COLLEGE of AMERICAN PATHOLOGISTS

Testing for Pheochromocytoma/Paraganglioma

Version: 2.0
Original Version Published April 2021; Reviewed and Updated May 4, 2023

Authors
Anthony J. Guidi, MD, FCAP, Larry W. Massie, MD, FCAP, Joseph A. Tworek, MD, FCAP
*Lead author, Newton-Wellesley Hospital, Newton MA

Editors
Richard W. Brown, MD, FCAP*, Brian R. Jackson, MD, FCAP, Barbara Blond, MBA, Thomas Long, MPH
*Lead editor: Memorial Hermann Health System, Houston, TX

SYNOPSIS AND RELEVANCE

Laboratory testing for pheochromocytomas and paragangliomas can be complicated by numerous available tests. Accurate interpretation of results may be hindered by assay limitations and preanalytical variables. Adherence to the strategies in this module will:

1. Ensure that appropriate testing for pheochromocytomas and paragangliomas is performed, both screening and confirmatory tests.
2. Ensure that appropriate steps are taken to avoid erroneous test result interpretations due to assay limitations or preanalytical variables.

OBJECTIVES

1. Understand pertinent clinical features regarding pheochromocytomas and paragangliomas and the compounds they secrete in order to better understand appropriate assay selection.
2. Recognize the utility of plasma free fractionated metanephrines and urinary total fractionated metanephrines in the diagnosis of pheochromocytomas and paragangliomas.
3. Understand and minimize the influence of preanalytical variables on testing for plasma free fractionated metanephrines for appropriate test interpretation.

BACKGROUND

Pheochromocytomas and paragangliomas are uncommon histologically identical tumors that occur with a peak incidence in the 4th and 5th decades, and arise from chromaffin cells in adrenal and non-adrenal locations, respectively. The tumors are associated with an annual incidence of 0.6 cases per 100 thousand-person years.[1] Recognition and accurate diagnosis of these tumors is important as they may be potentially lethal if untreated (more common with paragangliomas). However, diagnosis may be difficult because signs and symptoms of excessive catecholamine secretion occur in less than 50% of patients and can also be seen in multiple non-neoplastic medical conditions. Moreover, patients may present in many different ways, including clinical symptoms (headaches, palpitations, and profuse sweating represent the classic triad), incidental findings on imaging studies, or discovery following identification of genetic test results or clinical syndromes associated with these lesions.[2-4] While most tumors are sporadic, hereditary forms occur in 10 clinically relevant syndromes (including multiple endocrine neoplasia type 2, neurofibromatosis type 1, and von Hippel-Lindau disease). At least 19 specific susceptibility genes have been associated with these tumors.[2] Accurate diagnosis and treatment requires an understanding of the clinical features (including associated syndromes), selection and interpretation of appropriate laboratory tests and imaging studies, and genetic factors. While this module is focused on laboratory testing, several recent reviews provide excellent summaries of the pertinent clinical, imaging and genetic considerations.[2-4]

Catecholamines (epinephrine, norepinephrine and dopamine) constitute important neurotransmitters and endocrine hormones, and laboratory measurements of catecholamine metabolites in blood or urine are very useful in the diagnosis of pheochromocytomas and paragangliomas. The major catecholamine produced by pheochromocytomas is epinephrine while the major catecholamine produced by paragangliomas is norepinephrine. Dopamine may rarely be secreted by these tumors, but it is more typically secreted by neuroblastomas. The leakage of catecholamines and their subsequent metabolism represents the primary source of catecholamine metabolites found in blood and urine. Metanephrine and normetanephrine, collectively referred to as "metanephrines," are the key metabolites and represent the most sensitive assay targets to detect pheochromocytomas and paragangliomas. Metanephrine is derived from epinephrine and normetanephrine from norepinephrine. Metanephrines can be further metabolized to vanillylmandelic acid (VMA), but this compound is not used in the diagnosis of pheochromocytomas or

