



Haemoglobin A_{1c} in the diagnosis and monitoring of diabetes mellitus

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ABSTRACT

Haemoglobin A_{1c} (HbA_{1c}) is due to celebrate its 40th birthday. Many people would argue that the clinical studies relating the test to diabetes complications while in its late 20s are likely to be its finest ever achievement. However, this article looks at how HbA_{1c} has matured since then and discusses in detail how its many strengths and idiosyncrasies as a marker of glycaemic risk have, as a 30-something, become more clearly understood. As HbA_{1c} approaches middle age, this paper also describes how the test appears to be developing a mid-life crisis, as debate over how its results should be expressed seems likely to divide opinion among clinicians for some time to come.

A BRIEF EARLY HISTORY OF HbA_{1c}

In 1962, Huisman and Dozy reported an increase in one of the minor fractions of haemoglobin in four of their patients with diabetes.¹ They attributed this to all the subjects taking the oral hypoglycaemic drug tolbutamide, but attempts to reproduce this phenomenon in vitro proved unsuccessful. Five years later Rahbar rediscovered this fraction in two patients with diabetes being screened for abnormal haemoglobins. Further investigation found another 47 cases of the abnormal band, all occurring in patients with poorly controlled diabetes, and thus the finding of a “diabetic haemoglobin component” was reported in 1968.² Soon it was demonstrated that the diabetic component had a chromatographic characteristic similar to that of haemoglobin A_{1c} (HbA_{1c}), which is a minor haemoglobin component described by Schnek and Schroeder in 1961 and found in non-diabetic adults in a proportion of 1–4%.³ Structural studies later established that the haemoglobin found in patients with diabetes was indeed identical to HbA_{1c}.⁴

HbA_{1c} AS AN INDICATOR OF GLYCAEMIC CONTROL

The relationship between HbA_{1c} and mean blood glucose

It took almost a further decade before clinical studies started to emerge suggesting that the increased proportions of HbA_{1c} in diabetes patients could be used as a reliable index of glycaemic control over the preceding weeks and months. It is indicative of the tools available at that time to assess glycaemic control that glycated haemoglobin was compared with 24-hour urinary glucose excretions,⁵ plasma “glucose brackets”,⁶ daily mean plasma glucose⁷ and the area under the curve of the glucose tolerance test.⁸ Following these studies there was rapid acceptance of

glycated haemoglobin as a useful tool to objectively assess the prior glycaemic control of patients with type 1 and type 2 diabetes.

In some respects the general approval given to the use of the test at that time was ahead of evidence that it reliably reflected the mean glucose of patients with diabetes. However, the relationship was able to be more clearly defined by both the feasibility study of the Diabetes Control and Complications Trial (DCCT) and the subsequent full trial that followed.^{9–10} This was because the patients in the two treatment groups (intensively treated and conventionally treated) not only had HbA_{1c} recorded at quarterly intervals, but had a 7-point (pre- and postprandial) glucose profile collected and subsequently measured by a laboratory.¹¹ As the 1441 patients participated in the trial for an average of 6.5 years, it was possible to make 26 056 comparisons between HbA_{1c} and a full 7-point profile.¹² The linear relationship found (that mean plasma glucose (MPG, mmol/l) = 1.98 × HbA_{1c} (DCCT) – 4.29, *r* = 0.82) has since been used as the most accurate guide to clinicians and other healthcare workers when discussing glycaemic control with their patients.¹² Nevertheless, the scatter of patients around this regression line meant that an individual with a MPG of, say, 10 mmol/l, could have an HbA_{1c} anywhere between about 6% and 11%, and this has obvious implications when using solely HbA_{1c} to set glycaemic targets. More recently, the single linear relationship between MPG and HbA_{1c} has been called into question in that it appeared different between the two treatment groups of the DCCT such that the MPG was 1.2 mmol/l lower at 7% HbA_{1c} in intensively treated patients than in conventionally treated patients, with the difference becoming 4.6 mmol/l at 11% HbA_{1c}.¹³ This inferred that the relationship between MPG and HbA_{1c} may not constant but could differ depending on the glycaemic control of the population being studied.

