European Journal of Endocrinology (2002) **147** 85–94 ISSN 0804-4643

CLINICAL STUDY

The value of plasma markers for the clinical behaviour of phaeochromocytomas

E van der Harst, W W de Herder[1], R R de Krijger[2], H A Bruining, H J Bonjer, S W J Lamberts[1], A H van den Meiracker[1], T H Stijnen[3] and F Boomsma[1]

Departments of Surgery, [1]Internal Medicine, [2]Pathology and [3]Biostatistics, Erasmus University Hospital and MCRZ location Clara, Rotterdam, The Netherlands

(Correspondence should be addressed to E van der Harst, Department of Endocrine Surgery, MCRZ location Clara, PO Box 9119, 3007 AC Rotterdam, The Netherlands; Email: harstE@mcrz.nl)

Abstract

Objective: Phaeochromocytomas (PCCs) are widely known for their clinical unpredictability. This study intends to define predictive plasma markers for their variable postoperative behaviour. Furthermore, the diagnostic accuracy of these plasma tests was determined.
Design and Methods: A retrospective correlative study was performed in a series of 83 operated and four autopsied patients in order to correlate preoperative catecholamine (CAT) levels of 103 PCCs with their clinical behaviour. In a subset of cases, chromogranin-A (Chr-A) and enzymes/precursors of the CAT biosynthesis were studied for their predictive value.
Results: Basal CAT levels were elevated in 81/87 instances (sensitivity: 93%). Four of six cases with normal measurements showed only medullary hyperplasia. Larger PCCs, particularly those showing necrosis, capsular and vascular invasion, secreted higher CAT levels. Bilateral, hereditary tumours were less productive than their unilateral counterparts. Extra-adrenal PCCs secreted significantly lower levels of epinephrine (EPI) than intra-adrenal tumours. Fourteen patients developed metastases. According to Kaplan–Meier estimations, patients with higher levels of dopamine, norepinephrine (NE) and aromatic L-amino acid decarboxylase as well as lower ratios of EPI/EPI + NE, had significantly shorter metastases-free intervals. Existence of preoperative hypertension, left ventricular hypertrophy and measured blood pressures showed significant positive relationships with CAT levels, but not with Chr-A.
Conclusions: These data showed that plasma CAT measurement is a sensitive method in the diagnostic work-up of PCCs. Those tumours producing normal levels are commonly small and asymptomatic. Furthermore, certain secretion patterns are indicative of the presence of metastases as well as the size and site of sporadic and syndrome-related PCCs.

European Journal of Endocrinology **147** 85–94

Introduction

Phaeochromocytomas (PCCs), catecholamine (CAT)-secreting tumours of the sympathoadrenal system, have fascinated clinicians for decades. Symptoms due to variable hypersecretion of CATs range from almost absent in incidentally discovered PCCs to life-threatening conditions in large degenerating masses. In the absence of metastases, which occur in 10–25% of PCCs, no distinction can be made between benign and malignant tumours (1–5). Hereditary PCCs are usually intra-adrenal and commonly bilateral. Extra-adrenal PCCs (paragangliomas) are frequently multifocal (6). The cornerstone of diagnosis is biochemical evidence of excessive CAT production. For screening purposes, determination of urinary metanephrine excretion appears to be the most sensitive test (7). Recently, measurement of plasma metanephrines has been reported to be highly sensitive (8, 9) in screening patients with Von Hippel–Lindau disease (VHL) or multiple endocrine neoplasia type 2 (MEN2) (10). Nevertheless, the separate measurements of norepinephrine (NE), epinephrine (EPI) and dopamine (DA) remain of importance, since specific secretion patterns appear to be indicative of different clinical entities. Predominantly EPI-secreting PCCs, which do not lead to elevation of vanillylmandelic acid (VMA) (11), are relatively small and more common in cases of MEN2 (12, 13). They are rarely extra-adrenal in localisation (14) and tend to degenerate (15). Furthermore, separate measurement of CATs is useful for planning perioperative pharmacological management. High plasma NE levels have been associated with the highest degree of intraoperative haemodynamic instability, whereas absence

© 2002 Society of the European Journal of Endocrinology

of hypertension has been reported for tumours that secrete exclusively DA (16) or EPI (11, 17).

It is not clear whether specific secretion patterns are predictive for a more aggressive behaviour of PCCs. DA secretion has been reported to be indicative of malignancy (2, 18, 19). Furthermore, a reduced ratio of EPI and the sum of EPI plus NE was found to be predictive for ectopic localisation, recurrence and metastases (20). A high level of DA represents a more immature secretion pattern. Therefore, the study of enzymes and CAT precursors in the biosynthesis cascade is of interest. DA-β-hydroxylase (DBH), the enzyme that converts DA to NE, has been reported to be elevated in benign PCCs, but has not been studied in malignant tumours (21–24). 3,4-Dihydroxyphenylalanine (DOPA) and aromatic L-amino acid decarboxylase (ALAAD) appear to be useful prognostic markers for the recurrence in neuroblastomas (25), but have not been investigated in PCCs. Usefulness of chromogranin-A (Chr-A) determination in PCCs is a matter of controversy, since high rates of false-negative and false-positive results have been reported (26). As yet, the usefulness of Chr-A measurements in the diagnosis of malignant PCCs has not been established.

In this study, we report our experience with the determination of basal plasma CAT, DOPA, ALAAD, DBH and Chr-A concentrations in 87 patients with PCC diagnosed between 1983 and 1999. This study intends to define predictive plasma markers for the variable postoperative behaviour of PCCs. Furthermore, the diagnostic accuracy of these plasma tests was determined. Plasma levels were therefore correlated with malignancy, site and size of the tumour, tumour syndromes, and several macroscopic and microscopic features, which have been claimed to be associated with specific tumour behaviour. In addition, demographics, symptoms at presentation, and pre- and perioperative features in relation to CAT production were evaluated in order to define PCC subgroups with specific patterns of secretion.

Materials and methods

Basal plasma CAT assay

For adequate sampling, an intravenous cannula was inserted in a forearm vein. Subsequently, patients rested for 30 min in the supine position. Blood (20 ml) was collected into two chilled 10 ml heparinised polystyrene tubes containing 12 mg glutathione and plasma was stored at −70 °C. Plasma NE (normal: <600 pg/ml), EPI (normal: <100 pg/ml) and DA (normal: <100 pg/ml) were measured by high-performance liquid chromatography with fluorimetric or electrochemical detection (27). For the determination of plasma Chr-A (normal: <30 U/l) a commercially available ELISA kit was used as described previously (26). ALAAD (normal: <59 mU/l) was determined by its ability to form DA from the substrate 3,4-DOPA in the presence of pyridoxal-5-phosphate as cofactor (28). DOPA (normal: 3250 pg/ml) was determined after conversion to DA (29). DBH (normal: <67 U/l) was measured using a functional assay as described previously (30).

