Opinion Paper

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Glycemic control in the clinical management of diabetic patients

Abstract

In clinical practice, glycemic control is generally assessed by measuring and interpreting glycated hemoglobin levels, however, this test should be run under standardized conditions. We focus here on the crucial steps to ensure IFCC standardized HbA$_{1c}$ results, pointing out several residual weak points, mostly relating to the laboratory end-user (calibration, quality control materials, and EQAS). We also review the use of HbA$_{1c}$ for diagnosing diabetes and the various indicators useful for assessing glucose variability because in some cases they seem to represent a patient's glucose profile more accurately than one-off HbA$_{1c}$ assays. Finally, the potential utility of glycated albumin and the glycation gap, the costs involved and the laboratory management issues are briefly discussed.

Keywords: glycated albumin; glycated hemoglobin; glycation gap; HbA$_{1c}$ glucose variability; standardization.

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Introduction

Diabetes is considered a health problem of epidemic proportions. The IDF Diabetes estimated that 285 million people around the world had diabetes in 2010, representing about 7% of the world's adult population [1]. The ADA recommends screening for type 2 diabetes and assessing the risk of future diabetes in asymptomatic adults of any age who are overweight or obese and have one or more additional diabetes risk factors, and also in asymptomatic adults with no risk factors ≥45 years of age [2]. In the light

of the findings of an International Expert Committee, the ADA recently recommended using the HbA$_{1c}$ test to diagnose diabetes, adopting a threshold of 6.5% (48 mmol/mol) [3]. These recommendations have also been adopted by the American Association of Clinical Endocrinologists/American College of Endocrinology [4] and, to some degree, by the IDF [5].

This document reviews some of the topics, in the field of laboratory medicine, relevant to the diagnosis and monitoring of diabetes, paying particular attention to the use of HbA$_{1c}$, glucose variability indexes and glycated albumin.

Critical issues for ensuring standardized HbA$_{1c}$ measurements

Many papers have been written about the various analytical aspects relevant to a correct HbA$_{1c}$ measurement, making it difficult to add anything new. It is worth mentioning some excellent reviews on the topic [6, 7], and also a recent contribution [8] in which we have tried to group together the analytical interferences that could be overcome by appropriate sample handling or by choosing the most appropriate method for measuring HbA$_{1c}$, from those in whom some particular physiological or pathological conditions may be present, usually not known a priori (see table 1 in [8]).

Something that warrants further attention, in our opinion, is the standardization of the methods at the end-user level. Some recent papers emphasize that this issue needs to be considered carefully. For instance, Elder et al. pointed out that further assay standardization would be advisable in the UK, either because internal data are obtained by different analytical systems, or because of an unacceptable variability in the data collected through the UKNEQAS program, in which the results were quite often excessively variable for clinical purposes [9]. A similar warning was published by Lenters et al. in a more recent work, in which they showed an unacceptable

Actions	Tools
Choice of method	Assess the IFCC certificate
	(ask the manufacturer)
Calibration	Enter the IFCC target values provided by the manufacturer
	(ask the manufacturer for traceability to the IFCC reference system)
Reporting the HbA$_{1c}$ result	Use the mmol/mol units (possibly converting them afterwards into % units)
	Report decisional limits (not reference intervals)
Monitoring long-term imprecision	Internal Quality Control with materials at normal and high HbA$_{1c}$ content
	Calculate the CVs monthly (or over a longer time interval)
Evaluating trueness	Regular participation in EQAS exercises
	(commutable materials, IFCC target values assigned by the IFCC reference measurement procedures)

Table 1 Essential steps in order to provide IFCC standardized HbA$_{1c}$ test results.

level of imprecision for one in five laboratories involved in a Dutch/Belgian external quality assessment scheme (EQAS) with 220 laboratories participating [10]. Analytical problems also seem to arise if certain point-of-care testing (POCT) systems are used to measure HbA$_{1c}$ instead of using a conventional laboratory technique [11, 12]. Finally, recent data from a German EQAS exercise again seem to indicate that inter-laboratory variation is not entirely acceptable, and that the overall mean still differs significantly from the one obtained by the IFCC reference measurement procedure, or by a newly-developed LC-ID-MS procedure [13].

To shed some light on this issue, we would like to retrace the metrological chain for the traceability of laboratory activities, and to repeat that achieving a global standardization involves duties and responsibilities at various levels, from the scientific societies and metrological institutes to the diagnostics manufacturers, and finally to the end-user clinical laboratories [14, 15]. In the specific case of HbA$_{1c}$, the whole chain has been defined [16], primary materials have been prepared and characterized [17], an IFCC-approved reference method has been developed [18], and a network of reference laboratories has been created [19]. Close cooperation between the manufacturers and the IFCC Network has been in place for several years with a dedicated EQAS, the IFCC Monitoring Program [20]. Master equations were developed and published for converting the results obtained using methods calibrated with the IFCC reference system into those obtained by using NGSP-aligned methods [21], and an international consensus on the implementation of the IFCC reference system was signed [22], and renewed 3 years later [23]. An agreement with the manufacturers on the implementation of the reference system has also finally been reached [24]. Analytical goals for imprecision, bias and total error have been defined, based either on clinical needs [25] or on biological variations [26], and it

has ultimately been demonstrated that these goals are different when the HbA$_{1c}$ results being reported are expressed in different units [27].

Coming back to the laboratory operators, we feel that these professionals should essentially accomplish all the steps outlined in Table 1 in order to be IFCC standardized.

