Malignant Pheochromocytoma
Chromaffin Granule Transmitters and Response to Treatment

Fangwen Rao, Harry R. Keiser, Daniel T. O’Connor

Abstract—Chromaffin granule transmitters such as chromogranin A and catecholamines have been used in the diagnosis of pheochromocytoma, but the diagnostic and prognostic value of chromogranin A have not been explored in malignant pheochromocytoma. We evaluated these transmitters in patients with pheochromocytoma (n=27), both benign (n=13) and malignant (n=14). Patients with benign pheochromocytoma were studied before and after surgical excision (n=6), whereas patients with malignant pheochromocytoma were evaluated before and after combination chemotherapy with regular cycles of cyclophosphamide/dacarbazine/vincristine (nonrandomized trial in n=9). During treatment, patient responses to chemotherapy were divided according to anatomic and clinical criteria: responders (n=5) versus nonresponders (n=4). Plasma chromogranin A rose progressively (P<0.0001) from control subjects (48.0±3.0 ng/mL) to benign pheochromocytoma (188±40.5 ng/mL) to malignant pheochromocytoma (2932±960 ng/mL). Parallel changes were seen for plasma norepinephrine (P<0.0001), though plasma epinephrine was actually lower in malignant than benign pheochromocytoma (P=0.0182). In bivariate analyses, chromogranin A, norepinephrine, and epinephrine discriminated between pheochromocytoma and control subjects (all P<0.0001), whereas in a multivariate analyses, norepinephrine was the best discriminator (P=0.011). Chromogranin A was significantly different in benign versus malignant pheochromocytoma on both bivariate (P=0.0003) and multivariate (P=0.011) analyses. After excision of benign pheochromocytoma, chromogranin A (P=0.028), norepinephrine (P=0.047), and epinephrine (P=0.037) fell to values near normal. During chemotherapy of malignant pheochromocytoma (n=9), plasma chromogranin A (P=0.047) and norepinephrine (P=0.02) fell but not epinephrine. In 5 responders to chemotherapy, there were significant declines in chromogranin A (P=0.03) and norepinephrine (P=0.03) but not epinephrine; in 4 nonresponders, none of the transmitters changed. Plasma chromogranin A varied longitudinally with tumor response and relapse. We conclude that plasma chromogranin A is an effective tool in the diagnosis of pheochromocytoma, and markedly elevated chromogranin A may point to malignant pheochromocytoma. During chemotherapy of malignant pheochromocytoma, chromogranin A can be used to gauge tumor response and relapse. (Hypertension. 2000;36:1045-1052.)

Key Words: plasma ■ chromogranins ■ norepinephrine ■ epinephrine

Malignant pheochromocytoma presents several difficulties in management, both in diagnosis and in effective treatment. Even after excision, the diagnosis of malignant pheochromocytoma cannot be made reliably on histological grounds; thus, extensive evaluations of tumor location are required to ascertain the presence of local or distant invasion or metastases by either surgical exploration, imaging, or regional venous blood sampling. Specific biochemical diagnosis of malignant pheochromocytoma has received only limited attention. Once diagnosed, metastatic pheochromocytoma may be effectively treated. In analogy to neuroblastoma, combination chemotherapeutic regimens involving cyclophosphamide/dacarbazine/vincristine have been developed for malignant pheochromocytoma: when regularly scheduled and dose-escalated to the threshold of hematological toxicity, such regimens may result in effective (though temporary) remission in more than half of such patients; during these regimens, urinary catecholamine excretion has previously been used to estimate clinical response.

Chromogranin A is an acidic protein costored and coreleased by exocytosis, along with catecholamines from chromaffin granules of normal adrenal medulla and pheochromocytoma. In this study, we evaluated the utility of plasma concentrations of chromaffin granule transmitters (chromogranin A, norepinephrine, and epinephrine) in the management of pheochromocytoma, both diagnostic and therapeutic. Our results indicate that measurement of plasma chromogranin A is useful in the diagnosis of pheochromocytoma, and a markedly elevated chromogranin A may suggest the diagnosis of malignant pheochromocytoma. Chromogranin A may also assist in ascertaining the response to chemotherapy in malignant disease.
Methods