Between 2006 and 2007, patients have been recruited to the “mean blood glucose study”, which aims to establish the relationship between HbA_{1c} and blood glucose as definitively as possible.¹⁴ At the European Association for the Study of Diabetes conference in September 2007, preliminary results of the study in 427 patients were presented. The study used continuous glucose monitoring and traditional pre- and postmeal glucose meter testing to establish the mean glucose of the patients, and used a National Glycohemoglobin Standardization Program (NGSP)-certified method to measure HbA_{1c}. The relationship between MPG and HbA_{1c} was found

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to be closer than in the DCCT, with a correlation coefficient of 0.91, and more in keeping with the findings of a smaller recent study ($r = 0.90$) that also used continuous glucose monitoring.¹⁵ However, it remains to be seen from any final publication how representative these mean blood glucose study patients are of the diabetes population as a whole, since there were many exclusion criteria to the study that could have removed the very people who might account for much of the scatter around the regression line.

Time course of HbA_{1c} formation

Glycation of haemoglobin occurs over the entire 120-day lifespan of the red cell,¹⁶ but within this 120 days recent glycaemia has the largest influence on the HbA_{1c} value.¹⁷ Indeed, theoretical models and clinical studies suggest that a patient in stable control will have 50% of their HbA_{1c} formed in the month prior to sampling, 25% in the month before that, and the remaining 25% in months 2–4.¹⁸ This explains why, traditionally, HbA_{1c} has been thought to represent average glycaemia over roughly the last 6–8 weeks.¹⁹

Effect of glucose variability on HbA_{1c}

Until recently there has been very little evidence to establish whether or not two patients with the same mean blood glucose but very different glucose variability would have similar HbA_{1c} values. However, two recent studies—one using DCCT data—have shown that glucose instability seems to have little influence on the HbA_{1c} result, and rather that it is the mean glucose that appears to be the main determinant, not how that mean is arrived at.^{20 21}

HbA_{1c} AND DIABETES COMPLICATIONS

The clinical utility of HbA_{1c} as a tool to assess the risk of diabetes complications was cemented by the publication of the results of the aforementioned DCCT¹⁰ and also the United Kingdom Prospective Diabetes Study (UKPDS),²² these studies set out to establish the effect of intensive (as compared with conventional) glycaemic control on the development of microvascular complications in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) patients respectively. The original findings from these studies in relation to HbA_{1c} have been reviewed in this journal previously²³ and are only summarised here. Developments since then are included in more detail.

Microvascular complications

The microvascular (small vessel) complications of diabetes comprise retinopathy, nephropathy and neuropathy. Patients with diabetes who develop these conditions constitute a large proportion of all subjects who develop blindness, renal failure and/or require limb amputation. The DCCT found that when 1441 patients with type 1 diabetes were randomised to “intensive” rather than “conventional” treatment, their median HbA_{1c} was 7.3% compared with 9.1% throughout the 6.5 years average follow-up period. The subsequent risk of developing retinopathy in the intensively treated group was reduced by 76%, the risk of developing proteinuria was reduced by 54%, and the risk of clinical neuropathy was reduced by 60%.¹⁰ Looked at from the perspective of HbA_{1c}, the risk of microvascular complications in the two patient groups rose exponentially as the HbA_{1c} value increased,²⁴ with no threshold—short of normal glycaemia—below which patients with type 1 diabetes did not develop microvascular complications at all.²⁵

The publication of the UKPDS in 1998 confirmed that a relationship between HbA_{1c} and microvascular complication risk existed in 3867 patients with type 2 diabetes.²² The difference in HbA_{1c} between the intensive and conventional treatment groups was not as large as in the DCCT (HbA_{1c} 7.0% versus 7.9% over 10 years), but there was still a 25% reduction in microvascular risk. A subsequent analysis of the data has shown that when the two treatment groups are combined, then a similar exponential relationship between rising HbA_{1c} and rising microvascular risk exists as in the DCCT.²⁶

After the end of the DCCT, 96% of the patients in the original study agreed to continue to be followed up in a new study known as the Epidemiology of Diabetes Interventions and Complications study.²⁷ They were no longer in two separate treatment groups. In fact, following the outcomes of the DCCT, it was recommended that all patients follow an intensive treatment regime. It was therefore interesting that, out of a clinical trial scenario, the HbA_{1c} of the previously intensively treated patients rose to an average of approximately 8%, while that of the conventionally treated group tightened up to a similar value.²⁷ Long-term follow-up of these patients has shown that the benefits of improved glycaemic control during the DCCT on the risk of microvascular complications are maintained in the long term despite the convergence of glycaemia at the end of the original DCCT trial.^{28–30} This observation—that glycaemia from several years previously influences subsequent long-term complication risk—has since been dubbed “metabolic memory”³¹ and has reinforced the importance of good glycaemic control as soon as possible after the diagnosis of diabetes in order to avoid subsequent problems.