First of all, a valid method should be chosen, and this entails conducting a careful analysis of the IFCC certificate. This document (see the facsimile in Figure 1) is obtainable directly from the manufacturer and certifies to the method's alignment with the IFCC reference system thanks to the manufacturer's participation in the above-mentioned monitoring program [28]. In the example shown in Figure 1, the trueness of the measurements is reported for three HbA$_{1c}$ levels (30, 60 and 90 mmol/mol): on the whole, this performance is very good, showing only a slight underestimation at low HbA$_{1c}$ values, balanced by a slight overestimation at the highest HbA$_{1c}$ concentrations. A bias of ±1 mmol/mol (approx. equivalent to ±0.1%, expressed in NGSP units) is perfectly acceptable. As for the reproducibility, the reported value of 2.4% is also acceptable, the goal being within 2.8% [26]. The report also shows a figure for linearity, and this should theoretically always be ≥0.999.

A second point concerns the method's calibration: this should be done by inserting the IFCC target values provided by the manufacturer directly in the apparatus, but in our experience, this is not always what happens in the field. In some cases, calibration is done in percentage units, and then reporting is handled in mmol/mol units using the 'master equation', which is not really right!

Then there is a third point, reporting. According to the consensus statement on the implementation of the IFCC reference system [22, 23], this has to be done in mmol/mol, and possibly also in a second unit. Various national societies have taken a position on this aspect, and some European countries have the IFCC units already in place, while
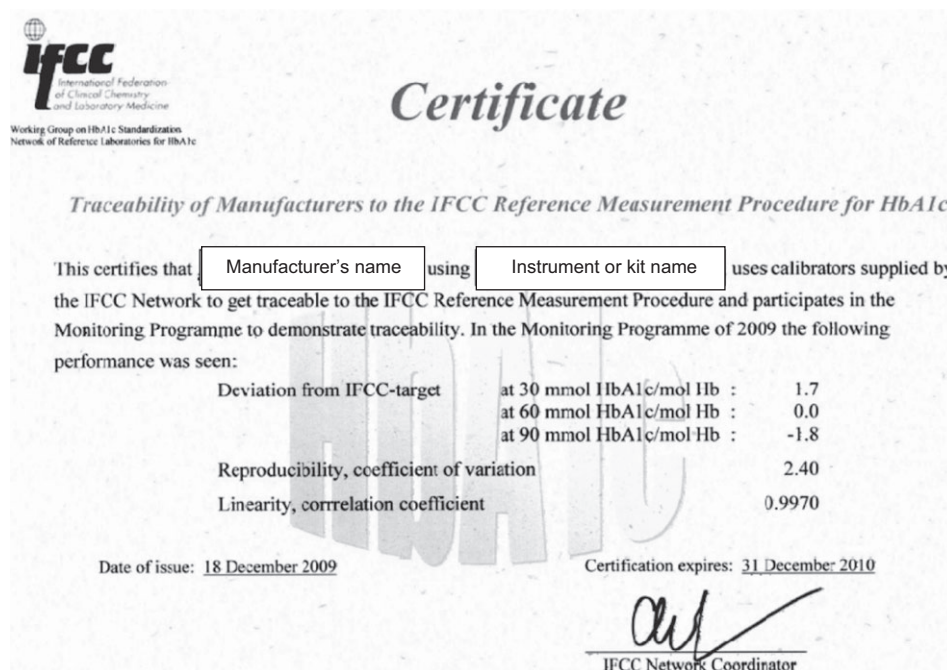
Figure 1 Example of a certificate released to a manufacturer by the coordinator of the IFCC network, reporting the results of the Monitoring Program (reprinted with the Editor's permission from [28]) modified.

others have double reporting systems, and an IFCC integrated project is now dedicated to this topic at global level [29]. It is not our intention to discuss the above-mentioned consensus statement in the present document, but simply to make the point that it becomes complicated, in the long run, to retain both units because:

1) the analytical goals differ for mmol/mol and percentage units [27]; 2) only IFCC units should be used, given the relatively poor specificity of the NGSP reference system, as illustrated by the following considerations. Let us consider the famous DCCT study, and particularly the relationship between mean plasma glucose and HbA_{1c} [30]. As we all remember, a lesson learned from that study was that a worsening plasma glucose control is associated with a higher risk of microvascular complications, and possibly also with a greater risk of macrovascular and neurological complications. It was demonstrated, moreover, that a 1% (10 mmol/mol) increase in HbA_{1c} correlated with an average increase in mean plasma glucose of approximately 36 mg/dL, as confirmed by a later re-assessment of the DCCT data (see figure 1 in [31]). Figure 2 shows the relationship between mean plasma glucose and HbA_{1c} emerging from the DCCT study (Figure 2A), and we have plotted both axes starting from the origin for both glucose and HbA_{1c}. We have also plotted a confidence interval of ±15% glucose values, as proposed at the time for the ADAG study [32]. There is a clearly evident negative intercept of the regression line, due essentially to the limited specificity of the

NGSP method. In Figure 2B, we have plotted the regression line of the same data in the DCCT study processed by Rohlfing et al. [31], this time by converting the NGSP-HbA_{1c} values into IFCC-HbA_{1c} values using the master equation published by Hoelzel et al. [21], adapted so as to convert the percentage units into mmol/mol units [i.e., NGSP-HbA_{1c}=0.0915 (IFCC-HbA_{1c})+2.15%]. Clearly, the regression line now passes through the origin of both axes, as one might logically expect based on practical biochemical considerations (no glucose, no HbA_{1c}).