Patients
The study was approved by the institutional review boards, and patients gave informed consent. Plasma samples were obtained from 14 patients with malignant pheochromocytoma. Malignancy was documented by metastatic disease to the retropertioneum, bone, lung, mediastinum, lymph nodes, spleen, or other sites. The mean age was 40.4 years (range, 16 to 64), and there was an 11:3 male/female predominance. The primary tumor arose from the adrenal gland(s) in 7 patients and from extra-adrenal sites in 7 patients. One patient had Von Hippel-Lindau syndrome. Nine of these patients underwent combination chemotherapy (see below), with mean and median intervals between the initial pathological diagnosis and the first chemotherapy treatment of 48.2 and 26 months, respectively.

Thirteen patients with benign pheochromocytoma were also evaluated, with postsection data available on 6. The mean age was 36.5 (range, 16 to 60) years, and there was a 10:3 female/male predominance (11 white, 2 black). Eleven benign tumors were intra-adrenal: 6 unilateral (4 right, 2 left) and 5 bilateral. The other 2 benign tumors were extra-adrenal (1 in the urinary bladder and 1 bilateral and extra-adrenal). Two benign tumors were familial, both with multiple endocrine neoplasia type 2. One patient had Cushing’s syndrome, which subsided on resection of the pheochromocytoma. In benign pheochromocytoma, treatment consisted of surgical resection.

Forty-nine normal control subjects (for chromogranin A) and 178 other normal control subjects (for norepinephrine and epinephrine) were studied.

The diagnosis of pheochromocytoma was made clinically, biochemically, and histologically.4,5 Because no reliable gross or microscopic features distinguish benign from malignant pheochromocytoma, the diagnosis of malignant pheochromocytoma was based on the presence of regional or distant metastases.4,5,6 Some features of 12 of these 14 malignant pheochromocytoma patients were reported previously.4

Assays
The plasma concentration of chromogranin A was measured by a rapid modification of a soluble-phase, double-antibody radioimmunoassay (11 white, 2 black). Plasma catecholamines were measured by high-performance liquid chromatography with electrochemical detection.17,18 The lower limit of detection of plasma epinephrine was 5 pg/mL. (To convert chromogranin A from mg/mL to μg/L, multiply by 1.0. To convert epinephrine from pg/mL to pmol/L, multiply by 5.458. To convert norepinephrine from pg/mL to nmol/L, multiply by 0.0005911.)

Treatment of Malignant Pheochromocytoma
Before treatment with combination chemotherapy, specific antihypertensive therapy was administered to patients to maintain normal blood pressure. Initial treatment consisted of oral propanolol or atenolol. The catecholamine synthesis inhibitor α-methyl-para-tyrosine was also administered orally, as previously described.3,4

Combination chemotherapy3,4 was administered to 9 of the 14 patients with malignant pheochromocytoma. The combination chemotherapy regimen consisted of intravenous cyclophosphamide, 750 mg/m² body surface area on day 1; vincristine, 1.4 mg/m² on day 1; and dacarbazine, 600 mg/m² on days 1 and 2. The combination was repeated on a 21-day cycle; hematologic toxicity (leukopenia) prompted either a treatment delay of 1 week or appropriate downward dosage modifications. If there was no significant hematologic toxicity, the doses of cyclophosphamide and dacarbazine were increased by 10% at each cycle until myelosuppression was seen. To monitor for possible hemodynamic or endocrine complications of chemotherapy, all patients received their first treatment while hospitalized. Subsequent treatment cycles were on an outpatient basis. The patients’ responses to therapy were divided by 2 criteria: tumor (anatomic) response and biochemical response. Anatomic response was judged according to modified standard criteria3,4 and classified as complete response (complete regression of all clinical evidence of disease, including resolution of all palpable and radiologic abnormalities); partial response (≥50% reduction of all measurable tumor); minimal response (≥25% but not >50% reduction of all measurable tumor); no change; and progression (the appearance of a new lesion or an increase of 25% in any measurable tumor). Change in tumor size was determined from 2 measurement periods at least 4 weeks apart and calculated by the product of 90° cross-perpendicular diameters at their greatest length. Biochemical responses were initially based on 24-hour urinary determinations of free catecholamines, metanephrines, and vanillylmandelic acid and were defined as complete response (return to normal values); partial response (≥50% reduction); minimal change; and progression (an increase of ≥25% in all 3 measurements). The patients were followed longitudinally until evidence of progressive disease, at which time therapy was discontinued.

