Author's response to reviews

**Title:** Measurement of Fractionated Plasma Metanephrines for Exclusion of Pheochromocytoma: Can Specificity be Improved by Adjustment for Age?

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**Author's response to reviews:** see over
To the Editors and Reviewers of BMC Endocrine Disorders,

Thank you for recently reviewing our manuscript, titled “Measurement of Fractionated Plasma Metanephrines for Exclusion of Pheochromocytoma: Can Specificity be Improved by Adjustment for Age?” We appreciate the thoughtful comments from Drs. Nogueira and Roden. We have revised the manuscript, in keeping with the suggestions and enclosed the revised manuscript for your review. We have also responded to the comments and questions of the reviewers in the following pages. If you have any questions or concerns about this manuscript, please do not hesitate to contact Anna Sawka. Thank you for your consideration.

Sincerely,

AM Sawka, MD, MSc
Responses to Reviewer 1 – Dr. Nogueira:

We appreciate the positive comments of the reviewer and the thoughtful suggestions.

1. The reviewer has inquired why the idea of a prediction rule for diagnosing pheochromocytoma has not been tried before. Although we cannot be certain of the cause, we suspect that a multi-attribute prediction rule may not have previously been tried in this area because clinical endocrinologists are generally accustomed to interpreting the results of hormone measurements (in blood or urine) rather than considering multiple attributes in a mathematically-based formula.

2. We appreciate the positive comments about the Methods section.

3. The reviewer has requested clarification on whether those without the disease were known as not having the disease. We have added the following the Study Populations section of the Methods: “All patients with pheochromocytoma had histologic confirmation of the diagnosis and those without pheochromocytoma had an alternative diagnosis assigned at the completion of their evaluation based on a combination of other biochemical test results (such as normal 24-hour urinary fractionated metanephrine and catecholamine measurements with or without normal imaging of the adrenals in the form of computerized tomography scanning [CT] or magnetic resonance imaging [MRI]).”

We have also acknowledged in the third paragraph of the Discussion that without autopsy confirmation (which is obviously unethical in live patients), we cannot be absolutely certain of the absence of a pheochromocytoma or paraganglioma:

“Furthermore, without autopsy confirmation, one cannot be absolutely certain that individuals labeled as not having a pheochromocytoma did not have an occult paraganglioma or pheochromocytoma. However, we believe that reasonable clinical criteria were used in excluding pheochromocytoma in our study.”
4. The reviewer has inquired about why age was adjusted for in the current analysis, and why other variables such as gender or body mass index were not adjusted for. We have added information on how age was selected for adjustment in the multivariable model in the *Statistical Methods* section of the **Methods**: “Clinical characteristics of subjects in the derivation set who did not have pheochromocytoma but had measurements of the normetanephrine or metanephrine fraction above the upper reference limits (false positive tests using traditional positivity criteria) were compared to those without pheochromocytoma who had true negative tests (χ² was used for categorical variables, and Student’s t-test for independent samples was used for continuous variables). Variables which were different between both groups at a significance level of 0.1 were then entered into a multivariable logistic regression model predicting pheochromocytoma. Age was the only variable of statistical significance distinguishing true positive from false positive fractionated plasma metanephrine measurements in the derivation set. Thus, we forced age with measurement values of normetanephrine and metanephrine fractions in a multivariable logistic regression model predicting pheochromocytoma in the derivation set (SPSS 10.0, Chicago, ILL).”

We have also added more information on this issue in Table 2 as well as in the **Results Section** under *Findings in the Derivation set*: “Baseline characteristics of individuals in the derivation set without pheochromocytoma were compared for individuals who had true negative fractionated metanephrine measurements (n = 264) to those who had false positive results (n = 46) (Table 1). The individuals with false positive fractionated plasma metanephrine measurements in the derivation set were significantly older than those with true negative measurements (p = 0.007), whereas blood pressure, antihypertensive medication use, and rates of obstructive sleep apnea were not significantly different between these groups. Thus, age was chosen as an important
variable to adjust for in interpretation of fractionated plasma metanephrines and an age-adjusted metanephrine score was developed from the derivation set data using logistic regression (as described in the Methods).”

Unfortunately, we did not collect information on all patients in terms of body mass index so we were unable to examine the impact of adjusting for this variable and have listed this limitation in the third paragraph of the Discussion: “…limited clinical data on each studied individual were collected so variables that could be of interest such as: body mass index, creatinine-clearance, and rates of diabetes mellitus were not recorded.”

5. We appreciate the positive comments of the reviewer with respect to the title and writing style.
Responses to Reviewer 2 – Dr. Roden:

We greatly appreciate the positive feedback from Dr. Roden in considering our approach interesting. We also appreciate the many insights that he has offered, particularly with respect to his own extensive research in his area.

1. Dr. Roden has brought to our attention that the method of measurement of fractionated plasma metanephrines that his group has developed is not subject to interference with acetaminophen. We have modified the second sentence in the second paragraph of the Background section as follows: “It is known that acetaminophen may interfere with measurements of fractionated plasma metanephrines using the Lenders’ method [8], so this drug has traditionally been avoided prior to testing.”

   In the third paragraph of the Discussion we have also acknowledged the limitation of our study in using only the Lenders method (which may be subject to interference by acetaminophen): “Another limitation is that we used an assay for measurement of fractionated plasma metanephrines that may be have been subject to interference with acetaminophen [8], whereas other assays, such as the one described by Roden et al, could have been preferable due to lack of acetaminophen interference [21].”