Still on the subject of reporting, most clinical laboratories report reference intervals and we feel that this custom should be abandoned, for at least a couple of reasons. First of all, Braga et al. demonstrated that between-subject variability is much greater than within-subject variability [26], with a mean CV_i (2.5%) lower than the figure (3.4%) reported by Ricòs et al. [33], and a slightly higher mean CV_G (7.1%) compared with the one published in the database (5.1%). We do not want, to this regard, to discuss which number is more correct. However, it is important to remember that the biological variability is an incompressible component of the total variability, due to a set of physiological and biochemical events not easily distinguishable separately from each other. In particular, previous works have established that there is some heterogeneity in red cell life span as well in non-diabetic subjects recruited from the general population, as in type 1 and type 2 diabetic subjects [34]. Mean RBC ages were found between 38 and 60 days in non-diabetic
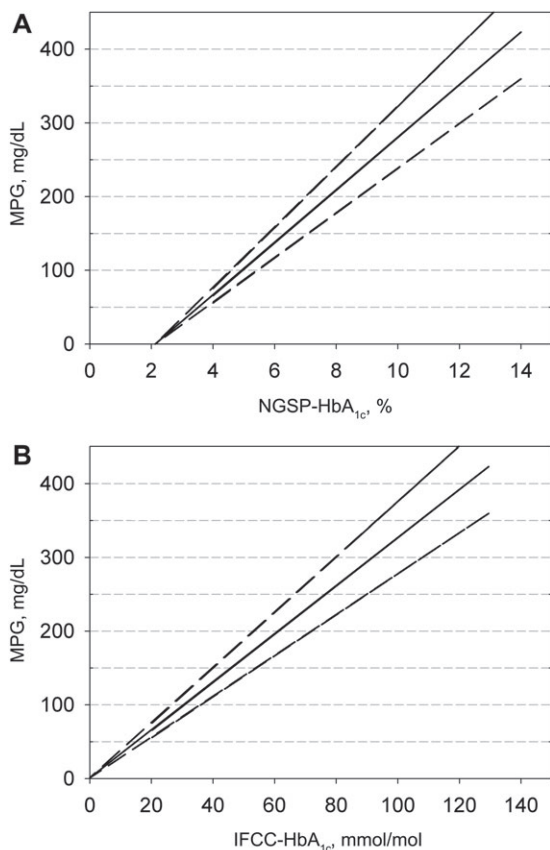
Figure 2 Relationship between mean plasma glucose calculated from seven-point blood glucose profiles and glycated hemoglobin in the Diabetes Control and Complications Trial (DCCT).
(A) Original data, reproduced from [30]. (B) Same data, with HbA$_{1c}$ expressed in SI units.

controls and between 39 and 56 days in diabetic subjects, and the impact of red cell age on HbA$_{1c}$ was such that three patients with similar glucose control could have very different HbA$_{1c}$ levels, i.e., between 7.5% and 9.9% (58–85 mmol/mol, respectively), if they would have mean RBC ages between 38 and 60 days. In other words, variation of red cell life span could result in variation of ±12% (CV) in the HbA$_{1c}$ values, at the same extent of glucose control [34]. Another potential source of between subjects variability in HbA$_{1c}$ could be related also to differences in glucose transport/gradient across the red cell membrane, as clearly proven by an elegant study with radioactive glucose analogues [35]. In this investigation, the authors speculated that differences between two different individuals showing various gradients in glucose concentrations between outside and inside the red cells could result in a 25% difference in the level of hemoglobin glycation.

Therefore, in our opinion, in the same way as for other analytes, it is preferable to use subject-based reference intervals in such cases [36] or, if such values are unavailable, then clinical decisional limits (diagnostic cut-offs) should be adopted. ADA also seems to recommend this [37]. It is worth bearing in mind that, given the small value of intra-individual variation, only two specimens are needed to determine an individual's homeostatic HbA$_{1c}$ set point to within 5% of the true mean value [26]. There is evidence of HbA$_{1c}$ values being different in different ethnicities, and some data indicate an effect of age (see later on). Indeed, the latest version of the AACC guidelines for laboratory analyses in the diagnosis and treatment of diabetes mellitus [38] also contain a rather generic comment on the reference intervals (For NGSP-certified assay methods, reference intervals should not deviate substantially (e.g., >0.5%) from 4% to 6% (20–42 mmol/mol). For these various reasons, we feel that using cut-off values would be preferable to either lower limits [HbA$_{1c}$ levels below 4.3% (23 mmol/mol) are very rare in our experience] or higher limits [HbA$_{1c}$≥6.5% (48 mmol/mol)]. In Table 2, we propose some of the most useful decisional limits for orienting the interpretation of HbA$_{1c}$ test results. In particular, as concerns pregnancy, O'Connor et al. recently defined trimester-specific reference ranges for HbA$_{1c}$ using a HPLC method well-aligned with the IFCC [40]. As for the reference change values, which are useful for assessing the significance of changes in results obtained by analyzing serial samples from the same individual, an average CD of 10% can be used, as reported elsewhere [26].