The 9 patients with chemotherapy-treated malignant pheochromocytoma in this study were divided into 2 groups according to the clinical response to chemotherapy: a “no response” group (n=4, including minor or no response) and a “response” group (n=5, including complete or partial response). Plasma samples for chromogranin A, norepinephrine, and epinephrine were obtained both before treatment and after treatment, at the time of maximal tumor regression. Posttreatment biochemical nadir values were analyzed. Two patients with malignant pheochromocytoma were also subjected to surgical “debulking” of their malignant pheochromocytoma tumor masses; their results were not included in the analysis of chemotherapy response.

Statistical Analyses
Data are reported as mean±1 SEM. Several statistical methods were used, both parametric (1-way ANOVA, t tests) and nonparametric (Mann-Whitney U test, Wilcoxon signed rank test). After ANOVA, post hoc comparisons of 2 groups were performed by the Bonferroni procedure in SPSS (Statistical Package for the Social Sciences) to guard against inappropriate conclusions drawn from multiple comparisons. Nonparametric tests were used if the sample size was very small (<10) or if the data were not distributed normally. Two-tailed tests were used unless a prior directional hypothesis was tested. Probability values of ≤0.05 were considered significant. Simultaneous-model, multiple linear regression analysis was performed in SPSS to assess the relative diagnostic value of several independent variables; during stepwise multiple linear regression, criteria for retention or exclusion of an independent variable from the model were significance levels of α=0.05. For analysis of treatment (surgery of chemotherapy) effects, posttreatment nadir values were used. Analyses were performed in the programs Excel (Microsoft), InStat (GraphPad Software), or SPSS (Statistical Package for the Social Sciences).

Results
Diagnosis of Pheochromocytoma

ANOVA
Chromogranin A (F=20.3, P<0.0001), norepinephrine (F=107, P<0.0001), and epinephrine (F=41.2, P<0.0001) were markedly elevated in patients with pheochromocytoma (Table 1). For chromogranin A and norepinephrine, there were progressive rises in plasma concentration from normal control subjects (n=178) to benign pheochromocytoma (n=13), to malignant pheochromocytoma (n=14). For epinephrine, concentrations were highest in patients with benign pheochromocytoma (670±267 pg/mL).

Pheochromocytoma Versus Control
To test which biochemical variables best distinguished patients with pheochromocytoma (all n=27; n=13 benign plus
n=14 malignant) from control subjects without pheochromocytoma, we used both bivariate (simple) and multivariate methods. In bivariate analyses, pheochromocytoma patients differed from control subjects in chromogranin A (1611±558 versus 48.0±3.0 ng/mL, P<0.0001), norepinephrine (4912±955 versus 200±7.8 pg/mL, P<0.0001), and epinephrine (415±144 versus 18.0±1.5 pg/mL, P<0.0001) but not in the ratios of chromogranin A/norepinephrine (0.96±0.52 versus 0.15±0.04 ng/pg, P=0.44), chromogranin A/epinephrine (377±112 versus 51.5±23.7 pg/pg, P=0.103), or norepinephrine/epinephrine (233±104 versus 3.9±1.19 pg/pg, P=0.086).

In a multivariate analysis, incorporating the same 6 plasma biochemical independent variables (chromogranin A, norepinephrine, epinephrine, or their ratios [chromogranin A/norepinephrine, chromogranin A/epinephrine, norepinephrine/epinephrine]), only a marginally significant model emerged (multiple R=0.565, R²=0.319, adjusted R²=0.178, F=2.27, P=0.065). When stepwise multiple linear regression was allowed to select the model, the independent variable best predicting the distinction of pheochromocytoma versus control was norepinephrine (multiple R=0.417, R²=0.174, adjusted R²=0.149, t=2.67, F=7.14, P=0.011).

