HbA1c: An anaytical review

HbA1c: An accurate way in diagnosis and follow up of diabetic patients?

Dr. Stefaan Marivoet
18/01/2013
Stefaan Marivoet
Manager Clinical Affairs
Tosoh Europe
Diabetes

- Impairment of glucose regulation

- High glucose levels increase:
  - Heart disease
  - Stroke
  - Retinopathy
  - Kidney disease
  - Peripheral neuropathy
  - Death

- Tool:
  - Follow up treatment of diabetes
  - Diagnose diabetes

HbA1c
What is Haemoglobin A1c?

- Sugar (= glucose) in the blood attaches to the β chain of the haemoglobin molecule.

- Haemoglobin (= Hb) with glucose attached to it is called glycated haemoglobin (gHb).

- The higher the glucose level in the blood, the more haemoglobin that will be glycosylated.

- The longer an elevated glucose level in the blood, the more haemoglobin that will be glycosylated.

- Once it has become glycosylated, it stays that way for the rest of the life of the RBC that is ~ 120 days.

Conclusion:

- HbA1c measures the average blood glucose levels over the past two/three months.
Haemoglobin A1c versus glucose

- HbA1c measures the average blood glucose levels over the past two/three months

- Glucose value gives a picture of a moment.

Rohlfing et al., Diabetes care 25 (2002)
Why measuring HbA1c for follow up?

Two studies:

US Diabetes Control and Complication Trial (DCCT)

UK Prospective Diabetes Study (UKPDS)

Intensive treatment

Lower HbA1c

Lower HbA1c

Reduction of the risk for complications
Results of Blood Sugar Control

- 76 % Reduction of retinopathy
- 54 % reduction of kidney disease
- 60 % reduction of peripheral neuropathy
- 35 % reduction in cardiovascular risk

TARGET:

< 7 % HbA1c
Use of Glycated Hemoglobin in the diagnosis of Diabetes

Recommendation

HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement.

An HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes diagnosed using glucose tests.

WHO recommendation
PURPOSE FOR gHb TESTING

Measurement of glycohemoglobin (gHb):
- Powerful tool in the evaluation and management of patients with diabetes.
- Screening/Diagnosis for Diabetes

Concentration of gHb:
- Assessment of long-term glycemic control
- Correlation with risk for the development of chronic complication (DCCT/UKPDS)

BUT the gHb value reported must reflect the average glucose value over the last three months
HbA1c measurement

• The gHb value reported must reflect the average glucose value over the last three months:

• Analytical problems:
  • HbA1c value obtained is not the correct value and can cause wrong treatment

• Clinical problems:
  • HbA1c value obtained is the correct value but can cause wrong treatment because it does NOT reflect the average glucose value
HbA1c measurement

• Analytical problems:
  • HbA1c value obtain is not the correct value and can cause wrong treatment

• Interferences with other Hb:
  • Chemical modifications:
    • Labile A1c
    • Carbamylated:
      Hb reacted with Urea (Kidney patients)
    • Acetylated
      Reaction with f.e. aspirin; pregnant woman, alcohol
HbA1c measurement

- Analytical problems:
  - HbA1c value obtain is not the correct value and can cause wrong treatment

- Interferences with other Hb:
  - Chemical modifications
  - Genetically modifications
Normal adult individual

Hemoglobin (Hb)

- HbF ($\alpha_2\gamma_2$) (<2% of Hb)
- HbA ($\alpha_2\beta_2$) (95% of Hb)
- HbA2 ($\alpha_2\delta_2$) (2-3.5% of Hb)

HbA0 (90% of HbA)

HbA1 (6% of HbA)

- HbA1a (0.4% of HbA)
- HbA1b (0.4% of HbA)

HbA1c (5% of HbA)

HbA1a1 (0.2% of HbA)

HbA1a2 (0.2% of HbA)
Genetically Modified Haemoglobines

- Qualitative modification:
  - Presence of Hb Variants
## Interference of Hb Variants

