Longitudinal plasma metanephrines preceding pheochromocytoma diagnosis: a retrospective case–control serum repository study

S W Olson, S Yoon[†], T Baker¹, L K Prince, D Oliver and K C Abbott²

Department of Nephrology, Walter Reed National Military Medical Center, 8901 Rockville Pike, Bethesda, Maryland 20889, USA, ¹Department Chief, Joint Pathology Center, 606 Stephen Sitter Avenue, Silver Spring, Maryland 20910, USA and ²The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Bethesda, Maryland 20892, USA

[†](Dr S Yoon has moved on to private practice)

Correspondence
should be addressed
to S W Olson

Email
Stephen.w.olson.mil
@mail.mil

Abstract

Objective: Plasma metanephrines (PMN) are highly sensitive for diagnosis of pheochromocytoma, but the natural history of PMN before pheochromocytoma diagnosis has not been previously described. The aim of the study was to compare the progression of PMN before pheochromocytoma diagnosis to matched healthy and essential hypertension disease controls.

Design: A retrospective case–control Department of Defense Serum Repository (DoDSR) study.

Methods: We performed a DoDSR study that compared three longitudinal pre-diagnostic PMN for 30 biopsy-proven pheochromocytoma cases to three longitudinal PMN for age, sex, race, and age of serum sample matched healthy and essential hypertension disease controls. Predominant metanephrine (MN) or normetanephrine (NMN) production was identified for each case and converted to a percentage of the upper limit of normal to allow analysis of all cases together. PMN were measured by Quest Diagnostics.

Results: The predominant plasma metanephrine (PPM) was > 100 and 300% of the upper limit of normal a median of 6.6 and 4.1 years before diagnosis respectively. A greater percentage of pheochromocytoma patients had a PPM > 100 and > 300% of the upper limit of normal compared with combined healthy and essential hypertension disease controls <2, 2–8, and >8 years prior to diagnosis. For patients with a baseline PPM 90–300% of the upper limit of normal, a 25% rate of rise per year was 100% specific for pheochromocytoma.

Conclusions: PPMs elevate years before diagnosis which suggests that delayed diagnoses are common. For mild PMN elevations, follow-up longitudinal PMN trends may provide a highly specific and economical diagnostic tool.

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Introduction

Pheochromocytomas are rare catecholamine-secreting neuroendocrine tumors associated with significant morbidity and mortality. Patients with a high pre-test probability for secondary hypertension, an incidentally discovered adrenal adenoma (incidentaloma), or a known hereditary predisposition are all at elevated risk for pheochromocytoma (1). But suggestive clinical features of a pheochromocytoma are often initially unappreciated with up to 50% diagnosed only at autopsy (2, 3).

An unknown pheochromocytoma is prone to medical or mechanical stimulation of a catastrophic catecholamine storm (4). Therefore, a missed or delayed diagnosis can increase risk of end-organ damage from hypertensive emergency, perioperative death, perinatal death, and inoperable metastatic disease (5, 6, 7, 8, 9, 10).

Plasma metanephrines (PMNs) are an ideal screening test with near 100% sensitivity but an inadequate confirmatory test with as low as 85% specificity depending

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on compliance for adequate sampling (11, 12, 13, 14, 15, 16, 17, 18). Levels between one and three times normal are a diagnostic challenge. Multiple additional urine and serum tests only reduce overall specificity (19). Tumor growth over time has not been shown to differentiate between pheochromocytoma and benign adrenal adenoma (20). A metaiodobenzylguanidine scan is highly specific but with some variability (89–100%) in addition to being expensive and not universally available (21, 22, 23, 24).

The natural history of PMN prior to pheochromocytoma could better quantify diagnostic delay and/or improve diagnostic specificity but has not been reported. We utilized the Department of Defense Serum Repository (DoDSR) to compare chronologic PMN in pheochromocytoma patients before diagnosis to age, sex, race, and age of serum matched healthy and essential hypertension disease controls.