### Pheochromocytoma: Benign Versus Malignant

What biochemical features best distinguished malignant from benign pheochromocytoma? Among the 27 patients with pheochromocytoma (Figure 1), bivariate analyses indicated that chromogranin A was substantially higher in patients with malignant (n=14) than benign (n=13) tumors (2932±960 versus 188±40.5 ng/mL, P=0.0003), as was norepinephrine (6646±1608 versus 3044±727 pg/mL, P=0.0344), though epinephrine was actually lower in malignant pheochromocytoma (179±101 versus 670±267 pg/mL, P=0.0182). Malignant and benign pheochromocytoma patients also differed in the ratios of chromogranin A/epinephrine (440±187 versus 11.0±7.88 ng/pg, P=0.001) and norepinephrine/epinephrine (539±199 versus 156±81.7 pg/pg, P=0.0054) but not chromogranin A/norepinephrine (1.64±0.98 versus 0.18±0.08 ng/pg, P=0.0631).

---

### Table 1. Plasma Concentrations of Chromogranin A, Norepinephrine, and Epinephrine in Pheochromocytoma

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Chromogranin A, ng/mL</th>
<th>Norepinephrine, pg/mL</th>
<th>Epinephrine, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control subjects</td>
<td>27</td>
<td>48.0±3.0 (n=49)</td>
<td>200±7.8 (n=178)</td>
<td>18.0±1.5 (n=178)</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>27</td>
<td>1611±558*</td>
<td>4912±955*</td>
<td>415±144*</td>
</tr>
<tr>
<td>Benign</td>
<td>13</td>
<td>188±40.5*</td>
<td>3044±727*</td>
<td>670±267*</td>
</tr>
<tr>
<td>Malignant</td>
<td>14</td>
<td>2932±960*</td>
<td>6646±1608*</td>
<td>179±101*</td>
</tr>
</tbody>
</table>

1-Way ANOVA

*F=20.3, P<0.0001

F=107, P<0.0001

F=41.2, P<0.0001

---

**Figure 1.** Plasma concentrations of chromaffin granule transmitters (chromogranin A, norepinephrine, or epinephrine) in subjects with pheochromocytoma (n=27) stratified by tumor behavior, benign (n=13) versus malignant (n=14). Individual values are shown from samples obtained before treatment. Normal ranges (see Table 1): chromogranin A, 48.0±3.0 ng/mL; norepinephrine, 200±7.8 pg/mL; epinephrine, 18.0±1.5 pg/mL. Lower limit of detection for plasma epinephrine is 5 pg/mL. (To convert chromogranin A from ng/mL to µg/L, multiply by 1.0. To convert norepinephrine from pg/mL to pmol/L, multiply by 5.458. To convert norepinephrine from pg/mL to nmol/L, multiply by 0.005911.)
Chemotherapy for Malignant Pheochromocytoma

Nine of the 14 patients with malignant pheochromocytoma underwent multiple cycles of intravenous combination chemotherapy with cyclophosphamide/vincristine/dacarbazine under or near the normal ranges (Figure 3). Nine of the 14 patients with malignant pheochromocytoma (in 8 of 14 [57%] of subjects with malignant disease). By stepwise multiple linear regression, the most significant biochemical difference between malignant and benign disease was chromogranin A (multiple \( R = 0.482 \), adjusted \( R^2 = 0.202 \), \( F = 7.56, P = 0.011 \)).

Response to Treatment

Surgery for Benign Pheochromocytoma

Six patients with benign pheochromocytoma underwent resection of the tumor, with plasma sampling before and after operation (Figure 3); postoperative nadir values were analyzed (samples obtained at 4.1 ± 2.0 [range, 0.7 to 13.5] months after resection). Chromogranin A declined by \( \approx 79\% \) (from 191 ± 62 to 41 ± 6.2 ng/mL; \( P = 0.028 \)), norepinephrine by \( \approx 87\% \) (from 3500 ± 1439 to 439 ± 172 pg/mL; \( P = 0.047 \)), and epinephrine by \( \approx 78\% \) (from 288 ± 101 to 64 ± 41 pg/mL; \( P = 0.037 \)) to values within or near the normal ranges (Figure 3).