Patients and definitions

Basal plasma CAT levels were measured routinely in 87 patients treated for PCCs in our hospital between 1983 and 2001. Only cases with histologically proven intra-abdominal PCCs were included in this report. Solely extra-abdominal PCCs were excluded. *Malignant* PCCs were defined as metastasised tumours. *Metastases* were defined as the presence of PCC tissue at sites normally devoid of chromaffin tissue, either proven histologically or identified by ^{123}I-meta-iodobenzylguanidine (MIBG) scintigraphy and/or computed tomographic (CT) scans in conjunction with raised serum or urine levels of CAT levels or their metabolites. *Extra-adrenal site* was defined as the presence of a CAT-producing tumour below the diaphragm of the paraganglion system, the so-called visceral-autonomic and aortico-sympathetic phaeochromocytomas, including tumours of the organ of Zuckerkandl and the sympathetic chain. Follow-up consisted of regular history taking, physical examination and determination of urine or serum levels of CATs and/or their urinary metabolites. We investigated the possible correlation of preoperative basal plasma CAT levels with the following clinical and histopathological features.

Mode of presentation Patients presented with either typical paroxysms, sustained hypertension (taking antihypertensive drugs and/or blood pressure $\geq 180/95$ mmHg in the absence of typical paroxysms), malignant hypertension (hypertensive emergency with hypertensive fundus grade III or IV) (31), or were asymptomatic as in incidentalomas or familial cases during screening. The duration of the symptoms was registered. Symptoms that were recorded included headache, palpitations, spells of perspiration, nausea, blanching, tremor, vertigo and miscellaneous symptoms. Preoperative electrocardiograms were reviewed and the presence of left ventricular hypertrophy recorded, according to the voltage criteria of Sokolow & Lyon (32). Furthermore, it was noted whether patients presented with a cardiovascular emergency, necessitating acute intensive care treatment.

Syndromes/neurofibromatosis/sporadic PCCs Based on family history and, since 1994, on germline DNA analysis, tumour syndromes were classified as MEN2A (PCCs, medullary thyroid carcinoma and hyperparathyroidism), MEN2B (identical but more aggressive tumours of type 2A, Marphanoid habitus and mucosal ganglioneuromatosis), VHL (PCCs,

cerebellar haemangioblastomas, retinal angiomas and renal, pancreatic and epididymal tumours) or neurofibromatosis type 1 (NF).

Tumour characteristics Tumour site (intra-adrenal or extra-adrenal), the largest axis, volume and tumour weight were taken from the pathology reports. Tumours smaller than 2 cm were as a rule included *in toto*. Of larger tumours, two sections were taken per cm diameter. Histopathology of all primary tumours and metastases was reviewed by a neutral pathologist with substantial expertise in endocrine pathology (see Acknowledgements). Capsular invasion was scored negative if the capsule was intact or positive when tumour cells, mostly wedge shaped, invaded into or through the capsule. Vasoinvasion was scored either negative if absent or positive if invasion into intratumoural, capsular or surrounding vessels was present. In addition, existence of tumour degeneration was recorded as presented by intratumoural necrosis, haemorrhage or cysts.

Blood pressure measurements Preoperative measurements were performed non-invasively and highest blood pressure peaks recorded. During surgery, invasive systolic and diastolic measurements were recorded before induction and during tumour manipulation. Blood pressure peak levels and elevation from post-induction baseline values were recorded.

Statistical analysis

For comparing nominal variables between groups, the Chi-square test was used. The Mann–Whitney U test (for two groups) or the Kruskal–Wallis H test (for three or more groups) were performed to compare ordinal variables or continuous variables that were not approximately normally distributed. For quantifying associations between variables, Spearman's non-parametric correlation coefficient was used. The Kaplan–Meier procedure and log rank testing were performed to estimate and correlate postoperative PCC-free survival (metastases-free interval). For this purpose the occurrence of metastases was defined as the end-point. The end of follow-up or mortality due to unrelated or unknown causes were censored. P values less than 0.05 were considered significant.

Results

Patient characteristics (Table 1)

Seventy-one unilateral and 16 bilateral resections for 103 PCCs were executed. Median duration of symptoms was 38 (0–420) months. Longer delays were not associated with higher levels of plasma CATs or Chr-A. Nor did these levels show correlation with any of the presenting symptoms or signs. In 81 cases, 24-h urinary assays for CAT metabolites were also performed. Median follow-up on 1 September 2001 was 120 months. MEN2A was suspected and genetically confirmed in 12 cases. As part of a previous study (33), 36 additional patients with sporadic tumours had *RET* proto-oncogene analysis, revealing no other germline mutations. *VHL* tumour suppressor gene analysis was performed in 68 patients (34), revealing missense mutations in six.

In 14 of 87 patients at risk, metastases were diagnosed after a median disease-free interval of 29 months. Two of these 14 patients had only locoregional lymph node metastases in their resection specimens. In 12 other patients, localisation studies revealed distant metastases (multiple sites: $n = 7$; liver: $n = 2$; lung: $n = 3$). Elevation of plasma CAT and/or urinary metabolites recurred in eight cases and persisted at above normal values in three cases. In three cases, metastases were non-productive. Ten patients have

Table 1 Demographic characteristics. Values are expressed as median (range).

Demographic feature	Number
Patients (M/F)/PCCs	87 (39/48)/103
Malignant: pts/tumours	14/14
Benign (FU > 5 years): pts/tumours	60/75
Disease free (FU < 5 years): pts/tumours	13/14
Resections (unilateral/bilateral)	87 (71/16)
Laparotomy	62
Thoracolaparotomy	7
Lumbotomy	2
Laparoscopy	12
Autopsy	4
Age at resection	46 years (9–78)†
Follow-up	120 months (3–192)
Syndromes	
MEN2	12
VHL	6
NF	9
Symptoms at presentation*	
Hypertension	57/87
Palpitations	48/87
Perspiration	46/87
Headache	36/87
Blanching	36/87
Nausea	30/87
Tremors	19/87
Vertigo	1/87
Miscellaneous	5/87
Duration of symptoms	38 months (0–420)*
Urinary CAT metabolites (fold increase)[a]	
VMA	4.2 (0.7–25.0)‡
Normetanephrine	10.7 (1.0–59.0)¶
Metanephrine	9.0 (1.0–79)‖

*Correlations with CAT levels and Chr-A not statistically different; †statistically significant correlation with NE ($r = 0.245$; $P = 0.026$) and EPI ($r = 0.27$; $P = 0.017$); ‡statistically significant correlation with NE ($r = 0.64$; $P < 0.0005$) and DA ($r = 0.67$; $P < 0.0005$); ¶statistically significant correlation with NE ($r = 0.71$; $P = 0.01$) and DA ($r = 0.61$; $P = 0.04$); ‖statistically significant correlation with EPI ($r = 0.57$; $P = 0.05$).
[a] VMA, normetanephrine and metanephrine urinary levels were determined in 66, 15 and 15 patients respectively.
FU, follow-up; pts, patients.

died after a median postoperative interval of 68 months. Two patients are still alive despite widespread metastatic disease. Both patients with only locoregional metastases have been in complete remission since operation.

