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- This speaker has nothing to disclose.
Pheochromocytomas and Paragangliomas
Justin Caron, MD
Clinical Chemistry
2014-09-22
Learning Objectives

1. Discuss the physiology, production, and metabolism of catecholamines.
2. Describe the clinical presentation of pheochromocytomas and paragangliomas.
3. Select appropriate laboratory tests for screening and diagnosis of pheochromocytomas and paragangliomas.
4. Recognize common analytical and physiologic interferences seen in laboratory testing of pheochromocytoma.
1. INTRODUCTION

“…produce work worthy of your efforts.”
- Dr. William J. Mayo
Historical Perspective

- The adrenal glands went *unnoted* by early physicians and the great anatomists:
  - Galen
  - Leonardo da Vinci
  - Andreas Vesalius

- **Bartholomaeus Eustachius** (1563)

- **Thomas Addison** (1855)
  - The first physician on record to describe the importance of the adrenal glands

From: *De humani corporis fabrica libri sephem*, 1543
Andreas Vesalius
Historical Perspective

- **1886** Paul Mannase  
  **Chromaffin reaction**

- **1886** Felix Fränkel  
  First to describe adrenal medulla tumor

- **1912** Ludgwig Pick  
  Coined the term  
  “pheochromocytoma”

- **1926** Felix Roux  
  First surgical removal  
  (Europe)

- **1927** Charles H. Mayo  
  First surgical removal  
  (United States)

phaios (“dusky”)  
chroma (“color”)  
cytoma (“tumor”)
Historical Perspective

PAROXYSMAL HYPERTENSION WITH TUMOR OF RETROPERITONEAL NERVE

REPORT OF CASE *

CHARLES H. MAYO, M.D.
ROCHESTER, MINN.

JAMA 1927;89(13):1047-1050
2. ADRENAL GLANDS

"Begin your anatomy with a man fully grown; then show him elderly and less muscular; then go on to strip him stage by stage right down to the bones."

- Leonardo da Vinci (1452-1519)
Adrenal Gland Histology

Cortisol

Aldosterone

Sex hormones

Catecholamines (Epi, NE)

Campbell-Walsh Urology. 10th ed.
Histology

- Chromaffin cells (pheochromocytes) in tight clusters
- Basophilic, finely granular cytoplasm
- Delicate vascular supporting stroma

Histology for Pathologists. 4th ed.
“Chromaffin Cell Reaction”

- Oxidizing agents polymerize the catecholamine-containing granules, turning them brown
  - Potassium bichromate
  - Ferric chloride
  - Ammoniacal silver nitrate
  - Osmium tetroxide

This staining is called the **chromaffin reaction**

We can use this type of electrical activity (oxidation) in our testing
Adrenal Medulla Overview

- **Phenylethanolamine-N-methyltransferase:**
  - Converts *norepinephrine* (NE) to *epinephrine* (E)
  - Cortisol enhances enzyme activity

Epinephrine and norepinephrine belong to a class of molecules called catecholamines…
3. CATECHOLAMINES

“That stuff makes pure mescaline seem like ginger beer, man. Pure adrenochrome.”

- Dr. Gonzo, Fear and Loathing in Las Vegas
Catecholamines

- **Organic amines** produced by the body to serve as chemical signals for the nervous system

- **Consist of:**
  - **Catechol**...
  - Attached to an **amine**
Catecholamines

- Organic amines produced by the body to serve as chemical signals for the nervous system

- Consist of:
  - Catechol...
  - Attached to an amine

![Chemical structures of catecholamines]

- 3,4-dihydroxybenzene
- Epinephrine
- Norepinephrine
Biosynthesis

80%

20%

PNMT
Sites of Biosynthesis

- **Epinephrine** is produced by the adrenal medulla
  - 90% of circulating epinephrine
  - **PNMT** is expressed mainly by adrenal chromaffin cells

- **Norepinephrine** is produced in the CNS and sympathetic nervous system
  - 90% of circulating norepinephrine
“Fight or flight”

A diffuse systemic response; due to catecholamines:

- Systemic vasoconstriction: ↑ BP
- ↑ HR, contractility
- Pupil dilation
- **Bronchodilation**
- Stimulation of **glucose** release
- Decrease in blood flow to nonessential organs
- Inhibition of digestion
Adrenoreceptors

- $\alpha_1$
  - Smooth-muscle contraction
  - Vasoconstriction
  - $\uparrow$ BP

- $\alpha_2$
  - Cardiostimulation
  - $\uparrow$ HR
  - $\uparrow$ myocardial contractility

- $\beta_1$
  - Smooth-muscle relaxation
  - Vasodilation
  - Bronchodilation
  - $\downarrow$ PVR

- $\beta_2$

$t_{1/2} = 2\text{ min.}$
Adrenoreceptors

α₁
Smooth-muscle contraction
- Vasoconstriction
- ↑ BP

α₂

β₁
Cardiostimulation
- ↑ HR
- ↑ myocardial contractility

β₂
Smooth-muscle relaxation
- Vasodilation
- Bronchodilation
- ↓ PVR

Lots of catecholamines = lots of adrenergic stimulation!!

