HbA1c: a review of non-glycaemic variables

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ABSTRACT

Identification of the correlation between HbA1c and diabetic complications has yielded one of the most clinically useful biomarkers. HbA1c has revolutionised the diagnosis and monitoring of diabetes mellitus. However, with widespread adoption of HbA1c has come increasing recognition that non-glycaemic variables can also affect HbA1c, with varying clinical significance. Furthermore, the identification of a discrepancy between predicted and measured HbA1c in some individuals, the so-called 'glycation gap', may be clinically significant. We aimed to review the current body of evidence relating to non-glycaemic variables to quantify any significance and provide subsequent suggestions. A PubMed-based literature search was performed, using a variety of search terms, to retrieve articles detailing the non-glycaemic variables suggested to affect HbA1c. Articles were reviewed to assess the relevance of any findings in clinical practice and where possible guidance is given. A range of non-glycaemic variables have statistically significant effects on HbA1c. While the clinical implications are generally irrelevant, a small number of non-glycaemic variables do have clinically significant effects and alternative biomarkers should be considered instead of, or in addition to, HbA1c. There are a small number of non-glycaemic variables which have a clinically significant effect on HbA1c, However, the vast majority of non-glycaemic variables have no clinical relevance. While clinicians should have an awareness of those non-glycaemic variables with clinical significance, in the vast majority of clinical scenarios HbA1c should continue to be used with confidence.

INTRODUCTION

Produced at a rate dependent on substrate (glucose) concentration, HbA1c (a subfraction of glycated haemoglobin) is formed in vivo continuously by glucose forming a ketoamine on the N-terminus of the haemoglobin beta chain. Glucose enters erythrocytes at a rate proportional to the extracellular concentration through GLUT1 channels, which are constitutively active, therefore intracellular and extracellular glucose environments are almost equivalent. The unique microvascular complications of diabetes (nephropathy, neuropathy and retinopathy) occur in tissues which also express the GLUT1 channel and intracellular glucose toxicity is possibly causative. This may account for why HbA1c correlates so significantly with diabetic complications and is such a clinically useful biomarker of glucose homeostasis.

HbA1c was initially used to monitor glucose levels as it both correlates with extracellular glucose levels and provides an estimate of average glucose levels over approximately 120 days. Standardisation of HbA1c measurement led to the introduction of HbA1c as a diagnostic test in addition to its use in monitoring glycaemic control.[1] Diagnostic efficiency is high due to the aforementioned relationship with complications.[2 3] However, at diagnostic thresholds, HbA1c detects subtly different groups of people with dysregulated glucose homeostasis than fasting plasma glucose (FPG) and oral glucose tolerance tests (OGTT).[4]

Additionally, erythrocytes integrate glucose levels over their lifespan and therefore acute changes will have less influence on the total result. This reduces the utility of HbA1c in diagnosis when the onset is recent, namely in type 1 (T1DM) and gestational diabetes mellitus (GDM), as well as in acute pancreatic damage.

The widespread implementation of HbA1c for the diagnosis and monitoring of type 2 diabetes mellitus (T2DM) means non-glycaemic variables affecting HbA1c measurement must be understood to ensure results are interpreted and used correctly. We will describe both the analytical variables and non-glycaemic variables hypothesised or proven to influence HbA1c; attempt to quantify the relevance in clinical practice; and, where possible, give guidance (see table 1 and figure 1).

Clinically significant differences in outcomes occur with relatively small changes in HbA1c, depending on both the absolute value and the quantum of change. A 5.5 mmol/mol change in HbA1c is typically considered significant (equivalent to 0.5%) and therefore we will use this as our threshold of significance for non-glycaemic variables.[5] It should be noted however that patients may consider changes smaller than this significant, particularly at diagnostic thresholds.

METHODS

A PubMed-based literature search was performed in order to undertake a systematic review. A variety of search terms were used to retrieve articles detailing the non-glycaemic variables suggested to affect HbA1c, with further literature identified from reference lists. As above, clinically significant changes in HbA1c are defined for the purposes of this paper as 6 mmol/mol (5.5 rounded mmol/mol, 0.5%).