Methods

Design

We performed a case–control DoDSR study comparing pre-diagnostic PMNs for 30 pheochromocytoma cases to 30 matched healthy and 26 matched essential hypertension disease controls. Comprehensive DoDSR research methodology is described in our previous publications (25, 26, 27). Briefly, a query of the military electronic medical record for pheochromocytoma (ICD-9 code 227.0) revealed 96 cases from 2000 to 2009. After comprehensive chart review, 55 cases were excluded because they were dependents of active duty members without requirements to bank serum or they lacked documented tissue confirmation of pheochromocytoma. Of the 41 remaining cases, five bilateral, extra-adrenal, syndromic, and/or metastatic pheochromocytoma cases were excluded due to minimal power for independent analysis and to minimize confounding variables for solitary pheochromocytoma analysis. In total, 30 of the remaining 36 patients had multiple serum samples available in the DoDSR. Background clinical, laboratory, and imaging data were collected for each case when available.

The DoDSR was established in ~1985. Each active duty service member has serum banked at entry into the military after passing a comprehensive medical screening exam. Additional samples are banked approximately biannually at the time of HIV screening in addition to before and after deployments. Additional clinical information is documented at required yearly medical

screenings. Samples are stored at -70°C with no scheduled freeze/thaw cycles after banking. The freezers are monitored continuously. Approximately two million samples are now stored yearly, and the DoDSR currently has up to 60 million samples stored in Silver Spring, MD.

The DoDSR identified one age, sex, race, and age of serum sample matched healthy and essential hypertension disease control. Essential hypertension disease controls had an ICD-9 code for 401.9 without any secondary hypertension, malignant hypertension, or adrenal adenoma ICD-9 codes. The DoDSR then sent the oldest, second-to-last, and most recent 0.5 ml samples prior to pheochromocytoma diagnosis along with matching control samples to Quest Diagnostics Nichols Institute (Chantilly, VA, USA) for measurement of PMN. PMN measurements were made by liquid chromatography and tandem mass spectrometry which is more accurate than enzyme immunoassay (EIA) (28). The upper limit of normal for metanephrine (MN) and normetanephrine (NMN) were 57 and 144 ng/ml respectively. Inter- and intra-assay variability were both $<10\%$. Results were automatically calculated by Analyst Software (Quest Diagnostics). Data were analyzed for MN and NMN levels.

Statistical analysis

Each NMN and MN level was divided by 148 and 57 pg/ml respectively to establish comparable ‘normalized’ percent of the upper limit of normal values (%ULNV). Each pheochromocytoma case was then classified as NMN or MN predominant based on the highest %ULNV. MN and NMN predominant cases were then combined together for statistical analysis with only the predominant plasma metanephrines (PPMs) %ULNV data used for comparison with combined matching controls. A similar model (free light chain difference (FLC diff)) is widely accepted to study a combination of κ and λ predominant AL amyloidosis cases (29). But, the %ULNV is required to adjust MN and NMN because they do not have the same normal ranges. Healthy and disease controls were combined for data analysis because there was no statistically significant difference between the two controls in any time period.

The percent of pheochromocytoma cases with PPM >100 , 200, and 300% ULNV were compared to combined healthy and essential hypertension disease controls at all times, <2 , 2–8, and >8 years prior to diagnosis. Not all patients had samples available for each subgroup time period. When a patient had multiple serum samples during a given time period, highest PPM determined

Table 1 Background information presented for pheochromocytoma patients. Five patients were diagnosed after an adrenal mass was noted incidentally on imaging from an unrelated evaluation. Incidence of symptoms likely underestimated due to incomplete history available for patients who initially presented in the ICU and/or civilian hospitals. Information for each variable was not present in the electronic medical record for all study patients. Continuous variables (interquartiles), categorical variables (ratios).

