COLLEGE of AMERICAN PATHOLOGISTS

Testing for Carcinoid Syndrome

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SYNOPSIS AND RELEVANCE

There are several biomarkers including 5-hydroxyindoleacetic acid (5-HIAA), chromogranin A (CGA) and serotonin which are used for the evaluation of patients with suspected neuroendocrine tumors (NET) associated with carcinoid syndrome symptoms (eg, flushing and/or diarrhea) as well as for individuals who are asymptomatic with incidental findings that suggest a possible NET from endoscopic or imaging examinations. Application of laboratory systems and interventions for optimal test selection and performance is important to avoid interpretive errors and provide the best diagnostic outcomes in patients with carcinoid syndrome and NETs.

OBJECTIVES

1. Define appropriate testing for the initial evaluation of suspected NETs in symptomatic and asymptomatic patients.
2. Understand patient and test conditions that can affect tests for carcinoid syndrome and NETs.
3. Ensure appropriate testing for post-treatment surveillance of NETs.
4. Describe various interventions that could be applied, if needed, to improve the use of these tests.

BACKGROUND

Carcinoid syndrome is a condition which most commonly causes abdominal pain with recurrent episodes of flushing and/or diarrhea, symptoms due to secretion of vasoactive compounds such as serotonin from low grade neuroendocrine tumors (NET). Tumors that most commonly secrete serotonin arise in the distal small intestine or proximal large intestine (midgut) regions.[1] Symptoms generally occur only after tumors metastasize to the liver or other locations, since vasoactive peptides produced from localized tumors are metabolized in the portal circulation. NETs arising in the stomach and duodenum (foregut), distal colorectal (hindgut), or other regions (eg, lungs) are less likely to produce vasoactive peptides or be associated with flushing or diarrhea. Asymptomatic NETs are typically detected as incidental findings during endoscopic procedures or imaging examinations.

Biomarkers for Initial Evaluation and Diagnosis of Carcinoid Syndrome and NET

Primary Test

5-Hydroxyindoleacetic Acid (5-HIAA)

The single best biomarker for initial evaluation of patients with carcinoid syndrome symptoms is measurement of 5-HIAA in a 24-hour urine specimen. Twenty-four-hour urine 5-HIAA is the final product of serotonin metabolism and in the presence of carcinoid syndrome has greater than 90% sensitivity and specificity for NETs associated with carcinoid syndrome symptoms.[2] However, 24-hour urine 5-HIAA has less diagnostic value in patients with NETs that produce little or no serotonin, and who lack symptoms of carcinoid syndrome. Importantly, 24-hour urine 5-HIAA concentrations increase with certain serotonin-containing foods and pose a risk of diagnostic error if the patient is not properly prepared for testing. Therefore, patients should be instructed to begin dietary restrictions 3 days prior to and during collection of specimens (**Table 1**). Disorders associated with malabsorption such as celiac disease may also give rise to elevated 24-hour urine 5-HIAA concentrations. Repeat 24-hour urine 5-HIAA testing to confirm initial results is an acceptable practice.

Secondary Tests

Chromogranin A (CGA)

Chromogranin A, a serum biomarker, is elevated with most NETs and higher concentrations are associated with larger tumor burdens and worse prognosis. However, this is a non-specific marker. Daily serum chromogranin A concentrations vary widely in patients with NETs, and CGA is commonly elevated in many other conditions including endocrine, gastrointestinal, cardiovascular, renal, inflammatory, and neoplastic disorders. Certain drugs such as proton pump inhibitors (eg, omeprazole), which stimulate neuroendocrine cells, are nearly always associated with elevated serum chromogranin A levels. While persistently normal serum chromogranin A levels may help exclude NET tumor as the cause of symptoms, a positive result (eg, greater than 10 times the upper limit of normal) lacks specificity. While serum CGA has limited value for initial diagnosis compared to 24-hour urine 5-HIAA measurements for patients with carcinoid syndrome symptoms, its measurement is useful for asymptomatic patients with suspected NET based on incidental findings detected during endoscopy or imaging examinations. It is also useful at initial evaluation for prognosis and as a baseline for monitoring after a diagnosis of NET is made.

