Immunochromatographic Strip Test for the Detection of Alpha Thalassaemia

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Why do we care about alpha thalassaemia?
Why do we test for alpha thalassaemia in Hong Kong?

• $\alpha^0$-thalassaemia trait is very common

• ($--^{SEA}$) in 4.5% of our Chinese population

• Rarely ($--^{THAI}$) and ($--^{FIL}$)
Why do we test for alpha thalassaemia in Hong Kong?

- $\alpha^0$-thalassaemia trait is very common
- $(-^{SEA})$ 4.5% (90% of $\alpha$ thalassaemia with microcytosis)
- $(-\alpha^{3.7})/(-\alpha^{4.2})$ 5.1%
- $\beta$ thalassaemia 3.1%
- Hb E 0.3%
How do we screen for alpha thalassaemia?

- MCV is very reliable
How do we test for alpha thalassaemia?

- By definition, a genetic disease is confirmed by genotyping
How do we test for alpha thalassaemia?

• In practice, in places where alpha thalassaemia is prevalent, phenotypic testing is performed for routine carrier detection.

• Genotyping is performed when there is reproductive implication.
How do we test for alpha thalassaemia?

- Supravital staining for $\beta_4$ (Hb H) inclusion bodies
Routine strategy for alpha thalassaemia diagnosis in Hong Kong

- MCV < 82 fL
  - Supravital staining
    - Haemoglobin H disease
    - α thalassaemia trait
  - Genotyping
How accurate is supravital staining for $\alpha^0$ mutations?

- Sensitivity as low as 47% in Western countries

- At QMH (05/2014 to 04/2016)
  - 242 cases showed occasional red cells containing Hb H inclusions
  - Majority expected to be (---SEA)
  - All unexplained microcytosis sent for genotyping
  - 2 additional (---SEA) cases found missed by Hb H testing
    $\Rightarrow$ Sensitivity for (---SEA) over 90%
How is this achieved?

• Read the whole slide for 10 min
Time spent on Hb H testing in one year

- On microscopy  = 10 min x 1620 cases = 270 hrs
  = 270 hrs / 40 hrs = 6.75 weeks
Time spent on Hb H testing in one year

- On microscopy  = 10 min x 1620 cases = 270 hrs
  = 270 hrs / 40 hrs = 6.75 weeks
Alternative methods to detect alpha thalassaemia

Hb H
Alternative methods to detect alpha thalassaemia

Hb Bart’s

Hb H
Alterative methods to detect alpha thalassaemia
### Published performance of immunochromatographic (IC) strip tests

<table>
<thead>
<tr>
<th>IC strip test for Hb Bart’s</th>
<th>Sensitivity for $\alpha^0$</th>
<th>Specificity for $\alpha^0$</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>C Tayapiwatana <em>et al</em> 2009</td>
<td>100 %</td>
<td>86 %</td>
<td>$\alpha^+, \beta$ thal, normal</td>
</tr>
<tr>
<td>P Prayalaw <em>et al</em> 2014</td>
<td>100 %</td>
<td>73 %</td>
<td>$\alpha^+, \beta$ thal</td>
</tr>
<tr>
<td>P Winichagoon <em>et al</em> 2015</td>
<td>97 %</td>
<td>87%</td>
<td>$\alpha^+, \beta$ thal, normal</td>
</tr>
<tr>
<td>C Bunkall <em>et al</em> 2016</td>
<td>100 %</td>
<td>62 %</td>
<td>$\alpha^+, \text{normal}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IC strip test for zeta chains</th>
<th>Sensitivity for $\alpha^0$</th>
<th>Specificity for $\alpha^0$</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>L Wen <em>et al</em> 2012</td>
<td>100 %</td>
<td>98 %</td>
<td>normal</td>
</tr>
<tr>
<td>W Jomouii <em>et al</em> 2017</td>
<td>100 %</td>
<td>85 %</td>
<td>$\alpha^+, \beta$ thal</td>
</tr>
</tbody>
</table>
QMH study on Hb Bart’s IC strip

• Determine whether the high diagnostic sensitivity for (--SEA) can be obtained

• Assess the diagnostic specificity and its potential impact on workflow and workload

• Compare the test performance with supravital staining

• Design an algorithm for cost-effective incorporation
Pre-launch evaluation study

- 01/2016 to 03/2016
- 101 selected cases tested based on phenotypes
  - Hb H inclusion-positive alpha thalassaemia trait and Hb H disease
  - Beta thalassaemia trait
  - Hb E heterozygote
  - No phenotypic Hb abnormalities
## Genotypes of 101 selected cases

<table>
<thead>
<tr>
<th>Count</th>
<th>Genotype Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>$\alpha^0$ heterozygote [44 (-^{SEA}); 1 (-^{THAI}); 1 (-^{FIL})]</td>
</tr>
<tr>
<td>6</td>
<td>$\alpha^+$ heterozygote [4 (-^{\alpha^{3.7}}); 2 (-^{\alpha^{4.2}})]</td>
</tr>
<tr>
<td>2</td>
<td>Hb H disease [(-^{SEA}/-^{\alpha^{3.7}})]</td>
</tr>
<tr>
<td>16</td>
<td>$\beta^0$ heterozygote</td>
</tr>
<tr>
<td>2</td>
<td>Hb E heterozygote</td>
</tr>
<tr>
<td>29</td>
<td>Normal subject</td>
</tr>
</tbody>
</table>
Immunochromatographic (IC) results
# Results by genotype group - α thalassaemia