<table>
<thead>
<tr>
<th>Method</th>
<th>Interference from HbC</th>
<th>Interference from HbS</th>
<th>Interference from HbE</th>
<th>Interference from HbD</th>
<th>Interference from elevated HbF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Architect/Aeroset</td>
<td>Yes</td>
<td>Yes</td>
<td>@</td>
<td>@</td>
<td>$</td>
</tr>
<tr>
<td>Bio-Rad Variant II Turbo</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes &gt;5% HbF</td>
</tr>
<tr>
<td>Bio-Rad Variant II Turbo 2.0</td>
<td>No</td>
<td>No</td>
<td>No/Yes (conflicting reports)</td>
<td>No</td>
<td>Yes &gt;25% HbF</td>
</tr>
<tr>
<td>Roche Cobas Integra Gen.2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>$</td>
</tr>
<tr>
<td>Tosoh G7</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Tosoh G8</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Trinity (Primus) HPLC (affinity)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes &gt;15%</td>
</tr>
</tbody>
</table>
Genetically Modified Haemoglobines

• Qualitative modification:
  • Presence of Hb Variants

• Quantitative modification:
  • Elevated HbF/ HbA2
Normal adult individue

Hemoglobine (Hb)

HbA (α2β2)
(95% van Hb)

HbA0
(90% van HbA)

HbA1
(6% van HbA)

HbA1a
(0,4% van HbA)

HbA1a1
(0,2% van HbA)

HbA1a2
(0,2% van HbA)

HbA1b
(0,4% van HbA)

HbA1c
(5% van HbA)

HbA2 (α2δ2)
(2-3.5% van Hb)

HbF (α2γ2)
(<2% van Hb)

(95% van Hb)
Interference of elevated HbF

The Effect of Elevated Fetal Hemoglobin on Hemoglobin A1c Results

Five Common Hemoglobin A1c Methods Compared With the IFCC Reference Method

Curt L. Rohlfing, Shawn M. Connolly, Jack D. England, Steven E. Hanson, Christina M. Moellerling, MD, Janielle R. Bachelder, MD, and Randie R. Little, PhD
HbA1c measurement

- Analytical problems:
  - HbA1c value obtained is not the correct value and can cause wrong treatment

- Interferences with other Hb:
  - Chemical modifications
  - Haemoglobin variants
  - Elevated HbF

- Calibration:
  - Value obtained must be traceable to clinical studies < 7%
  - Traceable to IFCC reference method and NGSP
  - Can be verified in EQAS
CAP SURVEY

CAP GH2-A 2011 mid level (mean ± 2SD)

%HbA1c

NGSP Target ± 7%
IFCC Monitoring Program

Certificate

Traceability of Manufacturers to the IFCC Reference Measurement Procedure for HbA1c

This certifies that **TOSOH Corporation** using **HLC-723 series**, uses calibrators supplied by the IFCC Network to get traceable to the IFCC Reference Measurement Procedure and participates in the Monitoring Programme to demonstrate traceability. In the Monitoring Programme of 2012 the following performance was seen:

- Deviation from IFCC-target
  - at 30 mmol HbA1c/mol Hb: 1.0
  - at 60 mmol HbA1c/mol Hb: 1.1
  - at 90 mmol HbA1c/mol Hb: 1.1

- Reproducibility, coefficient of variation: 0.21%
- Linearity, correlation coefficient: 0.9998

Date of issue: **18 December 2012**
Certification expires: **31 December 2013**

IFCC Network Coordinator
HbA1c measurement

• Analytical problems:
  • HbA1c value obtained is not the correct value and can cause wrong treatment

• Interferences with other Hb:
  • Chemical modifications
  • Haemoglobin variants
  • Elevated HbF

• Calibration

• Reproducibility:
  • Variation of HbA1c must reflect the variation of glucose and not due to variation of the test
HbA1c measurement

- Clinical problems:
  - HbA1c **value** obtained is the correct value but can cause wrong treatment because it does NOT reflect the average glucose value

- Medication:
  - Difference in glycation (vit E)
  - Difference in RBC life (anti viral drugs)
  - Increase RBC formation (Dapsone)
HbA1c measurement

• Clinical problems:
  • HbA1c value obtained is the correct value but can cause wrong treatment because it does NOT reflect the average glucose value

• Medication:
  • Difference in glycation (vit E)
  • Difference in RBC life (anti viral drugs)
  • Increase RBC formation (Dapsone)

• Variants:
Qualitative Modification

Genetic modifications:

- Substitution of one AA
- Substitution is possible in all chains
- Most known modification: HBS, HbC, HbD, HbJ and HbE

Note:

- Modification in $\alpha$ Chain: 25% of HbA affected
- Modification in $\beta$ Chain: 50% of HbA affected
- Possible reduction in transcription/translation
- Possible difference in RBC life

