

## Guidelines and recommendations for testing in diagnosis of diabetes mellitus: The role of HbA1c

Elizabeta Topic

Croatian Society of Medical biochemistry and Laboratory Medicine, Zagreb, Croatia

Corresponding author: elizabeta.topic@gmail.com

### Background

The first arbitrary criteria for diagnosis of diabetes mellitus (standardized by the World Health Organisation, WHO) appeared in 1980 (1). These criteria were based on blood glucose measurement fasting and 2 h after oral ingestion of a certain amount of glucose in non-pregnant adults (OGTT). In 1997, these criteria were supplemented with the value of fasting plasma glucose (FPG) more central to the diagnosis (2). Twelve years later, in 2009, the International Expert Committee for Diagnosis and Management of Diabetes recommended that HbA1c be used as the preferred test for diagnosing type 2 diabetes (T2D). The diagnosis of diabetes should be made solely on the basis of an HbA1c value 48 mmol/mol ( $\geq 6.5\%$ ) (3).

### HbA1c term

Although the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) and Nomenclature Commission of IUB (NC-IUB) drew attention to the issue of nomenclature related to glycosylated haemoglobin as early as 1984, explaining the difference between the two terms 'glycated' and glycosylated (or glucosylated) haemoglobin, unfortunately, the improper use of the term can still be found in the literature. The term glycated haemoglobin refers to haemoglobin that has been modified by the non-enzymatic addition of glucose, i.e. non-enzymatic reactions between glucose or other sugars and free amino groups of proteins. The compounds so formed result from the Schiff base formation followed by Amadori rearrangement.

The term glycosylated (glucosylated) is an enzymatic process resulting in glycoside (glucoside) compounds (4).

The currently acceptable term for glycation of haemoglobin in general is 'glycated haemoglobin' (GHb). HbA1c is a specific glycated species that is modified by glucose on the N terminus of the haemoglobin  $\beta$  chain. 'HbA1c' is also the internationally accepted term for reporting all GHb results.

### Methodology

Roughly 100 different GHb methods from low-throughput manual minicolumn methods to high-throughput automated systems for HbA1c measurement have been used for 35 years. Related to the principle, the methods can be classified mostly into two groups: methods that quantify GHb based on charge differences between glycated and nonglycated components (cation-exchange chromatography, agar-gel electrophoresis), and methods that separate glycated and nonglycated components based on structural differences (boronate affinity chromatography, immunoassay). Most charge-based and immunoassay methods measure HbA1c, whereas other methods quantify 'total glycated haemoglobin' (5-7).

It is not strange that GHb results reported for the same blood sample can differ considerably among methods.

In 1995, the IFCC established a working group (IFCC WG-HbA1c) to achieve international standardisation of HbA1c measurement by establishment of reference measurement procedure with purified primary calibrators, by development of a network of reference laboratories and implementation of traceability to the IFCC reference system. The analyte measured by IFCC reference method has been defined as  $\beta$ N1-deoxifructosyl-haemoglobin and recommended units are mmol/mol.

The IFCC WG-HbA1c recommended the term HbA1c to be used in clinical practice (8).

In 1996, the National Glycohemoglobin Standardisation Program (NGSP) initiated to standardise

GHb test results among laboratories to The Diabetes Control and Complication Trial (DCCT)-equivalent values (9). It was developed under the auspices of the AACC and endorsed by ADA recommendation that laboratories use only the methods that are certified by the NGSP as traceable to the DCCT reference. The manufacturers of HbA1c assays should also follow traceability to the IFCC reference method. The NGSP web site contains detailed information on the process of certification and maintains a list of certified methods (monthly updated) and factors known by now to interfere with specific methods (10).

In 1997, the IFCC formed a committee to develop a higher-order reference method and reference materials for HbA1c analysis; the method was approved in 2001. Since the preparing and measuring samples with this method is laborious, very expensive, and time-consuming, the method serves to manufacturers for standardisation of the method.

The advantage of a new reference method to standardize the HbA1c results, along with the anticipated documentation that the assay does indeed indicate average blood glucose resulted in a variety of proposed changes in the reporting of HbA1c test results worldwide.

Related to this, an international consensus agreement among the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), the International Diabetes Federation (IDF), and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) was signed in Milan, Italy, in 2007 (11).