Serotonin

Rarely, elevated urinary serotonin levels may be seen in patients with carcinoid syndrome symptoms who lack elevated 24-hour 5-HIAA concentrations. While not a primary biomarker for initial evaluation, measurement of serotonin in a 24-hour urine may be useful as a secondary test for the occasional patient with persistent carcinoid syndrome symptoms (eg, flushing, diarrhea) of unknown cause.[3] It may also be useful as an adjunct to chromogranin A for asymptomatic patients with suspected NET based on an incidental finding.

Biomarkers for Prognosis and Post-treatment Surveillance

Primary tests

Measurement of serum CGA at 3 to 12-month intervals for up to 10 years after treatment for gastrointestinal NET is recommended. Monitoring of 24-hour urine 5-HIAA concentrations is similarly recommended if originally elevated at diagnosis.

Secondary Tests

Serum neuron specific enolase and pancreastatin are commonly produced by NETs, and levels correlate with tumor mass and metabolic activity. Higher concentrations are associated with poorer prognosis and metastatic spread.

INSIGHTS

Twenty-four-Hour Urine 5-HIAA

1. Twenty-four-hour urine 5-HIAA is the primary screening test for carcinoid syndrome and suspected NET.
2. Repeat testing of 24-hour urine 5-HIAA to confirm previous results is appropriate practice.
3. Patients should be carefully instructed about dietary restrictions prior to and during collection of urine specimens for 24-hour urine 5-HIAA.[2]
4. Twenty-four-hour 5-HIAA is appropriate for post-treatment surveillance of NET at 3 to 12-month intervals for up to 10 years if elevated at initial diagnosis.

CGA

1. CGA should not be routinely ordered with 24-hour urine 5-HIAA for initial evaluation of patients with carcinoid syndrome symptoms.
2. Testing for serum CGA is appropriate for asymptomatic patients with suspected NET as an incidental finding on imaging or endoscopy examinations.
3. Elevated serum CGA concentrations are frequently associated with many other disorders, and these causes should be considered before attributing abnormal results to NET unless CGA is extremely high (eg, greater than 10 times upper limit of normal).
4. Patients on proton pump inhibitors must refrain from taking medication at least 2 weeks prior to CGA testing.
5. CGA testing every 3 to 12 months for up to 10 years is appropriate for post-treatment surveillance of NETs.

Serotonin

1. A 24-hour urine serotonin should not be routinely ordered with a 24-hour urine 5-HIAA for initial evaluation of patients with carcinoid syndrome symptoms.
2. Twenty-four-hour urine serotonin testing may be useful for patients with sustained carcinoid syndrome symptoms who have persistently normal 24-hour urine 5-HIAA levels.
3. Testing for 24-hour urine serotonin is appropriate for asymptomatic patients with suspected NET as an incidental finding.
4. A twenty-four-hour urine serotonin is not typically beneficial for post-treatment surveillance.

Neuron specific enolase, pancreastatin

1. Neuron specific enolase and/or pancreastatin may be useful as secondary prognostic and/or post-treatment biomarkers.

INTERVENTIONS

1. Establish local testing policies among medical staff for evaluation of carcinoid syndrome symptoms and suspected NET in asymptomatic patients.

2. Develop test algorithms appropriate for specific diagnostic evaluations depending on symptoms(see example in **Appendix A)**.

3. Develop written patient instructions for preparing for 24-hour urine 5-HIAA testing to explain dietary restrictions. This could be handed out by the clinician or by the laboratory at the time the patient is given a collection container (See example in **Appendix B**).

4. Develop a mechanism to determine if the patient is taking proton pump inhibitors prior to the collection of a specimen for CGA. This could be done by training phlebotomists, a pop-up alert at the time of order, or other methods.