Age (years)	43 (35, 50)
Gender (male)	93%
Race (Caucasian)	62%
Size (cm)	4.5 (3.4, 6.0)
Location (right)	54%
Diagnosis PPM %ULNV, <i>n</i> = 16	940% (392, 2212%)
Palpitations	68% (19/28)
Headache	57% (16/28)
Diaphoresis/flushing	32% (9/29)
Dizziness	29% (8/28)
Tremor	14% (4/28)
Pallor	11% (3/28)
Nausea/abdominal discomfort	18% (5/28)
Anxiety	14% (4/28)
Fatigue	21% (6/28)
Hyperglycemia	29% (8/28)
Hypertension before diagnosis (months), <i>n</i> = 22	27 (6, 48)
Sx before diagnosis (months), <i>n</i> = 18	6 (2, 31)
Multisystem organ failure/hypertensive emergency	14 (4/33)
Incidental discovery on imaging	15%

group assignment. The PPM percent change per year before pheochromocytoma diagnosis was compared to a combination of both healthy and essential hypertension disease controls. For example, percent change per year was calculated by dividing the most recent PPM (PPMr) by the oldest index PPM (PPMi), subtracting 1 and then multiplying by 100. This value was then divided by difference in days between the two samples $(T_l - T_i)$ and multiplied by 365 days/year $((PPMr/PPMi - 1) \times 100 / (T_l - T_i)) \times 365$. PPM doubling time was calculated by $((T_l - T_i) \times \log(2)) / (\log PPM_r - \log PPM_i)$. Because the data were not normally distributed, continuous variables for both cases and controls are reported with median values with interquartiles (25 and 75%), and Wilcoxon–Mann–Whitney test was used to evaluate statistical significance. Categorical variables for cases and controls were compared using the Fisher's exact test because some comparison groups had less than five subjects. PPM >100% ULNV receiver operating characteristic (ROC) curves were constructed for pheochromocytoma vs combined controls <2, 2–8, and >8 years before diagnosis. MN and NMN line graphs

were formed for all cases and combined controls. All data were analyzed with STATA 12.1 (Stata Corp., College Station, TX, USA). This study was approved by the Human Use Committee at Walter Reed National Military Medical Center, and informed consent was waived.

Results

Demographics

The majority of study cases were Caucasian males under 50 years old. The most common symptoms were palpitations, headache, and diaphoresis (Table 1). The longitudinal pre-diagnostic MN and NMN for each pheochromocytoma case and matched controls are presented via line graphs (Fig. 1). There were 21 pheochromocytoma cases that were NMN predominant and nine cases that were MN predominant.

Pheochromocytoma patients developed PPM >100, 200, and >300% ULNV years prior to diagnosis (Table 2). This delay between suggestive serologic diagnosis and clinical diagnosis is underestimated. The majority (16/30) of cases had PPM >100% ULNV in the oldest index sample, and there was a median 11 years between normal PPMi and elevated PPM in the second sample for the remaining 14 cases. Therefore, each case had an elevated PPM for a longer interval of unknown duration before diagnosis. A greater percentage of pheochromocytoma patients had an elevated PPM >100, 200, and 300% ULNV

Figure 1

Line graphs demonstrating longitudinal pre-diagnostic changes in MN for pheochromocytoma cases (A) and controls (B) as well as NMN for pheochromocytoma cases (C) and controls (D). A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-15-0651>.

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Table 2 Median time for PPM to surpass 100, 200, and 300% ULNV before pheochromocytoma diagnosis.

PPM (% upper limit of normal)	Time to surpass threshold (median years)	Interquartile range (25 and 75% years)
> 100% (n=30)	6.6	2.5, 10.8
> 200% (n=28)	5.2	2.1, 9.2
> 300% (n=25)	4.1	2.0, 8.3

compared with combined healthy and essential hypertension disease controls at any time, <2, 2–8, and >8 years before diagnosis (Table 3).

More pheochromocytoma cases had a PPM rate of rise per year >0% (100% vs 54%; $P \leq 0.001$), >10% (87% vs 23%, $P < 0.001$), >50% (53% vs 5%; $P < 0.001$), and >150% (33% vs 0%; $P < 0.001$) than combined healthy and essential hypertension disease controls. But, a rise in PPM over time within the normal range accounted for the majority of these positive controls. The longitudinal trend for PPMi within the diagnostically challenging range of 90–300% ULNV could have more practical clinical relevance. For patients with a PPMi between 90 and 300%, a subsequent rate of rise >25%/year was 100% specific for future pheochromocytoma (Table 4). The median PPM percent rise per year for pheochromocytoma cases was 38% (interquartiles 6 and 58%). The median time between index PPM 90 and 300% ULNV and a PPM >300% ULNV was 5 years. But, this is an overestimation because, all but one, the PPM became >300% ULNV between the oldest index and second-to-last sample which had the longest time interval. All six cases with available time intervals <3 years crossed the >300% ULNV value. The median doubling time for PPM was 3.1 years (interquartiles 2.0 and 5.8 years). In total, 50% of cases had a doubling time 2.0–4.5 years, 20% had a doubling time ≤ 1.5 years, and 17% of cases had a doubling time ≥ 8 years.