Language was limited primarily to English and the full text of any articles was acquired as necessary. Articles were reviewed to assess the relevance of any findings to clinical practice. Where possible, guidance was given by the authors with the aim to produce a useful but non-exhaustive summary.

ANALYTICAL AND PREANALYTICAL ISSUES

Method specific

The reference method, calibrated using glycated and non-glycated HbA standards, involves enzymatic cleavage; reverse-phase high-performance liquid

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Table 1 Summary of HbA1c non-glycaemic variables and significance of their effects

Variable	Subcategory	Effect	Comments
Haemoglobinopathies	N/A	Variable, possibly clinically significant	Consult NGSP website at http://www.ngsp.org/interf.asp
Circulatory source	N/A	No significant effect	All circulatory sources of blood acceptable
Anticoagulant	N/A	No significant effect	Consult manufacturer instructions and validate anticoagulant type locally
Storage	Routine analysis	N/A	Store sample at 4°C
	Long-term storage	N/A	Store sample at −80°C
Sample haemolysis	N/A	No effect—analytical methodologies are haemolysis independent	N/A
POCT	N/A	Variable dependent on specific POCT system	If POCT used quality assurance and clinical accreditation must be performed to ensure laboratory equivalence
Time of day	N/A	No significant effect	Sampling time does not need to be considered
Season	N/A	Non-clinically significant increase in HbA1c during winter	Season does not need to be considered
Age	Neonates	Insufficient data	Do not use HbA1c
	Paediatric	Variable, possibly clinically significant effects	Do not use HbA1c for diagnosis
	Adult	HbA1c increases with age	Consider additional/alternative biomarker in those aged>75
Ethnicity	N/A	Variable.	Consult dedicated research literature
Gender	N/A	Variable, but not clinically significant.	Gender does not need to be considered
Asplenism	N/A	May cause both a glycaemic and non-glycaemic mediated increases in HbA1c	Insufficient data to provide recommendation
Iron deficiency	N/A	Statistically but not clinically significant increase in HbA1c	Use HbA1c but caution in supplementation (see below)
Iron	Oral	Reports of clinically significant reduction in HbA1c	Consider use of additional biomarker until red cell indices stable
	Intravenous	Statistically but not clinical significant reduction in HbA1c	Consider use of additional biomarker until red cell indices stable
Vitamin B12	Deficiency	Statistically but not clinically significant increase in HbA1c	Limited data—interpret with caution
	Supplementation	Statistically significant reduction in HbA1c	Consider use of additional biomarker until red cell indices stable, for example, glucose or OGTT
Folic acid	Deficiency	Statistically but not clinically significant increase in HbA1c	HbA1c use acceptable
	Supplementation	Clinically significant reduction in HbA1c	Use additional biomarker until red cell indices stable, for example, glucose or OGTT
Dapsone	N/A	Variable, but potentially clinically significant	Do not use HbA1c
HIV	N/A	Variable	Insufficient data to provide recommendation
Antiretrovirals	N/A	Variable	Insufficient data to provide recommendation
Hydroxyurea	N/A	Potentially clinically significant effect	Consider use of additional biomarker, for example, glucose or OGTT
Acute changes in blood glucose	N/A	HbA1c not-reflective of acute glucose changes, but is reflective of previous mean glucose	Do not use HbA1c for diagnosis (however risk is false negatives, therefore if already raised indicates pre-existing diabetes)
Gestational diabetes mellitus	N/A	HbA1c may indicate pre-existing diabetes Studies suggest potential role in selecting those who may and may not need further testing, for example, OGTT	Insufficient data to suggest use in routine screening for new onset GDM
Hypothyroidism	Subclinical	Statistically significant increase in HbA1c—normalises after treatment	Do not use HbA1c as sole biomarker in hypothyroidism or unstable thyroid states, use glucose or OGTT
	Overt		
Hyperthyroidism	N/A	No significant effect	HbA1c use acceptable
Liver disease	N/A	Variable, clinically significant effects	Use frequent blood glucose monitoring in advanced liver disease
Chronic kidney disease	Stages 1–3	No clinically significant effect	N/A
	Stages 4–5	Variable, clinically significant effects.	Do not use HbA1c for diagnosis, cautious use for monitoring
	Erythropoietin	Clinically significant reduction	Do not use HbA1c
Acute inflammation	N/A	No clinically significant effect	Ensure follow-up testing if elevated HbA1c detected
Vitamin E	Supplementation in hypovitaminosis E in T2DM	Clinically significant reduction in HbA1 in subgroup analysis	Insufficient evidence at present, further research required

