Factors Affecting HbA1c results

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Objectives

At the end of the session, the participant should be able to:

1. Identify the reasons for HbA1c analysis.
2. Identify factors that affect the analytical performance of HbA1c methods.
3. Identify the demographic factors affecting HbA1c values.
4. Identify the clinical factors affecting HbA1c values.
Why measure HbA1c?

- **Traditional Use**
  - Monitoring response to therapy
  - Setting of treatment goals
  - Reinforce self monitoring of glucose (SMBG)
  - Reassure patient program is working
  - Question patient or glucometer reliability

- **Recommended Use**
  - Screening for diabetes recommended by all major diabetes societies

- **Possible Use**
  - Assessing cardiovascular risk in non-diabetics
  - Assessing 10 year all risk mortality
Methods available for HbA1c analysis

- **Methods based on charge differences**
  - HPLC
  - Capillary electrophoresis

- **Methods based on structural differences**
  - Immunoassay
  - Enzymatic
  - Boronate affinity
Methods available for HbA1c analysis

• Central laboratory
  ▪ HPLC
  ▪ Immunoassay
  ▪ Boronate affinity
  ▪ Enzymatic

• Large specialized clinic
  ▪ HPLC
  ▪ Immunoassay
  ▪ Boronate affinity
Methods available for HbA1c analysis

- POCT testing
  - (Doctor’s office, Patient self-testing)
  - Boronate affinity
Factors that Influence HbA1c Values

• Analytical
  ▪ precision
  ▪ presence of HbF
  ▪ presence of hemoglobin variants

• Demographic

• Clinical
Guidelines on HbA1c measurement

2011 National Academy of Clinical Biochemistry (NACB) Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus published as an electronic file at:

www.aacc.org/.../nacb/.../OnlineGuide/PublishedGuidelines
2011 NACB Guidelines

• Procedural guidelines
  ▪ Repeat testing
  ▪ Control frequency
  ▪ Participation in a proficiency testing program
  ▪ Discordant results

• Performance Guidelines
  ▪ Imprecision
  ▪ Presence of hemoglobin variants
2011 NACB Guidelines

Procedural guidelines- repeat testing

**Recommendation**
- Samples with HbA1c results below the lower limit of the reference interval or >15% HbA1c should be verified by repeat testing

**Response**
- Repeating the HbA1c test using the same method will likely give a similar result. The result is analytically correct but may not a true measure of HbA1c
- Repeating using a method based on a different analytical principle will help determine the presence of interferences
In our laboratory the glucose value is reviewed if HbA1c is greater than 12% or less than 4%. Also the CBC is reviewed when the HbA1c is <4%

**Patient is 40 year old male**

- HbA1c by HPLC is 19.9%
- Fasting glucose is 20.8 mmol/L (372 mg/dL) (3.1-6.1 mmol/L)
- Concordant results

**Patient is 16 y male**

- HbA1c is 13.6%
- SMBG results are consistently between 5.4 and 7.0 mmol/L (97 to 126 mg/L)
- Discordant result
Procedural guidelines - control frequency

**Recommendation**
- A laboratory should include 2 control materials with different mean values (high and low) at both the beginning and the end of each day’s run

**Response**
- The use of 2 different control levels is standard laboratory practice
Procedural guidelines-participation in a proficiency program

• **Recommendation**
  - Laboratories that measure HbA1c should participate in a proficiency-testing program, such as the College of American Pathologists (CAP) HbA1c survey, that uses fresh blood samples with targets set by the NGSP Laboratory Network

• **Response**
  - The main issue is the type of sample used in the proficiency program. If a whole blood sample is used then the right sample is used and the program has a good foundation
  - Other proficiency programs have tighter limits than CAP
Procedural guidelines-discordant results

• **Recommendation**
  - HbA1c values that are inconsistent with the clinical presentation should be investigated further

• **Response**
  - The use of eAG as a quality assurance tool
  - Checking glucose values against HbA1c values
  - Reviewing the CBC results
Performance goals for HbA1c analysis

Recommendation

- Several expert groups have presented recommendations for assay performance. Early reports recommended that the inter-assay CV be <5% at normal and diabetic GHb concentrations. Subsequent reports have suggested lower CVs [e.g., intralaboratory CVs <3% or <2%, and interlaboratory CVs <5%]

- Intraindividual CVs for healthy persons are very small (<2%), and many current assay methods can achieve intralaboratory and interlaboratory CVs of <2% and <3%, respectively

- We recommend an intralaboratory CV <2% and an interlaboratory of 3.5%
Precision requirements for HbA1c analysis

1. Sacks: Within laboratory 3%
2. Marshall: Between laboratory 5%
3. Kolatkar: Within laboratory CV ideally < 2 - 3%
4. Skeie: Patient-derived specifications
   3.1% (HbA1c increasing)
   3.2% (HbA1c decreasing)
5. Skeie: Physician response 3-5%
6. Edmonton Physicians: Preferably CV of < 3%
7. Phillipou: Practical CV of 3%
8. Goodall et al: Within laboratory 2%
   Between laboratory 3.3%
What is the justification for low HbA1c Precision?