paragangliomas. The metabolic end-product of dopamine can be further metabolized to homovanillic acid (HVA), a compound which is not a metabolite of metanephrines. VMA and HVA, while useful in the diagnosis of neuroblastomas, are insensitive tests for detecting pheochromocytomas and paragangliomas.[5-7]
High-performance liquid chromatography with electrochemical detection or tandem mass spectroscopy represent gold standards for testing catecholamines and their metabolites. Plasma free fractionated metanephrines or 24-hour urine total fractionated metanephrines are currently the most widely advocated tests for detecting pheochromocytomas and paragangliomas; testing for catecholamines does not improve the sensitivity for detecting these tumors. Random urine total fractionated metanephrines testing is discouraged due to a lack of diagnostic utility. Plasma free fractionated metanephrines has a mean sensitivity of 97% and a mean specificity of 93% across 15 studies.[2] Mild elevations in plasma free fractionated metanephrines are commonly seen in patients without pheochromocytomas or paragangliomas. Borderline positive test results require additional testing or additional clinical workup. A negative result for plasma free fractionated metanephrines is helpful to exclude pheochromocytomas and paragangliomas, with the exception of very early or limited disease. The sensitivity of 24-hour urine total fractionated metanephrines varies in the literature but is generally considered to be slightly lower than plasma fractionated metanephrines, while the specificity is generally considered to be higher. Whether testing should be performed on plasma or urine is debated, and there are regional and institutional differences in recommended testing approaches.[3-9]

Interpretation of test results requires an awareness of multiple pre-analytical factors that can significantly influence catecholamine release. Medications and drugs of abuse may increase metanephrine levels, including L-DOPA, alpha-methyldopa, monoamine oxidase inhibitors, antihypertensive medications, diuretics, alcohol, and cocaine. Medications should be tapered or discontinued at least 2 weeks prior to laboratory testing. Similarly, exercise, stress, renal failure, severe congestive heart failure, myocardial infarction and acute illness may increase metanephrine levels. Even the stress of a venipuncture and upright posture has been shown to increase plasma metanephrine levels; for this reason, it is recommended that patients remain supine for 30 minutes before collecting venipuncture samples for testing. If supine samples cannot be collected, use of an appropriate reference range (sitting vs supine) is recommended. Testing is discouraged in the inpatient setting. Lastly, conjugated metanephrines are excreted in the urine and renal impairment will decrease clearance; in these circumstances, testing plasma free fractionated metanephrines is recommended.[2-4]

INSIGHTS

1. Metanephrines are relatively specific markers for pheochromocytomas and paragangliomas, although there are many pre-analytic variables that require consideration when performing these assays and interpreting results.
2. Plasma free fractionated metanephrines and urinary total fractionated metanephrines are appropriate assays for the diagnosis of pheochromocytomas and paragangliomas.
3. Plasma and urine catecholamine levels have no significant role in testing for pheochromocytomas and paragangliomas.
4. Avoid random urine specimens for testing total (free and deconjugated) fractionated metanephrines.
5. It is recommended that patients remain supine for 30 minutes before collecting venipuncture samples for testing. If supine samples cannot be collected, use of an appropriate reference range (sitting vs supine) is recommended. Testing should be avoided in the inpatient setting.

INTERVENTIONS

1. Work with clinical colleagues to establish a preferred testing cascade: we recommend testing for plasma free fractionated metanephrines first. If negative, no further testing is required. If positive, appropriate imaging studies should be pursued. Provide educational information to medical staff about the use of an accepted testing algorithm for the diagnosis of pheochromocytoma and paraganglioma.
2. Review test panels for plasma free fractionated metanephrines and 24-hour urine total fractionated metanephrines to ensure that catecholamines, VMA, and HVA are not included. Modify or eliminate as needed to improve utilization. Consider adding comments to order forms or order entry systems to inform clinicians about proper use of these tests at the time of order based on policies developed by medical staff consensus committee. Given that HVA and VMA testing are rarely indicated, consider adding a pathologist gatekeeper role for approving these tests.
3. As pheochromocytoma and paraganglioma are tumors of adults, consider using order systems to develop soft or hard stops if the patient age is less than 20 years, as these tumors are rarely encountered in this age group.
4. Create a “pop-up” or other alert with message (soft-stop) (1) whenever plasma free fractionated metanephrines and 24-hour urine total fractionated metanephrines are ordered concurrently, and (2) whenever catecholamines,

MVA, or HVA are ordered concurrently with plasma free fractionated metanephrines or 24-hour urine total fractionated metanephrines.