5. Add comments to result reports with elevated 24-hour urine 5-HIAA concentrations to caution about interference from serotonin-containing foods (see **Appendix C**).

6. Add comments to result reports with elevated CGA concentrations to caution about other conditions that may cause these results (see **Appendix C**).

7. Perform random audits of patients with 24-hour urine 5-HIAA, CGA, and/or 24-hour urine serotonin orders for compliance with testing policies and provide feedback as needed (see Impact Analysis & **Appendix D**).

8. Restrict 24-hour urine serotonin ordering to designated specialists.

9. Implement a warning alert for 24-hour urine serotonin orders as not indicated for initial diagnosis of carcinoid syndrome .

10. Implement a warning alert for CGA orders as not indicated for initial diagnosis of carcinoid syndrome .

11. Establish a random or full review process by a pathologist or designated specialist for new serotonin or CGA orders. Check for prior or concurrent 24-hour urine 5-HIAA testing. Provide feedback as needed.

12. Establish a random or full review process by a pathologist or designated specialist to check for misapplication of 24-hour urine 5-HIAA, CGA, or 24-hour urine serotonin tests for evaluation of neuroblastoma (appropriate initial tests are urinary homovanillic acid [HMA] and vanillylmandelic acid [VMA]) or pheochromocytoma (appropriate initial tests are fractionated metanephrines in urine or blood). Provide feedback as needed.

13. Establish a registry of patients undergoing post-treatment surveillance for automatic approval of testing for CGA and, when indicated, 24-hour urine 5-HIAA levels.

14. Consider providing feedback to healthcare providers about ordering practices for specific cases that do not meet recommended local guidelines.

INTERVENTION ANALYSIS

<u>Perform Pre and Post Intervention Audits for Test Purpose and Guideline Compliance</u>

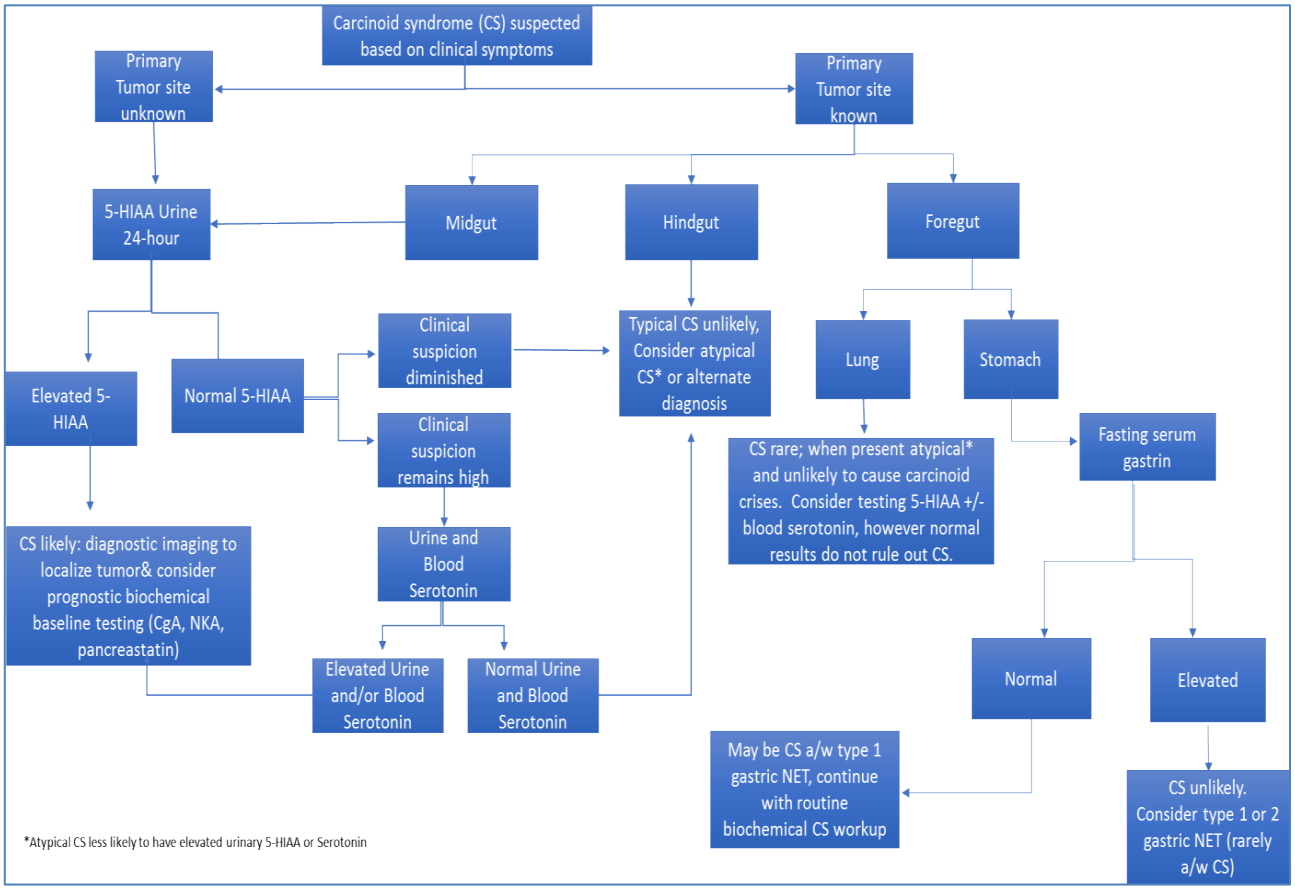
The number of inappropriate test orders before and after various interventions may be used to assess their effectiveness in improving ordering practices (see Appendix D). This can be done by sequential or random audits of orders for any or all of the various biomarkers described in this module (24-hour urine 5-HIAA, CGA, 24-hour urine serotonin) using the following steps:

1) Determine first if the test was ordered for diagnosis or for post-treatment surveillance, and next,
2) Determine if the indication for testing met your institution’s guidelines.

Perform Pre and Post Intervention Audits on Patient Preparation Before 24-Hour Urine 5-HIAA Testing

Track trends in percentages of patients who are not on appropriate dietary restrictions for 24-hour urine 5-HIAA testing, or who are on proton pump inhibitor medication for CGA testing based on questions asked by the blood collector (see **Appendix D**). As an example, determine the number of patients who are provided with dietary instructions either when the specimen containers are provided to the patient, or when the patients deliver their specimens to the laboratory staff. Likewise, determine the number of patients who are receiving proton pump inhibitor medication at the time their blood specimens are collected for CGA.

APPENDIX A: EXAMPLE OF A TEST ALGORITHM FOR SPECIFIC DIAGNOSTIC EVALUATIONS*



Abbreviation: Neurokinin A, NKA.

*Algorithm based on module references 3 and 4.

APPENDIX B: EXAMPLE OF PATIENT INSTRUCTIONS TO PREPARE FOR 24-HOUR URINE 5-HIAA TESTING

Your doctor ordered a test that may be affected by your diet. It is important for you to withhold eating any of the following foods at least two days before and during the period when collecting your urine specimen.

Table 1. Foods to avoid prior to and during 24-hour urine 5-HIAA Testing[2]

Avocado	Hickory nuts	Plantains
Banana	Kiwi fruit	Tomatoes
Butternut squash	Pecans	Walnuts
Eggplant	Plums	
Ethanol	Pineapple	

APPENDIX C: EXAMPLES OF COMMENTS THAT CAN BE INCORPORATED INTO REPORTS THAT HAVE ABNORMAL 24-HOUR URINE 5-HIAA OR CHROMOGRANIN A RESULTS

Twenty-four-hour urine 5-HIAA: Abnormal (high). While this value is consistent with a serotonin-producing tumor, other causes such as a diet containing foods with high serotonin content prior to or during specimen collection should be considered. Repeat testing for confirmation may also be useful prior to imaging or other diagnostic evaluations.

