Impact of Reference Measurement Systems on Clinical Evidence: HbA$_{1c}$ and Diabetes

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JCTLM Members and Stakeholders Meetings at BIPM
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Why is HbA$_{1c}$ important in diabetes mellitus?
Diabetes Mellitus: a "non infectious epidemic disease"

Prevalence in 2012
371 million patients

Estimation in 2030
552 million patients (+51%)

- Challenge: to prevent or delay severe degenerative long-term complications
- Necessity: optimal metabolic control (direct link between glycemic control and occurrence of complications)

HbA1c: a major tool
HbA$_{1c}$ : a glycated protein

Nonenzymatic glycation:
- Spontaneous binding of sugars (glucose) and by-products on aminogroups of proteins
- Cumulative and irreversible process related to red blood cell lifespan (120 days) and glucose concentration

Structural and functional protein alterations: participation in molecular protein ageing and involved in pathology
Nonenzymatic glycation of HbA

Hb A ($\alpha_2\beta_2$) $\alpha = 141$ aminoacids $\beta = 146$ aminoacids

Preferential glycation sites

$\beta$ - Val - 1

$\alpha$ - Lys - 16

$\beta$ - Lys - 66

$\beta$ - Lys - 17

$\alpha$ - Val - 1

$\alpha$ - Lys - 7

$\beta$ - Lys - 120
Heterogeneity of glycated HbA

HbA\(_{1a1}\) : fructose 1,6-bisphosphate
HbA\(_{1a2}\) : glucose-6-phosphate
HbA\(_{1b}\) : pyruvate
HbA\(_{1c}\) : glucose
The different theoretical forms of HbA$_{1c}$

Necessity of a strict definition for standardization purpose
HbA$_{1c}$ : the gold standard of diabetic survey

- Relationship HbA$_{1c}$ / degenerative complications of diabetes mellitus (DCCT and UKPDS large-scale studies)

HbA$_{1c}$ : retrospective and cumulative index of glycemic balance (4-8 weeks before sample)

- Reference values and therapeutic targets
  - Reference Range : 4 - 6% of total Hb
  - Good Glycemic Control : < 6.5% (T2D) < 7.0% (T1D)
  - Poor Glycemic Control : > 8.0%

Note : HbA$_{1c}$ values were established with reference to the NGSP standardization program (USA/international, non specific "reference method")
HbA$_1c$ use before standardization

Disorders in terminology and concepts

Glycated hemoglobin language: another tower of Babel

Variable quality of methods

First quality control assessment in France (1995)
The conditions of the international standardization of HbA$_{1c}$ assays

Prerequisite: Selection of robust field methods
Status of Hemoglobin A1c Measurement and Goals for Improvement:
From Chaos to Order for Improving Diabetes Care

Randie R. Little, Curt L. Rohlfing, and David B. Sacks for the National Glycohemoglobin Standardization Program (NGSP) Steering Committee
The conditions of the international standardization of HbA$_1^c$ assays

Prerequisite: Selection of robust field methods, achieved by using intensive proficiency testing and quality assurance schemes

Rationale: The NGSP standardization program previously used in most clinical studies (for establishing clinically meaningful values)

- was based on a non specific reference method for HbA$_1^c$ assay (ion-exchange chromatography)
- although having international activities, was only one national program (USA) among other standardization programs (Japan-Sweden)
- could not guarantee long-term traceability (valid permanent anchor)
The standardization process

- **International standardization (achieved by IFCC)**
- **Aim**: - Definition of the Hb species measured and of the measurand (HbA$_{1c}$ or glycated Hb ?)
  - Definition and validation of RMP
- **1990s–2000s**: IFCC Working Group on HbA$_{1c}$ standardization
- **2002**: The definitive IFCC Reference Method
Glycated species measured: $\text{HbA}_{1c}$

