Nutrition, Metabolism & Cardiovascular Diseases (2011) 21, 467–475

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/nmcd

Nutrition, Metabolism & Cardiovascular Diseases

VIEWPOINT

The general use of glycated haemoglobin for the diagnosis of diabetes and other categories of glucose intolerance: Still a long way to go

A. Lapolla [a,*], A. Mosca [b], D. Fedele [a]

[a] *Dipartimento di Scienze Mediche e Chirurgiche, Cattedra di Malattie del Metabolismo, Università degli Studi di Padova, Padova, Italy*
[b] *Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME), Dipartimento di Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Milano, Italy*

Received 26 July 2010; received in revised form 31 January 2011; accepted 6 February 2011

KEYWORDS
Diabetes;
Diagnosis;
HbA_{1c};
Standardisation

Abstract Glycated haemoglobin (HbA_{1c}) is considered the 'gold standard' for monitoring metabolic control in diabetes. An International Expert Committee recently recommended HbA_{1c} as a better method than measurement of glucose to use in the diagnosis of diabetes, based on its strong association with microvascular complications, a lower day-to-day variability and ease of use, not necessarily in the fasting state. These recommendations have been embraced by the American Diabetes Association (ADA), which stated in its Standards of Medical Care in Diabetes 2010 that "A_{1c}, fasting plasma glucose or the 2 h 75 g oral glucose tolerance test (OGTT) are appropriate for testing diabetes and assessing the risk of future diabetes," and that "a confirmed $A_{1c} \geq 6.5\%$ is diagnostic for diabetes." Measuring HbA_{1c} has several advantages over glucose measurements, but its exclusive use should only be considered if the test is conducted under standardised conditions and its limitations are taken into due account. The impact of its use on the epidemiology of diabetes and other categories of glucose intolerance, as seen from recent reports, is also discussed.

After Rahbar discovered a diabetic haemoglobin component in people with diabetes in 1968 [1], it was soon demonstrated that this component had chromatographic characteristics resembling those of HbA_{1c} (glycated haemoglobin), which is a minor haemoglobin component [2]. A series of clinical studies then demonstrated a close relationship between HbA_{1c} and mean plasma glucose in the months preceding the test [3,4], while the Diabetes Control and

* Corresponding author. Dipartimento di Scienze Mediche e Chirurgiche, Servizio di Diabetologia, Complesso Socio-Sanitario ai Colli, Via dei Colli 4, 35143 Padova, Italy. Tel.: +39 049 821 6857; fax: +39 049 821 6838.
E-mail address: annunziata.lapolla@unipd.it (A. Lapolla).

0939-4753/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.numecd.2011.02.006

Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) randomised trials revealed the link between glycaemic control (in terms of HbA_{1c}) and the risk of the onset and progression of chronic diabetic complications [5,6], thus confirming that HbA_{1c} can be considered the 'gold standard' for assessing medium- to long-term glycaemic control in diabetic patients. The most recent American Diabetes Association (ADA) Standards of Medical Care in Diabetes and other International Societies recommended a testing frequency and target values for this parameter according to patients' type of diabetes and glycaemic control [7–10].

HbA_{1c} and diagnosis of diabetes

Nowadays, diabetes is an important health problem in the world. The International Diabetes Federation (IDF) Diabetes Atlas has estimated that in 2010, 285 million people around the world have diabetes, representing nearly 7% of the adult world population [11]. It is well known that almost 30% of people with diabetes go undiagnosed [12], and nearly 25% of them have microvascular complications by the time their diabetes is diagnosed [13]. Every effort must consequently be made to diagnose diabetes and the other forms of glucose intolerance (impaired glucose tolerance (IGT) and impaired fasting glucose (IFG)) as promptly as possible [7]. Until now, diabetes has been diagnosed by measuring fasting plasma glucose (FPG) (threshold $\geq$7 mmol l^{-1}) and/or with the oral glucose tolerance test (OGTT) ($\geq$11 mmol l^{-1}), (see Appendix 1).

In agreement with an International Expert Committee suggestions, the latest ADA Standards of Medical Care in Diabetes 2010 state that "HbA_{1c} is appropriate for diabetes screening ...., a confirmed $HbA_{1c} > 6.5\%$ is diagnostic for diabetes," and that "mixing different methods to diagnose diabetes should be avoided" [7,14].

Advantages

Several considerations point in favour of the use of HbA_{1c} for the diagnosis of diabetes, the most important of which are listed in Table 1. First, patients must have fasted for at least 8 h before taking OGTT or FPG tests, but this does not apply to HbA_{1c} measurements. The HbA_{1c} test results are also less variable than glucose measurements, the within-individual day-to-day variability being <2% for HbA_{1c}, 12–15% for FPG [15,16] and 16.6% for OGTT [17]. For glucose measurements, we also have to consider errors due to the sample's treatment prior to its measurement (because even if blood samples are collected in sodium fluoride to avoid glycolysis, their storage at room temperature even for 1–4 h before the measurement is obtained results in a drop in glucose levels ranging from 0.2 to 0.6 mmol l^{-1} h^{-1}) [18]. In other works, a more dramatic drop in plasma glucose has been reported, on the average of 0.5 mmol l^{-1} h^{-1} (range 0.2–1.0 mmol l^{-1}), with stabilisation occurring after approximately 1 h [19]. HbA_{1c} values, on the other hand, are fairly stable after collection [20]. They are also unaffected by short-term lifestyle changes and, because they provide an estimate of the plasma glucose levels over preceding months, they can give a more accurate idea of an individual's previous glycaemia than FPG or OGTT. Finally, a real advantage stems from using a marker that has been related to the onset of diabetic complications (and microvascular complications in particular) [5,6], and the fact that interventional trials designed to reduce them have focused on HbA_{1c} [5,6,21,22]. HbA_{1c} is consequently familiar to clinicians in this setting because it is currently used to assess prior glycaemic control and to establish therapeutic strategies for diabetic patients.

