Testing Strategies for Evaluating Patients with Hereditary Causes of Erythrocytosis

James D. Hoyer, MD
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DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None
Learning Objectives

Erythrocytosis

• Discuss the causes of hereditary erythrocytosis.
• Utilize an algorithmic approach in the evaluation of erythrocytosis.
• 2 illustrative cases
# Erythrocytosis

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>18.5 g/dL</td>
<td>16.5 g/dL</td>
</tr>
<tr>
<td>Hct</td>
<td>52%</td>
<td>48%</td>
</tr>
</tbody>
</table>
Causes of Erythrocytosis

- Developed later (acquired)

- Lifelong (hereditary)
Acquired Erythrocytosis

- Polycythemia vera
  - JAK2 V617F
  - Other JAK2 mutations including exon 12
- Cardiac/pulmonary dysfunction
  - Congenital heart disease
  - Hypoventilation – sleep apnea/chronic lung disease
  - Smoking/chronic CO exposure
- Renal dysfunction
  - Renal artery stenosis, ESRD
  - Hydronephrosis, renal cysts
- Tumor-associated
  - Renal CA, hemangioblastoma, hepatoma
  - Paraganglioma, pheochromocytoma, somatostatinoma
- Androgen/Epo/transfusion abuse
- High altitude
Hereditary Mutations

- High oxygen affinity hemoglobin variants
- Methemoglobin Reductase/Hb M
- Bisphosphoglycerate mutase deficiency
- Epo receptor mutations
- Mutations in the oxygen sensing pathway
  - HIF$\alpha$ mutations
  - VHL mutations
  - PHD2 mutations
High Oxygen Affinity Hb variants

• Typically beta chain variants cause clinical symptoms.
• May be electrophoretically silent (neutral charge substitution).
• Must have high index of suspicion.
• Oxygen dissociation curve is almost always left shifted (p50 in mid teens).
Hb San Diego

HPLC

Capillary electrophoresis
Hereditary Mutations

- High oxygen affinity Hemoglobin variants
- Methemoglobin Reductase/Hb M
- Bisphosphoglycerate mutase deficiency
- Epo receptor mutations
- Mutations in the oxygen sensing pathway
  - HIFα mutations
  - VHL mutations
  - PHD2 mutations
Methemoglobin Reductase/Hb M

- Clinical picture is typically dominated by the cyanosis.
- Erythrocytosis is secondary.
- Met Reductase easy to diagnose by enzymatic methods.
- Hb M’s have unique patterns on HPLC.

Hb M-Saskatoon
Hereditary Mutations

- High oxygen affinity Hemoglobin variants
- Methemoglobin Reductase/Hb M
- Bisphosphoglycerate mutase deficiency
- Epo receptor mutations
- Mutations in the oxygen sensing pathway
  - HIFα mutations
  - VHL mutations
  - PHD2 mutations
Bisphosphoglycerate mutase deficiency

- Markedly decreased 2,3 BPG
- Oxygen dissociation curve left shifted
- Extremely rare
- Autosomal recessive

Glycolytic Pathway
Erythrocytosis Due to Bisphosphoglycerate Mutase Deficiency With Concurrent Glucose-6-Phosphate Dehydrogenase (G-6-PD) Deficiency

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3 Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California
Hereditary Mutations

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- Epo receptor mutations
- Mutations in the oxygen sensing pathway
  - HIFα mutations
  - VHL mutations
  - PHD2 mutations
Epo and Receptor

A. EpoR and JAK2 monomers and tyrosine (Y) residues

B. EpoR dimerization, JAK2 activation and effector signaling

C. Truncation of EpoR due to mutations

Nucleus

Gene transcription for cell proliferation, anti-apoptosis, etc.
EpoR Mutations

• Majority are truncation mutations (exon 8).
• Loss of cytoplasmic, intracellular domain.
• Loss of negative regulatory region.
• Epo levels are low.
• The only cause of a primary congenital erythocytosis.
Hereditary Mutations

- High oxygen affinity Hemoglobin variants
- Methemoglobin Reductase/Hb M
- Bisphosphoglycerate mutase deficiency
- Epo receptor mutations
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  - HIFα mutations
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O$_2$ Sensing Pathway

Hypoxia-inducible Factor
Prolyl Hydroxylase Domain
von Hippel Lindau

Alpha subunit half-life
↓ normal O$_2$
↑ hypoxia

Hypoxia-inducible Factor (HIFα)
Prolyl hydroxylation by PHD

HIFα - pVHL complex formation and ubiquitin-dependent proteasomal degradation of HIFα

Hypoxic preservation of HIFα

Increased transcription of HIF target genes
- erythropoiesis
- angiogenesis
- glucose metabolism

Nucleus
HIFα - pVHL complex and ubiquitin-depende degradation of HIFα.

