Detecting and Reporting Alpha Thalassemia In Newborns
T. Davis, C. Moore, L. Nayak, M.C. Dorley, M. del Pilar Aguinaga, M. Chan, J. Ubaike, C. Yusuf

- Alpha Thalassemia Screening Status in the US
- Clinical Presentation of Alpha Thalassemia
- Hemoglobin Bart’s Percentages
- Hemoglobin Constant Spring
- Hemoglobins on the RUSP
Alpha Thalassemia Screening in the US

All newborn screening programs are detecting alpha thalassemia.

How many programs are reporting it?

How is it being reported?  Hb H Disease, silent carrier, alpha thal trait, Hb Constant Spring, alpha thal major, Hb Bart’s %

As a workgroup, how can we improve the status of alpha thalassemia reporting in the US?
Alpha Thalassemia Survey Data

Response Rate (83%)
- Number of Programs that did not Respond = 9
- Number of Programs that Responded = 44

Number of States that Report Alpha Thalassemia (Hb Bart's)
- Number of Programs that Report Alpha Thalassemia (Hb Bart's) = 42
- Number of Programs that Do Not Report Alpha Thalassemia (Hb Bart's) = 2

95%
Alpha Thalassemia Survey Data

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Programs (N=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEF/HPLC</td>
<td>18</td>
</tr>
<tr>
<td>IEF Only</td>
<td>9</td>
</tr>
<tr>
<td>HPLC/IEF</td>
<td>8</td>
</tr>
<tr>
<td>HPLC Only</td>
<td>7</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
</tr>
</tbody>
</table>

Two Methods = 26
One Method = 16
# Alpha Thalassemia Survey Data

## Hb Bart’s Percentages Used

<table>
<thead>
<tr>
<th>Classification</th>
<th>Average</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent Carrier (N=3)</td>
<td>7</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Alpha Thal Trait (N=10)</td>
<td>9</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Hb H Disease (N=14)</td>
<td>22</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>Alpha Thal Major (N=9)</td>
<td>64</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Other (N=9)</td>
<td>15</td>
<td>3</td>
<td>25</td>
</tr>
</tbody>
</table>
Two-Part Webinar Series: Alpha Thalassemia

Part 1: Alpha Thalassemia: Clinical Aspects
Wednesday, May 31, 2017 | 2:00–3:00 pm ET

SPEAKERS

- Tim Davis, Chair, APHL Hemoglobinopathy Laboratory Workgroup, Microbiologist 3, State Department of Health, WA
- Maria del Pilar Aguinaga, PhD, DLM (ASCP), Co-Director, Sickle Cell Center, Meharry Medical College
- M. A. Bender, MD, Attending physician, Seattle Children’s Hospital, Director of Odessa Brown Comprehensive Sickle Cell Clinic

Part 2: Alpha Thalassemia Newborn Screening in the United States
Thursday, June 29, 2017 | 2:00–3:00 pm ET

SPEAKERS

- Tim Davis, Chair, APHL Hemoglobinopathy Laboratory Workgroup, Microbiologist 3, State Department of Health, WA
- Joseph Ubaike, Supervising Microbiologist, NBS Section, Department of Health, CT
- Ming Chan, PhD, Laboratory Director (retired), Department of Health, FL
- Christine Dorley, MSP, MT(ASCP), Newborn Screening Division Manager, Department of Health Laboratory Services, TN
<table>
<thead>
<tr>
<th>Alpha Genes Affected</th>
<th>Classification</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silent Carrier</td>
<td>Benign</td>
</tr>
<tr>
<td>2</td>
<td>Alpha Thal Trait</td>
<td>Mild anemia. Microcytosis.</td>
</tr>
<tr>
<td>3</td>
<td>Deletional Hb H Disease</td>
<td>Moderate anemia and microcytosis. Transfusion rarely required.</td>
</tr>
<tr>
<td>3</td>
<td>Non-Deletional Hb H Disease</td>
<td>Severe microcytic anemia and microcytosis with delayed growth. 20% require transfusion.</td>
</tr>
<tr>
<td>4</td>
<td>Alpha Thal Major</td>
<td>Fetal demise and high risk to mother.</td>
</tr>
</tbody>
</table>
Normal Newborn (Birth to approximately 3 weeks of age)

Fetal Hemoglobin (F) = $\frac{\alpha \gamma}{\gamma \alpha}$

Adult Hemoglobin (A) = $\frac{\alpha \beta}{\beta \alpha}$

The ratio of alphas to beta-like chains should be 1:1!