N/A, not available; OGTT, oral glucose tolerance test; POCT, point-of-care testing; T2DM, type 2 diabetes mellitus.

chromatography (HPLC) to separate the N-terminal hexapeptides; and their subsequent quantification by electro-spray ionisation-mass spectrometry or capillary electrophoresis. Routine clinical methods separate HbA1c from other haemoglobins based on charge or structure, meaning haemoglobin variants can interfere, for example, co-elution in cation exchange methods. Assay and haemoglobin-specific information is readily available and should be consulted by those performing analyses.[6 7] Additionally, methods without separation and graphical output (eg, chromatograms), such as immunoassay systems, may mean variants (eg, HbS) can be missed.[8] While modem immunoassays provide accurate results for most heterozygous variants, HbA1c should not be used in homozygous variants where erythrocyte lifespan is significantly abnormal. Other assay interferents such as carbamylation have largely been eradicated in modern assay systems. The analytical variation of most methods is less than the biological variation, therefore having limited overall effect on the total coefficient of variation.

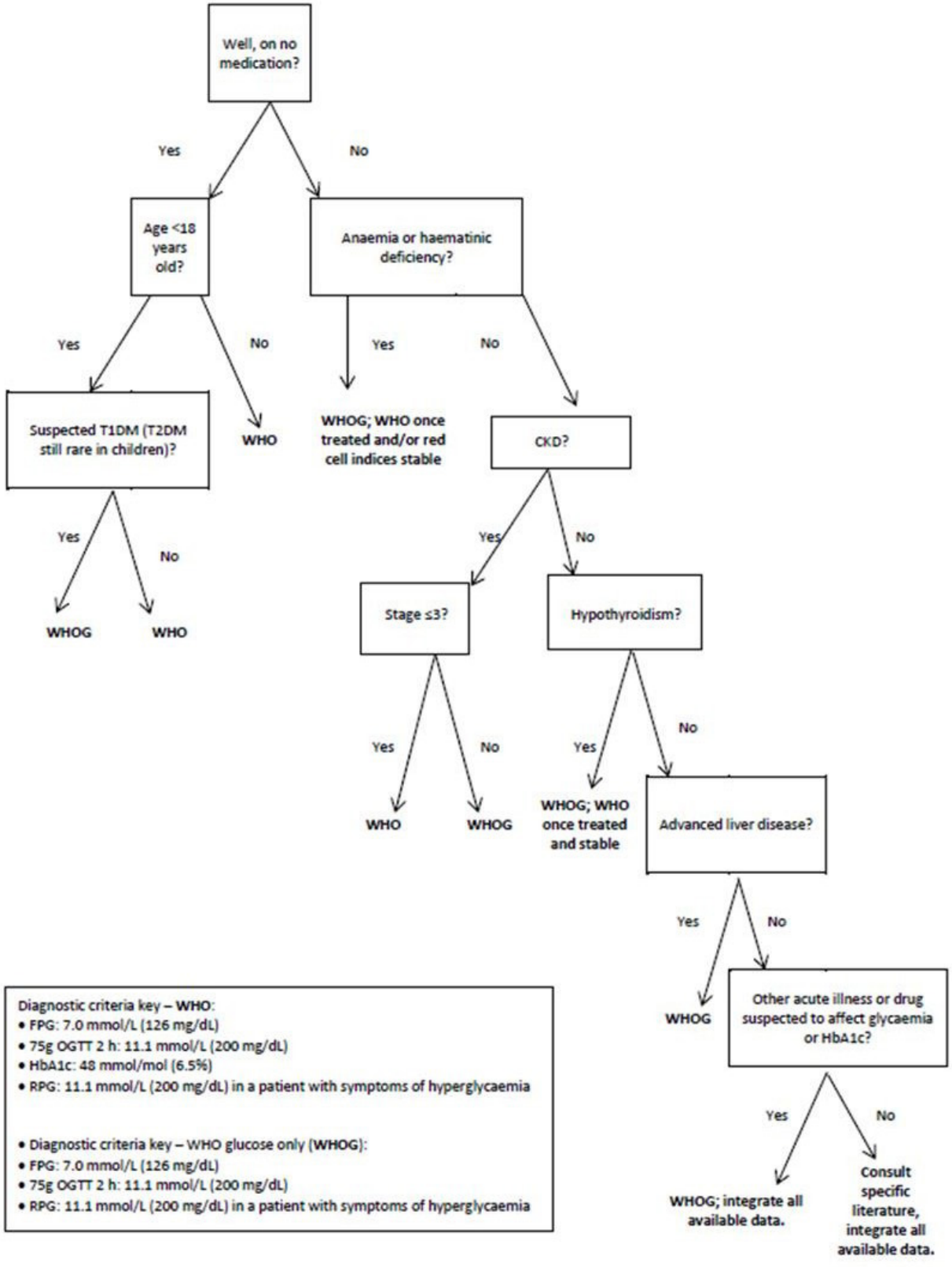

Figure 1 Flowchart for the selection of diagnostic strategy for T2DM

Sample type

Although theoretically there should be no difference between capillary and venous samples, one small study for one point-of-care method has shown a non-clinically significant difference.[9] No studies relating to arterial blood samples have been conducted to the authors' knowledge. Regardless of the source, whole blood is required, with EDTA and lithium heparin being common sample types. Other sample types can be used

with no data to demonstrate sample type affects measurement.[10] Regardless of sample type we suggest that manufacturer instructions are reviewed and sample and tube type are validated locally on assay introduction.

Sample stability and timing

