EXTERNAL QUALITY ASSESSMENT FOR HAEMOGLOBIN A$_2$

Dr Barbara Wild
The importance of Hb A₂ measurement

- Accurate and reliable measurement of Hb A₂ is essential for the diagnosis of beta thalassaemia trait
  
  *Small difference (if any) between normal & abnormal levels*

- Antenatal women should be screened for beta thalassaemia trait
  
  *Carriers: recommend partner testing prediction of genetic risk*

- Failure to detect condition *may* result in newborn with a medically significant condition
UKNEQAS: UK National External Quality Assessment Scheme

- All laboratories undertaking antenatal screening in England must participate in EQA scheme
  
  Required by: Accreditation bodies
  : National Screening Programme

- UKNEQAS’ Abnormal Haemoglobins Scheme
  issues 6 surveys per year, 3 samples per survey

- Specimens are accompanied by information on FBC, age, gender, ethnic group and clinical condition
UKNEQAS: UK National External Quality Assessment Scheme

- Participants are required to give analytical results and an interpretation.

- With increase in technologies:
  Results of Hb A$_2$ measurement related to methodology used.

*Identified differences in values obtained from different technologies and/or kits.*
National Sickle & Thalassaemia Screening Programme

• Established to provide a linked screening programme for antenatal women and newborn
• Universal screening
• Established laboratory standards
• Standardised reporting formats
• Standardised methodology (newborn)

• Decision algorithm (antenatal)
Review of Hb A$_2$ Data 2006-2008

- Review historic data for Hb A$_2$
  - Trends in Hb A$_2$ quantitation
  - Methodology changes
  - Differences in interpretation

- Develop and evaluate more sensitive indicators for monitoring performance

- Gather more information to evaluate the 3.5% cut-off for beta thalassaemia carrier status
Methodology 2000-2008

- Methodology for Hb A₂ measurement:
  - Changes in methods used (e.g. column chromatography, electrophoresis, HPLC)
  - Changes in analysers used (where HPLC analyser group is given)

Note: only UK data will be presented in this talk
UK Hb A₂ analysis methods (2000-2008)
Change in CV for Hb A\textsubscript{2} measurement 2000-2008
Distribution Curves

• Plotting the median Hb A₂ of the data set ± 3SDs

• Plotted all methods data and the data of the six largest method/analyser groups for all surveys from 2006 to mid-2008 to look for trends

• Later analysed the results of borderline Hb A₂ sample 0902AH1 (all methods trimmed mean = 3.7%)
Normal sample: Hb A2 2.6%
Beta thal trait sample: Hb A$_2$ 4.8%
Borderline sample: Hb A₂ 3.7%
Hb A₂ Assessment

• Hb A₂ assessment codes:
  - low, normal, high or uncertain

• Results ‘consensus’
  • if >85% of participants gave that answer

‘outwith consensus’ groups since 2006:
- Outwith consensus Hb A₂ result
- Transcription or assessment error
- Varying normal ranges between participants
Hb A$_2$ Assessment

- Outwith consensus result: 26.6%
- Varying normal range: 36.7%
- Transcription/assessment error: 5.1%
- Combination of reasons: 36.1%
Hb A₂ Reference Ranges
Beta thal trait specimens 2006 - 2008

‘No evidence of beta thalassaemia trait’

30 participants

- Out with consensus HbA2 result
- Transcription/interpretation error
- Combination of reasons including varying normal range
0604AH4:  
- 165 UK laboratories  
- All methods trimmed mean = 3.7%  
- Hb A₂ 3.5% or greater:  
  - 16 did not give an interpretation of beta thalassaemia carrier  
  - 34 did not categorise the Hb A₂ as ‘high’  
- 33 gave an Hb A₂ value of 3.4% or less

0902AH1:  
- 172 UK laboratories  
- All methods trimmed mean = 3.7%  
- Hb A₂ 3.5% or greater:  
  - 22 did not give an interpretation of beta thalassaemia carrier  
  - 37 did not categorise the Hb A₂ as ‘high’  
- 26 gave an Hb A₂ value of 3.4% or less
Table 2. Hb A₂ results for CPD-A1 blood (AH0501EX2)