Finally, particular attention should be paid to monitoring imprecision and trueness. This can be accomplished by a regular use of the IQC procedures to confirm and verify the manufacturer's declared performance of commercial systems, and by participating in the EQAS, using commutable control materials with target HbA$_{1c}$ values assigned by the reference measurement procedure, in order to be able to define the uncertainty of laboratory measurements [15]. Regarding the IQC, we would like to

HbA$_{1c}$ (%)	HbA$_{1c}$ (mmol/mol)	Utility	Reference
4.0	20	Lower limit of healthy subjects	[35][a]
5.4	36	Upper limit in pregnancy (1st and 2nd trimester)	[36]
5.7	39	Lower limit for prediabetes	[39]
6.4	46	Upper limit for prediabetes	[39]
6.5	48	Diagnosis of diabetes	[3–5]
7.0	53	ADA primary goal of therapy	[2]

Table 2 Possible decisional limits for HbA$_{1c}$.
[a]In our experience we very rarely find healthy subjects with HbA$_{1c}$<4.5% (<26 mmol/mol).

draw attention to the proper use of commercial control materials. In the package insert that comes with the materials, the assigned values are usually reported together with confidence intervals or ranges (depending on the manufacturer). In several cases we have found that these intervals are really too wide, and this can be very misleading in daily practice. For instance, just to quote two major diagnostics manufacturers, the leaflet for the Lyphochek Diabetes Control (Bio-Rad Laboratories) mentions a range from 8.8% (73 mmol/mol) to 11.2% (99 mmol/mol) for Level 2 control, and for the PreciControl HbA$_{1c}$ material (Roche) we find a range from 7.3% (56 mmol/mol) to 10.5% (91 mmol/mol). What if these were whole blood samples instead of control materials? How can we accept that a given patient could have an HbA$_{1c}$ of 7.3% (56 mmol/mol) according to a measurement obtained at one clinical laboratory and 10.5% (91 mmol/mol) according to another? We strongly urge the manufacturers to reduce the ranges of their control materials, at least enough to make them compatible with the proposed total error limit.

Trueness is best assessed by participating routinely in an EQAS program, providing that commutable materials are used and the HbA$_{1c}$ titer is assigned by the IFCC reference measurement procedure, or by reference laboratories. A list of the laboratories capable of providing such a service is available in the JCTLM database [41]. Unfortunately, these conditions are never met by lyophilized materials, which always suffer from non-commutability problems, as already demonstrated [39, 42].

HbA$_{1c}$ for diagnosis and monitoring

Background

Numerous studies have recently been published on this issue and some of them warrant consideration. In an unselected emergency department population, Menchine et al. found a very high proportion of adults with undiagnosed diabetes [22% of patients with a BMI≥30 kg/m^2 had an HbA$_{1c}$≥6.5% (48 mmol/mol)] and a high prevalence of suboptimally controlled diabetes (almost 45% of those with a history of diabetes). The authors consequently supported the use of simple diagnostic criteria, such as HbA$_{1c}$ tests, in emergency departments for screening purposes [43]. Other authors have supported the use of HbA$_{1c}$ measurements in acute care settings, since their value is uninfluenced by recent eating or acute illness, and the cut-offs used to screen for pre-diabetes and diabetes are similar to those used in outpatient settings [44]. A further

analysis on the 12,485 participants in the Atherosclerosis Risk in Communities (ARIC) study, and on a subpopulation of 691 participants in the NHANES III, identified a better performance of HbA$_{1c}$ assay [≥6.5% (≥48 mmol/mol)] in diagnosing prevalent diabetes and predicting incident diabetes than the use of single or repeated standard glucose measurements [45]. In a Dutch population, on the other hand, Riet et al. analyzed 2753 participants in the New Hoorn Study and found that considering HbA$_{1c}$ levels ≥6.0% (42 mmol/mol) as diagnostic would mean missing almost half of the people with diabetic glucose levels. The cut-off used to screen for diabetes was an HbA$_{1c}$ level of 5.8% (40 mmol/mol), which achieved a sensitivity of 72% and a specificity of 91%: this cut-off would detect 72% of patients with diabetes and 30% of cases at high risk of developing diabetes. Finally, 44% of patients with newly-diagnosed diabetes had HbA$_{1c}$ levels <6.0% (42 mmol/mol) [46] when tested using a standardized DCCT method (reverse-phase exchange chromatography).

Adding a glucose-based method to the process for diagnosing diabetes improves the performance of HbA$_{1c}$, as confirmed by other analyses [47, 48]. In 2332 oral glucose tolerance test (OGTT)-diagnosed diabetic subjects from a Chinese population aged 35–75 years, FCG (fasting capillary blood) measurements performed better than HbA$_{1c}$ as a screening tool for newly-diagnosed diabetes and pre-diabetes [49]. Using HbA$_{1c}$ as a single diagnostic tool for detecting type 2 diabetes may result in an overestimation of the cases by comparison with the OGTT-based method, as reported by Mostafa et al. in a study involving a multi-ethnic cohort [50].

Another analysis on 6890 adults without previously-diagnosed diabetes from the 1999–2006 NHANES study showed a reasonable consistency between HbA$_{1c}$ and fasting glucose measurements for the purposes of diagnosing diabetes (k=0.60; 95% CI 0.55, 0.64), although discrepancies were reported for non-Hispanic black patients [51].

Ethnicity

The question of whether HbA$_{1c}$ and glucose homeostasis differ among various ethnic groups has recently been debated. In particular, Chapp-Jumbo et al. conducted an in-depth analysis and found significantly higher HbA$_{1c}$ levels in healthy black adult offspring of parents with type 2 diabetes than in their white counterparts [5.68±0.033% vs. 5.45±0.028% (39±2 vs. 37±2 mmol/mol)], after adjusting for age, hemoglobin, hematocrit, BMI, fasting plasma glucose, and glucose AUC [52]. In an Italian cohort of 1019

Caucasian individuals, there was again a moderate consistency between HbA$_{1c}$ levels and FPG criteria in diagnosing type 2 diabetes (k coefficient=0.522), but 35.9% of the individuals in this study with diabetes according to the FPG criteria alone had HbA$_{1c}$ levels <6.5% (48 mmol/mol), confirming that different diagnostic criteria identify different diabetic populations [53].

