic mutation (HR = 4.59; 95% CI = 1.98−10.6; $p < 0.001$), with negative genetic testing (HR =0.50; 95% CI = 0.18−1.38; $p = 0.183$) and without performed genetic testing (long-rank