Of the 73 patients with benign PCCs, 60 patients have shown no signs of recurrence or metastases during a minimum follow-up of 5 years. Thirteen cases have so far been disease free, but follow-up has been less than 5 years. Postoperatively, plasma CAT and/or urinary CAT metabolites normalised in all but one benign case. This case had a benign recurrence in the contralateral adrenal gland 3 years after resection of an NE-secreting PCC.

Preoperative CAT secretion levels in benign and malignant PCCs (Table 2)

Basal plasma CAT levels were measured in 87 patients. Measurements of ALAAD, DOPA, DBH and Chr-A were available in 46, 42, 44 and 39 cases respectively. One or more basal plasma CAT levels were elevated in 81/87 instances (sensitivity: 93%).

In six cases, normal preoperative basal plasma CAT levels were recorded, despite histopathological proof of PCCs. None of these cases had elevated 24-h urinary CAT metabolites. Four of these six cases with normal CAT levels were subsequently shown to have PCC tumours smaller than 1 cm. Three of them were MEN2 cases, diagnosed during screening by positive localisation studies, whereas one sporadic case had a positive ^{123}I-MIBG scan, while plasma CAT levels became elevated only after glucagon stimulation. Two others were asymptomatic sporadic cases, incidentally discovered on CT scans.

Preoperative levels of NE, DA and ALAAD in malignant PCCs were significantly higher and more frequently elevated than in benign cases. In contrast, the ratio between EPI to EPI plus NE (EPI/EPI + NE) was significantly lower in the malignant group. Figure 1 shows Kaplan–Meier estimates of the postoperative metastases-free interval for patients at risk. This interval was significantly shorter in patients with lower than median preoperative levels of EPI/EPI + NE ($P = 0.002$ by the log rank test), higher levels of DA ($P = 0.002$), NE ($P = 0.0001$) and ALAAD ($P = 0.022$).

Basal CAT levels and clinical features (Table 3)

Patients with hereditary tumours (MEN2, VHL and NF) had lower preoperative NE and DA levels than patients with sporadic PCCs. Preoperative hypertension was present in 70% of cases. NE, EPI and DA levels were significantly higher in hypertensive than normotensive cases, whereas Chr-A levels did not differ between the two groups. NE and DA levels were significantly more increased in patients with left ventricular hypertrophy and in cases in whom cardiovascular emergencies had occurred before operation. Furthermore, these levels differed significantly between groups with particular modes of presentation. The highest levels were

Table 2 Preoperative plasma CAT levels in malignant, benign and disease-free tumours. Values are median (range).

				Benign PCCs		
PCC secretion	**Malignant PCCs**	**Benign PCCs**	***P****	FU > 5 years	FU < 5 years	*P*†
NE						
Elevation	14/14	51/73	0.003‡	44/60	7/13	0.004‖
Median level (pg/ml)	7629(3015–19 762)	1281 (115–155 240)	<0.0005†	1335 (236–155 240)	869 (115–17 044)	<0.0005¶
EPI						
Elevation	8/14	48/68	ns	40/55	8/13	ns
Median level (pg/ml)	294 (10–9100)	265 (14–11 200)	ns	266 (14–11 200)	225 (28–11 073)	ns
EPI/(EPI + NE)	2.12% (0.1–43.8%)	15.9% (0.3–80.5%)	0.001‖	15.4% (0.3–78.0%)	23.8% (1.1–80.5%)	0.003¶
DA						
Elevation	11/14	21/66	0.001‡	18/54	3/12	0.005‡
Median level (pg/ml)	232 (55–4330)	41 (1–3400)	<0.0005‖	43 (1–3400)	36 (5–472)	0.001¶
ALAAD						
Elevation	6/11	3/35	0.001‡	3/35	—	0.001‡
Median level (mU/l)	61 (15.6–330.8)	30 (12–236)	0.01‖	30 (12.0–235.0)	—	0.01‖
DOPA						
Elevation	6/11	15/31	ns	15/31	—	ns
Median level (pg/ml)	2852 (20–83 100)	3300 (898–77 900)	ns	3241 (898–77 900)	—	ns
DBH						
Elevation	6/11	24/33	ns	24/33	—	ns
Median level (U/l)	43 (13–362)	29 (7–232)	ns	31 (7.0–232)	—	ns
Chr-A						
Elevation	8/10	20/29	ns	20/29	—	ns
Median level (U/l)	151 (17.9–864)	46 (13–2500)	ns	46 (113.4–2500)	—	ns

*Malignant versus all benign cases; †malignant cases versus benign cases with postoperative follow-up (FU) periods longer and shorter than 5 years. ‡Chi-square test; ¶Kruskal–Wallis H test; ‖Mann–Whitney U test; ns, not significant.