HbA1c is relatively stable for prolonged periods, with delayed analysis unlikely to be clinically relevant. Storage at 4°C is preferable to −20°C for ion-exchange methods.[11] The optimum storage temperature is assay dependent, therefore manufacturer information should be consulted to determine exact storage conditions. For long-term storage, −70°C or lower is suggested.[11] Long-term storage at −80°C for up to 10 years has been demonstrated to have no effect.[12] HbA1c does not exhibit diurnal variation,[13] but may possibly exhibit seasonal variation (with elevated values in winter). However, this is debated, with any effect small in size, and potentially indicative of population movements, dietary variation, infection rates or other factors, rather than a true temperature or sunlight-related effect.[14 15]

Serum indices and drugs

Interferences from lipids, urea, glucose (and labile HbA1c), aspirin, vitamin C and bilirubin are often mentioned but are not significant in most systems/assays.[16–20] Lipids cause a significant negative relative bias in capillary electrophoresis once triglycerides exceed approximately 15 mmol/L and cholesterol 8.5 mmol/L.[18 19] Vitamin C is a positive interferent in some selected assay systems at levels ≥50 mg/mL but this is significantly higher than can be achieved by oral megadosage (<0.4 mg/mL) and the finding has not been consistent.[18 21–24] Icterus is not a significant interferent in most systems.[18 19] Aspirin is a significant positive interferent at lower HbA1c values in limited assays. However, these effects are only seen after high-dose therapy (1500 mg) so are unlikely to be clinically significant.[25] Aspirin may however also reduce glycation in vivo but the effect is small and unlikely to be clinically significant.[23 26]

Sample or in vitro haemolysis does not affect measurement as samples are haemolysed as part of analytical procedure.[12] Although the aforementioned interferents are not clinically significant, this list is not intended to be comprehensive. Therefore, we suggest that manufacturer literature should be consulted and method verification performed prior to clinical use to determine any significant interferents.

Erythocyte lifespan

Though erythrocyte lifespan is approximately 3 months, the most recent month's glucose levels affect 50% of value with an exponential-like decay with time.[27] Minimum repeat intervals quoted in guidelines reflect this stating that 2 months can indicate the direction of change if not the steady state.[28] This relationship is perhaps not so simple and gives no indication of variability.[29 30]