Chromosome 16

Chromosome 11
13.3% Hb Bart’s

Chromosome 11

Alpha Thalassemia Trait

Fetal Hemoglobin (F) = \( \frac{\alpha \gamma}{\gamma \alpha} \)

Adult Hemoglobin (A) = \( \frac{\alpha \beta}{\beta \alpha} \)

Hb Bart’s (B) = \( \frac{\gamma \gamma}{\gamma \gamma} \)

Chromosome 16

Chromosome 11
Hb Bart’s

- Hb Bart’s is a tetramer of gamma globin chains, along with acetylated and glycated forms.
- Bart’s is formed when there is a reduction in functional alpha globin chains.
- On isoelectric focusing gels, Bart’s presents as 2 to 3 bands.
- On cation exchange HPLC, Bart’s presents as 1 to 3 peaks.
- Discovered at St. Bartholomew’s Hospital in London.
- High levels of Bart’s are more common in Asian populations.
Deletional Hb H Disease

Fetal Hemoglobin (F) = \( \frac{\alpha_y}{\gamma_x} \)

Adult Hemoglobin (A) = \( \frac{\alpha_y}{\beta_x} \)

Hb Bart’s (B) = \( \frac{\gamma_y}{\gamma_y} \)  \( \rightarrow \) Hb H = \( \frac{\beta_y}{\beta_y} \)

Chromosome 16

Chromosome 11

Hb Bart’s = 34.3%
Reporting Hb Bart’s from HPLC

<table>
<thead>
<tr>
<th>Alpha Thalassemia Severity</th>
<th>Genes Affected</th>
<th>BioRad vnbs HPLC</th>
<th>Extended Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA Normal</td>
<td>0</td>
<td>0-6.4%</td>
<td>&lt; 4.9%</td>
</tr>
<tr>
<td>Silent Carrier</td>
<td>1</td>
<td>6.5-9.1%</td>
<td>4.9-6.4%</td>
</tr>
<tr>
<td>Alpha Thal Trait</td>
<td>2</td>
<td>9.2-24.9%</td>
<td>6.5-17.9%</td>
</tr>
<tr>
<td>Hb H Disease</td>
<td>3</td>
<td>&gt;=25%</td>
<td>&gt;= 18%</td>
</tr>
<tr>
<td>Alpha Thal Major</td>
<td>4</td>
<td>100%</td>
<td>100%</td>
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</tbody>
</table>

Factors that impact %

- Sample Matrix
- Resolution
- Software Integration
## HPLC Bart’s Percentages

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- **6.4% FA Normal**
- **6.7% Silent Carrier**
- **17.7% Alpha Thal Trait**
- **41.3% Hb H Disease**
HPLC Bart’s Percentages

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<td>&gt;=25%</td>
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<tr>
<td>Alpha Thal Major</td>
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<td>100%</td>
</tr>
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- 6.4% FA Normal
- 6.7% Silent Carrier
- 17.7% Alpha Thal Trait
- 41.3% Hb H Disease

Silent Carrier: 6.7%
Alpha Thal Trait: 17.7%
## HPLC Bart’s Percentages

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### Alpha Thalassemia Severity Images:
- **6.4% FA Normal**
- **6.7% Silent Carrier**
- **17.7% Alpha Thal Trait**
- **41.3% Hb H Disease**

### Alpha Thal Trait Image:
- **17.7%**

### Hb H Disease Image:
- **41.3%**
Hb Bart’s Percentages (N=4000) May 2016 through April 2017, Washington State

- 25.0%
- 9.2%
Hb Bart’s (N=4000) May 2016 through April 2017, Washington State

FREQUENCY

BART'S %

9.2%  25.0%

Alpha Thal Trait

Hb H
Hb Constant Spring

• Most common of the non-deletional alpha thalassemias.