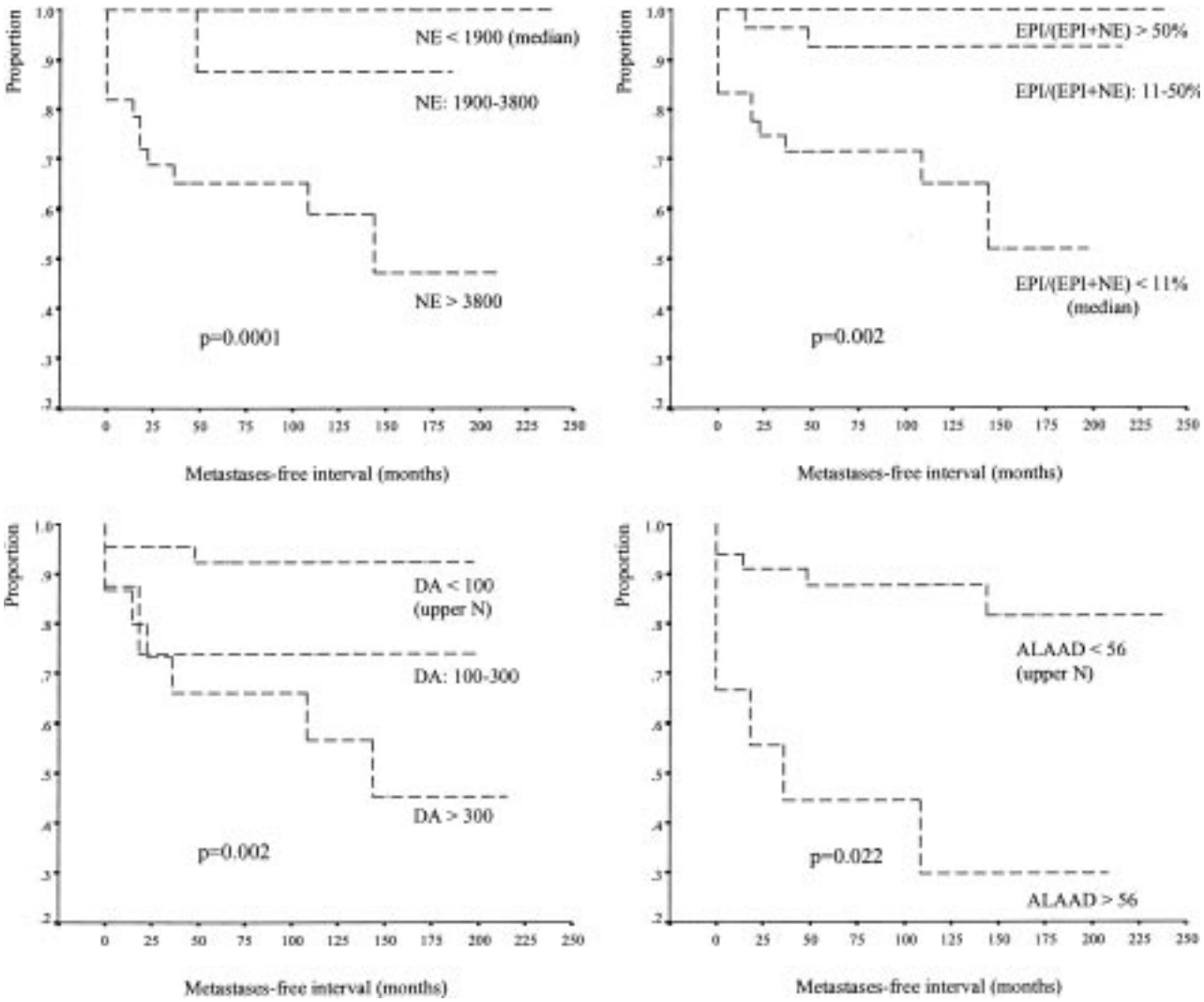

Figure 1 Metastases-free period (Kaplan–Meier graphs). Kaplan–Meier estimates of the metastases-free interval according to the percentage of EPI/EPI + NE and the levels of NE (normal: <600 pg/ml), DA (normal: <100 pg/ml) and ALAAD (normal: <59 mU/l).

measured in patients who were admitted for malignant hypertension, followed by patients analysed for sustained hypertension or for typical paroxysms. The lowest NE and DA levels were encountered in asymptomatic patients, whose tumours were more or less incidentally discovered or diagnosed in an early stage during a family screening programme.

Basal CAT levels and blood pressure measurements (Table 4)

Significant correlations were found for all CATs and the highest recorded preoperative diastolic and systolic blood pressures, prior to initiating α-adrenergic blockade. Despite α-adrenergic blockade, significant but slight intraoperative correlations with CAT levels were found in case of preinduction diastolic (NE), preinduction systolic (NE, DA) blood pressure measurements and the highest intraoperative systolic blood pressure peak (DA) during tumour manipulation.

Basal CAT levels and parameters of tumour size (Table 5)

Median tumour volume, largest axis and weight were $110.7\,cm^3$, 5.4 cm and 133.2 g respectively. All parameters of size were significantly correlated with CAT plasma concentrations and except for the largest axis also with Chr-A.

Basal CAT levels and tumour characteristics (Table 6)

Secretion of EPI was found to be lower in ectopic PCCs than in intra-adrenally located tumours. As opposed to unilateral tumours, bilateral tumours secreted lower

Table 3 Clinical features: correlations with basal CAT and Chr-A levels. Values are median (range).

Clinical feature	NE (pg/ml)	P	EPI (pg/ml)	P	DA (pg/ml)	P	Chr-A (U/l)	P
MEN2 or VHL or NF*								
Present ($n = 27$)	1201 (115–8332)		173 (17–4438)		39 (1–285)		36 (15.4–279)	
Absent ($n = 60$)	3505 (236–155 240)	0.03	336 (10–11 200)	ns	88 (4–4330)	0.006	65 (17.9–2500)	ns
Hypertension*								
Present ($n = 61$)	3385 (247–155 240)		442 (12–11 200)		106 (6–4330)		61 (13.4–2500)	
Absent ($n = 26$)	647 (115–28300)	0.002	80 (14–1351)	0.002	23 (1–303)	<0.0005	41 (15.4–175)	ns
Cardiovascular emergency*								
Present ($n = 12$)	7548 (575–155 240)		398 (37–11 021)		262 (25–763)		44 (15.4–864)	
Absent ($n = 75$)	1478 (115–27 600)	0.003	245 (10–11 200)	ns	49 (1–4330)	0.01	75 (17.0–2500)	ns
Left ventricular hypertrophy*								
Absent ($n = 62$)	1214 (247–155 240)		227 (12–11 073)		39 (1.0–1200)		40 (17.0–174)	
Present ($n = 25$)	6800 (583–27 600)	<0.0005	452 (10–11 200)	ns	271 (33–4330)	<0.0005	134 (13.4–2500)	0.02
Presentation†								
Asymptomatic ($n = 12$)	614 (236–2822)		57 (16–625)		16 (4.0–58.0)		99 (22.2–175)	
Hypertension analysis ($n = 16$)	3571 (746–12 469)		225 (10–9100)		105 (29.0–4330)		58 (26.6–362)	
Paroxysms ($n = 53$)	1920 (115–155 240)		452 (12–11 200)		60 (1.0–3400)		56 (13.4–2500)	
Malignant hypertension ($n = 6$)	11472 (1155–25 600)	0.004	304 (37–1170)	0.09	278 (160–378)	0.001	40 (15.4–864)	ns
Persistent hypertension*								
Absent ($n = 53$)	1742 (115–155 240)		172 (14–11 200)		35 (1.0–3400)		44 (20.7–277)	
Present ($n = 30$)	3505 (280–25 600)	ns	452 (10–11 200)	ns	161 (27–4330)	<0.0005	103 (15.4–2500)	ns

*Mann–Whitney U test; †Kruskal–Wallis H test; ns, not significant.

Table 4 Perioperative blood pressure correlations with CAT and Chr-A levels.