Analyzing the Leicester Ethnic Atherosclerosis and Diabetes Risk (LEADER) cohort showed that using HbA$_{1c}$ as a diagnostic test significantly increased the frequency of cases of diabetes, especially among the southern Asian population, while approximately one-third of the people previously identified as diabetic by OGTT were found non-diabetic using HbA$_{1c}$ assay [50]. In a Chinese population (2332 subjects aged 35–74 years), using the diagnostic cut-off of 6.5% (48 mmol/mol) for HbA$_{1c}$ generated a sensitivity of <30% in both men and women, i.e., lower than for plasma glucose. The optimal cut-off for detecting diabetes in this population was an HbA$_{1c}$ level of 5.6% (38 mmol/mol), i.e., significantly lower than in the new ADA recommendations [49], but this cut-off proved unsuitable for the South African population, for which Zemlin et al. identified a level of 6.1% (43 mmol/mol) as the optimal cut-off [54].

Finally, an analysis on six studies conducted from 1999 to 2009 in Denmark, the UK, Australia, Greenland, Kenya and India tested the probability of HbA$_{1c}$ levels being ≥6.5% (48 mmol/mol) among cases of diabetes diagnosed by means of OGTT. The prevalence of diabetes was lower in four of the six studies when the HbA$_{1c}$-based criteria were used. Indeed, the probability of HbA$_{1c}$≥6.5% (48 mmol/mol) among the OGTT-diagnosed cases ranged from 17% to 78%, with significant differences between different ethnic groups. Although methodological differences may have influenced these results, the impact of ethnicity on the different screening procedures needs to be clarified [55]. In this setting, an extensive analysis of the NHANES survey covering the years from 1988 to 2006 has shown that the prevalence of diagnosed diabetes has increased since 1988, while the percentage of undiagnosed diabetes has decreased and so has the prevalence of subjects at high risk of developing diabetes, though the latter two conditions remain common in the USA, and elderly and minority groups are disproportionately affected. The fact that HbA$_{1c}$ detects a much lower prevalence of diabetes than plasma glucose levels should therefore be borne in mind [56].

Age effect

When it comes to elderly patients, the use of HbA$_{1c}$ to diagnose diabetes is somewhat controversial. A cross-sectional

analysis on data from the Health, Aging and Body Composition study at year 4 (2000–2001) compared the sensitivity and specificity of HbA$_{1c}$-based diagnoses with those based on FPG in the elderly. The proportions of undiagnosed diabetes were 3.1% and 2.7%, respectively, using FPG≥126 mg/dL and HbA$_{1c}$≥6.5% (48 mmol/mol) [57]. Another study conducted on Asian patients using ROC curve analysis found that an HbA$_{1c}$ test result of 6.2% (44 mmol/mol) was the best cut-off for diagnosing diabetes, but the sensitivity and specificity of the area under the ROC curve deteriorated as the age groups increased [58]. In addition, there are several practical implications to consider when HbA$_{1c}$ is adopted as the preferred tool for diagnosing diabetes, as already explained [8].

Judging from the above-mentioned studies, it seems that using HbA$_{1c}$ alone to diagnose diabetes would lead to a number of diagnoses being overlooked, while the sensitivity and specificity of the assay could be improved by combining it with fasting blood glucose or the OGTT.

HbA$_{1c}$ and risk of diabetic complications

The preference for the use of HbA$_{1c}$ in the diagnosis of diabetes has also been supported on the strength of its supposed prediction of cardiovascular risk but, here again, published studies provide contradictory evidence. In a Spanish cross-sectional epidemiological survey, individuals without known diabetes underwent an OGTT and HbA$_{1c}$ assay, and several cardiovascular risk factors were assessed. The authors demonstrated that subjects newly diagnosed as diabetic according to the new criteria recommended by the ADA had a worse cardiovascular risk profile [a higher BMI and waist circumference, lower high-density lipoprotein (HDL) cholesterol concentrations, higher HOMA-IR and fibrinogen concentrations] than patients diagnosed using the glucose-based criteria [59]. In a study including approximately 1300 diabetic patients prospectively followed up for cardiovascular disease, Paynter et al. identified significant improvements in the ability to predict CVD risk using models that included HbA$_{1c}$ levels by comparison with classifications of diabetes as a cardiovascular disease equivalent. HbA$_{1c}$ has sometimes been shown to identify individuals at risk of diabetes in much the same way as fasting blood glucose, but is better able to predict cardiovascular events (multivariable-adjusted hazard ratio of 1.95 for HbA$_{1c}$≥6.5% [48 mmol/mol]), supporting the use of HbA$_{1c}$ assay as a test for diagnosing diabetes [60]. However, in a study conducted in 1015 patients with coronary artery disease (CAD) and no previous diagnosis of diabetes, the results of HbA$_{1c}$ measurements were

compared with those of an OGTT for the purposes of early diabetes diagnosis. The analysis demonstrated that OGTT identifies more patients with glucose impairments who undergo coronary angiography than the HbA_{1c} test using a cut-off of ≥6.5% (48 mmol/mol). In this set of patients, therefore, measuring HbA_{1c} alone seems to miss a substantial proportion of cases with hitherto-unknown diabetes. OGTT measurements were also found to correlate significantly with the severity of CAD (ρ=0.09, p=0.01), unlike the HbA_{1c} assay findings [61]. Moreover, as demonstrated in a study by Sturm et al., HbA_{1c} values were sometimes inversely associated with all-cause mortality in diabetic dialysis patients, in addition to presenting no significant associations with CVD events [62].