• Hb Constant Spring is a point mutation on the alpha 2 gene at stop codon 142 TAA → CAA (Term → Glutamine). Causes the alpha chain to be extended to 172 amino acids.

• Long peptide chain is unstable and there is a very low percentage of protein present.

• Detectable in very low levels on IEF and not on HPLC, except with whole blood.

• Seen more at early age on IEF. 24 hours vs 7 days.
HS-40 (Super Enhancer)

Normal

Deletional Hb H

Hb H/Constant Spring

\[\alpha_1 \alpha_2\]

\[\alpha_1 \alpha_2\]

\[\alpha_2^{CS}\]
Hb E trait and Hb H/Constant Spring

Chromosome 11

\[ \text{Hb E} = \alpha^E \beta \gamma \gamma \alpha \]

Chromosome 16

\[ \text{Hb Bart's} = 40.5\% \]

Fetal Hemoglobin (F) = \( \alpha^F \gamma \alpha \)

Adult Hemoglobin (A) = \( \alpha^A \beta \gamma \gamma \alpha \)

\[ \text{Hb Constant Spring} = \alpha^{CS} \gamma \alpha + \alpha^{CS} \beta \gamma \gamma \alpha \]

\[ \text{Hb Bart's (B)} = \gamma \gamma \gamma \gamma \]

\[ \text{Hb Constant Spring} = \gamma^A \gamma \gamma \gamma^A \gamma \]

\[ \text{Hb Bart's} = 40.5\% \]
Alpha Thalassemia Silent Carrier/Hb Constant Spring

Hb Constant Spring bands

Hb Bart’s

Hb Bart’s Peak Area = 7.6%
Human Alpha-2 Chain Sequence, 521 nucleotides (AF097635.1)

<table>
<thead>
<tr>
<th>wild type probe (VIC)</th>
<th>mutant type probe (FAM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb 0g</td>
<td>CTCAGCTTAACGGTATT</td>
</tr>
<tr>
<td>Hb 0g (inv)</td>
<td>AATACGTTAACGCTGAGAG</td>
</tr>
<tr>
<td>Forward Primer</td>
<td>CACCGTGCTGACCTCAA</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>CCATCGGGCAGGAAGGAA</td>
</tr>
</tbody>
</table>

Optics Graph

HPLC or IEF → Hb H

Hb H + Hb CS → Hb H
10 Years, 2005 - 2014

<table>
<thead>
<tr>
<th>State</th>
<th>Hb H Detected per 100,000 births</th>
<th>Hb H/Constant Spring per 100,000 births</th>
<th>% Asian Population 2010 Census</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washington</td>
<td>6.3</td>
<td>0.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Tennessee</td>
<td>2.7</td>
<td>N/A</td>
<td>1.4</td>
</tr>
<tr>
<td>Texas</td>
<td>4.8</td>
<td>N/A</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Hemoglobinopathies on the RUSP

Core Conditions

Secondary Conditions (Various Other Hemoglobinopathies)

- Beta Zero Thalassemia
- Sickle Beta Plus Thalassemia
- Hemoglobin E Disease
- Hemoglobin SV Disease
- Hemoglobin SE Disease
- Hemoglobin C Disease
- Hemoglobin D Disease
- Hemoglobin SD Disease
- Alpha Thalassemia Variants

Hemoglobin H Disease

Database of Human Hemoglobin Variants and Thalassemias
- Beta Gene Entries = 895
- Alpha 1 Gene Entries = 350
- Alpha 2 Gene Entries = 431
- Thalassemia Entries = 487

- Hemoglobin SE Disease
- Hemoglobin SD Disease
- Beta Thalassemia Variants
- Unstable Hemoglobin Variants
- Beta Gene Entries = 895
- Alpha 1 Gene Entries = 350
- Alpha 2 Gene Entries = 431
- Thalassemia Entries = 487

- Hemoglobin E Beta Zero Thalassemia
- Variants with High Oxygen Affinity
- Variants with Low Oxygen Affinity
- Beta Thalassemia Variants
- Unstable Hemoglobin Variants
- Alpha Thalassemia Variants
Sickling Disorders

Thalassemia Syndromes (α and β)

Unstable Hemoglobin Variants

Altered Oxygen Affinity Variants