↑↑ BP and HR
Adrenoreceptors

- $\alpha_1$
  - Smooth-muscle contraction
    - Vasoconstriction
    - $\uparrow$ BP

- $\alpha_2$

- $\beta_1$
  - Cardiostimulation
    - $\uparrow$ HR
    - $\uparrow$ myocardial contractility

- $\beta_2$
  - Smooth-muscle relaxation
    - Vasodilation
    - Bronchodilation
    - $\downarrow$ PVR

**Pre-operative blood pressure management:**

1. $\alpha$-blockade with **phenoxybenzamine**
2. $\beta$-blockade with **Labetalol**
Metabolism

Two major degradation pathways:

- **Monoamine oxidase (MAO)**
  - Responsible for the **oxidative deamination** of:
    - Norepinephrine and epinephrine to aldehydes

- **Catechol-O-methyl transferase (COMT)**
  - Responsible for **O-methylation** of:
    - Norepinephrine to normetanephrine
    - Epinephrine to metanephrine
Catecholamine metabolism

Norepinephrine

Epinephrine

Dihydroxymandelic aldehyde (DHM aldehyde)

Normetanephrine (NMN)

Metanephrine (MN)

Dihydroxyphenylglycol (DHPG)

Dihydroxymandelic acid (DHM acid)

3-Methoxy-4-hydroxy-mandelic aldehyde (MHM aldehyde)

Methoxyhydroxyphenylglycol (MHPG)

Vanillylmandelic acid (VMA)

MAO

COMT

reduction

oxidation

MAO

COMT

oxidation

Tietz Fundamentals of Clinical Chemistry, 5th edition
Catecholamine metabolism

Norepinephrine

EPINEPHRINE

Metanephrine (MN)

Normetanephrine (NMN)

Dihydroxymandelic aldehyde (DHM aldehyde)

Dihydroxymandelic aldehyde (VMA)

Dihydroxyphenylglycol (DHPG)

3-Methoxy-4-hydroxy-mandelic aldehyde (MHM aldehyde)

Dihydroxyphenylglycol (DHPG)

Vanillylmandelic acid (VMA)

Tietz Fundamentals of Clinical Chemistry, 5th edition
Catecholamine metabolism

Norepinephrine

Epinephrine

Metanephrine

Normetanephrine

Dihydroxymandelic aldehyde (DHM aldehyde)

Dihydroxymandelic acid (DHM acid)

3-Methoxy-4-hydroxy-mandelic aldehyde (MHM aldehyde)

Dihydroxyphenylglycol (DHPG)

Methoxyhydroxyphenylglycol (MHPG)

Vanillylmandelic acid (VMA)

“metanephrines”

Tietz Fundamentals of Clinical Chemistry, 5th edition
Catecholamine metabolism
Case 1:
The Music Teacher

History of present illness:

- A 30 year-old female music teacher presented to her physician complaining of attacks of dyspnea, headache, tachycardia, and vomiting

- These attacks are **paroxysmal** in nature and have been increasing in frequency and severity over the past year and a half

- During the attacks, she is prostrate with discomfort
Case 1: The Music Teacher

- Upon admission to the hospital
  - Physical examination:
    - Reveals a **pale** and **undernourished** woman but the exam is **otherwise unremarkable**
    - Vitals: normal heart rate; blood pressure: 130/82
    - **Labs**: CBC is normal
    - **Other studies**: EKG, CXR, Abdominal x-ray are all normal
Case 1: The Music Teacher

- Shortly after admission: the patient develops tachycardia; BP is measured at 280/180
- Symptoms: chest pain, headache, N&V, blurry vision, numb extremities
- EKG: tachycardia
- BP is repeated: 300/180
- Due to severe hypertension and intractable abdominal pain, an exploratory laparotomy was performed…
Case 1: The Music Teacher

...This is the presentation from the original case published by Dr. Charles Mayo in 1927

The diagnosis of pheochromocytoma did not exist as such-

- There were **no biochemical laboratory tests** for pheochromocytoma!!
- No **CT scans** or **MRI**
- No understanding of **how to control the blood pressure before and during surgery**

**Perioperative mortality rates were as high as 50%**
4. LABORATORY TESTING

“You know, I am sorry for the poor fellows that haven't got labs to work in.”