Macrovascular disease

Large vessel (macrovascular) disease remains the major cause of morbidity and mortality in patients with diabetes, with those having type 1 diabetes being at as high a risk as those with type 2.^{32–34}

In type 1 diabetes, the DCCT found an excess of macrovascular events in the conventional compared with the intensive group (40 versus 23), although this just failed to reach statistical significance ($p = 0.08$).³⁵ The small number of events were undoubtedly related to the young age (median 27 years at entry) of the study population. Subsequently, the Epidemiology of Diabetes Interventions and Complications study has shown that during a mean 17 years of follow up of these patients, 46 cardiovascular disease events occurred in 31 patients who had received intensive treatment in the DCCT, as compared with 98 events in the 52 patients who had received conventional treatment, meaning that intensive treatment reduced the risk of any cardiovascular event by 42% ($p = 0.02$).³⁶ Detailed analysis showed it was the mean HbA_{1c} value during the DCCT that explained a large part of this beneficial effect on cardiovascular risk. Again, like the development of microvascular complications, this showed how vital it is to have good glycaemic control early after a diagnosis of (at least) type 1 diabetes. Also, even though HbA_{1c} was unable to predict macrovascular complications during the original DCCT study period, it has recently been shown that mean blood glucose did predict macrovascular complications, and this again reasserts the need for early tight glucose management.³⁷

In the UKPDS, the event rate among the patients with type 2 diabetes was higher than in the DCCT, but the HbA_{1c} separation between the two groups was less marked. Nevertheless, there was a suggestion of more myocardial infarctions among conventionally treated patients

($p = 0.052$).²² In a subsequent analysis, where the two treatment groups were combined, there was an overall relationship between rising HbA_{1c} and increasing risk of myocardial infarction.²⁶

Even among individuals not known to have diabetes, a large epidemiological study has provided evidence that HbA_{1c} may also be a marker of cardiovascular risk within the general population.³⁸

Glycaemic variability and complication risk

As mentioned above, the fact that HbA_{1c} can give an integrated measure of mean glucose can be an advantage by balancing out the glycaemic excursions of an individual. However, since there is evidence from basic clinical research that glucose variability could, itself, be an independent risk factor for diabetes complications,^{39 40} there have been concerns that this may not be reflected by HbA_{1c} measurement alone.⁴¹ As it transpires, analysis of the DCCT dataset using the 7-point glucose profiles during the study have not confirmed any risk conferred by glycaemic variability above that already predicted by the mean glucose.^{42 43} In the UKPDS, although blood glucose was not measured throughout the day it might have been expected for insulin-treated patients to have greater glucose oscillations than non-insulin-treated patients and so have a different rate of complications, but such a difference did not appear to exist. Together, it means that this potential limitation of HbA_{1c} may not be as relevant as first envisaged. Since it would now be extremely difficult to perform a prospective study looking at the effect of glucose variability on complication risk that could adequately take account of other confounders, such as antihypertensive, antiplatelet and lipid lowering drugs, it may be that this particular question will never be definitively answered.