Chemotherapy for Malignant Pheochromocytoma

Nine of the 14 patients with malignant pheochromocytoma underwent multiple cycles of intravenous combination chemotherapy with cyclophosphamide/vincristine/dacarbazine. Spearman \( r = -0.378, n = 27, P = 0.026 \). To convert chromogranin A from ng/mL to \( \mu \)g/L, multiply by 1.0. To convert epinephrine from pg/mL to pmol/L, multiply by 5.458. To convert norepinephrine from pg/mL to nmol/L, multiply by 0.005911.)

On inspection of Figure 1, it can be seen that a chromogranin A value of >600 ng/mL was observed only in subjects with malignant pheochromocytoma (in 8 of 14 [57%] of subjects with malignant disease).

In patients with pheochromocytoma (benign plus malignant grouped together), chromogranin A correlated inversely with epinephrine (Figure 2; Spearman \( r = -0.378, n = 27, P = 0.026 \)).

In a multivariate analysis, we tested which of these 6 plasma biochemical independent variables (chromogranin A, norepinephrine, epinephrine, or their ratios [chromogranin A/norepinephrine, chromogranin A/epinephrine, norepinephrine/epinephrine]) differed between benign and malignant disease. By stepwise multiple linear regression, the most significant biochemical difference between malignant and benign disease was chromogranin A (multiple \( R = 0.482 \), adjusted \( R^2 = 0.202 \), \( F = 7.56, P = 0.011 \)).

Response of chromaffin granule transmitter concentrations to successful surgical resection of benign adrenal pheochromocytoma (n = 6 patients). Postoperative nadir values were analyzed (samples obtained at 4.1 ± 2.0 [range, 0.7 to 13.5] months after resection). Normal ranges (see Table 1): chromogranin A, 480.0 ± 3.0 ng/mL; norepinephrine, 200.0 ± 7.8 pg/mL; epinephrine, 180.0 ± 1.5 pg/mL. Paired comparisons were made by nonparametric Wilcoxon signed rank test. \( P < 0.05 \) compared with pretreatment. (To convert chromogranin A from ng/mL to \( \mu \)g/L, multiply by 1.0. To convert epinephrine from pg/mL to pmol/L, multiply by 5.458. To convert norepinephrine from pg/mL to nmol/L, multiply by 0.005911.)

Effects of combination chemotherapy (without surgery) on plasma concentrations of chromogranin A, norepinephrine, and epinephrine in patients with malignant pheochromocytoma (n = 9). Values represent mean ± 1 SEM. Post–chemotherapy nadir values were analyzed (samples obtained at 11 ± 1.8 [range, 4 to 22] months after initiation of treatment). After chemotherapy, in this group as a whole (n = 9) there were modest overall declines in the plasma concentrations of chromogranin A (from 2194 ± 1034 to 1752 ± 1095 ng/mL, \( P = 0.047 \)) and norepinephrine (from 7602 ± 2165 to 6386 ± 4563 pg/mL, \( P = 0.02 \)) but not epinephrine (from 178 ± 134 to 768 ± 711 pg/mL, \( P = 0.129 \)). In each case, the posttreatment values were substantially higher than the normal ranges (Figure 4).
Because the anatomic and clinical responses to chemotherapy were variable from patient to patient, we then analyzed biochemical responses in patient groups stratified by response (n = 5 responders, n = 4 nonresponders). In the chemotherapy responders (n = 5), there were significant declines (Figure 5) in chromogranin A (from 867 ± 439 to 78.4 ± 20 ng/mL, P = 0.03) and norepinephrine (from 9983 ± 3442 to 909 ± 304 pg/mL, P = 0.03) but not epinephrine (from 317 ± 234 to 70.4 ± 18 pg/mL, P = 0.31). In the 4 nonresponders to chemotherapy (Figure 5), none of the transmitters changed significantly (all P > 0.13). Once again, post–chemotherapy nadir values were analyzed (in the 4 nonresponders, samples were analyzed at 6.6 ± 1.1 [range, 4 to 9] months after initiation of treatment; in the 5 responders, samples were analyzed at 13.2 ± 2.5 [range, 6.5 to 22] months after initiation of treatment).

