Hemoglobin Variant Reporting in Newborn Screening

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Hemoglobinopathies Overview

- Hemoglobin disorders include sickle cell disease, alpha thalassemia, and beta thalassemia
- Prevalence of Hgb carriers between 5% and 20%
  - African American – 10% with sickle cell trait
- Newborn Screening began testing in the 1970s
- Utah began screening in 2001
Utah Hemoglobin Newborn Screening

- First screen – isoelectric focusing
- Second screen – isoelectric focusing and HPLC if IEF abnormal
- Utah reports FAU – Carriers of unidentified variants
  - Recommend complete blood count (CBC) and hemoglobin evaluation using HPLC between 6-9 months of age
Quality Improvement Study: Cases in 2017 identified with FAU

- 227 requests
- 120 responses
- 101 results
- 12 abnormal
Results

- 12 abnormal
  - Hb N-Baltimore
  - Hb I
  - Hb J-Baltimore
  - Hb J-Toronto
  - Hb J-Broussaid
  - Hb Manitoba
  - Hb Other (44%) – no further testing completed to identify
  - Hb Other (45.4%) – no further testing completed to identify
  - Hb Other (23.1%) – no further testing completed to identify
  - CBC – microcytosis referral to Hematology
  - 2 cases: persistent of fetal hemoglobin
Hemoglobin I

1\textsuperscript{st} NBS IEF – FU(Fast)AU(Fast)
2\textsuperscript{nd} NBS IEF – FU(Fast)AU(Fast)
HPLC - FA

Confirmatory/Diagnostic Testing Results

<table>
<thead>
<tr>
<th>CBC</th>
<th>Results</th>
<th>Ref range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>34.8</td>
<td>33-39</td>
</tr>
<tr>
<td>MCV</td>
<td>79.8</td>
<td>70-86</td>
</tr>
<tr>
<td>MCH</td>
<td>26.6</td>
<td>23-31</td>
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<tr>
<td>Hemoglobin A</td>
<td>75.8% (L)</td>
<td>86.1-97.2</td>
</tr>
<tr>
<td>Hemoglobin A2</td>
<td>2.3%</td>
<td>1.9-3.5</td>
</tr>
<tr>
<td>Hemoglobin F</td>
<td>1.4%</td>
<td>0.6-11.6</td>
</tr>
<tr>
<td>Hemoglobin Other</td>
<td>20.5% (H)</td>
<td>0-0</td>
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DNA results – \textit{HBA2}: c.49A>G; Lys16Glu – likely benign
Hemoglobin N-Baltimore

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2\textsuperscript{nd} NBS IEF – FU(Fast)A

HPLC - FA

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DNA results – \textit{HBB}: c.286A>G; p.Lys96Glu – likely benign
Conclusion

• Most Unidentified Hemoglobin Variants are benign, **however not all**
  • Associated with aberrant HbA1c values affecting diagnosis/treatment of diabetes
  • Hemolytic anemia/splenomegaly – Ex. Hb Brevedent
  • Hemolytic mild chronic anemia – Ex. Hb I-Toulouse

• Hb “Other” persistent at the 9 month period
Is HPLC a suitable primary screening method?
Considerations

• DNA analysis to confirm variant to determine clinic symptoms

• New updates to Utah NBS process
  • IEF as first screen
  • HPLC as second screen for abnormal results

• Alternatives
  • Repeat IEF on second screen if results are divergent
  • Sequencing

• Report obvious FAU bands with same recommendations