Prolyl hydroxylation of HIFα by PHD

Prolines 405 and 531
HIF2α \((EPAS1)\) Mutations

- Autosomal dominant inheritance
- Epo levels are normal or high.
- Missense mutations in exon 12 (proline 531)
- The amino acid substitution interferes with PHD2 hydroxylation.
- Mutations have recently been reported in exon 9 (proline 405).
PHD2 (EGLN1) Mutations

- Autosomal dominant inheritance
- Epo Levels are usually normal.
- Several different types of mutations, but common theme is disruption of the PHD2 catalytic domain.
VHL Mutations

- Autosomal recessive inheritance
- c.598 C>T, p.R200W (Arg to Trp)
- Chuvash polycythemia
- Markedly Elevated Epo levels
- Other mutations have now been found.
Methods

- DNA (Sanger) Sequencing:
  - *EPOR* - exon 8
  - *PHD2/EGLN1* - exons 1-5
  - *HIF2α/EPAS1* - exon 12, (exon 9?)
  - *VHL* - exons 1-3
Peripheral blood JAK2 V617F and serum Epo

- **JAK2 V617F positive**
  - P Vera
  - EPOR
    - Lab error
    - High O₂ affinity high
    - 2,3-BPG deficiency
    - Methemoglobinemia
  - p50
    - NL
    - Acquired erythrocytosis
      - PHD2
      - HIF2α
      - VHL
- **Epo low**
  - JAK2 exon 12
  - Lifelong erythrocytosis
    - p50
  - Acquired erythrocytosis
- **JAK2 V617F negative**
  - Epo normal/high
  - NL
Case 1

• 19-year-old American man – African heritage
• Erythrocytosis
• Headaches, chest pain “all his life”
• 3 cigarettes/day
• PE unremarkable
• Father and other family members with headaches and ruddy faces
• Cardiac W/U: R bundle branch block
### Case 1

<table>
<thead>
<tr>
<th>CBC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>20.6 g/dL</td>
<td></td>
</tr>
<tr>
<td>Hct</td>
<td>61.6%</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>$8.97 \times 10^{12}$/L</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>68.7 fL</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>$5.30 \times 10^9$/L</td>
<td></td>
</tr>
<tr>
<td>PLT</td>
<td>$252 \times 10^9$/L</td>
<td></td>
</tr>
</tbody>
</table>
Case 1

JAK2 V617F: Negative

Serum Epo: 21.4 mIU/mL (NL 3.7-31.5)
Peripheral blood JAK2 V617F and serum Epo

- JAK2 V617F positive
  - P Vera
  - EPOR (Lab error)
  - High O₂ affinity high
  - 2,3-BPG deficiency
  - Methemoglobinemia

- Epo low
  - JAK2 exon 12
  - p50
  - PHD2
  - HIF2α
  - VHL

- JAK2 V617F negative
  - Epo normal/high
  - Lifelong erythrocytosis
  - Acquired erythrocytosis
  - NL

- ±

Patnaik and Tefferi: Leukemia, 2009
Oxygen Dissociation Curve

\[ p_{50} = 12 \text{ mm Hg} \]
Case 1
Capillary Electrophoresis
Case 1

HPLC

Hb Lepore
# Case 1

<table>
<thead>
<tr>
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<th></th>
</tr>
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<tr>
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<td>20.6 g/dL</td>
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<tr>
<td>Hct</td>
<td>61.6%</td>
</tr>
<tr>
<td>RBC</td>
<td>$8.97 \times 10^{12}$/L</td>
</tr>
<tr>
<td>MCV</td>
<td>68.7 fL</td>
</tr>
<tr>
<td>WBC</td>
<td>$5.30 \times 10^9$/L</td>
</tr>
<tr>
<td>PLT</td>
<td>$252 \times 10^9$/L</td>
</tr>
</tbody>
</table>
• The presence of Hb Lepore explains the microcytosis but not the prominent erythrocytosis.

• The abnormal p50 indicates that further workup is needed to exclude a silent high $O_2$ affinity Hb variant.

• DNA sequencing and mass spectrometry identified Hb Johnstown ($\beta^{109}$ Val→Leu).
Case 2

- 12-year-old American girl – Spanish heritage
- Noted on 10 mo well baby exam
- Hgb 15-20 g/dL (NL 12.2-14.8)
- Asymptomatic “feels great”
- No known family history
- No cardiac or pulmonary disorders
### Case 2

<table>
<thead>
<tr>
<th>CBC</th>
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<tbody>
<tr>
<td><strong>Hgb</strong></td>
<td>19.4 g/dL</td>
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<tr>
<td><strong>Hct</strong></td>
<td>58.2%</td>
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<tr>
<td><strong>RBC</strong></td>
<td>$6.50 \times 10^{12}/L$</td>
</tr>
<tr>
<td><strong>MCV</strong></td>
<td>89.5 fL</td>
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<tr>
<td><strong>WBC</strong></td>
<td>$4.20 \times 10^{9}/L$</td>
</tr>
<tr>
<td><strong>PLT</strong></td>
<td>$310 \times 10^{9}/L$</td>
</tr>
</tbody>
</table>
Case 2