What initial transmitter values best predicted the subsequent response to chemotherapy in these 9 patients? In bivariate analyses, responders differed from nonresponders in epinephrine (317 ± 234 versus 4.75 ± 0.25 pg/mL, P = 0.02) and in chromogranin A/epinephrine ratio (28.7 ± 23.9 versus 773 ± 420 ng/pg, P = 0.03) but not chromogranin A (867 ± 439 versus 3867 ± 2102 ng/mL, P = 0.37), norepinephrine (9983 ± 3442 versus 4615 ± 1757 pg/mL, P = 0.20), or the ratios of chromogranin A/norepinephrine (0.09 ± 0.02 versus 4.10 ± 3.33 ng/pg, P = 0.45) or norepinephrine/epinephrine (503 ± 414 versus 923 ± 351 pg/pg, P = 0.14).

In multivariate analyses, the 6 biochemical independent variables (chromogranin A, norepinephrine, epinephrine, and their ratios) did not significantly predict the response to chemotherapy in these 9 patients (R = 0.751, R² = 0.564, adjusted R² = -0.742, F = 0.432, P = 0.820), and a better model was not obtained by stepwise multiple linear regression.

Figure 6 displays biochemical responses to chemotherapy in individual patients with malignant pheochromocytoma. One patient (Figure 6A) had a clinical and anatomic response to chemotherapy: Although his chromogranin A was initially elevated (619 ng/mL), it fell gradually in response to repeated cycles of chemotherapy, and by 4 to 6 months (6 to 9 chemotherapy cycles), it had stabilized at 45 to 55 ng/mL, within the normal range. Another patient (Figure 6B) displayed an initial anatomic response to chemotherapy, and although pretreatment chromogranin A was markedly elevated at 2753 ng/mL, by 13 to 17 months it had fallen to 60 to 66 ng/mL, values within the normal range; by 25 months, however, anatomic relapse occurred, with reelevation of chromogranin A to 2228 ng/mL.

Surgery for Malignant Pheochromocytoma

Two patients with malignant pheochromocytoma were subjected to operation (“debulking”). Both had declines in chromogranin A. In 1 patient, preoperative biochemistries were chromogranin A 10211 ng/mL, norepinephrine 5760 pg/mL, and epinephrine <5 pg/mL; 25-day postoperative values were chromogranin A, 800 ng/mL; norepinephrine, 4095 pg/mL; and epinephrine, <5 pg/mL. In 1 patient, preoperative biochemistries were chromogranin A, 2460 ng/mL; norepinephrine, 786 pg/mL; and epinephrine, <5 pg/mL; 17-day postoperative values were chromogranin A,
to malignant pheochromocytoma, with a parallel rise ($P<0.0001$) in plasma norepinephrine but higher epinephrine in benign than malignant pheochromocytoma (Table 1).

In the diagnosis of pheochromocytoma in general (benign or malignant), bivariate analyses found distinctions between control subjects and patients with pheochromocytoma (Table 1) for plasma chromogranin A ($P<0.0001$), norepinephrine ($P<0.0001$), and epinephrine ($P<0.0001$), whereas stepwise multiple linear regression identified plasma norepinephrine as the single most important variable in this diagnostic distinction (adjusted $R^2=0.149$, $F=7.14$, $P=0.011$). Bravo and Gifford have also emphasized the particular diagnostic value of plasma norepinephrine in pheochromocytoma.

**Benign Versus Malignant Pheochromocytoma**

In studying the characteristics of malignant versus benign pheochromocytoma (Table 1 and Figure 1), both plasma chromogranin A ($P=0.0003$) and norepinephrine ($P=0.0344$) were higher in malignant than benign pheochromocytoma, though plasma epinephrine was actually lower ($P=0.0182$) in malignant tumors. In a stepwise multivariate analysis of chromaffin granule transmitter concentrations, plasma chromogranin A elevation proved to be the most significant difference between benign and malignant tumor behavior (adjusted $R^2=0.202$, $F=7.56$, $P=0.011$). Indeed, a plasma chromogranin A concentration of $>600$ ng/mL (Figure 2) was found only in patients with malignant pheochromocytoma (and in the majority [8/14=57%] of such patients), although there was considerable overlap between the benign and malignant groups’ chromogranin A values (Figure 1).

