A1C – frequently asked questions

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BACKGROUND
Routine clinical measurements of glycated haemoglobin first became available during the early 1970s and are now accepted as the standard for estimated overall glycaemic exposure and risk of microvascular complications in diabetes.

OBJECTIVE
This article attempts to answer frequently asked questions concerning A1C, and provide guidance on how to make best use of A1C measurements in clinical practice.

DISCUSSION
Blood glucose gives immediate day-to-day information and A1C usually gives a reliable estimate of the average glycaemic exposure over the past 6–8 weeks. Both are important components of glycaemic monitoring. Discrepancies between these two estimates of glycaemia can usually be resolved by checking blood glucose and A1C techniques. Target A1C is less than 7%, but encouraging patients to aim slightly lower (eg. by 0.5%) on A1C can result in significant reduction in complication risk. There is a clear relationship between glycaemic control reflected by A1C and the progression of microvascular complications in both type 1 and type 2 diabetes.

In a general practice of 1000 patients there are typically 30–40 with existing diabetes (90% type 2) and in the next year a further 3–4 will be diagnosed. Most will not achieve the glycaemic targets for preprandial blood glucose (less than 6 mmol/L) and A1C (less than 7%).[1]

What do the different names mean?

Normal adult haemoglobin is made up of four chains of amino acids ($2\alpha 2\beta$). There are different forms of haemoglobin (A0, A1, F, C, S) and diseases that associate with each (eg. sickle cell anaemia with HbS). Like the amino acid substitutions of haemoglobin variants, glycation of haemoglobin can occur on the alpha or beta chain, and at different points in the chains. This results in a 'family' of glycated haemoglobins (*Figure 1*). Laboratory methods can measure different parts of this family: total glycated haemoglobin (GHb) includes all haemoglobin that has reacted with a sugar; HbA1 historically comprises the three subfractions (HbA1a, HbA1b and HbA1c); while HbA1c uniquely refers to N-(1-deoxyfructosyl) haemoglobin.

The commonly used terms 'glycosylated' or 'glucosylated haemoglobin' (which are incorrect and should not be used[2]) may refer to measurement of GHb or the HbA1c; to be certain one has to check the method used. Consequently, to prevent confusion with respect to terminology and to implement standardisation, several organisations[3] have proposed A1C as a more easily pronounced and remembered synonym for HbA1c.

Trials in 1993[4] and 1998[5] established a relationship between microvascular complications and the glycated haemoglobin levels in type 1 and type 2 diabetes respectively. Since these trials, measurements of glycated haemoglobin are generally expressed as a DCCT equivalent A1C value, allowing clinicians to directly use their laboratory value to estimate future risk of microvascular complications. The use of laboratory values requires international standardisation of different methods using special reference calibrators so that all laboratories report comparable results.

What is A1C?

A1C is the main glycated haemoglobin that forms when haemoglobin is exposed to glucose and undergoes a sequence of nonenzymatic reactions. The first is the rapid but reversible formation of an aldimine (or Schiff base), followed by the considerably slower formation of a stable ketoamine via a process known as the Amadori

rearrangement (*Figure 2*).[6] The ketoamine steadily accumulates over the life of the red cell and forms the bulk of the glycated haemoglobin measured by laboratories.

It is easiest to explain to patients that the haemoglobin in newly formed red cells (reticulocytes) has minimal A1C content, but as haemoglobin continuously reacts with glucose, A1C gradually builds up during the red cell life span more or less quickly, depending on the mean blood glucose level. Therefore, the oldest red cells will show the highest A1C levels.[7] However, the laboratory only measures A1C as a percentage of total haemoglobin in all red cells, thereby providing an index of blood glucose levels over the entire red cell lifetime. The International Diabetes Federation has recommended that A1C results should be reported in terms of the respective mean blood glucose (MBG) value they reflect, so that the results will be more meaningful to patients. However, appropriate prospective studies must first be carried out to establish the true relationship between A1C and MBG and the respective uncertainty associated with any predictive equation.[8]

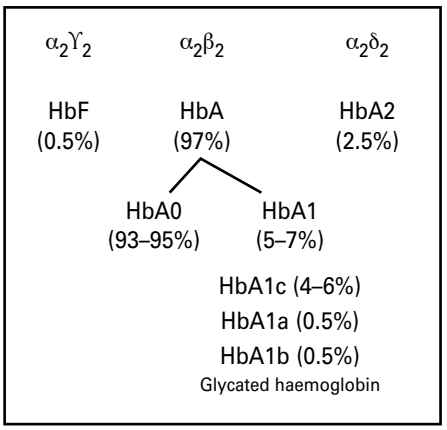

Figure 1. Haemoglobin components in nondiabetic adults

Table 1. Common causes of misleading blood glucose results

In decreasing order of frequency:

- inappropriate daily sampling protocol
- patient misreporting/manipulation
- meter or strip malfunction or operator error
- abnormal haematocrit, high vitamin C levels

At any one time, the A1C level signifies recent (rather than remote) glycaemic exposure, and has been shown to effectively approximate an exponentially weighted average of daily mean blood glucose concentrations, particularly during the preceding 30 days.[9] Accordingly, major changes in glycaemic control will be associated with significant changes in A1C within several weeks.

How does A1C relate to blood glucose?

It is believed that A1C reflects overall glycaemic exposure – fasting and postprandial highs and lows. Therefore, a stable patient with a blood glucose of 7 mmol/L will have about the same A1C as a patient who spends equal amounts of time at 12 and 2 mmol/L.

The relationship between A1C and blood glucose will depend on how 'average' blood glucose is defined. For example, the widely used 7-point profile (before and after three meals and once during the night) over represents day time glycaemia compared to a true 24 hour average. There may also be differences between population groups, and between those with type 1 and type 2 diabetes as blood glucose is more stable in type 2 diabetes.

There are many published estimates of a relationship between A1C and mean blood glucose, but an easy one to remember is:

MBG (mmol/L) = 2 x A1C(%) – 6.0.

Many patients (and perhaps some doctors) equate the A1C and blood glucose values. This is true at 6 mmol/L and 6% respectively. However, thereafter an A1C increase of 1% indicates an increase of about 2 mmol/L of blood glucose. Therefore, an A1C value of 8% (the threshold action level) reflects a MBG level closer to 10 mmol/L than 8 mmol/L.

Can A1C and blood glucose give different estimates of glycaemia?

Yes. For example:

Beverley's BGM: '... 4–6 mmol/L... but never over 8' A1C: 9.6%

John's BGM: '... rarely under 8... mostly teens' A1C: 7.4%.

According to our formula, Beverley's A1C should be much lower (under 6%) and John's much higher (over 10%). Why is this? Usually the A1C is more reliable and the problem is with the blood glucose measurement. There are four common causes for misleading blood glucose values (*Table 1*).

Beverley and/or John may only be testing at a certain time of the day. Recorded values may be lower (eg. fasting) or higher (eg. postprandial) than at other times. A1C generally reflects the mean blood glucose over the preceding 4 weeks and tests restricted to once or twice per day may not appropriately characterise the 24 hour average. Perhaps Beverley and/or John are

Table 2. Guidelines for glycaemic management based on HbA1c

HbA1c (%)	AACE 2002[12]	VA 2003[13]	ADA 2004[14]	DA/RACGP 2004[1]
4.0–5.9	Normal	Normal	Normal	Normal
6.0–6.5	Target			
6.6–6.9	Action	Target A[a]	Target	Target
7.0–7.9		Target B[b]	Action	Action
8.0–8.9		Target C[c]		
≥9.0		Action		

a = absent/mild microvascular complications and life expectancy >15 years

b = moderate microvascular complications and life expectancy at least 5 years, or absent/mild microvascular complications and life expectancy 5–15 years

c = advanced microvascular complications or life expectancy <5 years

not recording a particularly high or low result because they 'knew what caused it'. It is possible they are manipulating the recorded results. Blood glucose meters and strips are now very simple to use and very reliable, but occasionally the meter, strip or 'patient' malfunctions causing misleading results. High concentrations of vitamin C may affect results; also meters are affected by hypoxia and by the absolute level of haemoglobin (in the blood sample the concentration of glucose in plasma is much higher than in red cells. The lower the haemoglobin, the greater proportion of plasma and the more positive the bias of the result).

The A1C result may also be misleading. If there are more 'younger' red blood cells than normal which have a lower percentage of glycated haemoglobin (eg. after a haemorrhage), the A1C will be lower than expected for the corresponding blood glucose level. Blood transfused from someone without diabetes will have a markedly lower A1C causing an immediate and significant decrease in the patient's A1C level. Patients with haemolytic anaemia, receiving treatment for iron or vitamin B12 deficiency, or undergoing erythropoietin treatment, will all have decreased A1C levels, secondary to changes in the red cell age profile, and therefore A1C will underestimate the true glycaemic control.