Critical differences (reference change value) for HbA1c at different analytical CVs at a HbA1c of 7%

<table>
<thead>
<tr>
<th>Analytical CV %</th>
<th>Critical Difference %</th>
<th>Difference in HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7.2</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>9.5</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>11.9</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>14.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Clinically significant change in HbA1c

- An HbA1c change of 0.5% is considered a clinically significant change
  2011 NACB guidelines
HbA1c - Standardization issues

- Initially HbA1c was a peak on a C gram
- European directive requires better standardization
- IFCC first defined HbA1c then made calibrator
- IFCC %HbA1c values ~ 2% lower than NGSP % HbA1c values
- To avoid confusion between NGSP and IFCC HbA1c units in % the IFCC recommends the use of mmol/mol in reporting HbA1c.
- Europe appears to be moving to IFCC units whilst North America continues to use NGSP units
The inclusion of eAG on reports

The use of eAG (estimated Average Glucose)

- Suggested to be included on report to minimize confusion and to make reports more understandable to patients
- Based on a poorly executed study
- Patients are aware of the meaning of their HbA1c result
2011 NACB guidelines for HbA1c measurement

**Recommendation**

- Laboratories should be aware of potential interferences, including hemoglobinopathies, that may affect HbA1c test results, depending on the method used. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population.

- In addition disorders that affect erythrocyte turnover may cause spurious results, regardless of the method used.
“Hemoglobinopathies may interfere with GHb analysis independent of their effects on erythrocyte survival.”

“Moreover, hemoglobin variants cannot be identified by immunoassay.”

Case 1: 53 y Caucasian male. Fasting serum glucose 6.4 mmol/L (115 mg/dL)

<table>
<thead>
<tr>
<th>Method</th>
<th>HbA1c Result %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC1</td>
<td>4.7, 5.0</td>
</tr>
<tr>
<td>HPLC2 (Lab A)</td>
<td>9.9</td>
</tr>
<tr>
<td>HPLC2 (Lab B)</td>
<td>7.2</td>
</tr>
<tr>
<td>Immunoassay</td>
<td>5.2</td>
</tr>
<tr>
<td>HPLC3</td>
<td>4.1</td>
</tr>
<tr>
<td>Calculated HbA1c</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Presence of hemoglobin variant Hb Wayne
Case 2

- **HbA1c (HPLC)** 3.5%
- **HbA1c (Immunoassay)** 3.2%
  - Fasting glucose 14.8 mmol/L (267 mg/dL)
  - Total bilirubin 180 µmol/L (10.5 mg/dL)

- **NACB guideline suggest repeat**
  1) HbA1c value is below reference interval
  2) incongruent glucose/HbA1c results

59 y old with elevated reticulocyte count with diagnosis of autoimmune hemolytic anemia
Case 3

<table>
<thead>
<tr>
<th>Glucose mmol/L</th>
<th>HPLC</th>
<th>HbA1c Immunoassay 1</th>
<th>HbA1c Immunoassay 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 y male</td>
<td>5.9</td>
<td>0</td>
<td>5.0</td>
</tr>
<tr>
<td>107 mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 y female</td>
<td>5.1</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>93 mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two siblings with SC disease. No HbA hence no HbA1c. Decreased red call survival of 28 days.
Case 4

34 y male:

- Physician received two different HbA1c results from two laboratories
  - Lab A: 4.8% (3.6 – 6.0%)
  - Lab B: 6.5% (4.3 – 6.1%)

- Presence of δβ thalassemia with HbF of 28.9%
  - Lab A uses an immunoassay method
  - Lab B uses a HPLC method
Case 5

58 y male African American, fasting glucose 6.7 mmol/L (121 mg/dL)

<table>
<thead>
<tr>
<th>Method</th>
<th>HbA1c %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC (1)</td>
<td>0%</td>
</tr>
<tr>
<td>HPLC (2)</td>
<td>50.7%</td>
</tr>
<tr>
<td>Immunoassay</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

Presence of HbK Woolwich
Other factors that affect HbA1c results

Factors that affect erythrocyte survival

- Presence of HbH disease.
- Hemolytic anemia
- β thalassemia
- Chronic renal failure
- Iron deficiency
Demographic Factors that Influence HbA1c Values

- Ethnicity
- Age
- Gender
- Season/temperature change
Demographic Factors - Ethnicity