Because of metastases and local tissue invasion, malignant pheochromocytomas typically have much greater tumor mass than benign pheochromocytomas; hence, the extreme chromogranin A values seen in malignant pheochromocytoma (Figure 1) may simply reflect the greater tumor burden in these patients. Indeed, in patients with both benign and malignant pheochromocytomas, we have previously found that plasma chromogranin A correlates with the weight of the excised tumor.

There is little previous literature comparing plasma epinephrine in benign versus malignant pheochromocytomas. Because phenylethanolamine N-methyltransferase is a glucocorticoid-responsive enzyme, very large or extra-adrenal pheochromocytomas may be relatively deficient in epinephrine because the chromaffin cells in such tumors are not in close apposition to glucocorticoid-producing adrenal cortical cells. The quantity of catecholamine release by pheochromocytoma may be an unreliable gauge of tumor size because there is substantial intratumoral catecholamine metabolism, resulting in even some large pheochromocytomas with relatively modest catecholamine release.

The inverse correlation of plasma chromogranin A and epinephrine in pheochromocytoma, benign or malignant (Figure 2; Spearman $r=-0.378$, $n=27$, $P=0.026$), was initially unexpected because both chromogranin A and phenylethanolamine N-methyltransferase (the enzyme catalyzing the formation of epinephrine) are found in chromaffin cells, and each is glucocorticoid responsive. However, as noted above, chromaffin cells in malignant pheochromocytomas, by virtue

**Discussion**

Although pheochromocytoma is an unusual cause of hypertension, occurring in perhaps $\approx0.05\%$ of cases, it is among the best-understood causes of hypertension and may be successfully removed surgically in $\approx90\%$ of patients but will eventually be lethal in most untreated cases. Early diagnosis is therefore important, not only to avoid hypertensive complications but also because of the $10\%$ incidence of malignancy. Although urinary catecholamines are useful for the diagnosis of pheochromocytoma, combined biochemical tests on blood samples may offer the advantages of patient convenience and enhanced sensitivity.

**Diagnosis of Pheochromocytoma**

We found a progressive rise ($P<0.0001$) in plasma chromogranin A, from control subjects to benign pheochromocytoma...
of tumor size and metastases, are unlikely to be in close apposition to the adrenal cortex, the source of glucocorticoid delivery to the adrenal medulla; hence, shear tumor mass may be an overriding determinant of plasma chromogranin A in pheochromocytoma or neuroblastoma, whereas epinephrine secretion is likely to be prominent only from small intra-adrenal pheochromocytomas.

Other pheochromocytoma secretory products may aid in the distinction between benign and malignant pheochromocytoma: For example, substantial elevation in the plasma concentration of the catecholamine precursor DOPA (L-dihydroxyphenylalanine) may suggest malignancy in pheochromocytoma, raising the possibility of especially severe derangements in catecholamine biosynthesis (or relative loss of DOPA decarboxylase activity) within such tumors. Helman et al reported that neuropeptide Y mRNA is less frequently expressed in malignant than in benign pheochromocytoma, whereas de Senanayake et al found higher plasma neuropeptide Y concentrations in adrenal than in extra-adrenal pheochromocytomas. The role of plasma neuropeptide Y in distinguishing benign from malignant pheochromocytoma has not yet been explored. Plasma metanephrine concentration is a highly sensitive and specific approach to pheochromocytoma diagnosis, and metanephrines correlate with pheochromocytoma size; hence, plasma metanephrines might also be useful in discriminating malignant from benign disease.

Treatment of Pheochromocytoma

Benign Pheochromocytoma

In 6 patients with benign pheochromocytoma, surgical excision of the tumor resulted in normalization of all neurotransmitter concentrations (Figure 3).

Malignant Pheochromocytoma

When 9 patients with malignant pheochromocytoma were subjected to repeated cycles of combination chemotherapy (Figure 4), chromogranin A (P = 0.047) and norepinephrine (P = 0.02) but not epinephrine (P = 0.129) fell in the group as a whole, but the substantial clinical and anatomic heterogeneity of chemotherapy responses (Figure 4) prompted us to analyze the results in patients stratified by anatomic and clinical response to chemotherapy.