-Sir Ernest Rutherford (1871-1937)
Which analyte(s) should we choose?
Too Many Options!!

Biochemical testing options:
- Plasma metanephrines (HPLC/ECD)
- Urine metanephrines (GC-MS)
- Plasma catecholamines (HPLC/ECD)
- Urine catecholamines (LC-MS/MS)
- Urine vanillylmandelic acid (HPLC/ECD)
- Urine homovanillic acid (HPLC/ECD)
- Serum chromogranin A (EIA)
- Clonidine suppression test
PLUS confusion over the terms free, total and fractionated!
PLUS confusion over the terms free, total and fractionated!

FREE = unconjugated

Conjugated forms
PLUS confusion over the terms free, total and fractionated!

\[
\text{Total} = \text{metanephrines plus conjugated metabolites}
\]
PLUS confusion over the terms free, total and fractionated!

Fractionated = separate out normetanephrine and metanephrine
Current Recommendations

Endocrine Society Clinical Practice Guidelines (2014)

1. Initial biochemical testing should include:
   - Plasma free metanephrines and/or
   - Urinary fractionated metanephrines

2. Liquid chromatography using mass spectrometry or electrochemical detection is the preferred methods

3. For measurements of plasma metanephrines:
   - Draw samples with patient in supine position
   - Use reference intervals established in the same position
Diagnostic Considerations

- The most important consideration is the potential harm of a false negative.
- Pheochromocytomas have a high rate of morbidity and mortality if undetected.
- Therefore, sensitivity is a primary consideration for any testing strategy.
- If we have a negative test, can we trust the result to rule out the diagnosis?

**SNOUT** = Sensitivity rules **OUT**

**SPIN** = Specificity rules **in**
<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma-free metanephrines</td>
<td>99%</td>
<td>89%</td>
</tr>
<tr>
<td>Plasma catecholamines</td>
<td>84%</td>
<td>81%</td>
</tr>
<tr>
<td>Urinary catecholamines</td>
<td>86%</td>
<td>88%</td>
</tr>
<tr>
<td>Urinary-fractionated metanephrines</td>
<td>97%</td>
<td>69%</td>
</tr>
<tr>
<td>Urinary total metanephrines</td>
<td>77%</td>
<td>93%</td>
</tr>
<tr>
<td>VMA</td>
<td>64%</td>
<td>95%</td>
</tr>
</tbody>
</table>

Sensitivity values of all tests for familial phaeochromocytoma are lower than that for sporadic phaeochromocytomas; the reverse is the case for specificity values. Table adapted from reference 64.

**Table 3: Sensitivity and specificity of biochemical tests for diagnosis of phaeochromocytoma**

Lancet. 2005;365:665-675
Binary vs. Continuous Approach

Binary vs. Continuous Approach

Binary vs. Continuous Approach
Current Recommendations

Endocrine Society Clinical Practice Guidelines (2014)

1. Initial biochemical testing should include:
   - Plasma free metanephrines and/or
   - Urinary fractionated metanephrines

2. **Liquid chromatography using mass spectrometry or electrochemical detection is the preferred methods**

3. For measurements of plasma metanephrines:
   - Draw samples with patient in supine position
   - Use reference intervals established in the same position
Plasma Metanephrines

- **LC-MS/MS**
  - Best performance
  - Sensitivity 100%
  - Specificity 96%

- **LC-ECD**
  - Good performance
  - Sensitivity 96-100%
  - Specificity 85-100%

J Clin Endocrinol Metab. 2014 Jun;99(6):1915-42
Methods

- Plasma and urinary fractionated metanephrines: LC-MS/MS
Plasma Metanephrines

- Spike with IS
- Extraction:
  - **Weak cation exchange** (WCX) 96-well solid phase extraction plate
  - **Prep:** water and methanol
  - Add sample
  - **Wash:** water and methanol
- **Elute:** weak acid
  - Formic acid (2%) in acetonitrile

Ready for HPLC followed by TMS
Liquid Chromatography

- Separation by LC is based on distribution of the solutes between a liquid mobile phase and a stationary phase.
- High pressure pump pushes mobile phase through the solid phase (in the column).
- Separates analytes by time, charge, affinity.
- Each analyte will come off at a specific time.
HYDROPHILIC LIQUID INTERACTION CHROMATOGRAPHY (HILIC)

- Atlantis Silica HILIC Column
- Variation of normal phase chromatography
- The stationary phase is hydrophilic (likes water)
- High organic mobile phase
  - (>80% acetonitrile)
- Better retention of polar compounds

Tandem mass spectrometry
Urinary Metanephrines

- Metanephrines in urine are conjugated to glucuronic acid or sulfate

**Acid hydrolysis**
- Spike with IS
- Add acid (6 M HCl)
- Heat in water bath

- Hydrolyzed samples are pH adjusted
- Solid phase extraction (SPE)

Mass Spec II

Must be capped!!