5. Create a hold (hard-stop) on orders if (1) plasma free fractionated metanephrines and urine total fractionated metanephrines are ordered concurrently, and (2) if catecholamines, VMA, or HVA are ordered concurrently with plasma free fractionated metanephrines or urine total fractionated metanephrines, with additional action required (eg, additional justification, pathologist approval) as appropriate for your clinical setting.
6. If additional analysis shows a relatively high number of plasma or urine metanephrines with concurrent orders for catecholamines or VMA/HVA, attempt to identify specific clinicians or locations to uncover potential utilization problems. Alternatively, selective feedback to clinicians about ordering practices relative to peers may also lead to improved utilization.

INTERVENTION ANALYSIS

Collect data on catecholamine and catecholamine metabolite utilization patterns if available from laboratory or hospital information systems (**See Appendix A**). If access to data is limited, this information may be manually collected over a period of time determined by how frequently these tests are ordered. It is easiest to use the same time period for before and after the intervention to do the assessment, for example, 3 months for pre-intervention and 3 months post-intervention. A factor must be applied if different time periods are used..

APPENDIX A: CALCULATING THE INTERVENTION IMPACT

Collect data in the table below, preferably using the same measurement period of time before and after implementing interventions (eg, 3 months). Correct the volume accordingly if different time periods are used for pre-intervention and post-intervention studies.

Pre-Intervention Column

Prior to taking any interventions steps:

- Determine the number of plasma free fractionated metanephrines and 24-hour urine total (free and deconjugated) fractionated metanephrines ordered concurrently. (A1)
- Determine the number of potential unnecessary VMA and HVA tests ordered, including VMA or HVA tests ordered separately or as a panel. (B1 + C1)
- Determine the number of potential unnecessary urine and plasma catecholamine tests.

Post-Intervention Column

After the interventions steps are taken:

- Determine the number of plasma free fractionated metanephrines and 24-hour urine total (free and deconjugated) fractionated metanephrines ordered concurrently. (A2)
- Determine the number of potential unnecessary VMA and HVA tests ordered, including VMA or HVA tests ordered separately or as a panel. (B2 + C2)
- Determine the number of potential unnecessary urine and plasma catecholamine tests.

Volume Change Column: Calculate the volume of tests after interventions have been established for each row. (A3, B3, C3, D3, E3).

Percent Volume Change Impact (%) Column: Calculate the percent change in the pre-intervention and post-intervention test volumes to find the impact of the change(s) instituted by your laboratory. (A4, B4, C4, D4, E4)

Laboratory Test Volume Outcomes and Opportunities				
Description	**Pre-Intervention**	**Post-Intervention**	**Pre - Post Volume Change**	**Percent Volume Change Impact (%)**
Number of 24-hour urine total fractionated metanephrines ordered concurrently with plasma free fractionated metanephrines	A1	A2	A1 - A2 = A3	A3/A1 x 100% = A4%
Number of orders for HVA in patients older than 20	B1	B2	B1 - B2 = B3	B3/B1 x 100% = B4%
Number of orders for VMA in patients older than 20	C1	C2	C1 - C2 = C3	C3/C1 x 100% = C4%

Laboratory Test Volume Outcomes and Opportunities				
Description	**Pre-Intervention**	**Post-Intervention**	**Pre - Post Volume Change**	**Percent Volume Change Impact (%)**
Number of orders for plasma catecholamines	D1	D2	D1 - D2 = D3	D3/D1 x 100% = D4%
Number of orders for urine catecholamines	E1	E2	E1 - E2 = E3	E3/E1 x 100% = E4%

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE

Understand pertinent clinical features regarding pheochromocytomas and paragangliomas and the compounds they secrete in order to better understand appropriate assay selection.

QUESTION 1

Which of the following is TRUE regarding catecholamine secreting tumors?

A. A positive free metanephrine screening test for pheochromocytomas and paragangliomas does not require confirmation with a 24-hour urine total (free and deconjugated) fractionated metanephrine test.
B Neuroblastomas primarily secrete metanephrines.
C. Vanillylmandelic acid (VMA) and Homovanillic acid (HVA) levels are not clinically useful in the work-up of pheochromocytomas and paragangliomas.
D. Spot urine samples are recommended for testing metanephrine levels for pheochromocytomas and paragangliomas.