Point-of-care testing

Point-of-care testing (POCT), using finger-prick capillary or venous blood samples, is used as both a diagnostic and screening tool. Systematic review of POCT against laboratory methods suggests unacceptable imprecision with an overall negative bias in some POCT devices limiting their use in both diagnosis and management.[31] However, precision varies depending on platform with potentially some analysers performing adequately though the American Diabetes Association still does not recommend POCT for diagnosis of DM.[32 33] This is potentially controversial given laboratory-equivalent performance of some POCT systems and as long as quality assurance testing and clinical accreditation is conducted.[34] POCT HbA1c is used already for community and clinic monitoring of HbA1c in known diabetes in the UK allowing an immediate discussion with the patient with attendant benefits; also, the use of capillary samples makes this an ideal methodology for paediatric services.[35] As long as the required standards are met and clinical effectiveness and cost efficiencies are achieved, then we would suggest that POCT HbA1c could also be used for diagnosis.[36]

Diagnostic protocol

Diagnostic tests for DM include FPG, OGTT, HbA1c or a random glucose level in those exhibiting symptoms. All literature agrees that using either FPG, OGTT or HbA1c detects different individuals, with some overlap, who all have DM. While there is no generally superior test (though OGTT has greatest sensitivity),[37] some may be better in specific clinical situations.[38] Furthermore, pre-diabetes trials have only examined those with impaired glucose tolerance (IGT), therefore the evidence for primary prevention may not be easily applicable for those with pre-diabetes according to FPG or HbA1c criteria.[38] OGTT is generally considered the gold standard but we would suggest all modalities are available for diagnostic work as each have their strengths based on the pathophysiology of the underlying diabetes type, for example, HbA1c for T2DM, OGTT for GDM and plasma glucose for T1DM with the single most appropriate selected by each service.

NON-GLYCAEMIC BIOLOGICAL VARIABLES

Age

While HbA1c is a well-established biomarker in adults and, to a lesser extent, children, neonatal research is limited with no agreed reference range. However, given that the change from fetal to adult haemoglobin begins at 24 weeks, it is possible that neonatal HbA1c may have some utility in indicating in utero glucose exposure during the last trimester. While dried blood spot methods are feasible,[39] further work is required to establish ranges and clinical utility in this cohort, and as such, we do not currently suggest the use of HbA1c for diabetes diagnosis in neonates.

T2DM, once relatively scarce, is becomingly increasingly common in children, therefore HbA1c may be a suitable tool for screening overweight and obese children if the diagnostic threshold is adapted. A value of 42 mmol/mol (6%) has been shown to achieve a diagnostic sensitivity of 94% and specificity of 93% in those aged 7–17 years.[40]

Increasing age in adulthood may also affect interpretation. A positive correlation between age and HbA1c in a racially heterogeneous population has been shown ($p<0.0001$) and is thought, in part, due to changes in glycation rates with increasing age.[41] A large Chinese study demonstrated the diagnostic efficiency of HbA1c was significantly lower in those aged >75 compared with the 45–54 aged cohort (receiver operating characteristic (ROC) AUC 0.755 vs 0.878, $p<0.001$). When adjusting for erythrocyte count the negative association disappeared. The authors concluded that the diagnostic efficiency of HbA1c decreased with age secondary to lower erythrocytes count with HbA1c an unsuitable diagnostic tool in this specific patient cohort,[42] a recommendation not necessarily supported widely. We would suggest the use of HbA1c for monitoring and diagnosis in older adults. The treatment targets for T2DM are higher than T1DM reflecting the older age and increasing comorbidities in these

patients, therefore any problems occurring due to a possible negative effect of age when using HbA1c to titrate diabetes therapy are hopefully ameliorated.[43 44]

Ethnicity

Ethnicity affects absolute levels of HbA1c irrespective of mean blood glucose (MBG) through, as yet, unclear mechanisms. A meta-analysis of non-diabetic participants demonstrated statistically significantly higher levels of HbA1c in black (2.8 mmol/mol, 95% CI 0.18 to 0.33), Asian (2.6 mmol/mol, 95% CI 0.16 to 0.33) and Latino cohorts (0.9 mmol/mol, 95% CI 0.06 to 0.10) compared with Caucasian.[45] In a prospective study using continuous glucose monitoring and comparing 104 black and 104 white patients with known T1DM over 12 weeks, black patients had on average an HbA1c higher by 0.4% than whites for comparable average glucose measures.[46] While these differences are not of definitive clinical significance, the results do suggest further work should be undertaken to better understand the impact of ethnicity on HbA1c. Currently, however, we suggest ethnicity does not need to be considered when performing HbA1c for both diagnosis and monitoring but acknowledge that there is a potential small risk of overdiagnosis and overtreatment in non-Caucasian ethnic groups.