The pheochromocytoma vs combined healthy and essential hypertension disease controls PPM %ULNV area under the ROC curve was 1.00 for <2 years, 0.95 for 2–8 years, and 0.77 for >8 years before diagnosis (Fig. 2).

Discussion

Our results describe the natural history of PMN prior to pheochromocytoma for the first time and provide multiple important observations.

First, the PPM became elevated and even surpassed the 300% ULNV threshold for near 100% diagnostic

specificity, years prior to clinical diagnosis. Consistent with previous literature, this observation strongly suggests that a diagnostic delay is common and that timely consideration of the disease is paramount for a prompt diagnosis. A missed or delayed diagnosis can result in substantial morbidity and mortality particularly from mechanical or medicinal stimulation and wide-spread inoperable metastasis. The ‘false positive’ PPMs documented in the healthy and disease controls were likely due to non-optimal lab draw conditions. Non-compliant sampling results in ~50% higher PMN in normal controls, but has no effect in pheochromocytoma patients (30). However, any overestimation of PPM in our controls would have only reduced the statistical significance of our results.

Secondly, the median PPM doubling time was ~3 years. To our knowledge, this is the first description of PPM doubling rates for apparently sporadic pheochromocytomas. Interestingly, there were three temporal subgroups that accounted for the majority of cases (≤ 1.5 , 2–4.5, and ≥ 8 years). One interpretation is that pheochromocytoma genetic profiles impact tumor growth rates. Amar *et al.* (31) found that succinate dehydrogenase B gene mutation was associated with a shorter MN doubling time and worse survival in a cohort of metastatic pheochromocytomas. A future serum repository study that compares longitudinal pre-diagnostic PPM in pheochromocytomas with a multiple genetic profiles is required to address this hypothesis.

Table 3 Percent pheochromocytoma cases vs a combination of essential hypertension disease and healthy controls with PPM >100, 200, and 300% of the upper limit of normal (>57 ng/ml for MN and >148 ng/ml for NMN) at all times, <2, 2–8, and >8 years before diagnosis.

PPM (% upper limit of normal)	Cases (n=30) (%)	Combined controls (n=56) (%)	P value
(> 100%): all time prior	100 (30/30)	29 (16/56)	<0.001
<2 years	100 (21/21)	10 (4/41)	<0.001
2–8 years	100 (20/20)	11 (4/37)	<0.001
>8 years	50 (12/24)	20 (9/45)	0.01
(> 200%): all time prior	93 (28/30)	2 (1/56)	<0.001
<2 years	100 (21/21)	0 (0/41)	<0.001
2–8 years	90 (18/20)	3 (1/37)	<0.001
>8 years	38 (9/24)	0 (0/45)	<0.001
(> 300%): all time prior	83 (25/30)	0 (0/56)	<0.001
<2 years	95 (20/21)	0 (0/41)	<0.001
2–8 years	80 (16/20)	0 (0/37)	<0.001
>8 years	29 (7/24)	0 (0/45)	<0.001

Table 4 A comparison between the percentage of pheochromocytoma cases with a baseline PPM between 90 and 300% of the upper limit of normal (> 57 pg/ml MN and > 148 NMN) that subsequently rise at rates above specified thresholds when compared to matched controls.