- Clearly defined biochemical structure
- Nonenzymatic binding of glucose
- $\text{N}$-terminal extremity of $\text{HbA} (\alpha_2\beta_2) \beta$ chains
- Amadori rearrangement (glucose $\rightarrow$ deoxyfructose)
- $\text{HbA}_{1c} = \text{N}-(1\text{-deoxyfructose-1-yl}) \beta$ chain of hemoglobin

  = $\text{DOF-hemoglobin (DOF-Hb)}$

Primary reference materials: Purified $\text{HbA}_0$ and $\text{HbA}_{1c}$
Global IFCC standardization

Reference method (IFCC): HPLC/MS or HPLC/CE

Measurand: β-N-terminal hexapeptide [(glycated vs non glycated (mmol HbA₁c/mol HbA₀ + HbA₁c)]

Effect on standardization on long-term stability

- RMP maintained by an international IFCC network of approved laboratories: a valid anchor (especially for calibration of field methods by manufacturers)
- More than 10 years of experience

Missions of the IFCC Network HbA$_{1c}$

- Guarantee continuity of the IFCC Reference Measurement Procedure (IFCC-RMP)
- Make HbA$_{1c}$ assays worldwide traceable to the IFCC-RMP
IFCC Network Labs HbA$_{1c}$ in 2013
16 approved laboratories

**Asia**
- China: Shanghai
  - Prof. Ju Yi
- China: Beijing
  - Prof. Wenxiang Chen
- Japan: ReCCS
  - Dr. Violeta Raneva
- Japan: Tokyo
  - Prof. Izumi Takei
- Japan: Kanagawa
  - Dr. Tadao Hoshino
  - Dr. Yashihiro Hishinuma
- India: Calcutta
  - Dr Bhaskar Bhattacharya
- South Korea: CDC
  - Dr Junghan Song

**Europe**
- Italy: Universita di Milano
  - Prof Andrea Mosca
- Netherlands: Isala Klinieken
  - Dr. Robbert Slingerland
- Netherlands: Queen Beatrix Hospital
  - Dr. Cas Weykamp, Coordinator
- Germany: Roche
  - Dr. Angela Puhlmann
  - Dr Roland Thiele
- Germany: INSTAND e.v
  - Dr. Patricia Kaiser
- France: Reims
  - Prof. Philippe Gillery

**America**
- USA: CDC
  - Dr. Maria Ospina
- USA: Siemens, Norwood, MA
  - Dr. Yuanfang Deng
- USA: Univ. Columbia, MO
  - Prof. Randie Little

+ 2 candidate laboratories

www.ifcchba1c.net
Stability

Master Equation IFCC – NGSP over 10 years
Green = Low HbA$_{1c}$ level  Blue = High HbA$_{1c}$ level

Master Equation IFCC – JDS/JSCC over 10 years
Green = Low HbA$_{1c}$ level  Blue = High HbA$_{1c}$ level
Master Equation IFCC vs other standardization programs

NGSP/DCCT HbA\textsubscript{1c} = (0.915 \times \text{IFCC HbA}_{1c}) + 2.15

Effect on result expression and reporting of units

IFCC reference method for $\text{HbA}_{1\text{c}}$ is more specific than NGSP reference procedure and thus provides lower values in % (e.g. 4 - 6% NGSP correspond to 2.0 - 4.2% IFCC).

- **Keep the previous units (NGSP / DCCT)?**
  - **Pro:** well known and clinically meaningful values
  - **Con:** not "true" value

- **Change to IFCC units in %?**
  - **Pro:** "true" values
  - **Con:** risk of destabilisation and of clinical unefficiency (not realistic)

- **Use another expression mode?**
  - Other units for $\text{HbA}_{1\text{c}}$: mmol $\text{HbA}_{1\text{c}}$/mol Hb ("IFCC units" or "SI units")?
1. HbA$_{1c}$ results should be standardized worldwide, including the reference system and results reporting

2. The new IFCC reference system for HbA$_{1c}$ represents the only valid anchor to implement standardization of the measurement

3. HbA$_{1c}$ results are to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation

4. - HbA1c conversion tables easily accessible to the diabetologic community
   - Report in both SI and NGSP/DCCT units in scientific journals
   - HbA$_{1c}$ is the reportable term (A$_{1c}$ may be used in guidelines and educational materials)
IFCC standardization of HbA$_{1c}$ assays : What remains to be done ?