Limitations

The use of HbA_{1c} has its limitations, as summarised in Table 2, where we have differentiated between the interferences that can in some way be overcome, from the other ones.

As regards the first group, high bilirubin levels may affect some point-of-care testing (POCT) systems, and high triglyceride levels may be a problem in some immunochemical methods, with which high white blood cell counts may also interfere [23]. Some immunochemical methods have overcome this problem by including a detergent capable of lysing the leucocytes in their reagents. An abnormal haemoglobin level may influence the HbA_{1c} measurements, depending on the method used, and not all assays can correct for and/or distinguish between pathological and glycated haemoglobin [24]. The frequency of haemoglobin disorders varies among different races and different countries; for example, almost 10% of African-Americans have a haemoglobin C trait that could interfere with HbA_{1c} assay [24]. The web site of the National Glycohemoglobin Standardization Program (NGSP, http://www.ngsp.org/interf.asp) provides up-to-date information on how the more common haemoglobin variants (HbS, HbC, HbD and HbE) interfere with 20 methods most often used to measure HbA_{1c}, and some methods are totally unaffected by the presence of these variants. Of course, if the patient is a homozygous or double heterozygous for a haemoglobin

Table 1 Advantages in the use of HbA_{1c} for screening and diagnosis of diabetes.

Patient does not need to be fasting
HbA_{1c} concentration is related to the development of complications
HbA_{1c} has a smaller intra-individual biological variability (within 2%) respect to that of plasma glucose
HbA_{1c} is not influenced by sudden glycaemic variations (such as under stress) and reflects the mean plasma glucose levels over the last 2–3 months
HbA1c suffers a limited influence from common drugs known to influence glucose metabolism
HbA_{1c} is already used as an important target for therapy and is familiar to clinicians
The majority of manufactuers of HbA_{1c} kits is already standardized to the current reference systems

Table 2 Limitations in the use of HbA_{1c} for screening and diagnosis of diabetes.

Factors	Risk in the diagnosis
Analytical interferences (could be overcomed by appropriate sample handling or by choosing the most appropriate method for HbA_{1c} quantification)	
a) Hyperbilirubinaemia	overdiagnosis
b) Elevated serum triglycerides	overdiagnosis
c) Increased WBC	overdiagnosis
d) Presence of some hemoglobin variants (HbS, HbC, HbD, HbE)	misdiagnosis
In vivo effects due to physiological conditions (cannot be handled a-priori)	
a) Pregnancy	misdiagnosis
b) Seasonal variations	over- or mis-diagnosis
c) Age	overdiagnosis
d) Genetic determinants (including race)	over- or mis-diagnosis
e) Presence of other hemoglobin variants and/or thal. major	over- or mis-diagnosis
In vivo effects due to pathological conditions (cannot be handled a-priori)	
a) Type 1 diabetes under rapid development	misdiagnosis
b) Malaria	misdiagnosis
c) Haemolytic anaemia	misdiagnosis
d) Iron deficiency	overdiagnosis
e) Recent blood loss, recent transfusion	misdiagnosis
f) Splenectomy	overdiagnosis
g) Renal failure	overdiagnosis
h) HIV positive patients on anti-retroviral therapy	overdiagnosis
i) Erythropoietin and other drugs interacting with erythropoiesis	misdiagnosis
j) Chronic alcohol abuse	misdiagnosis
Other reasons	
a) HbA_{1c} may not be easily available in some countries	
b) Higher imprecision respect to plasma glucose determination	

variant, then HbA_{1c} cannot be measured by any method, simply because there is none to measure in the red blood cells (RBCs).

As for the second group, the glycated haemoglobin levels are lower in pregnancy than in women who are not pregnant [25]; hence, the cut-off of 6.5% currently adopted cannot be applied to these subjects. Few data are available as yet on seasonal variations in HbA_{1c} levels, but it seems they may account for a variation of approximately 7%, with 6-monthly cycles [26]. Age can cause an increase in HbA_{1c} levels (inducing an increase of approximately 0.1% HbA_{1c} units per decade from 40 years onwards), as reported by Pani et al., who also found levels 0.4% higher in 70-year-olds than in 40-year-olds with the same glucose tolerance [27]. Race has also been implicated in variations in HbA_{1c} [28,29], the levels in Afro-Caribbeans being 0.4% higher than in Europeans, [28] and this can affect diabetes diagnosis [8].