The correct answer is C. Vanillylmandelic acid (VMA) and Homovanillic acid (HVA) levels are NOT useful in the work-up of pheochromocytomas and paragangliomas and are frequently ordered incorrectly in their work-up. VMA and HVA are useful in the work-up of neuroblastomas.

A is incorrect. Since the incidence of pheochromocytoma and paraganglioma is very low, most positive plasma metanephrine tests will be falsely positive and should be confirmed with a 24-hour urine total (free and deconjugated) fractionated metanephrine test.

B is incorrect. Neuroblastomas primarily secrete dopamine.

D is incorrect. A 24-hour urine collection for measurement of urine total (free and deconjugated) fractionated metanephrines is encouraged. Spot urine samples for metanephrine testing are not useful.

REFERENCES

1. Lenders JM, Quan-Yang D, Eisenhofer G, et al. Pheochromocytoma and paraganglioma: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2014;99:1915-1043. https://doi.org/10.1210/jc.2014-1498
2. Eisenhofer G, Kopin IJ, Goldstein DF. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev*. 2004;56(3):331-349. doi.org/10.1124/pr.56.3.1

QUESTION 2 OBJECTIVE

Recognize the utility of plasma free fractionated metanephrines and urinary total fractionated metanephrines in the diagnosis of pheochromocytomas and paragangliomas.

QUESTION 2

Which of the following metabolites is MOST useful to measure for the detection of paragangliomas and pheochromocytomas?

A. Dopamine
B. Epinephrine
C. Vanillylmandelic acid (VMA)
D. Homovanillic acid (HVA)
E. Metanephrines

The correct answer is E. Plasma free fractionated metanephrines or 24-hour urine total (free and deconjugated) fractionated metanephrines are currently the most widely advocated tests for detecting pheochromocytomas or paragangliomas.

A is incorrect. Although dopamine is the precursor for epinephrine and norepinephrine, it is not recommended in the detection of paragangliomas or pheochromocytomas.
B is incorrect. Epinephrine and norepinephrine levels are insensitive in the detection of paragangliomas or pheochromocytomas.
C is incorrect. Vanillylmandelic acid (VMA) levels are used in the detection of neuroblastomas.
D is incorrect. Homovanillic acid (HVA) levels are used in the detection of neuroblastomas.

REFERENCES

1. Lenders JM, Quan-Yang D, Eisenhofer G, et al. Pheochromocytoma and paraganglioma: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2014;99:1915-1043.https://doi.org/10.1210/jc.2014-1498
2. Eisenhofer G, Kopin IJ, Goldstein DF. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev*. 2004;56(3):331-349. doi.org/10.1124/pr.56.3.1
3. Kudva YC, Sawka AM, Young WF. The laboratory diagnosis of adrenal pheochromocytoma: the Mayo Clinic Experience. *J Clin Endocrinol Metab.* 2003;88:4533-4539. https://doi.org/10.1210/jc.2003-030720

QUESTION 3 OBJECTIVE

Understand and minimize the influence of preanalytical variables on testing for plasma free fractionated metanephrines for appropriate test interpretation.

QUESTION 3

Which of the following is FALSE regarding catecholamine preanalytical testing issues?

A. Drugs of abuse may increase metanephrine levels.
B. Medications known to impact metanephrine levels should be tapered or discontinued 48 hours prior to testing.
C. Exercise, stress (including stress related to a venipuncture), renal failure, severe congestive heart failure, myocardial infarction, acute illness and upright posture may increase metanephrine levels.
D. Conjugated metanephrines are excreted in the urine, and renal impairment will decrease clearance.

The correct answer is B. Medications known to impact metanephrine levels (including L-DOPA, alpha-methyldopa, monoamine oxidase inhibitors, antihypertensive medications and diuretics) should be tapered or discontinued at least 2 weeks (not 48 hours) prior to testing.
A is incorrect. Substances of abuse (including alcohol and cocaine) may increase metanephrine levels.
C is incorrect. Each of these circumstances and underlying illnesses may lead to elevated metanephrine levels.
D is incorrect. Conjugated metanephrines are indeed excreted in the urine, and renal impairment will decrease clearance. In these circumstances, testing plasma free fractionated metanephrines is recommended.