PPM (% rise/year)	Cases (%)	Combined controls (%)	P value
($> 0\%$)	94 (15/16)	5 (1/20)	< 0.001
($> 25\%$)	81 (13/16)	0 (0/20)	< 0.001
($> 50\%$)	50 (8/16)	0 (0/20)	< 0.001

Finally, and possibly most significant, our data set suggests that diagnostically challenging patients with a PPM 90–300% ULNV can be followed with serial labs to achieve better diagnostic specificity. A $> 25\%$ PPM rise/year was 100% specific in our cohort. The time required for these cases to cross the $> 300\%$ ULNV threshold appeared to be < 5 years and potentially even far less. These findings could eventually apply for defined biochemical surveillance periods in cohorts with genetic risk, an incidentaloma, or concern for secondary hypertension.

Knowledge of specific genetic risk associated with pheochromocytoma is rapidly expanding. Up to 30% of apparently sporadic and non-syndromic pheochromocytomas have a known susceptibility gene, and this number will likely continue to grow with future discoveries (21, 32, 33). These patients may benefit from periodic biochemical surveillance for transformation to phenotypic disease. Incidentalomas are an additional clinical challenge. Up to 7% of people will develop an adrenal adenoma over their lifetime, and $\sim 5\%$ of these are a pheochromocytoma (10, 34, 35, 36, 37). Because most incidentalomas are benign, a biochemical diagnosis is required. Also, $\sim 10\%$ of hypertension patients have a secondary cause (38, 39). Only 3–6% of these cases are diagnosed with pheochromocytoma (40, 41). The classic triad of symptoms is insufficient for diagnosis (42). Our pre-diagnostic PPM comparison data between pheochromocytoma cases and combined healthy/essential hypertension disease controls could provide a resource to better interpret ongoing serologic surveillance in these at-risk populations (43).

But, due to many limitations of this study, prospective confirmation with more accurate controls is required before any clinical implementation. The healthy controls in this study were not known to have any genetic or family risk factors. The essential hypertension disease controls were not known to have clinical concern for a secondary cause of hypertension. In addition, the intervals between the oldest index sample and the second oldest sample were

often long due to the limitation of three samples for each subject. This made temporal estimations of certain threshold PPM %ULNV less accurate. Serial biochemical studies in at-risk patients at fixed intervals over time would be superior.

Our study had generalized limitations related to retrospective case-control DoDSR studies covered in previous publications (25, 26, 27). There are also additional study-specific limitations. Seasonal variations, phlebotomy position, rest period prior to blood draw, temporal relationship to exertion, caffeine and food consumption, nicotine use, and acetaminophen or prescription medications can all alter PMN (19, 44, 45, 46). These potential confounders were not regulated or accurately accounted for at each blood draw. However, our matching criteria for age and race accounted for these known confounding variables (45, 47). We were also unable to link the temporal relationship between pre-diagnostic PPM elevation and onset of disease-related symptoms. We cannot rule out the possibility that a healthy or disease control subsequently developed a pheochromocytoma. But, in addition to being rare, a case misattributed, as a control would only lessen the statistical significance of our findings. Samples were stored at -80°C for months-years prior to evaluation. But, there is precedence for analysis of frozen samples, and there was no evidence of PMN degradation over time in our healthy control samples (14, 15). We were unable to assign an age, sex, race, and age of serum matched essential hypertension disease control for four cases. Reported PML rate of change over

Figure 2

PPM $> 100\%$ ULNV receiver operating characteristic (ROC) curves for pheochromocytoma vs combined controls < 2 years (A), 2–8 years (B), and > 8 years (C) before diagnosis.

A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-15-0651>.

time assumes a linear rise between oldest and most recent sample. This has not been proven. The scope of this study is limited by exclusion of metastatic, syndromic, extra-adrenal, and bilateral pheochromocytomas. But future investigation could include a comparison of pre-diagnostic serologies in benign pheochromocytomas to metastatic pheochromocytomas (48). This study could potentially help better identify metastatic risk in at-risk populations under serologic surveillance.

Early diagnosis and definitive treatment of a pheochromocytoma mitigates substantial risk of morbidity and mortality. The need to better define longitudinal pre-diagnostic biochemical trends for improved surveillance has been previously proposed (18, 24). This data set addresses this important clinical need, but follow-up prospective confirmation is required prior to broad clinical implementation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. The views expressed in the manuscript are those of the authors and do not reflect the official policy of the Department of the Army, Department of Defense, or the US government.

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