The hereditability of native HbA_{1c} is estimated to be approximately 50%, with an approximately balanced contribution from genetic factors directly involved in glycaemic control (e.g., β-cell function, insulin sensitivity and incretin physiology), and from non-glycaemic factors (e.g., RBC glucose transport, deglycation pathways and altered RBC turnover) [30,31]. Other genetic determinants for HbA_{1c} will probably be detected in the near future, but the discovery of such novel genetic contributors to HbA_{1c} regulation seems to be more challenging in treated patients than in native, non-diabetic subjects [32].

Concerning the third group of conditions listed in Table 2, there are also several factors that interfere with HbA_{1c} measurement to bear in mind. All conditions affecting RBC life and/or turnover, such as iron deficiency anaemia, haemolytic anaemia, chronic malaria, blood transfusion, high blood loss, renal failure or human immunodeficiency virus (HIV) treatment (HbA_{1c} is nearly 10% lower on anti-retroviral therapy), can make the results unreliable [33]. As for iron deficiency, a recent evaluation of the data collected for the National Health and Nutrition Examination Survey (NHANES) study from 1999 to 2006 showed that iron deficiency is common in women, and is associated with a shift in HbA_{1c} to higher levels, mainly from $<5.5\%$ to $>5.5\%$ [34]. Furthermore, correcting iron-deficient anaemia in type 1 diabetic patients determines a considerable decrease in HbA_{1c}, for the same glucose values, from a mean of $10.1 \pm 2.7\%$ before iron treatment to a mean of $8.2 \pm 2.7\%$ afterwards [35]. Finally, misdiagnoses are also possible in heavy alcohol drinkers, as alcohol consumption is associated with slightly lower HbA_{1c} concentrations [36].

The unavailability of HbA_{1c} tests in the poorer parts of the world is another obvious drawback to consider, as the currently available point-of-care tests may be useful, but suffer from a number of analytical problems [37]. Most HbA_{1c} assays are significantly less accurate than plasma glucose measurements (i.e., 5.0% vs. 1.0%, expressed as CV) [38], and we have recently emphasised that the method used to assay HbA_{1c} should not exceed the target of 2% for

long-term inaccuracy [39]. At present, however, the majority of the kits available are unable to achieve this target.

Other factors influencing HbA_{1c} levels and its use

HbA_{1c} and glucose variability

The acute glucose fluctuations occurring in diabetes are considered important in the onset of the chronic complication of this disease. Analysing glucose profiles obtained with continuous interstitial glucose monitoring systems (CGMSs) in subjects enrolled as normal controls in the A_{1c} Derived Average Glucose (ADAG) study, based on fasting plasma glucose, produced some interesting findings [40]. First, 93% of all subjects without diabetes exceeded the IGT threshold of 7.8 mmol l^{-1}, for a median 26 min during the day; 7% of them reached glucose concentrations higher than 11.1 mmol l^{-1} during CGMS; and the mean HbA_{1c} in these subjects was 5.3 $\pm$ 0.3%, that is, within the normal range according to the ADA recommendations [7]. Hence, glucose levels (both FPG and HbA_{1c} values) vary considerably even in people classified as normal [41], casting doubts on the capacity of these parameters to diagnose diabetes correctly. Further studies are therefore needed to establish whether the period of hyperglycaemia in people without diabetes can affect the risk of progression of diabetes.

HbA_{1c} and oxidative modifications

Applying mass spectrometry to the study of HbA_{1c} has provided precious information on the kinetics, entity of its glycation and occurrence of oxidative modifications [42].

It is well known that, by activating oxidative stress, hyperglycaemia plays a pivotal role in the development of chronic diabetes complications. Evidence suggests that not only sustained hyperglycaemia, but also postprandial hyperglycaemia and hyperglycaemic spikes induce superoxide overproduction, giving rise to oxidative stress (glyco-oxidation products) [43–45].

Using matrix-assisted ionisation mass spectrometry (MALDI), we showed that both α and β globins are glycated to a similar extent [42]. Glyco-oxidation products, as well as glycated products, are present on both globins, but more abundant in diabetic patients than in normal controls. The best linear relationship was observed in the study where HbA_{1c} values obtained using current high pressure liquid chromatography (HPLC) measurements were plotted against the whole set of glycated and glyco-oxidated species, as measured by MALDI. A subsequent study showed a clearly higher abundance of glyco-oxidation products in type 2 diabetic patients with chronic complications than in cases without complications [46]. The methods currently available for measuring glycated haemoglobin are therefore unable to distinguish between glycated and glyco-oxidated species on HbA_{1c} and, combined with a different individual proclivity to oxidation, this might affect the diagnosis of diabetes.

Cost analysis

The ADA Expert Committee on this topic has yet to report on any cost- and risk–benefit analyses.

The matter can be summed up as follows. In Italy, the National Fee Schedule for the Lombardy Region reports a reimbursement of €12.15 per each HbA_{1c} determination, € 1.70 for each glucose test and € 2.60 for each OGTT. Judging from these costs, HbA_{1c} is much more expensive than glucose testing. In addition, a complete whole blood and bilirubin count, plus a test such as HPLC to rule out any variant haemoglobins, would be needed to be sure that the HbA_{1c} result is free of the most common pre-analytical interferences. Similar considerations have recently been published by Lippi et al. [47].