The implications of the activities according to the agreement were as follows:

1. HbA1c test results should be standardized worldwide, including the reference system and reporting of results.
2. The new IFCC reference system for HbA1c represents the only valid anchor to implement standardization of the measurement.
3. HbA1c results are to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation.

4. If the ongoing 'average plasma glucose study' fulfils its *a priori* specified criteria, a HbA1c-derived average glucose (ADAG) value calculated from the HbA1c result will also be reported as an interpretation of the HbA1c results.
5. In clinical setting, glycemic goals are expressed in IFCC units and derived NGSP units.

Since June 2011, the way of HbA1c reporting has switched from percentage to mmol/mol as the equivalent to NGSP DCCT-aligned results. Both NGSP and IFCC units were recommended to be used, but the decision to report was left to the discretion of individual countries.

To make sense of the new HbA1c units and compare these with old units and *vice versa*, a converter has been developed based on so-called master equation (12).

### **International consideration and recommendation**

An International Expert Committee comprising members appointed by the ADA, EASD and IDF published their report on the role of HbA1c in diagnosis of diabetes in 2009 (13), in which they recommend that diagnosis in type 2 diabetes should be made solely on the basis of an HbA1c confirmed to be 48 mmol/mol ( $\geq 6.5\%$ ), without the need to measure plasma concentration in the subject. A sub-diabetic high risk state would exist for subjects with an HbA1c of 42-46 mmol/mol (6.0%-6.4%).

Kilpatrick and Vinocour in the article on the Association of British Clinical Diabetologist (ABCD) position statement on haemoglobin A1c for the diagnosis of diabetes, on behalf of the ABCD, endorsed by the Association for Clinical Biochemistry (ACB), emphasis the advantages and disadvantages of using HbA1c to diagnose diabetes (14). Advantages include non-fasting samples, low biological variability, the measure of previous (prior) glycaemia, as well as analytical postulates enabling to bring results from different laboratories closer together.

Disadvantages are abnormal haemoglobin, anaemias, age and ethnicity, as well as technological

limitations that may, despite strong advantages of using HbA1c in the diagnosis of diabetes, give a misleading indication of glycaemia in an individual and so lead to an inappropriate or missed diagnosis.

While the current glucose criteria for diagnosis are arbitrary and the testing process itself has well documented limitations, at the moment it seems that there also are unresolved concerns related to the HbA1c, i.e. it may appear that HbA1c is much more likely than glucose to completely misdiagnose an individual as having or not having diabetes. The possible requirement for using HbA1c alone in the diagnosis of diabetes mellitus is previous exclusion of the conditions such as anaemia or haemoglobinopathies, while taking in consideration the factors of patient age and ethnicity. In this way, diagnostic workup would be simplified considerably.

The National Academy of Clinical Biochemistry: Laboratory Medicine Practice Guidelines published in 2011 Guidelines and Recommendation for laboratory analysis in the diagnosis and management of diabetes mellitus by David Sacks, endorsed by AACC and ADA.

The Guidelines revealed important recommendations concerning HbA1c; HbA1c should be measured routinely in all patients with diabetes to document their degree of glycemic control. Laboratories should use only HbA1c methods that are certified by the NGSP as traceable to the DCCT reference. The manufacturers of HbA1c assays should also show traceability to the IFCC reference method. Related to the reference intervals, it is recommended that a laboratory should determine its own reference interval even if the manufacturer has provided one.

In clinical settings, patient samples with HbA1c results below the lower limit of reference interval or 140 mmol/mol (>15%) HbA1c should be verified by repeat testing, i.e. HbA1c values that are inconsistent with clinical presentation should be investigated further.

The Guidelines also emphasise the importance of HbA1c interpretation, which requires close laboratory-physician interaction.

## Use of HbA1c

### Use of HbA1c to diagnose diabetes

Although the proposed diagnostic cut-off value for HbA1c of 48 mmol/mol is recommended, many studies have implicated that fewer patients will be newly diagnosed if HbA1c at this level is used alone, as compared with plasma glucose value. The prevalence of undiagnosed diabetes using any glucose criterion (fasting or 2 h) was 5.1%, and upon including HbA1c it rose to 5.4%. So, the prevalence differs markedly between the Expert Committee (1.6%) and both WHO (5.1%), and ADA recommendation (5.4%).

Summarised, 25% of individuals with a 'positive' OGTT had 48mmol/mol, while 45% of individuals that exceeded both the fasting and 2 h glucose criteria were not diagnosed with diabetes using HbA1c. The additional effect of ethnicity and aging has an important role in these proportions.