Gender

Gender effects on HbA1c are contradictory. A 2016 study demonstrated a statistically but not clinically significantly higher HbA1c in males compared with females (0.165%, $p<0.0001$) but only between the ages of 30–59.[47] In contrast, a study of children with T1DM found that girls had a statistically significant increased HbA1c compared with boys at the time of diagnosis, potentially related to the age of onset of puberty.[48] Therefore, this effect may not be evident in other age groups. Currently, given the limited evidence of any gender-related effect we suggest it does not need to be considered when performing HbA1c for either diagnosis or monitoring.

ERYTHROCYTE TURNOVER

Anything which affects erythrocyte turnover will alter the age distribution of erythrocytes and subsequently affect HbA1c levels. Anecdotally, splenectomy is thought to increase HbA1c.[49] However, it is also potentially associated with an increased rate of diabetes.[50] There is currently insufficient evidence to make any recommendation relating to HbA1c in asplenic patients. Sudden alterations in an individual's erythrocyte turnover may well have significant effects on HbA1c though but will depend very much on the individual case. We would urge caution in those having acute haemolysis and transfusion, for example, with both diagnosis and monitoring of diabetes with HbA1c.

Haematinics and anaemia

Earlier papers, mostly small studies, produced conflicting reports in regards to iron status, anaemia and HbA1c. However, most showed slightly higher HbA1c in iron-deficiency anaemia and a reduction on treatment with iron which has been widely cited as an interferent.[51 52] More recent large studies using statistical adjustment for variables have provided data to support this. In a multivariable univariate analysis on 948 patient samples, HbA1c was positively associated with reductions in ferritin and mean cell volume (MCV) but an elevation in haemoglobin.[53]

Iron supplementation can lead to upregulation of haematopoiesis and therefore reduction in HbA1c levels as the proportion of young erythrocytes increases.[54] In a paediatric cohort, enteral iron resulted in a clinically significant reduction in HbA1c (92 mmol/mol (10.6%±2.6%) to 67 mmol/mol (8.3%±2.6%)) with no change in MBG.[55] In adults with T2DM and chronic kidney disease (CKD), intravenous iron therapy results in a statistically but not clinically significant reduction in HbA1c.[56] Rafat *et al* showed a reduction from 33 mmol/mol (5.2%) to 32 mmol/mol (5.1%) on iron supplementation which correlates well with a large (8296 participants) and adjusted study by Ford *et al* which showed an absolute average difference in HbA1c of 1 mmol/mol (0.1%) in iron-replete and iron-deplete populations.[57 58] They do not recommend screening for iron deficiency when using HbA1c in diagnosis but advise caution with individuals with extremes of haemoglobin (<100 or >170 g/L).[57] We would agree with this recommendation that HbA1c can be used for diagnosis and monitoring in iron deficiency as the size of the effect is not clinically significant but would suggest caution using HbA1c, particularly to diagnose diabetes, in those patients receiving iron therapy, at least until red cell indices have stabilised.[59]

Vitamin B12 and folate deficiency have been associated with increases in HbA1c secondary to their haematpoietic effects; supplementation studies have demonstrated a statistically significant reduction.[60 61] Folate supplementation may lead to a reduction in homocysteine levels, thereby improving insulin resistance, suggesting glycaemic and non-glycaemic effects on HbA1c.[61] Data are however limited, but in cases of B12 and folate supplementation we would urge caution until red cell indices are stabilised, particularly when using HbA1c to diagnose diabetes.