Long-term glycemic control has proven to be one of the most important parameters for preventing chronic complications, and microvascular disorders, in particular. Identifying indicators better able to predict daily glycemic profiles has consequently been an important field of clinical research in recent years. HbA_{1c} represents the combination of both fasting and postprandial glucose variations over a 3-month period [63], proving very useful in providing background information to orient effective patient treatment decisions [64]. The importance of HbA_{1c} as a 'gold standard' for monitoring blood glucose in patients with diabetes was confirmed by the DCCT and UKPDS mega-trials [30, 65], which demonstrated the close relationship between HbA_{1c} values and the onset of chronic complications of diabetes in the long-term. In the follow-up of these trials, despite no further differences in the treatment administered, the advantage of intensive diabetes treatment persisted and even extended to a reduction of patients' micro- and macrovascular events, regardless of their HbA_{1c} values [66, 67]. This phenomenon is called 'metabolic memory' and has to do with an as yet unexplained HbA_{1c}-independent mechanism that contributes to the onset of chronic complications [68]. One of the most important determinants in this mechanism concerns the formation of advanced glycation endproducts (AGEs) and their accumulation in diabetic subjects, reflecting cumulative metabolic and oxidative stress, as reported elsewhere [69].

More recently, the A_{1c}-derived average glucose (ADAG) study described the relationship between HbA_{1c} and average blood glucose. This important work by Nathan et al. showed that a simple linear relationship exists between these two parameters, quite well-described by a simple regression equation. The conclusion was therefore that HbA_{1c} levels could be expressed as estimated average glucose (eAG, since it is a calculated average) in both DM1 and DM2 [32]. It should be noted, however, that some ethnic groups were under-represented in this trial (especially the Asian and African populations), and its findings are only applicable to populations in stable glycemic control with no suggestion of any erythrocyte disorders. A large inter-individual variability was also noticeable. As a matter of fact, the adoption of the eAG was not endorsed in the consensus statement on HbA_{1c} standardization [22].

In an effort to arrive at a comprehensive assessment of glycemic control, the term 'glucose triad' has also recently been introduced, meaning fasting glucose, postprandial blood glucose and HbA_{1c} [70]. A number of studies have investigated the contribution of these three components to overall 24-h hyperglycemia, often with contradictory results.

Monnier et al. [71] showed that a gradually worsening glycemic control follows a 'three-step' temporal sequence: the earliest changes occur in the three postprandial periods (after breakfast, lunch and dinner); at a later stage, hyperglycemia is more severe in the early morning (the so-called 'dawn phenomenon'); the last step corresponds to nocturnal hyperglycemia. Postprandial glucose levels thus contribute the most (70%) to the lower quintile (HbA_{1c} <7.3% [56 mmol/mol]), whereas fasting hyperglycemia appears to be the main contributor to the overall diurnal hyperglycemia in patients with poorly-controlled disease [HbA_{1c} >9.3% (78 mmol/mol)] [64]. According to the above-mentioned authors, postprandial hyperglycemia would be an early causal event in chronic glycemic damage, even in patients with well- or moderately-controlled diabetes. However, Borg et al. (again based on data from the ADAG study) showed more recently that, among all the SMBG measurements, preprandial glucose levels had a greater effect on HbA_{1c} than postprandial levels, although FBG levels alone failed to provide a clear indication of overall hyperglycemia [70].

To sum up, no single blood glucose measurement obtainable during the day accurately predicts HbA_{1c} [72], and that is why the contribution of pre- and postprandial glucose concentrations to overall glycemic control (HbA_{1c}), and the role of postprandial glucose targets in disease management are still being debated. As suggested in several reports, many patients do not reach the HbA_{1c} targets set by the published guidelines [73]. However, the goal is to cure the patient's diabetes, not their hyperglycemia, so an optimal glycemic control, as Ceriello suggested, 'equates to HbA_{1c} (at target)+fasting plasma glucose (to target)+postprandial glucose (to target) without hypoglycemia and weight gain' [74]. The low sensitivity of HbA_{1c} levels in describing glycemic profiles implies a need to diversify the use of indicators for monitoring diabetic patients in the long-term. The indicators of fasting glucose,

postprandial glucose and glucose variability each have their own pathophysiological value with a view to providing increasingly individualized treatment options.

Glucose variability

The acute glucose fluctuations that occur in diabetes have recently been considered an additional factor in the onset of chronic complications of the disease [75, 76]. In fact, a study by Monnier et al. [77] demonstrated that the urinary excretion of 8-isoprostane (a marker of oxidative stress) correlated positively with glycemic variability, as assessed by calculating the mean amplitude of glycemic excursion (MAGE). Ceriello et al. [78] also emphasized the role of acute hyperglycemic spikes in the physiopathology of micro- and macrovascular complications of DM, showing in particular that more limited hyperglycemic excursions coincide with lower levels of some oxidative stress markers. These data were not confirmed by Kilpatrick et al. [79], however, when they retrospectively analyzed the findings of the DCCT study: while mean blood glucose was predictive of microvascular complications, the within-day glucose variability (calculated as the standard deviation around the mean of a seven-point glycemic profile) was unrelated to chronic complications.