Ready for HPLC followed by TMS
Tandem Mass Spectrometry

- Electrospray ionization -> Q1 (select m/z) -> Q2 = collision cell (inert gas) -> Q3 (look for fragments with specific m/z)

**m/z:**
- NM 166
- M 180
- NM 166     134, 106
- M 180      148, 120
- NM 183
- M 197
Current Recommendations

Endocrine Society Clinical Practice Guidelines (2014)

1. Initial biochemical testing should include:
   - Plasma free metanephrines and/or
   - Urinary fractionated metanephrines

2. Liquid chromatography using mass spectrometry or electrochemical detection is the preferred methods

3. For measurements of plasma metanephrines:
   - Draw samples with patient in supine position
   - Use reference intervals established in the same position
Influence of Reference Intervals

Upper limit of reference intervals in supine position:

- **NMN = 0.65 nmol.L**
  - Noise from sympathoadrenal system: 0.30
  - Signal from Tumor: 0.35
  - Result: Positive result tumor detected
  - Upper limit of reference intervals in supine position: 0.61 nmol/L

Upper limit of reference intervals in seated position:

- **NMN = 0.75 nmol.L**
  - Noise from sympathoadrenal system: 0.39
  - Signal from Tumor: 0.35
  - Result: Negative result tumor detected
  - Upper limit of reference intervals in seated position: 0.79 nmol/L

Adapted from Tietz Textbook of Clinical Chemistry 5th ed.
Analytical Interferences

<table>
<thead>
<tr>
<th></th>
<th>HPLC assays: plasma catecholamines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee (including decaffeinated coffee)</td>
<td>Spectrophotometric and fluorometric assays: urinary catecholamines and metanephrines;</td>
</tr>
<tr>
<td>Labetalol</td>
<td>HPLC assays: plasma catecholamines</td>
</tr>
<tr>
<td>Sotalol</td>
<td>HPLC assays: urinary metanephrines</td>
</tr>
<tr>
<td>Buspirone</td>
<td>HPLC assays: plasma-free metanephrines</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>HPLC assays: catecholamines and metabolites</td>
</tr>
<tr>
<td>Levodopa</td>
<td>HPLC assays: catecholamines</td>
</tr>
<tr>
<td>( \alpha )-methyldopa</td>
<td>Spectrophotometric and fluorometric assays: plasma and urinary catecholamines</td>
</tr>
<tr>
<td>Sympathomimetics (eg, amphetamines, ephedrine)</td>
<td></td>
</tr>
</tbody>
</table>

Lancet. 2005;365:665-675
# Drug Interferences

## Table 7. Major Medications That May Cause Falsely Elevated Test Results for Plasma and Urinary Metanephrines

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NMN</td>
<td>MN</td>
</tr>
<tr>
<td>Acetaminophen (^a)</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Labetalol (^a)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sotalol (^a)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(\alpha)-Methyldopa (^a)</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Tricyclic antidepressants (^b)</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Buspirone (^a)</td>
<td>−</td>
<td>++</td>
</tr>
<tr>
<td>Phenoxybenzamine (^b)</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>MAO-inhibitors (^b)</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Sympathomimetics (^b)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cocaine (^b)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sulphasalazine (^a)</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Levodopa (^c)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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*J Clin Endocrinol Metab. 2014 Jun;99(6):1915-42*
5. PHEOCHROMOCY TOMAS AND PARAGANGLIOMAS
Pheochromocytomas

- Tumors arising from chromaffin cells
  - Prove one or more catecholamines:
    - Epinephrine, norepinephrine
    - Rarely, these tumors are biochemically silent
- The majority are benign
- Distribution:
  - Adrenal medulla (80-85%)
  - Paragangliomas (15 to 20%)
Paragangliomas

- Tumors derived from **extra-adrenal chromaffin** cells
  - Sympathetic paravertebral ganglia of thorax, abdomen, and pelvis
  - Parasympathetic ganglia located along nerves in the neck and at the base of the skull
Clinical Presentation

- The dominant presentation is **paroxysmal hypertension**

- Classic triad:
  - Tachycardia/palpitations
  - Headache
  - Sweating

- Others: pallor, nausea, fatigue, weight loss, hyperglycemia
Background Information