Chromogranin A: Abnormal (high). While this value is consistent with a neuroendocrine tumor, other causes such as use of proton pump inhibitors, renal insufficiency, or certain gastrointestinal, cardiovascular, inflammatory, and neoplastic disorders should be considered.

APPENDIX D: PRE AND POST INTERVENTION AUDIT FOR GUIDELINE COMPLIANCE AND PATIENT PREPARATION

Pre-Intervention		
Patients with a CGA order which is not in compliance with guidelines	A1	
Patients with a 24-hour urine 5-HIAA order which is not in compliance with guidelines	B1	
Patients with a 24-hour urine serotonin order which is not in compliance with guidelines	C1	
Patients who are on an inappropriate diet for 24-hour urine 5-HIAA testing when a urine specimen is received	D1	
Patients who have taken a proton pump inhibitor medication within 2 weeks prior to blood collection for CGA	E1	
Post Intervention		Percent Change
Patients with a CGA order which is not in compliance with guidelines	A2	(A1-A2)/A1 X100% = A3%
Patients with a 24-hour urine 5-HIAA order which is not in compliance with guidelines	B2	(B1-B2)/B1 X100% = B3%
Patients with a 24-hour urine serotonin order which is not in compliance with guidelines	C2	(C1-C2)/C1 X100% = C3%
Patients who are on an inappropriate diet for 24-hour urine 5-HIAA testing when a urine specimen is received	D2	(D1-D2)/D1 X100% = D3%
Patients who have taken a proton pump inhibitor medication within 2 weeks prior to blood collection for CGA	E2	(E1-E2)/E1 X100% = E3%

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE

Understand patient and test conditions that can affect tests for carcinoid syndrome and NETs.

QUESTION 1

A 56-year-old woman with recent onset of mild abdominal pain and diarrhea with negative stool examinations for bacterial and parasitic pathogens was evaluated for a neuroendocrine tumor as a potential cause of her symptoms. She has no symptoms of flushing or weight loss. A 24-hour urine specimen for 5-HIAA was slightly elevated, and the serum CGA level was within the normal reference range. These findings are most likely a false positive 5-HIAA result due to:

A. Diet, since she reports eating guacamole almost every day.
B. Diet, since she has recently eaten shellfish.
C. Medication, since she is taking omeprazole (Prilosec), a proton pump inhibitor.
D. Medication, since she is taking fluoxetine (Prozac), a serotonin reuptake inhibitor.
E. Vigorous exercise since she runs at least 3 miles per day.

The correct answer is A. Ingestion of avocados raises serotonin and its metabolite (5-HIAA).
B is incorrect because ingestion of shellfish does not raise the level of serotonin or its metabolite (5-HIAA).
C is incorrect because proton pump inhibitors do not affect serotonin metabolism.
D is incorrect because serotonin reuptake inhibitor medications do not affect plasma serotonin concentration.
E is incorrect because exercise does not affect plasma serotonin concentration.

REFERENCE

O'Toole D, Grossman A, Gross D, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: biochemical markers. *Neuroendocrinology*. 2009; 90:194-202.

QUESTION 2 OBJECTIVE

Understand patient and test conditions that can affect tests for carcinoid syndrome and NETs.

QUESTION 2

A 36-year-old man with long standing upper gastrointestinal pain shows a single well-enhanced intraluminal polypoid lesion in the duodenal bulb on CT examination which is also seen in double-contrast barium examination of the upper gastrointestinal tract. He has no symptoms of flushing, diarrhea, or weight loss. The patient's 24-hour urine 5-HIAA was normal, and the serum CGA level was slightly increased. These findings are most likely a false positive CGA due to:

A. Diet, since he reports eating fish almost every day.
B. Diet, since he is a vegetarian and eats nuts of various kinds each day.
C. Medication, since he is taking omeprazole, a proton pump inhibitor.
D. Medication, since he is taking fluoxetine (Prozac) a serotonin reuptake inhibitor.
E. Vigorous exercise since he swims at least one hour each day.