Some other factors need to be taken into account, however. First, there are the indirect costs for patients, such as loss of income, which could be estimated at nearly 1 h off work to measure fasting glycaemia and at least 3 h for an OGTT. Second, it is worth bearing in mind that, as the demand for HbA_{1c} testing increases, its cost will probably drop.

HbA_{1c} and other categories of glucose intolerance

The International Expert Committee used retinopathy as the outcome for defining the HbA_{1c} level to be taken into account as diagnostic for diabetes, considering cross-sectional epidemiologic studies on Pima Indians, Egyptians and NHANES populations. In these studies, it was shown that the prevalence of retinopathy was increasing substantially with HbA_{1c} values rising from 6% to 7% [48]. Moreover, the recent analysis drawn from the Evaluation of screening and early detection strategies for type 2 diabetes and impaired glucose tolerance (DETECT-2) trial showed, using receiver operating characteristics (ROC) curve analysis, that the optimal cut-off for detecting retinopathy was an HbA_{1c} of 6.5% [14]. The relationship between HbA_{1c} and macroangiopathy, a very well known and worrying complication of diabetes, is less clear. While some studies found no relationship between these two variables, and others found a capacity to predict cardiovascular disease only at values $\geq$6.5%, and only in women [49,50], a few prospective studies have described a positive, continuous relationship between HbA_{1c} levels and cardiovascular disease [51]; in particular, a recent study by Selvin et al., with a 6–16-year follow-up of non-diabetic individuals, found HbA_{1c} associated with the diagnosis of diabetes in the same way as FPG, and associated even more strongly with the risk of cardiovascular disease and death from any cause [52]. It should be emphasised, however, that HbA_{1c} was measured in whole blood samples that had been frozen for more than 10 years and using a DCCT-standardised assay, while traceability to the DCCT only applies to fresh blood samples [53]. Other studies are needed to confirm this relationship, moreover, because risk factors other than glucose are involved in this diabetic complication, as also shown by a recent *post hoc* analysis of the Action to Control in Diabetes (ACCORD) trial [54].

The International Expert Committee, considering that, as for glucose measurement also for HbA_{1c} levels, a continuum of risk for the development of diabetes has been demonstrated [55], identifies people with HbA_{1c} levels between 6.0% and 6.4% as being at high risk of diabetes and recommends that

these people should receive clear effective preventive interventions; but there is no information about the metabolic characteristics of this high-risk class. The only report from Lorenzo et al. [56] involving 855 subjects participating in the Insulin Resistance Atherosclerosis Study, showed that HbA_{1c} is less sensitive in detecting individuals at risk of diabetes than FPG or OGTT, and that it has a lower correlation with insulin resistance and secretion than FPG or 2-h PG. The possibility that the consistency between PG and HbA_{1c} is the same as the level of agreement between FPG and 2-h PG, as demonstrated by the Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe (DECODE) study, also needs to be confirmed [57].

Furthermore, a low-risk class has been added, that is, below the threshold of 6.0%.

However, what is the real risk of diabetes developing later on in these two new risk populations? Indeed, recent studies have reported a greater risk of diabetes developing at lower glycaemic levels than at those considered normal [58,59]. A recent systematic review of seven studies examining incident diabetes across a range of HbA_{1c} levels between 5% and 6.5% showed that HbA_{1c} levels between 6.0% and 6.5% are associated with a 25–50% of diabetes over 5 years, while levels between 5.5% and 6.0% are associated with a 9–25% incidence and levels between 5.0% and 5.5% with an incidence of <9% [60]. The review did not compare potential differences in the incidence of diabetes associated with different glycaemia tests (i.e., HbA_{1c}, FPG and OGTT); hence, further studies will be necessary to establish whether HbA_{1c} is more suitable for predicting diabetes than the other tests currently used.

HbA_{1c} and diagnosis of diabetes: epidemiological studies

A number of studies have recently been published on this issue and some of them warrant consideration (Table 3, Appendix 2). 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 the OGTT.

HbA_{1c} standardisation

The effort to standardise the HbA_{1c} test dates back at least 30 years. The most recent and comprehensive approach is the one promoted by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) that

Table 3 A summary of the clinical utility of HbA_{1c} testing in the diagnosis of diabetes, as from the recent literature available.

Author, year of publication	N°/study	Country	Ref test	HbA_{1c} cut off	Sensitivity	Specificity
Saudek et al., 2008 [61]	NHANES 1999–2004	US	FPG	**≥6.5**	**44.3%**	**99.6%**
	NHANES III	US	FPG	**≥6.5**	**42.8%**	**99.6%**
Ng et al., 2010 [62]	1135	UK	OGTT	19.9% of positive OGTT present HbA1c <6.0%		
Lu, 2010 [66]	2494 MP	Australia	OGTT	**≥6.5**	–	**88.8%**
	6014 Aus Diab	Australia	OGTT	**≥6.5**	–	**99.9%**
Van't Riet, 2010 [63]	2753 new Hoorn Study	Netherland	OGTT	**≥6.5**	**24%**	**99%**
Carson, 2010 [65]	6890 subgroup of NHANES 99-06	US	FPG	**≥6.5**	**49.9%**	**99.5%**
Kramer, 2010 [64]	2107 Rancho Bernardo Study	US	OGTT	**≥6.5**	**44%**	**79%**
	Age quartiles		OGTT	**≥6.5**	**52%**	**95%**
Zhou, 2010 [67]	2332	China	OGTT	**≥5.6**	**64.4% M**	**61.6% M**
				≥5.6	**62.3 % F**	**63.3% F**
Christensen, 2010 [68]	Inter99 Study	Denmark	OGTT	**≥6.5**	**42.6%**	–
	Whitehall II Study	UK	OGTT	**≥6.5**	**25%**	–
	Aus Diab	Australia	OGTT	**≥6.5**	**17%**	–
	Inuit Health in Transition Study	Greenland	OGTT	**≥6.5**	**29.6%**	–
	Black population	Kenya	OGTT	**≥6.5**	**20%**	–
	CURES Study	India	OGTT	**≥6.5**	**78%**	–
	23094	All	OGTT	**≥6.5**	**43.5%**	–
Olson, 2010 [69]	SIGT (1581), NANHES III (2057), NANHES 2005-2006 (1154)	US	OGTT	**≥6.5**	**30 %**	**88-97%**