Highest BP (median±S.D.)	NE	P	EPI	P	DA	P	Chr-A	P
Preoperative								
Systolic BP (170±41 mmHg)	$r = 0.58$	<0.0005	$r = 0.29$	0.02	$r = 0.64$	<0.0005	$r = 0.10$	ns
Diastolic BP (103±25 mmHg)	$r = 0.61$	<0.0005	$r = 0.33$	0.009	$r = 0.71$	0.0005	$r = -0.06$	ns
Preinduction								
Systolic BP (150±32 mmHg)	$r = 0.30$	0.01	$r = 0.03$	0.82	$r = 0.33$	0.01	$r = 0.34$	ns
Diastolic BP (83±17 mmHg)	$r = 0.28$	0.02	$r = 0.02$	0.88	$r = 0.23$	0.07	$r = 0.12$	ns
Tumour manipulation								
Systolic BP (186±40 mmHg)	$r = 0.20$	ns	$r = 0.22$	0.07	$r = 0.28$	0.02	$r = 0.16$	ns
Diastolic BP (99±22 mmHg)	$r = 0.20$	ns	$r = -0.02$	ns	$r = 0.24$	ns	$r = -0.12$	ns
Increase of systolic BP (61±38 mmHg)	$r = 0.01$	ns	$r = 0.10$	ns	$r = 0.22$	ns	$r = 0.07$	ns
Increase of diastolic BP (27±17 mmHg)	$r = 0.08$	ns	$r = 0.03$	ns	$r = 0.08$	ns	$r = 0.03$	ns

r, correlation coefficient; BP, blood pressure; ns, not statistically significant.

Table 5 Tumour size correlations with CAT and Chr-A levels.

Size (median±S.D.)	NE	P	EPI	P	DA	P	Chr-A	P
Largest axis (5.4±3.1 cm)	$r = 0.42$	<0.0005	$r = 0.22$	0.05	$r = 0.44$	<0.0005	$r = 0.18$	ns
Volume (110.7±305.6 cm^3)	$r = 0.38$	0.001	$r = 0.35$	0.002	$r = 0.37$	0.001	$r = 0.34$	0.04
Weight (133.2±265.1 g)	$r = 0.47$	0.003	$r = 0.50$	0.002	$r = 0.45$	0.007	$r = 0.67$	0.006

Tumour volume was calculated according to the formula: $D_1 \times D_2 \times D_3 \times \pi/6$, where D_{1-3} represent the three dimensions of the resected tumours.
r, correlation coefficient; ns, not significant.

amounts of DA and EPI. Intratumoural necrosis was more frequently present in tumours producing higher levels of all sorts of CATs. Ingrowth or full capsular perforation of the tumour correlated with higher levels of NE and DA. No differences were found between secretion patterns of tumours with or without angioinvasion. In PCCs with haemorrhagic or cystic degeneration, DA, ALAAD and DBH were significantly more elevated.

Discussion

The present study intended to correlate endocrine activity of PCCs with clinical characteristics, tumour morphology and tumour behaviour. Overall, basal CAT levels were elevated in 93% of cases. In only six cases were these levels normal, despite histopathological diagnosis of PCCs. Notably, all of these latter cases had normal urinary CAT metabolites as well, but PCCs were more or less incidentally diagnosed by CT and MIBG scans in three sporadic cases and during family screening in three MEN2 cases. In four of these six cases, tumours were smaller than 1 cm, this has been arbitrarily referred to as micronodular medullary hyperplasia, the PCC-precursing lesion in the adrenal medulla in MEN2 (35) and sporadic (36, 37) cases. The following correlations of plasma markers and clinicopathological features of PCCs could be established.

Malignancy and CAT levels

Histopathological analysis of a primary tumour cannot reliably distinguish malignant from benign PCCs. Unambiguous proof of distant or nodal metastases is the only reliable evidence of malignancy. For practical purposes, massive local tumour invasion, resulting in irresectability, can be regarded as a criterion for malignancy. As effective treatment modalities are lacking, prognosis becomes very poor as soon as metastases have occurred (5-year survival: 23%) (20). Obviously, persistent or recurrent high levels of CATs or their metabolites should alert clinicians to recurrent or metastasised disease. Therefore, predicting malignancy before the occurrence of metastases would be of considerable clinical importance. In this study, the secretory profiles of PCCs that turned out to be malignant differed significantly from benign tumours. DA production, representing more premature CAT secretion, was confirmed to be predictive of the presence and future occurrence of metastases (16, 19, 38, 39). Furthermore, we have found malignant PCCs to secrete statistically higher levels of NE. Although this has been occasionally reported by others (20), in most studies no NE secretion differences were reported between benign and malignant PCCs (4, 19). The ratio EPI/EPI + NE, which serves as an index of tumour differentiation, has been claimed to have predictive impact for the presence of malignancy (20). In the present study, the prognostic value of this ratio was confirmed. Theoretically, other constituents of the CAT biosynthesis pathway would be better markers for an immature pattern of CAT secretion. This has been the first study to establish ALAAD as a preoperative marker of malignancy in PCCs. We found DOPA levels, which like ALAAD have been associated with tumour recurrence in resected neuroblastomas (25), to be similar in benign and malignant PCCs. Furthermore, neither Chr-A nor DBH proved to be associated with malignant behaviour.

Mode of presentation, symptoms, clinical work-up and CAT secretion pattern

Symptoms associated with PCC are often vague, and the correct diagnosis is frequently delayed. A long delay before PCC was properly diagnosed has been associated with a higher chance of developing metastases (19). In the present study, the CAT production profile was not related to the duration or the particular symptoms of PCC paroxysms. Other investigators have reported correlations between predominant EPI secretion and paroxysmal hypertension (40). In our study, significantly higher basal plasma levels of NE and DA were detected in patients presenting with malignant hypertension, whereas values were lower in patients presenting with only paroxysms or sustained hypertension. The lowest levels were found in PCCs which were incidentally discovered or diagnosed in an early stage through familial screening.

Data on correlations between CAT production and left ventricular hypertrophy are scarce. A recent paper reports its presence in 25% of PCC cases (41). Our data indicate left ventricular hypertrophy to be associated with higher plasma levels of NE, DA and Chr-A. Some authors have suggested that CATs exert a direct effect on myocardial growth (42). Others were, however, unable to show correlations between NE and EPI plasma levels and the presence of left ventricular hypertrophy (43).

Perioperative blood pressure measurements and CAT levels

Not surprisingly, before the start of α-adrenergic blockade, positive correlations were established between levels of CATs and the highest recorded preoperative diastolic and systolic blood pressures. Correlation coefficients were the highest for NE and DA. Chr-A levels did not show an association with measured blood pressure values. In other studies, PCCs with predominant NE secretion have been associated with the highest degree of haemodynamic instability during resection (12). We did not find NE levels to correlate with intraoperative blood pressure peaks. Only levels of DA, of which the haemodynamic effects are less

Table 6 Tumour characteristics: associations with CATs, ALAAD, DOPA, DBH and Chr-A levels. Values are median (range).