⇒ **Strategy of implementation** of the new international standardization (IFCC Integrated Project) : reporting of units different according to the countries : dual reporting (e.g. France), SI reporting only (e.g. UK, Italy) …. or NGSP reporting only (e.g. USA, Canada)

⇒ **Validation of new numbers** by large-scale clinical studies

<table>
<thead>
<tr>
<th>mmol HbA$_{1c}$/mol Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Reference Range       : 4 - 6% of total Hb  20 - 42</td>
</tr>
<tr>
<td>✓ Good Glycemic Control : &lt; 6.5% (T2D) &lt; 48</td>
</tr>
<tr>
<td>✓ Poor Glycemic Control : &gt; 8% &lt; 53</td>
</tr>
<tr>
<td>✓ Good Glycemic Control : &lt; 7% (T1D) &lt; 64</td>
</tr>
</tbody>
</table>
Standardization: Effect of result expression on patient outcome

A first experience with percentages

Psychological Impact of Changing the Scale of Reported HbA$_1c$ Results Affects Metabolic Control

Ragnar Hanas, MD, PhD

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Address correspondence to Ragnar Hanas, MD, PhD, Department of Pediatrics, Uddevalla Hospital, S-451 80 Sweden. E-mail: ragnar.hanas@bls.se.
Aim: To evaluate the effect on a diabetic patient population of raising the reference scale up to the DCCT level in 1992 and then down to the Swedish National Standard in 1997.

Lab situation

**Before 1992**: Samples sent to central lab (Mono S HPLC method, Pharmacia, reference range 3.0 - 4.6%)

**1992**: POCT use of DCA 2000, Bayer, reference range 4.1 - 5.7% DCCT reference

**1997**: DCA 2000 calibration adjusted to the Swedish National Standard (reference range 3.1 - 4.6%)

Patients

- Diabetes onset at least 3 years after the change in 1997
- Follow-up for at least 2 years after the change
- 49 children and adolescents (born 1971 to 1985)
- Intensive insuline therapy
1992:  - Expected: 1.4% higher
  - Observed: after 9 - 12 months, mean HbA$_1c$ value decreased $\approx 0.5$
    (i.e. glycemic control improved)
1997:  - Expected: 1.1% lower
  - Observed: after transient decrease, mean HbA$_1c$ values increased again (i.e. glycemic control deteriorated)

Conclusions

- Psychological impact of absolute numbers very high when small changes are made to reference levels.
- Be careful with changes of units ("IFCC perspective").
UK experience

- June 1\textsuperscript{st}, 2009: dual reporting (% DCCT and SI units)
- October 1\textsuperscript{st}, 2011: results reported solely in SI units (mmol/mol)
- 12 months evaluation
Fig. 1. Monthly mean Hb A1c throughout period of study.
<table>
<thead>
<tr>
<th>All samples</th>
<th>Year before unit change 2010-2011</th>
<th>Year after unit change 2011-2012</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>21 880</td>
<td>22 841</td>
</tr>
<tr>
<td>HbA(_{1c})</td>
<td>%</td>
<td>7.5 (6.6, 8.7)</td>
<td>7.5 (6.5, 8.7)</td>
</tr>
<tr>
<td></td>
<td>mmol/mol</td>
<td>58 (49, 72)</td>
<td>58 (48, 72)</td>
</tr>
<tr>
<td>HbA(_{1c}) initially &gt; 8% (64 mol/mol)(^b)</td>
<td>( n )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HbA(_{1c}) change</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>- 0.2 (-0.9, 0.3)</td>
<td>- 0.2 (-0.8, 0.3)</td>
</tr>
<tr>
<td></td>
<td>mmol/mol</td>
<td>- 2 (10.3)</td>
<td>- 2 (9.3)</td>
</tr>
<tr>
<td>Days between HbA(_{1c}) samples</td>
<td>99 (64, 147)</td>
<td>98 (64, 147)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

\(^a\) All data expressed as median (25th, 75th) centiles.