Some ion exchange HPLC A1C assays cannot adequately resolve A1C and the increased levels of carbamylated haemoglobin (a product of urea and haemoglobin) associated with renal failure. Haemoglobin variants may also significantly increase or decrease the A1C value. Laboratories rarely document the shortcomings of their particular A1C method, so it is worthwhile repeating (using a different assay method) a measurement that doesn't seem to make sense.

If A1C and blood don't agree, how am I to determine which is right?

Checking the blood glucose result

The 7-point profile is the accepted best means of approximating average blood glucose control, until clinical availability of 24 hour blood glucose monitoring increases. Beverley was only measuring fasting glucose, while John was testing values 2 hours after meals. Patients may be given different recommendations as to when they should test and it is always useful to confirm when the test was done in relation to meals, activity, and last dose of medication.

Scrolling through the memory of the meter will identify tests not recorded or misrecorded. Many patients don't perform quality control checks and it is often useful to ask the patient to check finger prick blood glucose immediately before and after (within minutes) blood is taken for blood glucose and other tests (eg. yearly cholesterol check). The difference between before and after values indicates test variability; the average is assessed by comparing the average and laboratory value.

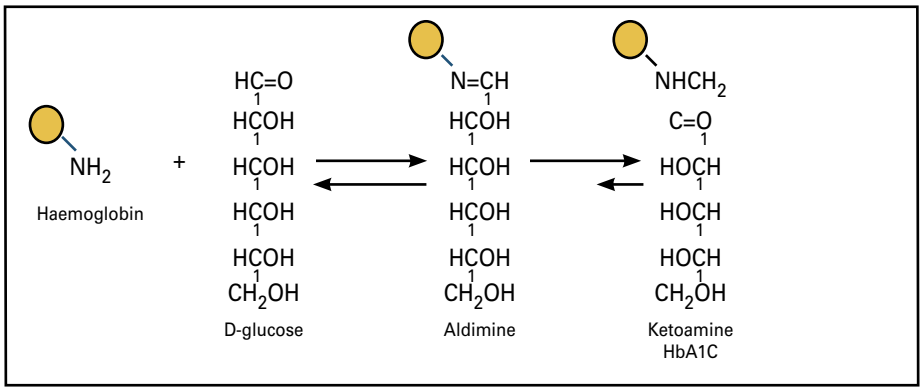

Figure 2. Nonenzymatic formation of HbA1c from haemoglobin and glucose

Checking the A1C

Ask the laboratory if uraemia or haemoglobin variants might affect the patient's A1C assay. If so, ask them to arrange testing by a method that is not commonly affected by factors of interference. Suggest the Bayer DCA 2000 A1C assay when possible to resolve such discrepancies. While the DCA 2000 is a point of care testing instrument, it has been extensively documented as a very selective assay for A1C estimation.[10]

Another independent measure of medium term glycaemic exposure is provided by the fructosamine value, which reflects glycation of all plasma proteins but primarily albumin.

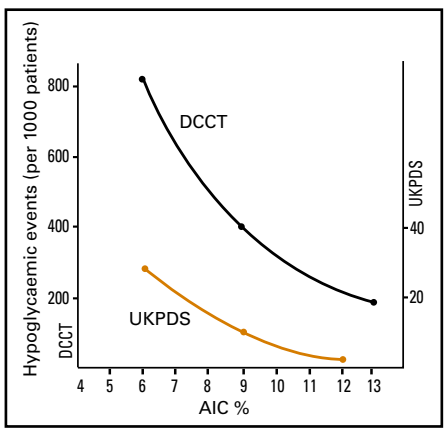

Figure 3. Hypoglycaemic risk and glycaemic control

Compare the A1C and fructosamine levels with respect to their respective reference ranges. Discuss any significant discrepancies with the laboratory.

How does A1C relate to complications?

Both the Diabetes Control and Complications Trial (DCCT) in type 1, and the United Kingdom Prospective Diabetes Study (UKPDS) in type 2 diabetes gave similar results; per 1% A1C increase there was an average 30% increase in the risk of new microvascular complications or the progression of existing ones (eg. new retinopathy or significant progression of retinopathy). In practical terms this means that the higher the A1C, the greater the absolute benefit of A1C reduction. For example, an A1C reduction from 10 to 9% gives an absolute risk reduction from 10 to 5.6 steps in retinopathy progression per 100 patient years (4.4 steps). Whereas a similar reduction from 7 to 6% gives the

same relative reduction (30%) but a much smaller absolute reduction from 1.8 to 0.5 (1.3 steps).