More than 1000 characterized Hb Variants:

- The vast majority of these variants have little known clinical consequences
- Variant database: [http://globin.cse.psu.edu/globin/hbvar/](http://globin.cse.psu.edu/globin/hbvar/)
Quantitative Modification

Elevated HbF
- Age?
- Persistent HbF
- Thalassaemias
- Haematological disease

Elevated A2
- Thalassaemias
HbA1c measurement

- Clinical problems:
  - HbA1c value obtained is the correct value but can cause wrong treatment because it does NOT reflect the average glucose value

- Medication:
  - Difference in glycation (vit E)
  - Difference in RBC life (anti viral drugs)
  - Increase RBC formation (Dapsone)

- Variants:
  - Difference in glycation
  - Difference in RBC life

- Haematological diseases:
  - Iron deficiency:
    - HbA1c falsely high due to longer RBC life
  - Differences in RBC life
gHb measurement solutions

- HbA1c value reflect average glucose:
  - it is measured as a relative values glycated HbA vs total HbA:

- Immunochemical method:
  - Interferences with HbF depending on the test
  - Specificity depending of the ab against glycated which Hb?
  - Measured the glycated HbV
  - CV
  - No indication of Haematological issues and HbV
gHb measurement solutions

- HbA1c value reflect average glucose:

- it is measured as a relative values glycated HbA vs total HbA:

- Immunochemical method

- Affinity method
  - Ratio between total glycated vs total Hb
  - Measured total gHb
  - Interference of HbF
  - No indication of Haematological issues and HbV
gHb measurement solutions

- HbA1c value reflect average glucose:
- it is measured as a relative values glycated HbA vs total HbA:
- Immunochemical method
- Affinity method
- Ion exchange chromatography/electrophoresis:
  - “purification”/quantification
  - No interference Variant
  - No Interference HbF
  - Indications of Haematological diseases.
Tosoh measurement solution

- HPLC system
- Cation exchange HPLC

- Change in Positive charge = extra peak
  - $\text{NH}_3^+$ to $\text{NH}_2^-$ glucose
  - GLY to ARG

- No change in charge:
  - Net charge =
  - LEU to ILE
G8 Variant mode

4 step elution gradient

Interaction with resin

Strength

- HbC
- HbS
- HbD
- HbA0
- s-HbA1c
- L-HbA1c
- HbF
- HbA1b
- HbA1a

Weakness
Tosoh System Overview

- On board, user friendly software
- Non-porous cation exchange resin column
- 90 sample autoloader
- Primary tube cap piercing with automatic dilution

Elution Buffer Set
Haemolysis/Wash solution
STAT position
HbA1c results

- No interference from:
  - Carbamylated Hb (UREA)
  - Acetylated Hb
  - Labile HbA1c
Interference of I\(\text{A1c}\) on Tosoh G8

Effect of labile \(\text{A1c}\)

- \(\text{L-A1c+ (%)}\)
- \(\text{s-A1c (%)}\)

Control Concentration (g/L)
Effect of IA1c Fraction

**GLYCOHEMOGLOBIN REPORT**

2001/08/23 10:39

TOSOH CORPORATION V01.03

NO: 0007 SL
ID: 0001-07
CALIB Y = 1.2833X - 0.6415

<table>
<thead>
<tr>
<th>NAME</th>
<th>%</th>
<th>TIME</th>
<th>AREA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>A1A</td>
<td>0.2</td>
<td>0.31</td>
<td>2.41</td>
</tr>
<tr>
<td>A1B</td>
<td>0.8</td>
<td>0.43</td>
<td>8.52</td>
</tr>
<tr>
<td>F</td>
<td>0.5</td>
<td>0.53</td>
<td>4.96</td>
</tr>
<tr>
<td>LA1C+</td>
<td>1.5</td>
<td>0.62</td>
<td>15.74</td>
</tr>
<tr>
<td>SA1C</td>
<td>5.3</td>
<td>0.74</td>
<td>49.39</td>
</tr>
<tr>
<td>AO</td>
<td>92.4</td>
<td>1.10</td>
<td>951.24</td>
</tr>
</tbody>
</table>