Table 4 Recommendations for the implementation of international standardization of HbA_{1c} in Italy.

Topic	Recommendation
Analytical error limits laboratory practice	The goal of the total allowable error on a singe HbA_{1c} measurement should not exceed $\pm$ 6.7% (as a percentage fraction of the absolute HbA_{1c} value). The long-term imprecision of the method should be no more than 2%. An adequate internal Quality program has to be used to ensure that this imprecision goal be met by the HbA1c method. Participating in EQAS programs, in which commutable materials are used, and adopting HbA_{1c} values assigned using the IFCC reference measurement procedure, are the best way to establish whether measurements that are obtained meet the standards for total error stated above.
Calibration	HbA_{1c} should be measured using methods calibrated to the IFCC reference system.
Reporting units	The result should be reported in mmol/mol and % units derived using the master conversion equation [43].
Timing	Beginning 01 January 2010, HbA_{1c} results will be expressed in units aligned with the DCCT system (%) and in standardized IFCC units (mmol/mol). Next, beginning on 01 January 2012, HbA_{1c} results will be expressed in IFCC units (mmol/mol) only.

addressed this issue in 1995 by creating a working group committed to this topic (IFCC $WGHbA_{1c}$). Thanks to the activities of the $WGHbA_{1c}$, a reference measurement procedure is now available [70], two primary reference materials have been produced [71], an international network of reference laboratories for HbA_{1c} measurements has been created [72] and master equations have been developed to convert the findings obtained with the IFCC reference system into results aligned with the system of the NGSP [73].

The standardised measurement of HbA_{1c} is now a well-defined matter, agreed on a worldwide basis since the consensus document was signed in 2007 by officers from the ADA, the European Association for the Study of Diabetes (EASD), the IDF and the IFCC [74]. This document, like the one that followed (published by representatives of the IFCC and the companies that manufacture this test) [75], states that all manufacturers of HbA_{1c} diagnostic tests should have been aligned to the IFCC reference system by 31 December 2009, at the latest.

What now remains to be accomplished is the deployment of this reference system at the end-user level (at public health laboratories and diabetes treatment centres, amongst general practitioners and patients). Different actions have been taken in different countries, and no comprehensive report on this topic is available as yet. A working group was created in Italy for this purpose, and it has issued some recommendations [39], the most important of which are listed in Table 4.

In the near future, a great effort will be made to promote an information campaign intended for all stakeholders (medical laboratories, general practitioners, diabetologists and diabetic patients and all medical specialists involved in managing diabetic patients) to ensure a synchronous and smooth transition to the new IFCC reference system throughout the country. Various indicators will be used in the long term to ensure that all (or at least most) laboratory professionals use the IFCC standardised method within the target margins of error. Laboratory professionals should ask the manufacturers of diagnostic kits for evidence of their alignment to the IFCC reference system to ensure consistency with the reference system and to establish the related uncertainty right from the production process onwards.

Conclusions

Looking back at the balance between the pros and cons of using HbA_{1c} in the diagnosis and screening of diabetes, the weaknesses seem to outweigh the benefits. For the time being, the lack of a cost—effect analysis, the limited availability of the assay in the less developed countries, the epidemiological studies showing small numbers of people being diagnosed with diabetes when this method was used, and racial variability, make it difficult to use HbA_{1c} to diagnose diabetes all over the world. Moreover, evidence of the standardisation of the HbA_{1c} assay may be clear in some countries, but not in others, wherein independent quality audits are not mandatory.

We would therefore discourage the use of HbA_{1c} for diagnosing diabetes for the time being, particularly with the proposed cut-off of 6.5%, unless co-morbidities can be ruled out, the person is under 70-years-old and the method is IFCC standardised and/or NGSP aligned. The HbA_{1c} test should not be used to diagnose diabetes in pregnancy.

We are nonetheless convinced that using HbA_{1c} to rule out diabetes in subjects at risk of developing the disease could significantly reduce the number of other tests (e.g., OGTT) performed and result in a better diagnostic sensitivity and specificity (providing the assay is standardised). It is also worth noting that HbA_{1c} can be higher in 'normal' individuals (FPG < 126 mg dl^{-1} and 2 h OGTT < 140 mg dl^{-1}) with an altered daily glucose profile (41), suggesting that it might be an earlier marker of dysglycaemia than FPG or OGTT.