- Benign (~85%) or malignant (~15%)
- Sporadic or familial
- Can occur at any age (4th - 5th decade most common)
- Rare: 2-8 per 1 million persons per year
- 0.1% of hypertensive patients have pheochromocytoma

Of 15,984 total autopsies
Inherited Syndromes

- von Hippel-Lindau syndrome (VHL)
- Multiple endocrine neoplasia (MEN) type 2A & 2B (RET)
- Neurofibromatosis type 1 (NF1)
- Paraganglioma syndromes (SDH)
Clinical Importance

- **High mortality rate** if untreated or not recognized
  - **Hypertensive crisis** is a threat to life and/or organs
  - Enlarging masses can compress vital structures
  - ~15% of cases are malignant

- Surgical resection is **curative** in most cases
Clinical Importance

- Elevated catecholamines may acutely precipitate:
  - Congestive heart failure
  - Pulmonary edema
  - Myocardial infarction
  - Ventricular fibrillation
  - Cerebrovascular accidents
Case 2:
An Inherited Syndrome

- A 45-year-old female with neurofibromatosis type 1 and severe kyphoscoliosis
- **HPI**: palpitations, rapid heart rate (140s) and persistent headache
- **Meds**: Ritalin and methadone
- Symptoms attributed to Ritalin
Case 2: An Inherited Syndrome

- **PMH**: remote history of a stroke 23 years ago
  - She has had dizziness and palpitations for years
  - Biopsy for a thyroid nodule (2009) - benign
  - A CT scan performed during that work up revealed a right adrenal mass thought to be a likely neurofibroma

- **Family history**: multiple first degree relatives who have died from complications of NF-1
Case 2: An Inherited Syndrome

- She is unable to tolerate an MRI and undergoes a CT scan instead.
- The radiologist finds a 6 x 2 cm mass c/o pheochromocytoma.
- A 24-hour urinary fractionated metanephrines is ordered confirming the diagnosis.
  - Urine metanephrine 794 ug/d (30-350)
  - Urine normetanephrine 752 ug/d (50-650)
Case 2: An Inherited Syndrome

- The patient was scheduled for surgery
- SBP 120’s at admission
  - α-blockade started 10 days pre-op (phenoxybenzamine)
  - β-blockade started after a week of α-blockade (propranolol)
- A transperitoneal right robotic adrenectomy was planned
ROBOT-ASSISTED LAPAROSCOPIC RIGHT ADRENALECTOMY

Surgeons console

Slave unit

Anesthesiologist

Assistant

Campbell-Walsh Urology. 10th ed.
Operative Course

- Attempted right robotic-assisted laparoscopic adrenalectomy aborted
  - Dense abdominal adhesions and poor access to retroperitoneum due to unusual liver anatomy
- Stopped the operation, sewed up the port sites, scrubbed in again
- Converted to right flank open adrenalectomy
Surgical incision over 11\textsuperscript{th} rib for flank adrenalectomy
Case 2: Surgical Specimen

Adrenal gland with two masses:
5.5 x 2.8 x 1.5 cm
1.5 x 1.3 x 1.0
Zellballen
“Normal” sized nerve

Plexiform neurofibroma
Neurofibromatosis type 1

- Neurofibromatosis type 1 (NF 1): first described pheochromocytoma associated syndrome
- Autosomal dominant (1/3000) individuals in all populations
- The expression is highly variable but penetration is nearly 100%
  - Multiple neurofibromas
  - Café au lait spots
  - Axillary freckling of the skin
  - Lisch nodules of the iris
  - Optic nerve gliomas
  - Skeletal dysplasias
Diagnostic Work Up

- **Biochemical testing**: cornerstone of the diagnosis!!
  - Should minimally include measurements of plasma free and/or urinary fractionated metanephrines

- Imaging:
  - CT and/or MRI
  - Consider functional imaging

- Genetic testing
  - **Endocrine Society Guidelines (2014)** recommend considering genetic testing for all patients
  - Familial or syndromic presentation merits high priority on genetic testing
Summary

- Pheochromocytoma is a tumor of the chromaffin cells of the adrenal medulla.
- The classic presentation is tachycardia, headache, and sweating but the dominant sign is hypertension.
- Most are curable if detected, deadly if not.
- The recommended biochemical testing is:
  - Plasma free metanephrines and/or
  - Urinary fractionated metanephrines.
Acknowledgements

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References


5. Mayo CH. Paroxysmal hypertension with tumor of retroperitoneal nerve. JAMA. 1927;89(13):1047-1050


References


References