**TOTAL AREA** 1072.25

HbA1c 5.3%

**GLYCOHEMOGLOBIN REPORT**

2001/08/23 14:55

TOSOH CORPORATION V01.03

NO: 0041 SL
ID: 0001-04
CALIB Y = 1.2833X - 0.6415

<table>
<thead>
<tr>
<th>NAME</th>
<th>%</th>
<th>TIME</th>
<th>AREA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>A1A</td>
<td>0.4</td>
<td>0.32</td>
<td>13.37</td>
</tr>
<tr>
<td>A1B</td>
<td>0.9</td>
<td>0.43</td>
<td>28.93</td>
</tr>
<tr>
<td>F</td>
<td>1.2</td>
<td>0.53</td>
<td>36.31</td>
</tr>
<tr>
<td>LA1C+</td>
<td>9.8</td>
<td>0.62</td>
<td>307.94</td>
</tr>
<tr>
<td>SA1C</td>
<td>5.4</td>
<td>0.74</td>
<td>146.89</td>
</tr>
<tr>
<td>AO</td>
<td>83.0</td>
<td>1.09</td>
<td>2602.50</td>
</tr>
</tbody>
</table>

**TOTAL AREA** 3133.93

HbA1c 5.4%
HbA1c results

- No interference from:
  - Carbamylated Hb (UREA)
  - Acetylated Hb
  - Labile HbA1c

- No interference from most common Hb variants (99.5%)
HbA1c Calculation  normal sample

\[ X = \left( \frac{\text{area (sA1c)}}{\text{total area}} \right) \times 100 \]

\[ y (HbA1c) = ax + b \]

\[ X = \left( \frac{39.91}{937.83} \right) \times 100 \]
\[ Y = (HbA1c) = 4.2556 \times 12.4025 - 19.0939 \]
\[ Y = 33.68 \text{mmol/mol} = 34 \text{mmol/mol} \]

HbA1c %

\[ (0.09148 \times \text{IFCC}) + 2.152 \]
\[ (0.09148 \times 34) + 2.152 = 5.26\% \]
HbA1c calculation “known” variant

\[ X = \left( \frac{\text{area (sA1c)}}{\text{total area} - \text{area H(VAR)}} \right) \times 100 \]

\[ y (HbA1c) = ax + b \]

\[ X = \left( \frac{39.88}{1331.37 - 465.13} \right) \times 100 \]

\[ Y(HbA1c) = 4.604 \times 12.4025 - 18.0939 \]

\[ Y = 39.00 \text{mmol/mol} \]

HbA1c %

\[ (0.09148 \times \text{IFCC}) + 2.152 \]

\[ (0.09148 \times 39) + 2.152 = 5.72\% \]
Separation of Hb Variants

**HbD**

**HbS**

**HbC**

**TOSOH BIOSCIENCE**

The Chemistry of Innovation
Detection of HbAE

Presumptive glycated E fraction separated and indicated as P-HV3.

Presumptive glycated E fraction integrated together with HbA0.

HbA1c underestimated in both cases, and will not be reported.
HbA1c results

- No interference from:
  - Carbamylated Hb (UREA)
  - Acetylated Hb
  - Labile HbA1c

- No interference from most common Hb variants (99.5 %)

- Presumptive identification D, S, C
HbA1c results

- No interference from:
  - Carbamylated Hb (UREA)
  - Acetylated Hb
  - Labile HbA1c

- No interference from most common Hb variants (99.5 %)

- Presumptive identification D, S, C

- No interference from high HbF:
  - Elimination of HbF Area HbF subtracted from total area
  - Identification of a high HbF
# Precision

## Intra- Precision (n=10)

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.26</td>
<td>7.75</td>
<td>10.47</td>
</tr>
<tr>
<td>SD</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.56</td>
<td>0.28</td>
<td>0.19</td>
</tr>
</tbody>
</table>

## Inter- Precision (n=10)

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.31</td>
<td>10.51</td>
</tr>
<tr>
<td>SD</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>CV</td>
<td>0.60</td>
<td>0.37</td>
</tr>
</tbody>
</table>
CONCLUSION

Reporting the correct HbA1c value:

- With high level of accuracy
- With a low Imprecision (CV)
- With no interferences with other Hb:
  - Labile A1c, Carbamylated, Acetylated
  - With no Interferences with Hb Variants incl HbF

Measuring HbA1c:

- The value obtained reflect the long term glucose
- or gives an indication of haematological problem
HbA1c?

The accurate way in diagnosis and follow up of diabetic patients!

When using the correct method

Thank for your attention