### Using HbA1c to identify the risk of microvascular complications

It seems that the International Expert Committee agreed that HbA1c 48mmol/mol could be at least predictive in identifying patients at risk of developing microvascular complications, particularly retinopathy.

### Using HbA1c to identify the risk of macrovascular complications

One of the most common macrovascular complications is cardiovascular system (CV) involvement. Several risk factors along with hyperglycaemia should be managed in diabetes patients; so many patients are prescribed antihypertensives and lipid lowering drugs. At the HbA1c 48 mmol/mol cut-off, this treatment would automatically be considered in fewer individuals. The HbA1c is also known to be poor in the group of patients with impaired glucose tolerance and in identifying patients with impaired fasting glucose. With regard to CV risk prediction, there is evidence that HbA1c may be superior to fasting glucose alone in predicting future CV events. There is evidence for a relationship between the increasing HbA1c and increasing CV risk.

## Using HbA1c to identify type 1 diabetes

The ADA makes HbA1c applicable to patients with type 1 diabetes, but there is a concern that rapidly evolving hyperglycaemia in these patients may not be immediately reflected in a raised HbA1c. So, it could result in delaying the diagnosis of type 1 diabetes.

## Using HbA1c in monitoring of diabetes

The HbA1c testing should be performed at least biannually in all diabetic patients and quarterly in patients whose therapy has changed or who failed to meet treatment goals. These testings are recommended for non pregnant patients with either type 1 or type 2 diabetes.

When using HbA1c in monitoring of diabetic patients, it is of utmost importance to consider the race- and age-specific HbA1c target, as well as dif-

ferent causes leading to misinterpretation of HbA1c, such as haemoglobinopathies, anaemia, renal failure, HIV infection, etc.

## Using HbA1c in emergency

Point-of-care (POC) HbA1c is not sufficiently accurate to use it for the diagnosis of diabetes. Although several POC HbA1c assays are NGSP certified, due to the lack of objective and ongoing documentation of performance proficiency testing POC HbA1c instruments should not be used for diagnosis or screening.

In conclusion, the existence of formal recommendations is crucial for standardization of the criteria, methods and procedures in various clinical conditions, however, a number of questions remain that require additional research for the recommendations to resolve all the shortcomings observed to date.

## References

1. World Health Organisation. *Diabetes Mellitus report of WHO Study Group (Tech Rep Ser. No 727)*. Geneva: WHO, 1985.
2. *Report of the Expert Committee on the diagnosis and classification of diabetes mellitus*. *Diabetes Care* 1997;20:1183-97.
3. *International Expert Committee Report on the role of the HbA1c assay in the diagnosis of diabetes*. *Diabetes Care* 2009;32:1327-34.
4. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), and Nomenclature Commission of IUB (NC-IUB). *Arch Biochem Biophys* 1984;229:237-45.
5. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycated hemoglobin: methodologies and clinical applications. *Clin Chem* 1986;32:B64-70.
6. Sacks DB. Diabetes mellitus. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz textbook of clinical chemistry and molecular diagnostics*. 5<sup>th</sup> ed. St. Louis: Elsevier Saunders, 2012.
7. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, Sacks DB. Tests of glycemia in diabetes. *Diabetes Care* 2004;27:1761-73.
8. Mosca A, Goodall I, Hoshino T, Jeppsson JO, Garry John W, Little RR, et al. *Clin Chem Lab Med* 2007;45:1077-80.
9. The Diabetes Control and Complication Trial research group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977-86.
10. *Harmonising Haemoglobin A1c testing*. Available at: <http://www.ngsp.org>. Accessed on 28th August 2014.
11. Hicks JMB, Müller MM, Panteghini M, John WG, Deeb L, Buse J et al. Consensus Statement on the Worldwide Standardization of the Hemoglobin A1c Measurement. *Diabetes Care* 2007;30:2399-400.
12. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. *Clin Chem* 2008;54:240-8.
13. Kilpatrick E, Bloomgarden Z, Zimmet P. Is haemoglobin A1c a step forward for diagnosing diabetes? *BMJ* 2009;339:1288-90.
14. Kilpatrick ES, Vinocour PH. ABCD position statement of haemoglobin A1c for the diagnosis of diabetes. *Pract Diab Int* 2010;27(6):1-5.
15. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. *Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus*. *Diabetes Care* 2011;34:e61-e99.