However, the DCCT and UKPDS show very different relationships between A1C and the rate of severe hypoglycaemia (*Figure 3*) which is defined as hypoglycaemia where help was required from another person. Overall, the risk from hypoglycaemia at any A1C value for type 2 diabetes is approximately 10 times less than the risk for type 1 diabetes. Many patients with type 2 diabetes are over concerned about hypoglycaemia. It is unusual for someone with type 2 diabetes to require hospital attendance/admission for hypoglycaemia and the majority of such episodes are for type 1 diabetes.

This means that the patient with type 2 diabetes and an A1C of 8.8% (the Australian average) can reduce the long term risk of microvascular complications without significantly increasing the risk of hypoglycaemia. The higher the A1C, the greater the benefit and the lesser the risk of reduction. In patients with A1C levels exceeding 8% (the action level) more active management can be considered.

How variable are A1C measurements?

Variability includes several components.[11] For example, intra-individual (how much does A1C vary over time in Beverley or John), intralaboratory (within or between batch in one laboratory) or interlaboratory (the variability between laboratories using the same or different methods). Often laboratories report their within batch variation, which is reassuringly low (eg. 1%), but may greatly underestimate the total assay variability (~6%). The 'least significant difference' is the change that is likely to be caused by a real change in the patient's A1C rather than variability within the patient and/or laboratory and/or between laboratories.

Generally the least significant change is greater than 2.3 times the coefficient of variation, which gives a 90% expectation that the change is real and not caused by intra-individual or laboratory variation. The total coefficient of variation can be expected to be less than 7% and the least significant change to be 15% (ie. where the initial A1C level is 10%, follow up A1C values lower or higher than 8.5% and 11.5% respectively, reflect a true change).

For different tests, different laboratories and different individuals, the proportion of variations from the method, laboratory and individual can vary widely. To determine how much, contact the laboratory and ask for the total variability of the test result over the period since it was last measured.

What are the targets for A1C?

As with all targets in medicine, these depend on the potential cost (eg. in terms of risk of hypoglycaemia and weight gain, or extra effort required) and the benefit (eg. the reduction of symptoms in the short term and of complications in the long term). As noted, there is a clear relationship between glycaemic control reflected by A1C and the development or progression of microvascular complications in both type 1 and type 2 diabetes (~30% risk reduction per 1% A1C reduction). There is also a clear relationship between the A1C and risk of hypoglycaemia but this risk is much higher absolutely for type 1 diabetes than for type 2 (~10 times higher).

If the patient has symptoms attributable to hyperglycaemia or glycosuria (eg. tiredness, particularly after meals, polyuria, recurrent thrush), most patients are prepared to try a little harder with their self care or consider additional medication. Similarly, if there are progressive microvascular complications and the A1C is over 8%, the benefit of a 1% A1C reduction is a considerable absolute risk reduction. For example, in the DCCT, a change from 10 to 9% gave a risk reduction of 4.4 steps and a very small increase in the risk of hypoglycaemia (from 10 to 15 events per 100 patient years).

On the other hand, if the patient's life expectancy is short, and they have no microvascular complications or symptoms, improving glycaemic control may offer no benefit in terms of longevity or quality of life and may actually reduce quality of life by increasing the complexity of medical and self care.

Table 2 lists some of the targets recommended by various authorities. Most recommend an A1C under 7% as the absolute risk of microvascular complications increases with higher values. Targets lower than 6% (ie. within the nondiabetic range) are usually very difficult to achieve, often require quite complex medical and self care schedules, and are associated with an increased risk of hypoglycaemic events.

Often the message to patients is to aim slightly lower than 7%, as many have A1C values well above the ideal (the median A1C for Australians with diabetes is 8.5%, meaning considerably more than 50% of patients are over the action level of 8%). Getting the A1C a little bit lower on several occasions over time can result in significant improvements in overall glycaemic control and significant reductions in complication risk (eg. a series of four decreases of 0.5% in A1C over a 1 year period).

Conclusion

A1C usually gives a reliable estimate of the average glycaemic exposure over the preceding 6–8 weeks. Differences between A1C and blood glucose estimates of glycaemia can usually be resolved by checking blood glucose and A1C techniques. Glucose gives immediate, day-to-day information and A1C gives medium to long term information about glycaemic control. Both are important. Targets may need to be adjusted because of health problems (eg. progressive diabetic nephropathy or microalbuminuria) or psychosocial reasons (eg. a difficult transition through teenage years). Combining the long term and immediate/day-to-day aspects of A1C and blood glucose levels enables people to manage diabetes more effectively.

Conflict of interest: none declared.

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