In contrast to glucose variability, analysis of the Pittsburgh Epidemiology of Diabetes Complications Study has suggested that HbA_{1c} variability itself could contribute to an increased macrovascular risk amongst patients with type 1 diabetes, perhaps indicating that long-term fluctuations in glucose control influence complications more than short-term fluctuations.⁴⁴

HbA_{1c} and hypoglycaemia

Hypoglycaemia is the main barrier that prevents patients with diabetes achieving “normal” glucose control.⁴⁵ Unfortunately, it remains a very significant cause of physical morbidity⁴⁶ and mortality⁴⁷ in patients, and recurrent episodes are often associated with psychological, quality-of-life, driving and employment issues.⁴⁸ Perhaps surprisingly, few studies have been able to establish a relationship between low HbA_{1c} values and an increased risk of hypoglycaemia, but the DCCT certainly demonstrated an exponentially increasing risk as HbA_{1c} values fell.^{10 49 50} A recent analysis of the same study has also shown that the mean glucose value and the degree of glucose variability in a patient each independently added to the predictive ability of HbA_{1c}.⁵¹ It should be noted, however, that there is much more to hypoglycaemia risk than just these glycaemic markers, with features such as a prior history of hypoglycaemia or prolonged duration of diabetes also exerting a large influence.

HbA_{1c} TARGETS

The DCCT and the UKPDS have allowed a more “evidence-based” approach to be taken to the target recommendations for HbA_{1c} in patients with type 1 and type 2 diabetes. Prior to these

studies, European guidelines tried to account for the lack of standardisation in glycated haemoglobin measurement by comparing patients using the number of standard deviations that their HbA_{1c} result lay from the non-diabetic mean value of their particular assay.⁵² The fact that the two studies used the same HbA_{1c} method has allowed these standard deviation targets to be dispensed with (see Standardisation of HbA_{1c}). However, as the relationship between HbA_{1c} and microvascular complication risk is exponential with no obvious “threshold” value, it means that targets aimed for are still to some extent arbitrary.⁵⁵ Indeed, there has been a steady creep towards lower target values in both Europe and the USA. For example, the European Diabetes Policy Group guidelines in 1999 recommended that type 1 and type 2 patients aim for a DCCT-equivalent assay value of $\leq 7.5\%$ ^{53 54} and at that time the USA recommended achieving $<7.0\%$, with values $>8\%$ suggesting that additional action be taken.⁵⁵ The current UK guidelines suggest a target between 6.5% and 7.5% (depending on the presence of complications or high arterial risk),^{56 57} and in the USA a value of $<7\%$ is now recommended in all situations.⁵⁸ This trend to lower targets seems to be continuing with the draft of new UK guidelines in type 2 diabetes encouraging values below 6.5%.⁵⁹

HbA_{1c} AS A SCREENING TEST FOR DIABETES

Interest in using HbA_{1c} as a possible replacement for fasting glucose or the oral glucose tolerance test (OGTT) in diagnosing diabetes seldom abates. The appeal is understandable since it would obviate the need for the patient to attend fasting and, if an OGTT was required, would address the problem with poor reproducibility of the 2-hour glucose value. However, repeated studies have shown that the limitation to its use is usually not because a high HbA_{1c} result does not indicate diabetes, but that a “normal” one does not exclude it.⁶⁰ Thus, as a diagnostic tool, it is specific but lacks sensitivity.⁶¹ Also, from an analytical perspective, HbA_{1c} is not the most precisely measurable analyte; therefore, an assay showing a 3% coefficient of variation at the critical 6.0% HbA_{1c} value can show a difference in excess of 0.7% HbA_{1c} within the same individual. Added to this is the fact that it has the unenviable task of trying to identify a condition that has two means of being diagnosed (ie, the fasting glucose value and the 2-hour OGTT value), so it is never likely to be able to satisfy the two criteria. There has therefore been a focus on using the test in addition to measuring fasting glucose to either diagnose diabetes or to screen for patients who do or do not need to progress to a full OGTT.^{62–64} In other words, HbA_{1c} is being used as a surrogate for the 2-hour glucose value of the OGTT. Used in this way, the specificity of a raised HbA_{1c} is being exploited in a population already at high risk of having glucose intolerance because of a borderline fasting glucose. One study of individuals at high risk of diabetes found that 43% of those with a fasting plasma glucose <7.0 mmol/l, but a diabetic response to the OGTT, had an HbA_{1c} above the upper limit of the reference interval.⁶⁵ Presumably, however, the percentage identified would drop substantially in a lower risk population.

Whatever the technique used to incorporate HbA_{1c} into any diagnostic criteria, it would certainly be feasible to produce an HbA_{1c} threshold that would give a similar proportion of patients the diagnosis of diabetes as the glucose criteria currently do,^{61 65} but it is likely to be a different group of individuals who are identified. Until this group can be shown to be at the same risk of micro- and macrovascular complications as those diagnosed by traditional criteria, and the cost of HbA_{1c}

becomes compatible with its use in less affluent countries, then glycaemic cut-offs are likely to remain for the foreseeable future.