Another relevant point, in our opinion, remains the weakness of adopting more than one diagnostic criterion to diagnose diabetes. It would be advisable to use just one test to diagnose diabetes the world over because this would enable a better comparison of epidemiological data; it would seem unfeasible, however, because diabetes is a very heterogeneous disease. The only recommendation that can feasibly be made is therefore that patients be tested for diabetes using only one test (i.e., FPG), and that the results be confirmed using the same test at any future visits to avoid the difficulty of interpreting different test results.

Acknowledgement

This work was supported by a grant from the Italian Ministry for the University and Research (PRIN 2008).

Appendix
Supplementary material

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.numecd.2011.02.006.

References

[1] Rahbar S. An abnormal haemoglobin in red cells of diabetes. Clin Chim Acta 1968;22:296—8.
[2] Schnek A, Schroder W. The relation between the minor components of whole normal human adult haemoglobin as isolated by chromatography and starch block electrophoresis. J Am Chem Soc 1961;83:1472—8.
[3] Gabbay K. Glycosylated haemoglobin and long-term blood glucose control in diabetes mellitus. J Clin Endocrinol Metab 1977;44:859—64.
[4] Boronat M, Saavedra P, Varillas VF, Nòvoa FJ. Use of confirmatory factor analysis for the identification of new components of the metabolic syndrome: the role of plasminogen activator inhibitor-1 and haemoglobin A_{1c}. NMCD 2009;19:271—6.
[5] DCCT 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.
[6] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837—53.
[7] American Diabetes Association. Standards of medical care in diabetes 2010. Diabetes Care 2010;33(Suppl. 1):62—9.
[8] AMD, Sid, Diabete Italia. Standard Italiani per la cura del diabete mellito. Linee guida e raccomandazioni. Infomedica Ed, 2009—2010.
[9] International Diabetes Federation. Global Guideline for type 2 diabetes. 2005.
[10] Ceriello A, Colagiuri S, Gerich J, Tuomilehto J. Guideline for management of postmeal glucose. NMCD 2008;18:S17—33.
[11] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4—14.
[12] Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelau MM, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health and Nutrition Survey 1999—2002. Diabetes Care 2006;29:1263—8.
[13] Harris MI. Undiagnosed NIDDM: clinical and public health issues. Diabetes Care 1993;16:642—52.
[14] The International Expert Committee. International Expert Committee report on the role of the A_{1c} assay in the diagnosis of diabetes. Diabetes Care 2009;33:1327—34.
[15] Ollerton RL, Playe R, Ahmed K, Dunstan FD, Luzio SD, Owens DR. Day-to-day variability of fasting plasma glucose in newly diagnosed type 2 diabetic subjects. Diabetes Care 1999;22:394—8.
[16] Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2002;48:436—72.
[17] Selvin E, Craniceanu C, Brancati F, Coresh J. Short term variability in measures of glycemia and implications for the classification of diabetes. Arch Intern Med 2007;167:1545—51.
[18] Bruns DE, Knowler WC. Stabilization of glucose in blood samples: why it matters. Clin Chem 2009;55:850—2.
[19] Gambino R. Glucose: a simple molecule that is not simple to quantify. Clin Chem 2007;53:2040—1.
[20] Little RR, Rohlfing CL, Tennill AL, Connolly S, Hanson S. Effects of sample storage conditions on glycated haemoglobin measurements; evaluation of five different high performance liquid chromatography methods. Diabetes Technol Ther 2007;9:36—42.
[21] Nathan DM, Zinman B, Cleary PA, Backlund JY, Genuth S, Miller R, et alDiabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group. Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complication experience (1983—2005). Arch Intern Med 2009;169:1307—16.
[22] Holman RR, Sanjoy KP, Bethel MA, Matthews DR, Neil AW. 10-Year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577—89.
[23] Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycosylated haemoglobin: methodologies and clinical applications. Clin Chem 1986;32(10b):B64—78. Proceedings of the 9th Annual Arnold O. Beckman Conference. Diabetes Mellitus: from theory to therapy.
[24] Roberts WL, Safa-Pour S, De BK, Rohling CL, Weykamp CW, Little RR. Effects of haemoglobin C and S traits on glycohemoglobin measurements by eleven methods. Clin Chem 2005;51:776—8.
[25] Mosca A, Paleari R, Dalfrà M, Di Cianni G, Cuccuru I, Pellegrini G, et al. References Intervalsf or hemoglobin A_{1c} in pregnant women: data from an Italian Multicenter study. Clin Chem 2006;52:1138—43.
[26] Garde AH, Hansen AM, Skovgaardt LT, Christensen JM. Seasonal and biological variation of blood concentrations of total cholesterol, dehydroepiandrosterone sulfate, hemoglobin A_{1c}, IgA, prolactin and free testosterone in healthy women. Clin Chem 2000;46:551—9.
[27] Pani L, Korenda L, Meigs J, Driver C, Chamany S, Fax C, et al. Effect of aging on A_{1c} levels in individuals without diabetes: evidence from the Framingham Offspring study and the national health and nutrition examination survey 2001—2004. Diabetes Care 2008;31:1991—6.
[28] Herman W, Ma Y, Uwaifo G, Haffner S, Kahn S, Horton E, et al. Differences in A_{1c} by race and ethnicity among patients with impaired glucose tolerance in the diabetes prevention program. Diabetes Care 2007;30:2453—7.
[29] Ziemer DC, Kolm P, Weintraub WS, Vaccarino V, Rhee MK, Twombly JG, et al. Glucose-independent, black-white differences in haemoglobin A_{1c} levels: a cross-sectional analysis of 2 studies. Ann Intern Med 2010;152:770—7.
[30] Kera PK, Joiner CH, Carruthers A, Lindsell CJ, Smith EP, Franco RS, et al. Evidence for interindividual heterogeneity in the glucose gradient across the human red blood cell membrane and its relationship to haemoglobin glycation. Diabetes 2008;57:2445—52.
[31] Delpierre G, Veiga-da-Cunha M, Vertommen D, Buysschaert M, Van Schaftingen E. Variability in erythrocyte fructosamine 3-kinase activity in humans correlates with polymorphisms in the FN3K gene and impacts on haemoglobin glycation at specific sites. Diabetes Metab 2006;32:31—9.
[32] Florez JC. A genome-wide association study of treated A_{1c}. A genetic needle in an environmental haystack. Diabetes 2010;59:332—4.