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total case</th>
<th>Hb H inclusion +</th>
<th>Immunochromatography +</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₀ heterozygote</td>
<td>46</td>
<td>42</td>
<td>46 (4 weak +)</td>
</tr>
<tr>
<td>α⁺ heterozygote</td>
<td>6</td>
<td>0</td>
<td>1 (weak +)</td>
</tr>
<tr>
<td>Hb H disease</td>
<td>2</td>
<td>2 (numerous)</td>
<td>2</td>
</tr>
</tbody>
</table>

- All α₀ were detected (−SEA); (−THAI); (−FIL) by strip but not by staining
- Hb H disease were directly diagnosable by staining but not by strip
Results by genotype group - β thalassaemia

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total case</th>
<th>Hb H inclusion +</th>
<th>Immunochromatography +</th>
</tr>
</thead>
<tbody>
<tr>
<td>β₀ heterozygote</td>
<td>16</td>
<td>0</td>
<td>1 (weak +)</td>
</tr>
<tr>
<td>Hb E heterozygote</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- False positivity in β₀ heterozygote by strip but none by staining
## Results by genotype group - normal

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total case</th>
<th>Hb H inclusion +</th>
<th>Immunochromatography +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>29</td>
<td>0</td>
<td>1 (weak +)</td>
</tr>
</tbody>
</table>

- Rare false positivity by strip but none by staining
Interference by high Hb F

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Total case</th>
<th>Hb H inclusion +</th>
<th>Immunochromatography +</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Hb F</td>
<td>7</td>
<td>0 of 6</td>
<td>6 (1 weak +)</td>
</tr>
</tbody>
</table>

- Hb F ranges from 5.7% to 75%
  - Strip negative 5.7%
  - Strip positive 7.5 – 75%

- Presence of a small amount of Hb Bart’s in these cases
### Diagnostic performance for $\alpha^0$ mutations

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity for $\alpha^0$</th>
<th>Specificity for $\alpha^0$</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC strip</td>
<td>100% (some weak +)</td>
<td>94%</td>
<td>$\alpha^+, \beta$ thal, normal (all weak +)</td>
</tr>
<tr>
<td>Supravital staining</td>
<td>91%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>
Incorporation of IC strip in diagnostic algorithm

• Started 01/2017
Immunochromatographic strip for Hb Bart’s (Hb F <5%)
Immunochromatographic strip for Hb Bart’s (Hb F <5%)
Immunochromatographic strip for Hb Bart’s (Hb F <5%) → Scan supravital staining → positive → Hb H disease → α thalassaemia trait
Immunochromatographic strip for Hb Bart’s (Hb F <5%) 

Exam supravital staining 

Scan supravital staining 

α thalassaemia trait 

Hb H disease
Immunochromatographic strip for Hb Bart’s (Hb F <5%) 

- Exam supravital staining
  - weakly positive
  - positive

- Scan supravital staining
  - positive

- Hb H disease

- $\alpha$ thalassaemia trait
Immunochromatographic strip for Hb Bart’s (Hb F <5%)

Scan supravital staining

- positive
  - Exam supravital staining
    - weakly positive
      - Unexplained
      - Genotyping
    - positive
      - α thalassaemia trait
    - negative
      - Hb H disease

- positive
  - α thalassaemia trait
Immunochromatographic strip for Hb Bart’s (Hb F <5%)

Unexplained

Examination of supravital staining

- Positive
  - α thalassaemia trait
  - Unexplained
- Negative
  - Weakly positive
    - Hb H disease
    - Unexplained
- Negative
  - Unexplained

Genotyping
Post-launch extended evaluation

- Confirm its high sensitivity for $\alpha^0$ thalassaemia detection

- Determine its ability to detect $\alpha^0$ thalassaemia when there is co-existing $\beta$ thalassaemia

- Evaluate the specificity of weakly positive results
Confirm its high sensitivity for $\alpha^0$ thalassaemia detection

- 67 microcytosis cases - IC strip negative, Hb $A_2$ normal

- Alpha genotyping results:
  - 2 (--SEA) (1 Hb H inclusion -)
Determine its ability to detect $\alpha^0$ thalassaemia when there is co-existing $\beta$ thalassaemia

- 77 $\beta$ thalassaemia trait cases

- Alpha genotyping results:
  - 4 (--SEA) (3 Hb H inclusion -) (1 IC strip -) not overlap
Evaluate the specificity of weakly positive results

• 54 out of 390 (14%) IC strip tests gave a weakly positive result

• Alpha genotyping results:
  – 6 (--SEA) (2 Hb H inclusion -)
  – 9 (−α^{3.7})/(−α^{4.2})
  – 39 normal alpha genotype
Insights from the post-launch extended evaluation

- Both IC strip test and supravital staining has a very high sensitivity for $\alpha^0$ thalassaemia, but not at 100%
  - False negative cases largely do not overlap (only 1 of 7 cases negative by both)

- IC strip test can better detect $\alpha^0$ thalassaemia in co-existing $\beta$ thalassaemia when compared to supravital staining
  - Both tests together detect all 4 double heterozygous cases

- The rather frequent weak positivity seen in non-$\alpha^0$ thalassaemia subjects means that IC strip test has a lower specificity than supravital staining
An integrated approach for $\alpha^0$ thalassaemia detection

- Quick, simple and highly sensitive IC strip test as first line screen

- Highly specific supravital staining as a second line test to improve diagnostic accuracy

- Save manpower, maintain high sensitivity without losing specificity
Immunochromatographic strip for Hb Bart’s (Hb F <5%)

Unexplained

Exam supravital staining

Unexplained

Genotyping

Scan supravital staining

α thalassaemia trait

Hb H disease

α thalassaemia trait
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