FACTORS INFLUENCING HbA_{1c} MEASUREMENT

Abnormal haemoglobins

Normal adult HbA₀ glyicates to form HbA_{1c}, but if an abnormal haemoglobin is present then a patient is likely to form other glycated products such as HbS_{1c}, HbC_{1c} and so on, either in addition to or instead of HbA_{1c}.⁶⁶ Many glycated haemoglobin analysers can now at least identify the non-glycated portion of these abnormal fractions,^{67, 68} but there can still be difficulty in trying to make sense of what a patient's HbA_{1c} would be were it not for their haemoglobinopathy. This is even more difficult in patients who have homozygous haemoglobinopathies where there will be no HbA present at all. Some instruments that base their analysis on affinity chromatography can more easily identify glycation on any form of haemoglobin molecule as can immunoassay methods, but even then there is the suggestion that some abnormal haemoglobins glycate at a different rate to native HbA and so may give rise to misleading results.^{68, 69}

Small proportions of fetal haemoglobin persisting into adult life used to cause considerable problems with some glycated haemoglobin methods because they would co-migrate or co-elute with the glycated haemoglobin fraction leading to an overestimation of the HbA₁ or HbA_{1c} result.⁷⁰ Modern instrumentation is now able to account for fetal haemoglobin and so this issue should now be only of historic interest.⁷¹

Anaemia

Iron-deficiency anaemia can lead to rises in HbA_{1c} of up to 2% that can then subsequently be reversed by iron treatment.⁷²⁻⁷⁶ The reason for this rise is not fully known, but since iron deficiency is such a common finding, especially in premenopausal women, it could influence the diabetes management of many patients. There is also some evidence in premenopausal women without overt anaemia that a low mean cell haemoglobin is also associated with higher HbA_{1c} values.⁷⁷ Reassuringly, however, the overall relationship between MPG and HbA_{1c} in the population of premenopausal women participating in the DCCT was no different to that of men, suggesting that overt anaemia needs to be present to have marked effects on HbA_{1c} measurement.⁷⁸

Haemolytic anaemia has the opposite effect to iron deficiency by reducing HbA_{1c} in affected individuals. This is simply a consequence of reduced red cell survival, meaning a reduction in the availability of haemoglobin for glycation.⁷⁹ All causes of this form of anaemia (including immune haemolytic anaemia, haemoglobinopathies and chronic renal failure) are affected.

Effect of drugs and health conditions

Any drug which gives rise to haemolytic anaemia will have the same effect. High-dose aspirin, by forming acetylated haemoglobin, can lead to spurious rises in HbA_{1c} using some methods, but the effect is usually only apparent at doses (4 g/day) that are well in excess of that prescribed normally.⁸⁰

Renal failure can have complex influences on HbA_{1c} formation and measurement. Patients can be iron deficient, exhibit haemolytic anaemia and have altered red cell survival. Compounding the problem is the fact that urea-derived isocyanate can lead to the formation of carbamylated haemoglobin, which can be indistinguishable from HbA_{1c} when using some glycated haemoglobin methods.⁸⁰ However, for most

patients the overall effect does not seem too substantial with most HbA_{1c} methods.⁸¹

HOW SHOULD HbA_{1c} VALUES BE EXPRESSED?

Standardisation of HbA_{1c}

In the 1980s and 1990s an important issue for HbA_{1c} measurement was the lack of standardisation of the assay, and this meant that different analysers could have widely differing reference intervals and give varying results with patient samples.⁸²

As the DCCT and the UKPDS used the same method of analysis in their studies, this was felt to be a useful method to harmonise results against. It also had the added attraction that it meant patients could have their HbA_{1c} results compared directly with those of the subjects who participated in the two trials.⁸³ In order to develop this international harmonisation an extensive network of reference laboratories was established by the NGSP based in the USA. In the last decade this development has made great strides in making the HbA_{1c} results reported from different laboratories much more comparable.⁸³⁻⁸⁵