\(^b\) Change in HbA\(_{1c}\) represents the difference between 2 successive DCCT/SI values (before unit change) and 2 successive SI-only values (after unit change) in samples with initial values > 8% (64 mmol/mol)
Use of SI units

- No influence of unit change on quality of glycemic control
- To be confirmed on a longer period
Standardization: Effect on analytical goals

The Analytical Goals for Hemoglobin $A_{1c}$ Measurement in IFCC Units and National Glycohemoglobin Standardization Program Units Are Different

Clinical Chemistry 57:8 (2011) 1205
Result expression and analytical goals

- Could the change of units for reporting HbA$_{1c}$ results impact analytical goals?

- Example: Repeatability study

<table>
<thead>
<tr>
<th>NGSP (%)</th>
<th>IFCC (mmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA$_{1c}$</td>
<td></td>
</tr>
<tr>
<td>6.8</td>
<td>51</td>
</tr>
<tr>
<td>6.5</td>
<td>48</td>
</tr>
<tr>
<td>7.2</td>
<td>55</td>
</tr>
<tr>
<td>7.0</td>
<td>53</td>
</tr>
</tbody>
</table>

Mean 6.88
SD 0.30
CV 4.3%

51.8
3.0
5.8%
Why this difference?

Analogy with temperature

Unit variation equivalent to 1°C
- Celsius (°C, Europe)
- Fahrenheit (°F, USA) \((°F = 1.8°C + 32)\)
- Kelvin (°K, official unit) \((°K = °C + 273)\)

<table>
<thead>
<tr>
<th>Unit</th>
<th>Variation</th>
<th>Variation in percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celsius</td>
<td>37°C</td>
<td>1°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1/37 \times 100 ) = 2.7%</td>
</tr>
<tr>
<td>Fahrenheit</td>
<td>99°F</td>
<td>1.8°F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.8/99 \times 100 ) = 1.8%</td>
</tr>
<tr>
<td>Kelvin</td>
<td>310°K</td>
<td>1°K</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1/310 \times 100 ) = 0.3%</td>
</tr>
</tbody>
</table>

Could the conclusion be: « Temperature variation is lower in scientists and higher in Europeans »?
Why this difference?

- In both case, the conversion equation from one unit system to another is $y = ax + b$, where $b$ (y intercept) is not equal to zero.
- Variation across metrologic systems cannot be compared in terms of relative percentages when $b$ is different from zero.
- A higher y-intercept value has a greater impact ($^\circ F = 1.8^\circ C + 32$ and $^\circ K = ^\circ C + 273$)
- In clinical chemistry, it means that the specificity of both systems is different
- Case of HbA$_{1c}$
  - Master equation: NGSP/DCCT = (0.0915 IFCC + 2.15)
  - $b$ (2.15%) represents the difference of specificity between the two methods
- (NB: HbA$_{1c}$ peak in ion-exchange chromatography is not pure)
Result expression and analytical goals

- Result expression mode (IFCC or NGSP units) modifies analytical goals, even when crude results are the same.
- Target values, estimated performance, CVs are concerned.
- Different expressions, different goals

*Weykamp et al., Clin. Chem., 2011, 57, 1204-1205*
Conclusions

The international standardization of HbA\textsubscript{1c} assays has brought

- a valid anchor for all methods
- a long term stability
- significant changes in unit use and result reporting that necessitate a global strategy of implementation

This strategy allows the optimal use of a valuable biological test in an important context of public health (and of new indications of HbA\textsubscript{1c} assay: e.g. diagnosis of diabetes mellitus)
Thank you for your attention!