	NE	*P*	EPI	*P*	EPI/(EPI + NE)	*P*	DA	*P*	ALAAD	*P*	DOPA	*P*	DBH	*P*	Chr-A	*P*
Ectopic site*																
IA ($n = 93$)	1486 (115–155 240)		343 (14–11 200)		15.4% (0.3–80.5%)		46.5 (1–3400)		30.3 (12.0–235.5)		3182 (20–83 100)		32.6 (6.6–362.4)		55.1 (20.7–2500)	
EA ($n = 10$)	6429 (280–25 600)	ns	68.0 (10.0–1178)	0.02	9.8% (0.1–28.1%)	0.003	287.0 (55–4330)	0.001	37.7 (15.6–330.8)	ns	3500 (1226–68 500)	ns	20.8 (12.7–145.8)	ns	96.2 (13.4–864)	ns
Bi–/unilateral*																
BL ($n = 16$)	927 (115–13 968)		107 (16–3500)		15.8% (0.3–73.2%)		34.5 (1–306)		35.5 (16.7–98.8)		2703.5 (1233–15 200)		21.4 (7.8–96.1)		35.7 (15.4–278)	
UL ($n = 71$)	2256 (236–155 240)	ns	436 (10–11 200)	0.04	12.4% (0.1–80.5%)	ns	76.0 (4–4330)	0.01	31.2 (12.0–330.8)	ns	3400 (20–83 100)	ns	32.6 (6.6–362.4)	ns	61.3 (13.4–2500)	ns
Necrosis*																
Absent ($n = 67$)	1234 (115–25 600)		171 (14–4438)		15.3% (0.3–80.5%)		50 (1.0–2300)		28.2 (12.0–55.6)		3182 (898–77 900)		29.4 (6.6–114.6)		44.1 (21–864)	
Present ($n = 36$)	5900 (303–155 240)	0.003	609 (12–11 200)	ns	9.5% (0.2–77.8%)	ns	120 (9.0–4330)	0.05	61.1 (22.3–235.5)	<0.0005	2403 (20–83 100)	ns	70.9 (11.9–362.4)	0.04	172.4 (15–2500)	ns
Capsular ingrowth*																
Absent ($n = 49$)	630 (115–27 600)		126 (16–11 200)		15.9% (0.3–80.5%)		29.5 (1.0–340)		30.3 (15.2–69.5)		2200 (900–4899)		29.4 (7.8–114.6)		46.1 (25–134)	
Present ($n = 54$)	3412 (236–155 240)	0.003	452 (12–11 021)	ns	12.4% (0.2–62.9%)	ns	106 (16–2000)	<0.0005	32.6 (12.0–235.5)	ns	2876 (20–83 100)	0.04	32.6 (6.6–362.4)	ns	49.6 (15–2500)	ns
Angioinvasion*																
Absent ($n = 66$)	1281 (115–155 240)		225 (14.0–11 200)		15.4% (0.3–77.8%)		55.0 (1–3400)		32.6 (12.0–204.9)		2779 (20–77 900)		31.2 (7.8–362.4)		57.7 (22–864)	
Present ($n = 37$)	1908 (236–28 300)	ns	266 (16.0–4438)	ns	10.9% (0.3–80.5%)	ns	60.0 (6–4330)	ns	30.0 (15.2–235.5)	ns	2900 (898–83 100)	ns	37.0 (6.6–99.1)	ns	60.0 (15–362)	ns
Degeneration*																
Absent ($n = 42$)	1224 (115–25 600)		136 (14–3500)		15.4% (0.3–73.4%)		43.5 (5.0–2000)		30.1 (12.0–55.6)		3041 (898–77 900)		21.5 (6.6–99.1)		39.2 (21–864)	
Present ($n = 61$)	3571 (280–155 240)	ns	266 (12–11 200)	ns	12.4% (0.2–80.5%)	ns	106 (1.0–4330)	ns	42.2 (15.2–235.5)	0.04	3103 (20–83 100)	ns	57.8 (11.9–362.4)	0.03	109.2 (15–2500)	ns

IA, intra-adrenal; EA, extra-adrenal; BL, bilateral; UL, unilateral.
*Mann–Whitney U test.

strongly affected by α-adrenergic blocking, showed a slight correlation with systolic blood pressure peaks during tumour manipulation.

Tumour size and CAT levels

A positive linear correlation was established between preoperative plasma CAT levels and tumour size. Although this association makes sense and has been previously reported for urinary CAT metabolites (44), the literature on this subject is confusing. In two papers reporting large series of over 50 cases, no correlation between the tumour size and concentrations of CATs excreted in urine (45) and plasma (40) was established. Others observed positive relationships between tumour mass and plasma (nor-)metanephrines, but not with plasma NE and EPI (8, 9, 46). Therefore, tumour size was claimed to be a determinant for the rate of metabolisation of CATs into free metanephrines, but not of CAT secretion itself (8). In these investigations, however, DA levels were not determined.

Morphological aspects of PCCs and CAT levels

We found no correlations between basal CAT levels and PCCs showing angioinvasion. NE and DA levels were, however, significantly higher in locally aggressive PCCs showing capsular invasion and in malignant PCCs. Others have found capsular invasion to be predictive of malignancy (47). Necrosis within resected PCCs has been established as an unfavourable prognostic factor (5, 47). As in malignant PCCs, we found NE, DA and ALAAD levels to be significantly higher in PCCs with areas of necrosis. Degeneration in PCCs with pseudocyst formation is often present (5). It has been correlated with higher EPI levels (15) and appears to be causative for the paroxysmal release of CATs and sudden rise of blood pressure. In the present study, higher levels of DA, ALAAD and DBH were detected in tumours with cystic degeneration, but EPI levels were not different.

Ectopic and syndrome-related PCCs and CAT levels

Preoperative differentiation between extra-adrenal and intra-adrenal tumours remains troublesome, particularly for juxta-renal ectopic PCCs (48). Even in experienced hands almost 20% of the extra-adrenal PCCs remained undetected using contrast-enhanced CT (49). Extra-adrenal PCCs are multicentric in up to 25% of cases and non-functional ectopic localisations are not exceptional (48). Furthermore, extra-adrenal PCCs are more likely to be malignant than those found within the adrenal gland (48). Therefore, additional parameters to differentiate extra-adrenal from intra-adrenal PCCs preoperatively would be helpful, in order to focus attention on localising multifocal

tumours and metastases. In the present study, median EPI production was found to be five times lower in extra-adrenally located PCCs than in intra-adrenal tumours ($P = 0.008$). This finding may be at least partly attributed to MEN2-related PCCs, which are commonly intra-adrenally located (50) and predominantly EPI secreting (44). However, even considering only sporadic PCC cases ($n = 60$), a statistically significant positive correlation between intra-adrenal site and EPI production remained present ($P = 0.014$, data not shown). Unlike other investigators, we have been unable to establish a positive association between NE production and extra-adrenal site (20, 51).