However, the HbA_{1c} results reported by this means were not the "true" HbA_{1c} values, but simply the best that 1980s technology could deliver when the DCCT study was conceived. In order to rectify this situation, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a reference method for HbA_{1c} that first established reference material of purified HbA_{1c} and HbA₀, and then a highly specific reference method for their measurement.⁸⁶⁻⁸⁸ Given this increased specificity it is perhaps not surprising that the results using this technique are between 1.5% and 2% HbA_{1c} lower than the NGSP results that relate to the DCCT.⁸⁹ As a result, the move over to using these numbers has been resisted in some quarters because of a fear of confusion between the two sets of values,⁹⁰ with some evidence from previous changes in HbA_{1c} numbers that clinicians may either under treat or over treat patients because they are still using the older targets.⁹¹

This led to the suggestion that rather than moving to these IFCC values, there could be a wholesale change to expressing HbA_{1c} as a "mean plasma glucose equivalent",⁹² or, as it has recently been coined, "estimated average glucose" (eAG). The main purported reason for making this change is that patients would be able to equate this result more closely to that obtained from their blood glucose meter readings, although this move inherently means there will not be a single global result, as the eAG value will be different depending on whether glucose is expressed as mmol/l or mg/dl. In order for eAG to be a suitable alternative to HbA_{1c} in the first place, there has to be certainty that HbA_{1c} can truly give an accurate reflection of mean blood glucose in most situations. The mean blood glucose study described above is being used for that purpose. Little has yet been published on this study, but the a priori criteria for acceptable agreement is that at least 90% of patients should have a mean glucose that is within 15% of that derived from the simple regression of MBG on HbA_{1c} for the study population (ie, within 15% of the eAG). However, even if this criterion is fulfilled it equates to 99% of the population being within $\pm 24%$ of the estimated average glucose, meaning that in two individuals with an eAG of 10 mmol/l, one patient could have an actual mean glucose of 7.6 mmol/l and another 63% higher at 12.4 mmol/l. Thus, the final results of the study will need to be examined closely to be sure that eAG is not adopted to the detriment of these types of patients.

Since this proposal, the IFCC has taken steps to make sure there is little chance of confusion between its results and those

of DCCT values by changing its units from percentages to being expressed as mmol HbA_{1c}/mol HbA₀.^{93, 94} The relationship between percentage and mmol/mol is one of a 10-fold change meaning that, for example, 7% by the IFCC reference system equates to 70 mmol/mol. This would obviously be a large upheaval for users of the test although it is sweetened, perhaps, by the fact that HbA_{1c} results could no longer be confused by patients as being their glucose result when expressed in SI units. Unfortunately, for those countries who express glucose as mg/dl it introduces this problem where it did not exist previously.

So where from here? Following a meeting on 4 May 2007, a joint consensus statement was issued by the European Association for the Study of Diabetes, the American Diabetes Association, the IFCC and the International Diabetes Federation. It recommended that HbA_{1c} results be reported worldwide in IFCC units (mmol/mol) and NGSP (ie, DCCT) units (percentage) and, if the ongoing mean blood glucose study fulfils its a priori criterion then the eAG will also be reported as an interpretation of the HbA_{1c} result.⁹⁵

This author is bemused at how a single test can end up having to be reported in three different ways (four if glucose units are included) on the same report. It therefore seems inevitable that there will be a re-evaluation of this consensus at some stage, with the fear that there may be less rather than more global harmonisation of results in the future if individual groupings of countries choose to take different paths.

CONCLUSIONS

The benefits associated with using HbA_{1c} to monitor glucose control in diabetes are now being fully realised. However, it is important for clinicians to be aware that there is always likely to be a significant proportion of patients in whom results from the test need to be interpreted with caution.

Take-home messages

- ▶ For most patients, haemoglobin A_{1c} (HbA_{1c}) measurement has become the most important means of assessing their glycaemic control.
- ▶ Despite methodological improvements and the recent harmonisation/standardisation of measurement there remains inherent limitations with the test that healthcare staff and patients need to be aware of.
- ▶ There is currently debate about whether HbA_{1c} should be expressed as a percentage, in millimoles per moles, as an "estimated average glucose", or as a combination of all three. We are therefore likely to be going through a period of considerable change for the test.

Competing interests: None.

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