Haemoglobin variants

Normal adult haemoglobin consists of four globin chains, two α and two β, and fetal haemoglobin (HbF) two α and two γ. HbF is usually mostly undetectable by 6 months but can persist in those with haemoglobinopathies. The most common haemoglobinopathies are caused by single amino acid substitutions in the β chain and include HbS, HbE, HbC and HbD. Not only can the changes cause alterations in erythrocyte lifespan but affect glycation, the charge of the molecule and interfere with specific assays such as coelute with the HbA1c fraction. (Little) Hb Raleigh is associated with acetylation which can reduce glycation and hence HbA1c in some methods and cause positive interference in others.[7 62] Similar discrepancies occur with Hb Okayama.[63] Hb Görwihl is a good example of an asymptomatic mutation that considerably slows glycation resulting in an HbA1c significantly lower than would be expected for prevailing glycaemia.[64] It may be that quantification of glycohaemoglobin isoforms, other than HbA1c, may be useful in some of these carriers, such as Hb Rambam.[65] Due to variety of preanalytical and analytical variation and plethora of Hb variants described, each laboratory will need to review their assay for the nature and degree of any interference. Complexities arise particularly when the haemoglobinopathy is asymptomatic and undiagnosed.

Pregnancy

OGTT is the gold standard and recommended screening test for diagnosis of GDM, though there a number of diagnostic strategies recommended throughout the world.[66] A number of studies have demonstrated that pregnancy-specific HbA1c reference ranges could be developed to reduce those patients requiring OGTT.[67] In a 2015 study, a threshold of 5.8% had a sensitivity of 26.4% and specificity of 94.9% with 38% of participants diagnosable based on HbA1c alone.[68] Furthermore, use of HbA1c in this manner may facilitate screening a wider population, thereby detecting patients with GDM who fail to complete or are not

detected by an OGTT.[69] A further study has demonstrated the threshold of >41 mmol/mol (>5.9%) identified all women with GDM (and those at increased risk of adverse outcomes).[70] While these studies support the case for developing pregnancy-specific HbA1c diagnostic thresholds, currently we suggest adherence to local guidance, for example, the National Institute for Health and Care Excellence guidelines in the UK.[65] However, HbA1c may provide useful information in addition to the OGTT.[71] HbA1c may also indicate undiagnosed pre-existing diabetes during pregnancy and is used routinely to monitor those with known diabetes very effectively.

Thyroid disease

Thyroid status affects haematopoiesis and may influence HbA1c levels, independently of MBG.[72] Several studies have demonstrated statistically but not clinically elevated HbA1c levels in subclinical and overt hypothyroidism, which normalise following thyroxine therapy and establishment of euthyroid status.[72–74] Notably, while the effects were statistically but not clinically significant, there were patients who at baseline had HbA1c values>39 mmol/mol (5.7%), that is, could be classified as 'pre-diabetic',[33] whose HbA1c fell below this level following thyroxine therapy. Although current study sizes are small, given the increasing body of consistent evidence demonstrating this effect, larger-scale studies are indicated. Currently, we suggest using a second biomarker, such as OGTT or glucose, in addition to HbA1c in untreated hypothyroidism for both monitoring and diagnosis until euthyroid status is well established.

A 2017 study with a hyperthyroid arm demonstrated no statistical difference in HbA1c between hyperthyroid and matched euthyroid controls at baseline or following treatment.[75] Based on this small study, we suggest stable hyperthyroidism does not need to be considered when performing HbA1c.

Liver disease

The liver plays a central role in glucose metabolism, with liver cirrhosis promoting glucose intolerance and diabetes. While these glycaemic changes would affect HbA1c, liver disease may also have non-glycaemic effects on HbA1c, for example, through associated anaemia and decreased protein synthesis.[76] HbA1c is therefore recognised as an unreliable marker of MBG in diabetic patients with chronic liver disease.[77] A study of 15 patients with liver cirrhosis demonstrated that 40% of patients had an HbA1c result below the reference range, with fructosamine results within or above the diabetic range.[78] A subsequent study of 82 patients supported the conclusion that HbA1c is not an adequate indicator of MBG in chronic liver disease.[79] While the small sample sizes limit the power of these studies, the discrepancies in glycaemic markers certainly warrant further study. Therefore, we suggest the use of frequent blood glucose monitoring as the most reliable biomarker in this patient cohort.