Finally, glucose variability has been seen as a plausible candidate for predicting severe hypoglycemia, because this condition is preceded by blood glucose disruptions [80]. Several studies have reported a decline in the occurrence of hypoglycemia episodes coinciding with a lower glucose variability [81].

Numerous glucose variability indicators [82] have been developed, but none of them have so far come to be considered the 'gold standard' for assessing glycemic variability in diabetic individuals (Table 3). There is also still no consensus as to which indicator is preferable for assessing glucose variability and for how long glycemic variability measurements should be continued.

In a study of ours, glucose variability indicators were recorded over at least 48 h using a continuous glucose monitoring (CGM) system in patients with type 1 diabetes, patients with type 2 diabetes taking multiple daily injections of insulin therapy, and type 2 patients treated with OHA and/or basal insulin. Baseline and postmonitoring HbA_{1c} levels were also measured. HbA_{1c} correlated significantly only with average glucose (AG) levels and the high blood glucose index (HBGI), and only in type 1 diabetic patients. There was greater glucose variability in patients with longstanding type 1 diabetes, irrespective

of their HbA_{1c} levels. An insulin regimen with MDI correlated strongly with HbA_{1c}, but was unassociated with glucose variability. HbA_{1c} values therefore identify states of sustained hyperglycemia and seem to be unaffected by hypoglycemic episodes or marked glucose variability, thus revealing shortcomings as a 'gold standard' indicator of metabolic control. Glucose variability indicators adequately represent the glucose profile of type 1 patients and identify any worsening glycemic control (typical of longstanding diabetes) more accurately than one-off HbA1c tests [83].

Judging from the above considerations, it is clear that what we know about the relationships between glycemic variability and the onset of diabetic complications is very limited, so prospective randomized trials specifically addressing this topic are needed to further strengthen the evidence in the literature.

Glycated albumin

The use of other proteins, such as glycated albumin, and the measurement of advanced glycation endproducts as predictors of the risk of chronic diabetic complications have not been mentioned in the guidelines, not even the most recent, and this is essentially because of the lack of sufficiently strong evidence to support their application [63]. We are nonetheless of the opinion that such markers should be used more. We would particularly like to stress the importance of measuring glycated albumin when interpreting HbA_{1c} measurements could be hampered by certain pre-analytical conditions (e.g., in cases of chronic hemolytic anemia or hemoglobin variants). It is also worth bearing in mind that glycated albumin can be measured with a specific, robust and reliable enzymatic method that overcomes the limitations of the previous fructosamine test [84]. It could prove extremely interesting to apply this new test in re-assessing the data used to calculate the glycation gap (GG), proposed for the first time by Cohen et al. [85], whose work proved that GG is reproducible. In a cohort of 40 type 1 diabetic patients with a history of the disease spanning more than 15 years, the authors found that a 1% increase in their GG was associated with a three-fold increase in the cases of nephropathy, and this parameter correlated better with the severity of the nephropathies than either HbA_{1c} or fructosamine. Cohen et al. subsequently demonstrated that GG is at least 70% inherited, as are HbA_{1c} levels (60% inherited) [86]. Apparently, a third of the heritability of HbA_{1c} is shared with that of GG, while the remainder is HbA_{1c}-specific [87]. It has

Indicator	Meaning	Advantages	Disadvantages
Mean glucose (MG):	Mean of all glucose values	Simple and straight forward to interpret; it provides the best correlation with HbA$_{1c}$ values	It does not attribute more importance to hypo- or hyperglycemia
Standard deviation (SD):	SD of all glucose values	Simple to determine, the most classical and well-known method used by physicians	It is affected by extremely low and high glucose values, but fails to capture the rapidity and magnitude of glycemic spikes (not applicable to asymmetrical non-Gaussian distributions); it is affected by mean glucose
Interquartile range (IQR):	75th percentile – 25th percentile of glucose values		
J index	Combining information from mean glucose and SD→0.001×(mean+SD)^2	Combining information from the two most used CGM indicators; strongly correlated with time spent within target range	Almost unknown and not used in clinical practice; sensitive to hyperglycemia but relatively insensitive to hypoglycemia
Coefficient of variation (% CV):	(100×SD)/MG	Simple, unaffected by mean glucose	
Percentage within target range	Defined as time spent within a given glucose range (the most often used ranges are: 80–200 mg/dL, 70–180 mg/dL, 70–140 mg/dL)	Simple and straightforward to interpret, effective (sensitive to clinical intervention)	The choice of an arbitrary target range may not be optimal
Percentage below target range	Time spent below a given cut-off (the most often used cut-offs are: <80 mg/dL and <50 mg/dL)	Simple and straightforward to interpret, sensitive in detecting hypoglycemic events during the day	It does not attribute more weight to extremely low values
Percentage above target range	Time spent above a given cut-off (the most often used cut-offs are: >250 mg/dL, >200 mg/dL, >180 mg/dL, >140 mg/dL)	Simple and straightforward to interpret, sensitive in detecting hyperglycemic events during the day	It does not attribute more weight to extremely high values
Mean amplitude of glycemic excursion (MAGE)	Calculated as the arithmetic mean of the differences between consecutive glycemic peaks and nadirs, only considering changes in glycemic values of more than 1 SD	Describes major fluctuations; directly proportional and strongly correlated with the SD of all glucose values	Rarely used in clinical practice; statistically less efficient than SD
Mean of daily differences (MODD)	Mean of the absolute differences between glycemic gaps observed during the same time interval on 2 consecutive days, as an expression of 'between-days' glucose variability	The only indicator to describe between-days variability	Originally defined only for 2 consecutive days, assuming similar meals, physical activity and therapy on both days
Continuous overlapping net glycemic action (CONGAn)	The SD of the glycemic differences recorded between a specific point on the CGM profile and a point n hours previously (where n=1,2,3,4,…)	Sensitive in capturing 'short-term' glucose variability; opportunity to compare variability during fasting (CONGA *night*) and in postabsorptive period (CONGA *day*); strongly correlated with SD for certain n values (when n~2.5)	Difficult to interpret for physicians who do not use CGM systems very often
High blood glucose index (HBGI)	27.695×(log$_e$ [glucose]$^{1.084}$−5.381), for glucose values >112.5 mg/dL	Greater weight attributed to hyperglycemic values	Does not predict hypoglycemic events or low glucose trends; obscure mathematical derivation