**JAK2 V617F:** Negative

**Serum Epo:**  14.4 mIU/mL  (NL 9-28)
Peripheral blood JAK2 V617F and serum Epo

- JAK2 V617F positive
  - P Vera
  - EPOR
    - Lab error
      - High O₂ affinity high
      - 2,3-BPG deficiency
      - Methemoglobinemia

- Epo low
  - JAK2 exon 12
    - p50
      - ↓
        - NL
      - PHD2
        - HIF2α
        - VHL
      - Lifelong erythrocytosis
      - Acquired erythrocytosis

- JAK2 V617F negative
  - Epo normal/high
Oxygen Dissociation Curve

Oxyhemoglobin (%)

p O2

p50 = 27 mm Hg
Case 2

HPLC

CE
Case 2

**PHD2:** Normal

**HIF2α:** P534R, 1601 C → G

**VHL:** Normal
## Summary (ISLH, May 2014)

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Hb</th>
<th>RBC</th>
<th>p50</th>
<th>Epo</th>
<th>Gene</th>
<th>Mutation</th>
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</thead>
<tbody>
<tr>
<td>11</td>
<td>M</td>
<td>19.1</td>
<td>6.70</td>
<td>28.5</td>
<td>NP</td>
<td>EPOR</td>
<td>c.1161_1186del;p.P388HfsTer3</td>
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<tr>
<td>11</td>
<td>F</td>
<td>18.1</td>
<td>5.86</td>
<td>NP</td>
<td>1.6</td>
<td>EPOR</td>
<td>c.1362C&gt;A;p.W454Ter</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>17.1</td>
<td>5.64</td>
<td>23</td>
<td>5.0</td>
<td>PHD2</td>
<td>c.494delC;p.P165QfsTer9</td>
</tr>
<tr>
<td>66</td>
<td>M</td>
<td>18.4</td>
<td>6.20</td>
<td>26</td>
<td>NP</td>
<td>PHD2</td>
<td>c.1109G&gt;A;p.R370H</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>19.2</td>
<td>6.30</td>
<td>28</td>
<td>10.8</td>
<td>PHD2</td>
<td>c.1153G&gt;A;p.A385T</td>
</tr>
<tr>
<td>36</td>
<td>M</td>
<td>17.0*</td>
<td>5.60</td>
<td>29</td>
<td>9.1</td>
<td>PHD2</td>
<td>c.867C&gt;G;p.S289R</td>
</tr>
<tr>
<td>33</td>
<td>M</td>
<td>17.9</td>
<td>6.20</td>
<td>26</td>
<td>NP</td>
<td>PHD2</td>
<td>c.1167G&gt;T;p.W389C</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>19.4</td>
<td>6.50</td>
<td>27</td>
<td>14.4</td>
<td>HIF2A</td>
<td>c.1601C&gt;G;p.P534R</td>
</tr>
<tr>
<td>74</td>
<td>M</td>
<td>18.6</td>
<td>6.36</td>
<td>28</td>
<td>NP</td>
<td>HIF2A</td>
<td>c.1631C&gt;G;p.P544R</td>
</tr>
</tbody>
</table>

Hb=g/dL, RBC=x10^{12}/L, nl range: p50=24-30 mmHg, Epo=2.6-18.5 mIU/mL, NP=not performed, *recently phlebotomized
EPOR mutations

- c.1161_1186del;p.P388HfsTer3
- c.1362C>A;p.W454Ter

- Both of these mutations result in premature truncation of the Epo protein.
- Results in loss of the cytoplasmic domain and deletion of negative regulatory domain.
PHD2 Mutations (1)

- c.1109G>A; p.R370H
- c.1153G>A; p.A385T
- c.1167G>T; p.W389C

- Three highly conserved amino acids across species.
- All are predicted to be deleterious by in silico analysis or near a previously reported mutation (Percy, et al, Blood 2007, 110:2193).
PHD2 Mutations (2)

• c.494delC;p.P165QfsTer9
  • Causes a frameshift with a premature stop codon in exon 1.
  • Loss of the entire catalytic domain of the PHD2 protein.
PHD2 Mutations (3)

- c.867C>G; p.S289R

- Occurs in the latter half of exon 1.

- In silico analysis strongly predicts the creation of a new donor splice site.

- If used, would result in a premature stop codon in exon 2 at codon 324.

- Results in loss of a large portion of the PHD2 catalytic domain.
HIF2α mutations

- c.1601C>G; p.P534R
- c.1631C>G; p.P544R
- Both are predicted to interfere with hydroxylation of Proline 531.
- VHL cannot bind.
- HIF2α is not degraded.
- Similar to other reported mutations.
Summary

• Many of the causes of erythrocytosis have been discovered.

• In cases of secondary erythrocytosis, an algorithmic approach can be used to direct appropriate testing/

• This allow effective utilization of testing, and reduces unnecessary costs.
Summary (cont)

• But there is still a long way to go.
• 70 patients, cause of erythrocytosis found in only half of cases.
Questions & Discussion