[33] Gallagher EJ, Le Roith D, Bloomgarden Z. Review of haemoglobin A_{1c} in the management of diabetes. J Diabetes 2009;1:9—17.
[34] Kim C, McKeever Bullard K, Herman WH, Beckles GL. Association between iron deficiency and A_{1c} levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999—2006. Diabetes Care 2010;33:780—5.
[35] Tarim Ö, Küçükerdorgan A, Günay Ü, Eralp Ö, Ercan I. Effects of iron deficiency anemia on haemoglobin A_{1c} in type 1 diabetes mellitus. Pediatr Int 2002;41:357—62.
[36] Selvin E, Zhu H, Brancati FL. Elevated A_{1c} in adults without a history of diabetes in the U.S. Diabetes Care 2009;32: 828—33.
[37] Lenters-Westra E, Slingerland RJ. Six of eight haemoglobin A_{1c} point-of-care instruments do not meet generally analytical performance criteria. Clin Chem 2010;56:44—52.
[38] Sacks DB. The diagnosis of diabetes is changing: how implementation of haemoglobin A_{1c} will impact clinical laboratories. Clin Chem 2009;55:1612—4.
[39] Mosca A, Branca MT, Carta M, Genna ML, Giorda CB, Ghidelli R, et al. Recommendations for the implementation of International standardization of glycated hemoglobin in Italy. GLAD Working Group (A_{1c} delegates WG). Clin Chem Lab Med 2010; 48:623—6.
[40] Natahn DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine R, for the A_{1c} Derived Average Glucose (ADAG) Study Group. Translating the A_{1c} assay into estimated average glucose values. Diabetes Care 2008;31:1473—8.
[41] Borg R, Kuenen JC, Zheng H, Nathan DM, Heine RJ, Nerup J, et al. On behalf of the ADAG study group. Real-life glycaemic profiles in non-diabetic individuals with low fasting glucose and normal HbA_{1c}: the A_{1c}-Derived Average Glucose (ADAG) study. Diabetologia 2010;53:1608—11.
[42] Lapolla A, Fedele D, Traldi P. The role of mass spectrometry in the study of non-enzymatic protein glycation in diabetes. Mass Spectrom Rev 2000;19:279—304.
[43] Giugliano D, Ceriello A, Esposito K. Glucose metabolism and hyperglicemia. Am J Clin Nutr 2008;87(Suppl.):217S—22S.
[44] Ceriello A, Quagliaro L, Catone B, Pascon R, Piazzola M, Bris B, et al. Role of hyperglycemia in nitrotyrosine postprandial generation. Diabetes Care 2002;25:1439—43.
[45] Quagliaro L, Picone L, Assaloni R, Martinelli L, Motz E, Ceriello A. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of poly(ADPribose) polymerase. J Thromb Haemost 2004;2(8):1453—9.
[46] Lapolla A, Tubaro M, Fedele D, Reitano R, Aricò NC, Ragazzi E, et al. A matrix assisted laser desorption/ionization mass spectrometry study of the non-enzymatic glycation products of human globins in diabetes Rapid Commun. Mass Spectrom 2005;19:162—8.
[47] Lippi G, Targher G. Glycated haemoglobin (HbA_{1c}): old dogmas, a new perspective? Clin Chem Lab Med 2010;48:609—14.
[48] The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997;20:1183—97.
[49] Chonchol M, Katz R, Fried LF, Sarnak MJ, Siscovic DS, Newman AB, et al. Glycosylated hemoglobin and the risk of death and cardiovascular mortality in the elderly. Nutr Metab Cardiovasc Dis 2010;20:15—21.
[50] Cederberg H, Saukkonen T, Laakso M, Jokelainen J, Härkönen P, Timonen M, et al. Postchallenge glucose, HbA_{1c}, and fasting glucose as predictors of type 2 diabetes and cardiovascular disease: a 10-year prospective cohort study. Diabetes Care; 2010 [Publish ahead of print].
[51] Unwin N, Shaw J, Zimmet P, Alberti GMM. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. Diabetic Med 2002;19: 708—23.
[52] Selvin E, Steffes M, Zhu H, Matsushita K, Wagenknecht L, Pankow J, et al. Glycated hemoglobin, diabetes and cardiovascular risk in nondiabetic adults. New Engl J Med 2010;362: 800—11.
[53] National Glycohemoglobin Standardization Program. List of NGPS certified method (updated, March 2010) at http:www//ngsp.org/prog/index.html.
[54] Riddle MC, Ambrosius WT, Brillon DJ, Buse JB, Byington RP, Cohen RM, et al. For the Action to Control in Diabetes (ACCORD) Investigators. Epidemiologic relationships between A_{1c} and all-cause mortality during a median 3.4-year follow-up of glycemic treatment in the ACCORD trial. Diabetes Care 2010;33:983—90.