REFERENCES

1. Neumann HPH, Young WF, Eng C. Pheochromocytoma and paraganglioma. *N Engl J Med* 2019;381:552-565. doi: 10.1056/NEJMra1806651
2. Young WF. Clinical presentation and diagnosis of pheochromocytoma. In: Post TW, ed. *Up to Date*. UpToDate Inc. 2023. Literature review current April 2023. Updated October 3, 2022. Accessed May 4, 2023. https://www.uptodate.com/contents/clinical-presentation-and-diagnosis-of-pheochromocytoma?search=clinical-presentation-and-diagnosis-of-%20pheochromocytoma%20&source=search_result&selectedTitle=1~147&usage_type=default&display_rank=1
3. Young WF. Paragangliomas: epidemiology, clinical presentation, diagnosis and histology. In: Post TW, ed. *UpToDate*. UpToDate Inc. 2023. Literature review current April 2023. Updated April 19, 2022. Accessed May 4, 2023. https://www.uptodate.com/contents/paragangliomas-epidemiology-clinical-presentation-diagnosis-and-histology?search=paragangliomas-epidemiology-clinical-presentation-diagnosis-and-%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20histology&source=search_result&selectedTitle=1~59&usage_type=default&display_rank=1

REFERENCES FOR MODULE

1. Tischler AS, de Krijger RR, Gill AJ, et al. Pheochromocytoma: In: WHO classification of tumours of endocrine organs. 4th ed. Lyon, France: International Agency for Research on Cancer; 2017:183-190.
2. Neumann HPH, Young WF, Eng C. Pheochromocytoma and paraganglioma. *N Engl J Med* 2019;381:552-565. doi: 10.1056/NEJMra1806651
3. Young WF. Clinical presentation and diagnosis of pheochromocytoma. In: Post TW, ed. *Up to Date.* UpToDate Inc.2023. Literature review current April 2023. Updated October 3, 2022. Accessed May 4, 2023.

https://www.uptodate.com/contents/clinical-presentation-and-diagnosis-of-pheochromocytoma?search=clinical-presentation-and-diagnosis-of-%20pheochromocytoma%20&source=search_result&selectedTitle=1~147&usage_type=default&display_rank=1
4. Young WF. Paragangliomas: epidemiology, clinical presentation, diagnosis and histology. In: Post TW, ed. *UpToDate*. UpToDate Inc. 2023. Literature review current April 2023. Updated April 19, 2022. Accessed May 4, 2023. https://www.uptodate.com/contents/paragangliomas-epidemiology-clinical-presentation-diagnosis-and-histology?search=paragangliomas-epidemiology-clinical-presentation-diagnosis-and-%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20histology&source=search_result&selectedTitle=1~59&usage_type=default&display_rank=1
5. Lenders JM, Quan-Yang D, Eisenhofer G, et al. Pheochromocytoma and paraganglioma: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2014;99: 1915-1043. https://doi.org/10.1210/jc.2014-1498
6. Eisenhofer G, Kopin IJ, Goldstein DF. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev*. 2004;56(3):331-349. doi.org/10.1124/pr.56.3.1
7. Kudva YC, Sawka AM, Young WF. The laboratory diagnosis of adrenal pheochromocytoma: the Mayo Clinic experience. *J Clin Endocrinol Metab.* 2003;88:4533-4539. https://doi.org/10.1210/jc.2003-030720
8. Lenders JW, Pacak K, Walther MM, et al. Biochemical diagnosis of pheochromocytoma: which test is best? *JAMA*. 2002;287(11):1427. doi:10.1001/jama.287.11.1427
9. Grouzmann E, Drouard-Troalen L, Plouin PF, et al. Diagnostic accuracy of free and total metanephrines in plasma and fractionated metanephrines in urine of patients with pheochromocytoma. *Eur J Endcrinol* 2010;162: 951-960. doi: 10.1530/EJE-09-0996