2. Dr. Roden has requested clarification of why a minimal sensitivity of 90.9% was chosen in developing a cut-off value for the age-adjusted metanephrine score, as a higher sensitivity may be preferred. In the last two sentences of the first paragraph under Statistical Methods of the Methods, we have added the following explanation: “The sensitivity level of over 90% was chosen because such a sensitivity level was believed to be clinically reasonable and at this level, specificity was still acceptable. We were aware that the lower the cut-off, the higher the sensitivity, but this would be at the expense of specificity.”
Also, in the second last sentence of the third paragraph of the Discussion, we have acknowledged the subjective nature of our choice of positivity cut-off: “The cut-off that we chose for positivity of the age-adjusted metanephrine score was also arbitrary and use of a lower cut-off could have resulted in improved sensitivity, albeit with likely some expense of specificity.”

Of, when the age-adjusted metanephrine score was applied to an appropriate population of non-genetically predisposed individuals, the sensitivity was 100%, with specificity superior to that of traditional interpretation of fractionated metanephrines.

3. Dr. Roden has interpreted that a patient in the validation set had von Hippel-Lindau (VHL), which is in fact not correct as, no genetically predisposed individual was included in the validation set. The patient in question with VHL was actually in the derivation set. We apologize for any confusion in the former version of the manuscript and we have restructured the Results section into a section titled, Findings in the Derivation Set and Findings in the Validation Set, referring to the results of each of the respective data sets separately. Also, as requested by Dr. Roden, in the second sentence of the section, Findings in the Derivation Set, we have clarified the number of genetically predisposed individuals with pheochromocytoma included in the analysis: “In the derivation set, 8 of the 33 individuals had clinically-diagnosed genetic syndromes predisposing to pheochromocytoma (three familial malignant paraganglioma, two von Hippel-Lindau, one had Multiple Endocrine Neoplasia 2a, one had Multiple Endocrine Neoplasia 2b, and one had familial multiple benign paraganglioma).” In the second sentence under Findings in the Validation Set, we have indicated that no genetically predisposed individuals were included.

4. Dr. Roden has requested further information on the baseline characteristics, including age ranges and percentage of clinical symptoms. We have added the following information
in the **Results** under *Findings in the Derivation Set*: “The 316 individuals in the derivation set who did not have pheochromocytoma underwent such testing the following reasons: refractory hypertension (174, 55%), spells (periodic episodes of symptoms such as palpitations, headache, or sweating, 124, 39%), adrenal mass (45, 14%), previous pheochromocytoma or known genetic predisposition to pheochromocytoma (24, 8%). The mean age of subjects with pheochromocytoma was 48 years (SD 18 years, range 16 to 60 years), whereas the mean age of subjects without pheochromocytoma was 52 years (SD 15 years, range 10 to 73 years).”

We have also added the following information in the **Results** under *Findings in the Validation Set*: “The mean age of subjects with pheochromocytoma was 50 years (SD 16 years, range 16 to 83 years), whereas the mean age of subjects without pheochromocytoma was 55 years (SD 16 years, range 7 to 86 years). Of the 135 subjects without pheochromocytoma, 83 (62%) were women. Reasons for measurement of fractionated plasma metanephrines in the subjects without pheochromocytoma were as follows: hypertension (55, 41%), spells with or without hypertension (44, 33%), an incidentally discovered adrenal mass (20, 15%), and previously surgically cured pheochromocytoma (16, 12%).”

5. Dr. Roden has shared some information from a study in which his research group examined the relationship between hypertension and type 2 diabetes and the impact on normetanephrine measurements after exercise. Dr. Roden has asked that we examine the effect of hypertension and diabetes in our cohort. As shown in Table 1 of the revised manuscript, systolic and diastolic blood pressures were not significantly different between subjects with false-positive fractionated plasma metanephrine measurements and those with true-negative values (in the derivation set). Unfortunately, we did not collect information on rates of diabetes mellitus. We have added the following information in
the last four sentences of the second paragraph in the **Discussion**: “Of note, Raber et al have noted exaggerated increases in plasma normetanephrine after exercise in hypertensive individuals with type 2 diabetes, compared to normotensive individuals with or without diabetes [20]. Furthermore, Raber et al have suggested that the excessive response of plasma normetanephrine to exercise may serve as a marker of exaggerated sympathoadrenal function in hypertensive type 2 diabetics [20]. Fractionated plasma metanephrine measurements were performed only at rest in our study and we did not examine any potential relationship with diabetes. Systolic and diastolic blood pressures were not significantly different between individuals with false-positive fractionated metanephrine measurements and those with true-negative measurements in the derivation set in our study.”

We have highlighted the limitation of lack of information on diabetes in the second sentence of the third paragraph of the Discussion: “…limited clinical data on each studied individual was collected so variables that could be of interest such as: body mass index, creatinine-clearance, and rates of diabetes mellitus were not recorded.”

6. **Dr. Roden** has asked for us to assess or at least speculate on the impact of use of the age-adjusted metanephrine score on economic aspects of pheochromocytoma diagnosis. In response to this request, we have performed a formal Decision Analysis examining the cost implications (in terms of confirmatory imaging expenditures) of use of the age-adjusted metanephrine score compared to traditionally interpreted fractionated plasma metanephrine measurements – see **Methods** under *Economic Evaluation: Decision Analysis* and **Results** under *Imaging Cost Implications of Screening Strategies for Pheochromocytoma*. We have estimated that the use of the age-adjusted plasma metanephrine score for biochemical testing for sporadic pheochromocytoma in a hypothetical population of 100,000 tertiary care hypertensive patients could result in a
cost savings of 36.7 million dollars with equal detection of pheochromocytoma cases, relative to using the same biochemical testing but interpreting fractionated plasma metanephrine measurements in a traditional fashion.