[55] Droumaguer C, Balkau B, Simon D, Caces E, Tichet J, Charles MA. Use of HbA_{1c} in predicting progression to diabetes in French men and women data form an epidemiological study on the insulin resistance syndrome (DESIR). Diabetes Care 2006;29:1619—25.
[56] Lorenzo C, Wagenknecht LE, Hanley AJG, Rewers MJ, Karter AJ, Haffner SM. Hemoglobin A_{1c} between 5.7 and 6.4% as a marker for identifying pre-diabetes, insulin sensitivity and secretion, and cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study (IRAS). Diabetes Care; 2010 [Publish ahead of print].
[57] Hyvarinen M, Qiao Q, Tuomilehto J, Laatikainen T, Heine RJ, Stehouwer CDA, et al. Hyperglycemia and stroke mortality: comparison between fasting and 2-h glucose criteria. Diabetes Care 2009;32:348—54.
[58] Tirosh A, Shai I, Tekes-Manova D, Israeli E, Pereg D, Shochat T, et al. Normal fasting plasma glucose levels and type 2 diabetes in young men. N Engl J Med 2005;335:1454—62.
[59] Nichols G, Hillier TA, Brown JB. Normal fasting plasma glucose and risk of type 2 diabetes diagnosis. Am J Med 2008;121:519—24.
[60] Zhang X, Gregg EW, Williamson DF, Barker LE, Thomas W, Bullard McK K, et al. A_{1c} level and future risk of diabetes: a systematic review. Diabetes Care 2010;33:1665—73.
[61] Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look to screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab 2008;93:2447—53.
[62] Ng JM, Dawson AJ, Cox H, Atkin SL, Kilpatric ES. New recommendations in diagnosis of diabetes mellitus from the Department of Health: comparing the old and new. Diabetic Med 2010;27:244—5 .
[63] Van'r Riet E, Alssema M, Rijkelijkhizen JM, Kostense PJ, Nijpels G, Dekker JM. Relationship between A_{1c} and glucose levels in the general Dutch population. Diabetes Care 2010;33:61—6.
[64] Kramer CK, Araneta MRG, Barrett-Connor E. A_{1c} and diabetes diagnosis: the Rancho Bernardo study. Diabetes Care 2010;33: 101—3.
[65] Carson AP, Reynolds K, Fonseca VA, Munter P. Comparison of A_{1c} and fasting glucose criteria to diagnose diabetes among U.S. adults. Diabetes Care 2010;33:95—7.
[66] Lu ZH, Walker KZ, O'Dea K, Sikaris KA, Shaw JE. A_{1c} for screening and diagnosis of type 2 diabetes in routine clinical practice. Diabetes Care 2010;33:817—9.
[67] Zhou X, Pang Z, Gao W, Wang S, Zhang L, Ning F, et al. Performance of HbA_{1c} and fasting capillary blood glucose test for screening newly diagnosed and pre-diabetes defined by OGTT in Quigdao, China. Diabetes Care 2010;33:545—50.
[68] Christensen DL, Witte DR, Kaduka L, Jorgensen ME, Borch-Johnsen K, Mohan V, et al. Moving to an HbA_{1c} based diagnosis of diabetes has a different impact on prevalence in different ethnic groups. Diabetes Care 2010;33:580—2.
[69] Olson DE, Rhee MK, Herrick K, Ziemer DC, Twombly JG, Phillips LS. Screening for diabetes and prediabetes with proposed A_{1c}-based diagnostic criteria. Diabetes Care 2010;33:2184—9.

[70] Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, et al. Approved IFCC reference method for the measurement of HbA_{1c} in human blood. Clin Chem Lab Med 2002;40:78—89.
[71] Finke A, Kobold U, Hoelzel W, Weykamp C, Miedema K, Jeppsson JO. Preparation of a candidate primary reference material for the international standardisation of HbA_{1c} determinations. Clin Chem Lab Med 1998;36:299—308.
[72] Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC reference measurement system for HbA_{1c}: a 6-year progress report. Clin Chem 2008;54:240—8.
[73] Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, et al. IFCC reference system for measurement of hemoglobin A_{1c} in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166—74.
[74] Consensus Statement. Consensus statement on the worldwide standardization of the hemoglobin A_{1c} measurement. Diabetes Care 2007;30:2399—400.
[75] Report. Implementation of standardization of HbA_{1c} measurement. Clin Chem Lab Med 2008;46:573—4.