<table>
<thead>
<tr>
<th>Method</th>
<th>Median HbA₂ (%)</th>
<th>Est SD</th>
<th>CV%</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Chromatography (All)</td>
<td>2.70</td>
<td>0.22</td>
<td>8.2</td>
<td>61</td>
<td>2.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Electrophoresis &amp; elution (All)</td>
<td>2.65</td>
<td>0.13</td>
<td>4.9</td>
<td>6</td>
<td>1.9</td>
<td>3.1</td>
</tr>
<tr>
<td>HPLC (All)</td>
<td>2.50</td>
<td>0.30</td>
<td>11.9</td>
<td>103</td>
<td>1.8</td>
<td>3.1</td>
</tr>
<tr>
<td>BioRad (All)</td>
<td>2.60</td>
<td>0.15</td>
<td>5.8</td>
<td>56</td>
<td>1.9</td>
<td>3.1</td>
</tr>
<tr>
<td>BioRad Variant Classic</td>
<td>2.50</td>
<td>0.07</td>
<td>3.0</td>
<td>14</td>
<td>1.9</td>
<td>2.7</td>
</tr>
<tr>
<td>BioRad Variant II Dual</td>
<td>2.30</td>
<td>0.09</td>
<td>4.0</td>
<td>8</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>BioRad Variant II Beta</td>
<td>2.60</td>
<td>0.15</td>
<td>5.7</td>
<td>32</td>
<td>2.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Menarini HA-8160</td>
<td>2.20</td>
<td>0.19</td>
<td>8.1</td>
<td>11</td>
<td>2.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Menarini Hb Gold</td>
<td>2.20</td>
<td>0.22</td>
<td>10.1</td>
<td>17</td>
<td>1.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Primus Ultra 385</td>
<td>2.20</td>
<td>~</td>
<td>~</td>
<td>5</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>TOSOH G7</td>
<td>2.70</td>
<td>0.37</td>
<td>13.7</td>
<td>11</td>
<td>2.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>

UKNEQAS
Current developments

• Instrument calibration-use of calibrant(s)

• Development of new Hb A₂ reference material

• Target value for performance scoring:
  • all methods mean
  • method-specific mean – current target
  • submethod-specific mean
Causes of ‘raised Hb A₂’

- Beta thalassaemia trait
- Unstable beta globin variants
- HIV infection - usually treatment related
- Metabolic disorder
- Other haemoglobinopathies
- Artefact, eg HbS adducts
Sickle cell trait

Normal FBC

Hb S% : 35-45

Hb A₂ may be raised

Consider omitting A₂ value from report
Hb Sβ⁺thalassaemia

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>9.0*</td>
<td>---</td>
<td>1.11</td>
<td>153554</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.7</td>
<td>2.11</td>
<td>11443</td>
</tr>
<tr>
<td>Ao</td>
<td>---</td>
<td>16.2</td>
<td>2.47</td>
<td>273375</td>
</tr>
<tr>
<td>A2</td>
<td>6.5*</td>
<td>---</td>
<td>3.62</td>
<td>99298</td>
</tr>
<tr>
<td>S-window</td>
<td>---</td>
<td>68.1</td>
<td>4.50</td>
<td>1149258</td>
</tr>
</tbody>
</table>