Kidney disease

CKD is proven to affect HbA1c,[56 80] in varying degrees.[81] HbA1c should therefore not be used to diagnose T2DM in end-stage renal failure (CKD 5).[82] Data however support its use in milder CKD as, after adjustment for age, ethnicity, gender and haemoglobin, HbA1c is not associated with CKD stage 3.[83] A study of 15 patients with T2DM and CKD (3B/4) receiving erythropoietin demonstrated a clinically significant reduction in HbA1C values[56]; therefore, we suggest limiting HbA1c for diabetes diagnosis use to those with CKD 1–3 and use of alternative biomarkers in those with CKD 4/5 and/or receiving erythropoietin. HbA1c can continue to be used for monitoring therapy in stages 1–3 and glucose levels the preferred marker in those with CKD 4/5.

Acute inflammatory response

The rapid changes in blood glucose and albumin during an acute inflammatory response mean aberrant blood glucose and fructosamine values are of no use in diagnosing T2DM. However, a prospective study of 30 patients undergoing elective orthopaedic surgery demonstrated no significant change in HbA1c despite a clear systemic inflammatory response.[84] Therefore, we suggest that patients in whom an elevated HbA1c is found during an acute inflammatory episode undergo further screening for diabetes.

Other conditions

HIV, either the infection or specific treatments, can lead to a low-grade haemolytic state shown by some, but not all, to reduce HbA1c levels.[85 86] The antimetabolite, hydroxyurea, has been shown to lead to clinically significant aberrant HbA1c results.[87] There is currently not enough data on either to provide recommendations.

Specific conditions, for example, sepsis, and drugs (corticosteroids and antipsychotics) can cause acute, clinically significant changes in blood glucose which will not immediately affect HbA1c levels. Therefore, HbA1c levels should be interpreted with caution in these circumstances. Dapsone is reported to reduce HbA1c in case reports, in some to a large degree, by a variety of non-glycaemic mechanisms.[76] Although the body of evidence is small, the clinical significance of the effect warrants further investigation and currently we suggest considering alternative biomarkers in patients receiving Dapsone.

Despite an early study suggesting vitamin E has a glycation inhibiting effect,[88] a subsequent meta-analysis failed to demonstrate a significant change. However, interstudy heterogeneity led to further subgroup analysis, which revealed a significant reduction in HbA1c in hypovitaminosis E T2DM patients following supplementation.[89] However, as noted by the authors, the small datasets involved in this subgroup analysis mean further studies are required to corroborate this finding before any recommendations can be made.

Glycation gap

The disparity between predicted and actual HbA1c has been dubbed the 'glycation gap'.[90 91] The basis and significance of the glycation gap is still debated but likely genetic and/or red blood cell life spanvariation result in individuals demonstrating different glycation rates.[92–95] For example, in children and adolescents with T1DM 29% of participants had HbA1c levels statistically different (either lower or higher) than predicted based on the MBG,[90] a finding corroborated by others.[96] This suggests that MBG and HbA1c are not necessarily interchangeable measures, similar to non-comparability between different diagnostic tests. This effect would suggest that the relationship between HbA1c and MBG is likely to be different for each individual; however, it does not alter our suggestions as in the main initiation and alterations of diabetic therapies are almost never made based on an isolated HbA1c, particularly at levels close to the diagnostic threshold.

CONCLUSIONS

HbA1c is a proven test for both the diagnosis of T2DM and monitoring of DM in general. However, there is an increasing body of evidence that HbA1c is affected by a number of

non-glycaemic variables, including analytical and biological. However, the paucity of research data and the small cohort sizes in those studies available prevent the clinical significance from being definitively determined.

Once identified, analytical influences on HbA1c are predictable and can be detected and compensated for. The non-glycaemic biological variables, in general, assert their effect through influencing haematopoiesis. Therefore, while HbA1c continues to be a useful biomarker of MBG in most stable comorbidities and drug regimens, it should be used with caution in these circumstances. In particular cases, for example, CKD 4/5, hypothyroidism or in those receiving iron, erythropoietin or Dapsone, additional or alternative biomarkers should be considered. The identification of the glycation gap has raised yet another, even more fundamental, factor to consider when interpreting HbA1c and demonstrates that while HbA1c has proven utility, our understanding of it remains far from complete.

Therefore, while the evidence clearly demonstrates HbA1c is a generally robust marker of MBG, our overall suggestion is that in the diagnosis and management of DM clinicians should consider all available data, including those provided by other biomarkers.

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