After chemotherapy (Table 2 and Figure 5), the 5 responders showed declines in chromogranin A (by 91%, P = 0.03) and norepinephrine (also by 91%, P = 0.03) but not epinephrine (P = 0.31). By contrast, the 4 nonresponders showed no significant decline in any of the transmitters (all P = 0.13); indeed, each transmitter tended to rise, though the number of subjects was too small to achieve statistical significance.

Bivariate (but not multivariate) analyses identified 2 biochemical parameters that predicted a favorable response to chemotherapy: plasma epinephrine (317±234 versus 4.75±0.25 pg/mL, P = 0.02) and the ratio of chromogranin A/epinephrine (28.7±23.9 versus 773±420 ng/pg, P = 0.03).

The progress of 2 individual patients with malignant pheochromocytoma (Figure 6) is illustrative of how plasma chromogranin A and norepinephrine paralleled the clinical and anatomic response to chemotherapy. One patient (Figure 6A) whose chromogranin A and norepinephrine showed a sustained fall after treatment did well; however, a substantial rise in chromogranin A and norepinephrine after an initial fall was associated with disease relapse (Figure 6B).

Previously, we noted that plasma chromogranin A also conveys diagnostic and prognostic information in children with neuroblastoma: Plasma chromogranin A increased progressively with advances in disease stage (ie, tumor burden), and stratification by degree of plasma chromogranin A elevation predicted survival.

TABLE 2. Plasma Concentrations of Chromogranin A, Norepinephrine, and Epinephrine in Patients With Malignant Pheochromocytoma Before and After Combination Chemotherapy (Without Surgery) Stratified by Response to Chemotherapy

<table>
<thead>
<tr>
<th>Biochemical Variable</th>
<th>Response to Chemotherapy</th>
<th>Baseline (Pretreatment) Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=5)</td>
<td>No (n=4)</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Chromogranin A, ng/mL</td>
<td>867±439</td>
<td>78.4±20*</td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>9983±3442</td>
<td>909±304*</td>
</tr>
<tr>
<td>Epinephrine, pg/mL</td>
<td>317±234</td>
<td>70.4±18</td>
</tr>
<tr>
<td>CgA/NE, ratio</td>
<td>0.09±0.02</td>
<td>1.18±0.3*</td>
</tr>
<tr>
<td>CgA/Epi, ratio</td>
<td>28.7±23.9</td>
<td>0.10±0.02*</td>
</tr>
<tr>
<td>NE/Epi, ratio</td>
<td>503±414</td>
<td>13.9±4.5*</td>
</tr>
</tbody>
</table>

CgA indicates chromogranin A; NE, norepinephrine, Epi, epinephrine. (To convert chromogranin A from ng/mL to μg/L, multiply by 1.0. To convert epinephrine from pg/mL to pmol/L, multiply by 5.458. To convert norepinephrine from pg/mL to nmol/L, multiply by 0.005911.)

Values represent mean±1 SEM. *Versus pretreatment, P<0.05. Posttreatment nadir values were analyzed. In the 4 nonresponders, postchemotherapy samples were obtained at 6.6±1.1 (range, 4 to 9) months after initiation of treatment; in the 5 responders, postchemotherapy samples were obtained in 13.2±2.5 (range, 6.5 to 22) months after initiation of treatment. Normal ranges (see Table 1): chromogranin A, 48.0±3.0 ng/mL; norepinephrine, 200±7.8 pg/mL; epinephrine, 18.0±1.5 pg/mL. Paired comparisons were made by the nonparametric Wilcoxon signed rank test (1-tailed test).
In conclusion, plasma chromogranin A is an effective tool in the diagnosis of pheochromocytoma as well as in the distinction between benign and malignant pheochromocytoma. During chemotherapy of malignant pheochromocytoma, chromogranin A can be used to gauge tumor response and relapse.

Acknowledgments

This study was supported by the Department of Veterans Affairs, National Institutes of Health, and National Kidney Foundation of Southern California. The chromogranin A radioimmunoassay was performed by Annie Chen. Catecholamines were measured by Courtney Holmes.

References

Malignant Pheochromocytoma: Chromaffin Granule Transmitters and Response to Treatment
Fangwen Rao, Harry R. Keiser and Daniel T. O'Connor

Hypertension. 2000;36:1045-1052
doi: 10.1161/01.HYP.36.6.1045

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/36/6/1045

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/