We found significantly lower preoperative DA and EPI levels in patients with involvement of both adrenal glands than patients (so far) operated for a unilateral tumour. Patients with already established hereditary tumour syndromes had lower DA and NE levels than patients operated for sporadic PCCs. This indicates that PCCs in these patients are diagnosed at an earlier stage of disease than their unilateral, sporadic counterparts.

In conclusion, the present study has shown that determination of basal plasma CAT levels has an important place in the diagnostic work-up of PCCs. Overall, a sensitivity of 93% is reasonable and those tumours producing normal CAT levels ($n = 6$) were smaller than 1 cm in four cases and all but one was asymptomatic. In malignant PCCs, elevation of NE, DA and ALAAD levels occurred more frequently and values were significantly higher than in benign cases. In contrast, the ratio EPI/EPI + NE was significantly lower in malignant tumours. PCCs associated with hereditary tumour syndromes secreted lower levels of NE and DA. Furthermore, plasma CAT levels differed significantly, according to mode of presentation, the presence of left ventricular hypertrophy, perioperative blood pressures and the size of the resected PCCs. Different secretion patterns were also established for particular morphological changes in benign and malignant PCCs.

Acknowledgements

We thank Fred T Bosman (head of the Pathology Department of the University Hospital of Lausanne, Switzerland) for reviewing the histopathology of the primary tumours and their metastases.

References

1 Scott HW Jr & Halter SA. Oncologic aspects of phaeochromocytoma: the importance of follow-up. *Surgery* 1984 **96** 1061–1066.
2 Proye C, Vix M, Goropoulos A, Kerlo P & Lecomte Houcke M. High incidence of malignant phaeochromocytoma in a surgical unit. 26 cases of 100 patients operated from 1971 to 1991. *Journal of Endocrinological Investigation* 1992 **15** 651–663.
3 Modigliani E, Vasen HM, Raue K, Dralle H, Frilling A, Gheri RG *et al.* Phaeochromocytoma in multiple endocrine neoplasia type 2: European study. The Euromen Study Group. *Journal of Internal Medicine* 1995 **238** 363–367.
4 Mornex R, Badet C & Peyrin L. Malignant phaeochromocytoma: a series of 14 cases observed between 1966 and 1990. *Journal of Endocrinological Investigation* 1992 **15** 643–649.
5 Medeiros LJ, Wolf BC, Balogh K & Federman M. Adrenal phaeochromocytoma: a clinicopathologic review of 60 cases. *Human Pathology* 1985 **16** 580–589.
6 O'Riordain DS, Young WF Jr, Grant CS, Carney JA & van Heerden JA. Clinical spectrum and outcome of functional extraadrenal paraganglioma. *World Journal of Surgery* 1996 **20** 916–921.
7 Plouin PF, Duclos JM, Menard J, Comoy E, Bohuon C & Alexandre JM. Biochemical tests for the diagnosis of phaeochromocytoma: urinary versus plasma determinations. *British Medical Journal (Clinical Research Edition)* 1981 **282** 853–854.
8 Eisenhofer G, Keiser HR, Friberg P, Merzey E, Huynh TT, Hiremagalr B *et al.* Plasma metanephrines are markers of phaeochromocytoma produced by catechol-O-methyltransferase within tumours. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 2175–2185.
9 Lenders JWM, Keiser HR, Goldstein DS, Willemsen JJ, Friberg P, Jacobs MC *et al.* Plasma metanephrines in the diagnosis of phaeochromocytomas. *Annals of Internal Medicine* 1995 **123** 101–109.
10 Eisenhofer G, Lenders JWM, Linehan WM, Walther MM, Goldstein DS & Keiser HR. Plasma normetanephrine and metanephrine for detecting phaeochromocytoma in Von Hippel–Lindau disease and multiple endocrine neoplasia type 2. *New England Journal of Medicine* 1999 **340** 1872–1879.
11 Gerlo EAM & Sevens C. Urinary and plasma catecholamines and urinary catecholamine metabolites in phaeochromocytoma: diagnostic value in 19 cases. *Clinical Chemistry* 1994 **40** 250–256.
12 Col V, de Canniere L, Collard E, Michel L & Donckie J. Laparoscopic adrenalectomy for phaeochromocytomas: endocrinological and surgical aspects of a new therapeutic approach. *Clinical Endocrinology* 1999 **50** 121–125.
13 Hamilton BP, Landsberg L & Levine RJ. Measurement of urinary epinephrine in screening for phaeochromocytoma in multiple endocrine neoplasia type II. *American Journal of Medicine* 1978 **65** 1027–1032.
14 Von Euler US. Increased urinary excretion of noradrenaline and adrenaline in cases of phaeochromocytoma. *Annals of Surgery* 1951 **134** 929–931.
15 Ito Y, Obara T, Yamashita T, Kanbe M & Iihara M. Phaeochromocytomas: tendency to degenerate and cause paroxysmal hypertension. *World Journal of Surgery* 1996 **20** 923–926.
16 Proye C, Fossati P, Fontaine P, Lefebvre J, Decoulx M, Wemeau JL *et al.* Dopamine-secreting phaeochromocytoma: an unrecognised entity? Classification of phaeochromocytomas according to their type of secretion. *Surgery* 1986 **100** 1154–1162.
17 Bachmann AW, Hawkins PG & Gordon RD. Phaeochromocytomas secreting adrenaline but not noradrenaline do not cause hypertension and require precise adrenaline-measurement for diagnosis. *Clinical Experimental Pharmacology and Physiology* 1989 **16** 275–279.
18 Proye C, Vix M, Jansson S, Tisell LE, Dralle H & Hiller W. The phaeochromocytoma: a benign, intra-adrenal, hypertensive, sporadic unilateral tumour. Does it exist? *World Journal of Surgery* 1994 **18** 467–472.
19 John H, Ziegler WH, Hauri D & Jaeger P. Phaeochromocytomas: can malignant potential be predicted? *Urology* 1999 **53** 679–683.
20 Plouin P-F, Chatellier G, Fofol I & Corvol P. Tumour recurrence and hypertension persistence after successful phaeochromocytoma operation. *Hypertension* 1997 **29** 1133–1139.
21 Wocial B, Januszewicz W, Siedlecki I, Feltynowski T & Debinski W. Alterations in plasma dopamine-beta-hydroxylase and catecholamine concentrations during surgical removal of phaeochromocytoma. *Endokrinologie* 1982 **79** 131–139.