Total Area: 1686929

F Concentration = 9.0* %
A2 Concentration = 6.5* %

*Values outside of expected ranges

Analysis comments:
δ chain variant

Consider total Hb A₂

and

review red cell indices

Note:
also check for carry-over
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Origin</th>
<th>Usual mean Hb A₂ (%)</th>
<th>Usual mean MCH (pg)</th>
<th>Usual mean MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent β thalassaemia trait (normal MCV, MCH, and Hb A₂ %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−101 (C → T)</td>
<td>Mediterranean</td>
<td>3.3</td>
<td>28</td>
<td>85</td>
</tr>
<tr>
<td>−92 (C → T)</td>
<td>Mediterranean</td>
<td>3.5</td>
<td>28</td>
<td>82</td>
</tr>
<tr>
<td>IVSII-844 (C → G)</td>
<td>Mediterranean (Italian)</td>
<td>3.5</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>+33 C → G [64]</td>
<td>Mediterranean (Greek Cypriot)</td>
<td>3.0</td>
<td>29</td>
<td>86</td>
</tr>
<tr>
<td>+10 (−T) [65]</td>
<td>Mediterranean (Greek, one case)</td>
<td>2.6</td>
<td>32</td>
<td>97</td>
</tr>
<tr>
<td>+1480 C → G (termination codon +6 C → G)</td>
<td>Mediterranean (Greek)</td>
<td>2.7 [62]</td>
<td>28</td>
<td>88</td>
</tr>
<tr>
<td>Almost silent β thalassaemia trait (reduced MCV, MCH, normal Hb A₂ %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSII-6 (T → C)</td>
<td>Mediterranean</td>
<td>3.5</td>
<td>23</td>
<td>71</td>
</tr>
<tr>
<td>Codon 27 (G → T)</td>
<td>Mediterranean and Middle Eastern</td>
<td>2.1</td>
<td>25</td>
<td>71</td>
</tr>
<tr>
<td>(haemoglobin Knossos)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSII-5 (G → A) Corfu δβ⁺</td>
<td>Mediterranean</td>
<td>3.5</td>
<td>26</td>
<td>70</td>
</tr>
<tr>
<td>IVSII-128 (T → C)</td>
<td>Saudi</td>
<td>3.4</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>CAP +1 (A → C)</td>
<td>South Asian</td>
<td>1.6⁺</td>
<td>23.5⁺</td>
<td>76⁺</td>
</tr>
<tr>
<td>Mutation not linked to β globin gene cluster [43]</td>
<td>Italian</td>
<td>3.9</td>
<td>23.5</td>
<td>79</td>
</tr>
<tr>
<td>+22 G → A [66]</td>
<td>Turkish, Bulgarian</td>
<td>3.9</td>
<td>23.5</td>
<td>79</td>
</tr>
<tr>
<td>Indices typical of thalassaemia trait but Hb A₂ % normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β Thalassaemia caused by deletion of the locus control region</td>
<td>Various</td>
<td>Normal</td>
<td>Typical of β thalassaemia</td>
<td>Typical of β thalassaemia</td>
</tr>
<tr>
<td>γδβ Thalassaemia</td>
<td>Various</td>
<td>Normal</td>
<td>Typical of β thalassaemia</td>
<td>Typical of β thalassaemia</td>
</tr>
</tbody>
</table>
Risk assessment

The following conditions will be missed:

• **Silent or near silent beta thalassaemia carrier**

• Possible beta thalassaemia carrier obscured by severe iron deficiency

• Alpha zero thalassaemia occurring outside of the defined at-risk family origins

• Dominant haemoglobinopathies where the woman has no haemoglobinopathy

• Any significant variant not detected by HPLC
Borderline / normal Hb A₂ value and beta thalassaemia

Normal Hb A₂ thalassaemia

- Borderline / normal Hb A₂ (3.2% – 3.8%)
- Reduced red cell indices
  eg CAP+1 A>C

Silent beta thalassaemia

- Normal Hb A₂ (2.5% – 3.8%)
- Normal red cell indices
  eg -101C>T
Measurement of Hb A₂
ICSH recommendations ISLH Oct 2011

Previous ICSH recommendations written in 1978

- Hb A₂ is measured as a percentage of haemoglobin present relative to any other haemoglobin present – not an absolute value

- Therefore analytically important to measure the A₂ and any other fractions present – separation, resolution and integration crucial

- In the presence of an Hb A₂ variant, it is the total of the normal and abnormal Hb A₂ which is significant
ICSH recommendations ISLH Oct 2011

- Fraction separation by
  - Electrophoresis with elution or microcolumn chromatography
  - Quantification by spectrophotometry at 415nm

- HPLC

- Capillary Zone Electrophoresis

- Capillary Isoelectric Focusing

*DNA analysis is required for the characterization of beta thalassaemia mutations*
ICSH recommendations ISLH Oct 2011

• Measurement of the Hb A\(_2\) alone cannot absolutely confirm or exclude the carrier state as there may be little difference between A\(_2\) in normals and some beta thalassaemia carriers.

• Precision levels should be +/- 0.1% of the final answer (SD 0.05%).

• Common beta thalassaemia trait Hb A\(_2\) = 4.0 - 6.0%
• Beta thalassaemia trait overall usually Hb A\(_2\) = 3.5 - 7.0%
• Normal subjects usually Hb A\(_2\) = 2.2-3.3%
Considerations

- Use of different normal ranges –
  variation even within same instrument group

- Use of a universal cut-off
  Instrument bias – impact on borderline values

- Examination of chromatograms

- Varying causes of a raised Hb $A_2$
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Insights on the diagnosis of hemoglobin disorders

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