- > 2000 patients with Type 2 diabetes
  - Mean HbA$_{1c}$ level:
    - Other ethnic/racial 9.7%
    - Hispanics 9.4%
    - Asians 9.2%
    - Caucasians 8.9%

- A1c may not be valid for assessing and comparing glycemic control across racial and ethnic groups
  (Herman et al. Diabetes Care 2007;30:2453-7)
Diabetes Prevention Program
>3000 patients with impaired glucose tolerance

Mean HbA1c:
- non-Hispanic white 5.78%
- Hispanic 5.93%
- Asian 6.00%
- American Indian 6.12%
- African American 6.18%

Herman et al  Diabetes Care 2007;30:2453-7
• Increased red cell turnover in patients with β thalassemia minor (94 day rbc survival)

• In α thalassemia red cell turnover dependent on genotype

• In individuals with HbS median HbA1c is 0.4% higher
Demographic factors - Age

- A1c levels are positively associated with age in non-diabetic populations.

- None of these groups showed significant correlation between HbA1c and age.

- Other factors than age must be retained to explain HbA1c variability.
Figure 1—Mean A1C by age categories in the FOS nondiabetic population (A), the NHANES 2001–2004 nondiabetic population (B), and the FOS NGT population (C). The number of subjects in each age-group is shown in Table 1. Tests for trend were significant at $P < 0.0001$ for both the FOS and NHANES 2001–2004. ◆, All; ■, women; ▲, men.
Demographic Factors- Gender

- Girls had significantly higher mean A1c than boys during adolescence

- Increasing hemoglobin A1c may be more strongly related to increased all-cause cardiovascular mortality in women than in men.
Demographic factors - Season

HbA1c is higher in the colder months than the warmer months.

*HbA$_{1c}$ (normal <5.2%) among all the months for all visits to outpatient clinic since 1987 - Asplund J. Journal of Diabetes Care 1997;20:234*
Demographic factors – Temperature Change

Change in median $\text{HbA}_{1c}$
Clinical Factors that Influence HbA1c Values

- Iron status
- HIV status
- Chronic renal failure
- Smoking status
- Glycation phenotype
- Hemodialysis
- Red Cell turnover
Clinical factors - Iron status

- Iron deficiency must be corrected before any intervention or therapeutic decision is made based on HbA1c.
  Coban et al Effect of iron deficiency on the levels of hemoglobin A1c in non-diabetic patients. Acta haematol 2004;112:126-8

- The red cell survival is longer in patients with iron deficiency which increases the HbA1c value.
Clinical factors - HIV Status

• HbA\textsubscript{1c} underestimates glycemia in HIV-infected patients and is related to NRTI use. Fructosamine may be more appropriate in this setting.

Clinical factors - Chronic Renal Failure

- Analytical interference in modern HbA1c methods from carb Hb is minimal

- Shortened red cell survival resulting in decreased HbA1c by about 1.5%
Clinical Factors – Smoking status

- Multiple logistic regression analysis disclosed that smoking was independently associated with elevated HbA1c... in both type 1 and type 2 diabetics.

Nilsson et al. Smoking is associated with increased HbA1c values and microalbuminuria in patients with diabetes-data from the national diabetes Register in Sweden. Diabetes Metabol 2004;30:261-8

- It can be concluded that poor metabolic control together with smoking increases teeth loss.

Clinical Factors – genetic phenotype

- Mean blood glucose and HbA1c are not necessarily interchangeable estimates of glycemic control and that hemoglobin glycation phenotype may be important for the clinical assessment of diabetic patients.


Clinical Factors - Hemodialysis

- In diabetic hemodialysis patients, hemoglobin A1c levels significantly underestimate glycemia control

- Assessment of glycemic control by HbA1c in hemodialysis patients might lead to underestimation likely as a result of the increasing proportion of young erythrocytes by the use of erythropoietin.
Both epoetin alfa and darbepoietin alfa may artificially lower $A_1c$ levels in patients with diabetes not undergoing dialysis

Drug Interaction

- Ribavirin used to treat hepatitis C infections causes a reduction in A1c

Vitamin D and HbA1c

- Vitamin D concentrations are inversely associated with HbA1c levels.

Hutchinson MS et al Serum 25-hydroxyvitamin D levels are inversely associated with glycated haemoglobin (HbA1c). The Tromsø Study. Scan J Clin Lab Invest 2011;71:399-406.
Vitamin C and E

- **Vitamin C levels are inversely related to HbA1c levels due to inhibition of glycation.**
  

- **Vitamin E lowers HbA1c**
  
Conclusion

- HbA1c requires accurate, precise measurement
- Analytical, demographic and clinical factors impact HbA1c values
- Laboratorians and clinicians need to be aware of these factors