(Table 3 Continued)

Indicator	Meaning	Advantages	Disadvantages
Low blood glucose index (LBGI)	$7.695 \times [\log_e (\text{Glucose})^{1.084} - 5.381]$, for glucose values <112.5 mg/dL	Greater weight attributed to hypoglycemic values	Does not predict hyperglycemic events or high glucose trends; obscure mathematical derivation
Average daily risk range (ADRR)	Average sum of |HBGI for maximum glucose|+|LBGI for minimum glucose| for each day	Combines information from HBGI and LBGI; moderate correlation with time spent within target range, determined by self-monitoring of blood glucose (SMBG)	Calculated using extreme values (min and max); more appropriate for SMBG than for CGM; for SMBG it takes 30 days, obtaining at least 4 values a day
Area under curve (AUC):	very similar to the percentage below or above a given target range. It can be used to describe the total glycemic exposure in a given glucose range or at a particular time of day, e.g., the *postprandial AUC* is calculated from the preprandial glucose levels to the highest peak over a 2-h period after a meal.		

Table 3 Glucose variability indicators.

also been suggested that an index derived from HbA_{1c} and glycated albumin [CLD-HbA_{1c}=0.5 (HbA_{1c}+AG/3)] could be more representative of mean blood glucose [88].

Diabetes and laboratory management

The most efficient medical management of diabetes mellitus, especially as regards the long-term degenerative complications, represents a key challenge not only for patients' well-being and quality of life, but also for economic reasons. The costs of screening, diagnosing diabetes, and ensuring optimal disease control have to be considered in view of the costs of the disease's treatment and the global social consequences for patients with severe degenerative complications, which greatly affect their independence and quality of life. Diabetes mellitus also has a substantial influence per se on the clinical outcomes and/or costs of managing patients with other pathologies, such as elderly patients with heart disease [89, 90].

It has been clearly demonstrated that the costs of improving glycemic control, and thereby delaying degenerative complications, in type 2 diabetic patients are lower than the costs generated by such complications [91]. Diabetic patients' participation in disease management programs is also associated with lower medical costs [92]. A recent paper by Driskell et al. highlighted that glycated hemoglobin assays were inappropriately prescribed in 50% of cases [93], and this justifies the correct use of markers of glycemic control such as HbA_{1c} for predicting all types of cost-generating complications. This applies to both short- and long-term costs [94]. The use of such markers is governed by various clinical practice guidelines, which should thoroughly consider the needs and characteristics of all sub-populations to ensure a better use of healthcare resources [95]. In the case of gestational diabetes mellitus, a recent meta-analysis reviewing relevant publications from 1975 to 2009 has shown that HbA_{1c} could become a credible alternative to OGTT, reducing the burden and cost of identifying women at risk of associated adverse outcomes [96].

However, our current knowledge of the pathophysiology of diabetes may be influenced by new concepts with an impact on screening strategies. To give an example, the identification of a particular amino acid profile could be a promising predictor of the onset diabetes mellitus [97]. Such new strategies might enable the earlier implementation of corrective measures before the disease becomes irreversible.

Conclusions

Much remains to be done in order to optimize the use of laboratory tests for assessing glycemic control in diabetic patients. To our mind, what we still have to do can be summarized as follows.

1. Some manufacturers need to improve their methods because there is an inacceptable bias and/or imprecision with respect to the desired goals.
2. Laboratory professionals should carefully analyze the IFCC certificates before choosing a method for measuring HbA_{1c}.
3. Several manufacturers should improve the information they provide together with their control materials. It is absolutely unacceptable to use control materials with assigned ranges that are wider than the total error limits for the quantity being measured.
4. EQAS providers need to demonstrate the commutability of the materials used in the inter-laboratory exercises; and the HbA_{1c} targets must be assigned by means of reference measurement procedures or by reference laboratory services, as stated by the JCTLM.
5. Further studies are needed to ascertain whether other glycated proteins, such as glycated albumin, are clinically useful for routinely monitoring patients' glycemic status.
6. The quality of glucose measurements obtained by POCT and CGM systems needs to be improved, to make most of the glucose variability indexes more robust and traceable to the laboratory data.
7. It would be advisable to be able to measure red cell age by a simple, automated procedure, in order to reduce a significant component of the between-subjects biological variability and to normalize the HbA_{1c} concentration with respect to cell age.
8. Further studies are warranted to assess the power of HbA_{1c} in diagnosing diabetes under well-controlled conditions (IFCC standardized assays and no confounding factors).

Conflict of interest statement

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