22 Feldman JM, Blalock JA, Zern RT & Wells SA. The relationship between enzyme activity and the catecholamine content and secretion of phaeochromocytomas. *Journal of Clinical Endocrinology and Metabolism* 1979 **49** 445–451.
23 Aunis D, Miras-Portugal MT, Coquillat G, Warter JM & Mandel P. Plasma dopamine beta-hydroxylase in a noradrenalin-secreting phaeochromocytoma. *Clinica Chimica Acta* 1976 **70** 455–458.
24 Feldman JM, Blalock JA, Farrell RE & Wells SA. Plasma and tumour dopamine-beta-hydroxylase activity in patients with familial phaeochromocytomas. *Metabolism* 1978 **27** 1797–1802.
25 Boomsma F, Ausema L, Hakvoort-Cammel FGA, Oosterom R, Man in't Veld AJ, Krenning EP *et al.* Combined measurement of plasma aromatic L-amino acid decarboxylase and DOPA as tumour markers in diagnosis and follow-up of neuroblastoma. *European Journal of Cancer and Clinical Oncology* 1989 **25** 1045–1052.
26 Boomsma F, Bhagoe UM, Man in't Veld AJ & Schalekamp MA. Sensitivity and specificity of a new ELISA method for determination of chromogranin A in the diagnosis of phaeochromocytoma and neuroblastoma. *Clinica Chimica Acta* 1995 **239** 57–63.
27 van der Hoorn FAJ, Boomsma F, Man in't Veld AJ & Schalekamp MADH. Determination of catecholamines in human plasma by high performance liquid chromatography comparison between a new method with fluorescence detection and an established method with electrochemical detection. *Journal of Chromatography* 1989 **487** 17–28.
28 Boomsma F, van der Hoorn FAJ & Schalekamp MADH. Determination of aromatic-L-amino acid decarboxylase in human plasma. *Clinica Chimica Acta* 1986 **159** 173–183.
29 Boomsma F, van der Hoorn FAJ, Man in't Veld AJ & Schalekamp MADH. Determination of 3,4-dihydroxyphenylalanine (DOPA) in plasma and cerebrospinal fluid by high performance liquid chromatography with electrochemical detection. *Clinica Chimica Acta* 1988 **178** 59–70.
30 Boomsma F, Meerwaldt JD, Man in't Veld AJ, Hovestadt A & Schalekamp MA. Treatment of idiopathic parkinsonism with L-dopa in the absence and presence of decarboxylase inhibitors: effects on plasma levels of L-dopa, dopa decarboxylase, catecholamines and 3-O-methyl-dopa. *Journal of Neurology* 1989 **236** 223–230.
31 Ahmed MEK, Walker JM, Beevers DG & Beevers M. Lack of difference between malignant and accelerated hypertension. *British Medical Journal* 1986 **292** 235–237.
32 Sokolov M & Lyon TP. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limbleads. *American Heart Journal* 1949 **37** 161–186.
33 van der Harst E, de Krijger RR, Bruining HA, Lambers SWJ, Bonjer HJ, Dinjens WNM *et al.* Prognostic value of *RET* proto-oncogene point mutations in malignant and benign, sporadic phaeochromocytomas. *International Journal of Cancer* 1998 **79** 537–540.
34 van der Harst E, de Krijger RR, Dinjens WNM, Weeks LE, Bonjer HJ, Bruining HA *et al.* Germline mutations in the *VHL*-gene in patients presenting with phaeochromocytomas. *International Journal of Cancer* 1998 **77** 337–340.
35 Carney JA, Sizemore GW & Sheps SG. Adrenal medullary disease in multiple endocrine neoplasia, type 2. *American Journal of Clinical Pathology* 1976 **66** 279–290.
36 Kurihara K, Mizuseki K, Kondo T, Ohoka H, Mannami M & Kawai K. Adrenal medullary hyperplasia. Hyperplasia-phaeochromocytoma sequence. *Acta Pathologica Japonica* 1990 **40** 683–686.
37 Qupty G, Ishay A, Peretz H, Dharan M, Kaufman N & Luboshitzky R. Phaeochromocytoma due to unilateral adrenal medullary hyperplasia. *Clinical Endocrinology* 1999 **47** 613–617.
38 Schlumberger M, Gicquel C, Lumbroso J, Tenenbaum F, Comoy E, Bosq J *et al.* Malignant phaeochromocytoma clinical, biological, histologic and therapeutic data in a series of 20 patients with distant metastases. *Journal of Endocrinological Investigation* 1992 **15** 631–642.
39 Tippett PA, McEwan AJ & Ackery DM. A re-evaluation of dopamine excretion in phaeochromocytoma. *Clinical Endocrinology* 1986 **25** 401–410.
40 Ito Y, Fujimoto Y & Obara T. The role of epinephrine, norepinephrine, and dopamine in blood pressure disturbances in patients with phaeochromocytoma. *World Journal of Surgery* 1992 **16** 759–763.
41 Shub C, CuetoGarcia L, Sheps SG, Ilstrup DM & Tajik AJ. Echocardiographic findings in phaeochromocytoma. *American Journal of Cardiology* 1986 **57** 971–975.
42 Cho T, Tanimura A & Saito Y. Catecholamine-induced cardiopathy accompanied with phaeochromocytoma. *Acta Pathologica Japonica* 1987 **37** 123–132.
43 Fouad-Tarazi FM, Imamura M, Bravo EL, Rossi G, Nagi HK, Lin WW *et al.* Differences in left ventricular structural and functional changes between phaeochromocytoma and essential hypertension. Role of elevated circulating catecholamines. *American Journal of Hypertension* 1992 **5** 134–140.
44 Stenstrom G & Waldenstrom J. Positive correlation between urinary excretion of catecholamine metabolites and tumour mass in phaeochromocytoma. *Acta Medica Scandinavica* 1985 **217** 73–77.
45 Liu TH, Chen GS, Nan C & He ZG. Clinicopathological and ultrastructural characteristics of phaeochromocytoma. An analysis of 55 cases. *Pathology, Research and Practice* 1984 **178** 355–362.
46 Crout JR & Sjoerdsma A. Turnover and metabolism of catecholamines in patients with phaeochromocytoma. *Journal of Clinical Investigation* 1964 **43** 94–102.
47 Linnoila RI, Keiser HR, Steinberg SM & Lack EE. Histopathology of benign versus malignant sympathoadrenal paragangliomas: clinicopathologic study of 120 cases including unusual histologic features. *Human Pathology* 1990 **21** 1168–1180.
48 Whalen RK, Althausen AF & Daniels GH. Extra-adrenal phaeochromocytoma. *Journal of Urology* 1992 **147** 1–10.
49 Moulton JS. CT of the adrenal glands. *Seminars in Roentgenology* 1999 **23** 288–293.
50 Pomares FJ, Canas R, Rodriquez JM, Hernandez AM, Parilla P & Tebar FJ. Difference between sporadic and multiple endocrine neoplasia type 2A phaeochromocytoma. *Clinical Endocrinology* 1998 **48** 195–200.
51 Crout JR & Sjoerdsma A. Catecholamines in the localisation of phaeochromocytomas. *Circulation* 1960 **22** 516–522.

Received 19 October 2001
Accepted 3 April 2002