The correct answer is C because proton pump inhibitors usually raise serum CGA levels and may cause diagnostic error.

A is incorrect because fish consumption does not affect CGA levels.
B is incorrect because CGA is not affected by diet.
D is incorrect because CGA is not affected by serotonin reuptake inhibitor medications.
E is incorrect because exercise does not affect serum CGA concentration.

REFERENCE

O'Toole D, Grossman A, Gross D, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: biochemical markers. *Neuroendocrinology*. 2009; 90:194-202.

QUESTION 3 OBJECTIVE

Define appropriate testing for the initial evaluation of suspected NETs in symptomatic and asymptomatic patients.

QUESTION 3

A 44-year-old man with a 6-month history of abdominal pain and diarrhea has recent onset of flushing symptoms that occur almost daily. A transverse CT examination shows a single well-enhanced intraluminal polypoid lesion in the distal ileum wall which is also seen on double-contrast barium examination. Which one of the following laboratory tests should be included in the initial evaluation for, and diagnosis of, a neuroendocrine tumor?

A. 24-hour urine serotonin
B. 24-hour urine 5-HIAA
C. Serum chromogranin A
D. Serum neuron specific enolase
E. Stool ova and parasite examination

The correct answer is B, because 24-hour urine 5-HIAA is the single best laboratory test for symptomatic (carcinoid syndrome-related) neuroendocrine tumors.

A is incorrect because serotonin is considered a secondary biomarker, especially in symptomatic patients.
C is incorrect because CGA is less specific and adds little value compared to 24-hour urine 5-HIAA in patients with carcinoid syndrome symptoms.
D is incorrect because neuron specific enolase is a prognostic biomarker and is too insensitive for initial diagnostic evaluation.
E is incorrect since symptoms are not consistent with an infectious cause of diarrhea.

REFERENCE

O'Toole D, Grossman A, Gross D, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: biochemical markers. *Neuroendocrinology*. 2009; 90:194-202.

QUESTION 4 OBJECTIVE

Define appropriate testing for the initial evaluation of suspected NETs in symptomatic and asymptomatic patients.

QUESTION 4

A 49-year-old woman with a 6-month history of persistent upper abdominal pain was evaluated with imaging examinations. Transverse arterial phase CT scan showed mild enlargement of the major duodenal papilla, and transverse CT examination showed dilatation of the extrahepatic bile duct along with multiple metastatic tumors in the liver. Both 24-hour urine 5-HIAA and serum CGA levels were markedly elevated. What additional test may benefit this patient?

A. 24-hour urine serotonin
B. Urine homovanillic acid (HMA)
C. Urine vanillylmandelic acid (VMA)
D. Serum neuron specific enolase
E. 24-hour urine histamine

The correct answer is D, because neuron specific enolase provides additional prognostic information when a neuroendocrine tumor is identified or strongly suspected.

A is incorrect because 24-hour urine serotonin is considered a secondary biomarker, and with elevated 24-hour urine 5-HIAA and CGA levels, provides little if any additional diagnostic value.

B is incorrect because HMA is primarily indicated for evaluating patients with suspected catecholamine-secreting tumors (eg, neuroblastoma), a condition that is inconsistent with the patient's symptoms or with her laboratory and imaging results.

C is incorrect because VMA is primarily indicated for evaluating patients with suspected catecholamine-secreting tumors (eg, neuroblastoma), a condition that is inconsistent with the patient's symptoms or with her laboratory and imaging results.

E is incorrect because urine histamine is a secondary biomarker that may be elevated in some patients with gastric neuroendocrine tumors, and the patient does not have symptoms (eg, itching, flushing) or imaging results consistent with this condition.

REFERENCE

O'Toole D, Grossman A, Gross D, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: biochemical markers. *Neuroendocrinology*. 2009; 90:194-202.

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