Determinants of HbA1c in non-diabetic children and adults

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Chapter 6

General Discussion

HbA_{1c} is not suitable for diagnosing diabetes

"Measure twice, cut once"

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Introduction

An international expert committee comprising members appointed by the American Diabetes Association, the European Association for the Study of Diabetes and the International Diabetes Federation recently recommended the use of HbA_{1c} for diagnosing diabetes[1]. Since then, dozens of articles have been published concerning this issue; some supporting this new application of HbA_{1c}[2], while others argue against it[3-5]. In this paper, we will discuss important limitations of HbA_{1c} as diagnostic test for diabetes.

Diagnose diabetes or its complications?

According to the International Diabetes Federation, diabetes is defined as: "a group of heterogeneous disorders with the common elements of hyperglycaemia and glucose intolerance, due to insulin deficiency, impaired effectiveness of insulin action, or both[6]". The chronic hyperglycaemia of diabetes is associated with long-term damage to various organs, especially the eyes, kidneys, nerves, heart and blood vessels.

Our comprehension of the pathophysiology of diabetes is limited, and there is no biological marker known to be unique to diabetes. Consequently, an important question to answer in the discussion around diagnosing diabetes is: what is diabetes? Is diabetes defined by the pathophysiology causing the hyperglycaemia (i.e. insulin deficiency, insulin resistance or both), is diabetes defined by the hyperglycaemia itself, or is it defined by the complications caused by hyperglycaemia?

Since diabetes is per definition characterized by hyperglycaemia, to diagnose diabetes, it seems to be logic to measure this hyperglycaemia. And indeed for many years, hyperglycaemia has been the leading diagnostic criterion for diagnosing diabetes. But around 1997, the focus of diagnosing diabetes changed to a more pragmatic approach, with diagnosis based on the development of complications caused by the hyperglycaemia instead of the hyperglycaemia itself. Epidemiological studies demonstrated glycaemic levels (fasting plasma glucose, 2-hour plasma glucose and HbA_{1c}) below which there was little prevalent retinopathy and above which the prevalence of retinopathy increased in an apparently linear fashion. Following these data, the Expert committee recommended a cut off value of 7.0 mmol/mol for fasting glucose, which is equivalent to a 2-hour post-load glucose concentration of 11.1 mmol/mol[7]. HbA_{1c} was not included in the guidelines because of lack of assay standardization.

An updated examination of the laboratory measurements of glucose and HbA_{1c} by the International Expert Committee in 2009 indicated that with advances in instrumentation and standardization, the accuracy and precision of HbA_{1c} assays at least match those of glucose assays[1]. They conclude that: "compared with the measurement of glucose, the HbA_{1c} assay is at least as good at defining the level

of hyperglycaemia at which retinopathy prevalence increases", and recommend the use of HbA_{1c} for diagnosing diabetes, in men and non-pregnant women.

"Diabetes should be diagnosed when HbA_{1c} is ≥ 6.5%"

The International Expert Committee declared that a large volume of data from diverse populations has established an HbA_{1c} level associated with an increase in the prevalence of moderate retinopathy and provides strong justification for assigning an HbA_{1c} cut point of ≥ 6.5% for the diagnosis of diabetes. This cut point is solely based on the detection of retinopathy, a microvascular complication of diabetes. However, microvascular as well as macrovascular complications are both highly relevant to type 1 and type 2 diabetes[8]. Available data on the relationship between HbA_{1c} and cardiovascular disease, an important macrovascular complication, may be less defined than those relating to retinopathy. But several studies have shown an association between HbA_{1c} and cardiovascular risk throughout the whole distribution of HbA_{1c}, so even in non-diabetic patients[9-11]. Khaw et al. concluded from their study to the relationship between HbA_{1c}, cardiovascular disease and total mortality in a general population aged 45-79 years that the relationship between HbA_{1c} and cardiovascular disease was continuous and significant throughout the whole distribution of HbA_{1c}. These findings suggest a continuous relation between HbA_{1c} and cardiovascular disease, and therefore strongly argue against the use of a cut point for diagnosing diabetes when it comes to macrovascular complications.

The relation between level of glycaemia and HbA_{1c}

Mismatches between blood glucose monitoring data and HbA_{1c} levels, also often seen in clinical practice, may imply that HbA_{1c} and glucose partly reflect different processes, especially in the non-diabetic range of glucose tolerance. The proposal to express HbA_{1c} as an estimated average glucose[12] is based on the assumption of a consistent mathematical relationship between glucose concentration and the extent of glycation. Consequently, two individuals that have identical glucose profiles should have identical HbA_{1c} levels. But this is not the case. Various studies found evidence for the existence of a "haemoglobin glycation index" or also called "glycation gap". This concept denotes that individuals glycate haemoglobin proteins at different rates[13,14]. Khera et al. confirmed the existence of a glucose gradient across the human erythrocyte membrane and demonstrated inter-individual heterogeneity in glucose gradients across the human erythrocyte membrane that may affect haemoglobin glycation[15]. There is also evidence for an association of the "glycation gap" with diabetes complications independent of the level of glycaemia[16]. Thus, HbA_{1c} may reflect both differences in blood glucose levels over time and the individual effects of additional biological factors that influence nonenzymatic protein glycation. This implies that HbA_{1c} is a better predictor of

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diabetes complications than other measures of glycaemia. But, since this susceptibility to glycation appears to be independent of glycaemic control, it is questionable whether this is specific to diabetes and therefore should be accounted for when diagnosing diabetes.

Though erythrocytes are freely permeable to glucose and the rate of formation of HbA_{1c} is thus expected to be directly proportional to the ambient glucose concentration, there is inter-individual variation in the relation between plasma glucose levels and HbA_{1c}[16]. And consequently, other factors besides glycaemia appear to determine HbA_{1c} levels. Substantial with-in person variability in the relation between mean glycaemia and HbA_{1c} level will have important consequences for the use of HbA_{1c} as diagnostic tool for diabetes.

Other factors determining HbA_{1c} in non-diabetic persons

The test for diagnosing diabetes will be used in persons without clear clinical signs of diabetes, including many persons without diabetes at the moment of the test. Consequently, if we want to use HbA_{1c} for diagnosing diabetes, it is important to know if there are other factors, besides plasma glucose levels, which determine HbA_{1c} in non-diabetic persons. Several studies investigating determinants of HbA_{1c} in non-diabetic persons, found evidence that HbA_{1c} is not only associated with levels of glycaemia but seems to be determined by other factors as well.

Firstly, HbA_{1c} is better correlated in monozygotic than dizygotic non-diabetic twin, demonstrating that there are genetic factors, possibly apart from glycaemic control, which explain a part of the variation in HbA_{1c}[17]. The heritability of HbA_{1c} nears 40% in the general population[18]. Two genome-wide association studies identified fifteen different single nucleotide polymorphisms (SNPs) to associate with HbA_{1c} in non-diabetic adults[19,20]. Some SNPs are considered to modulate glycaemic physiology[21], while others are supposed to regulate non-glycaemic factors like red blood cell function[22]. Thus, despite the lack of knowledge about the exact way the identified SNPs act on HbA^{1c}, it is clear that also genetic factors determine HbA_{1c}.

Because glycation of HbA begins during erythropoiesis and continues slowly throughout the lifespan of haemoglobin in the circulation, erythrocyte lifespan determines the duration of exposure of haemoglobin to glucose, and thereby also determines HbA_{1c} levels. Increased erythrocyte turnover, as observed in for example haemolytic anaemia, results in lower HbA_{1c} levels[23]. On the contrary, several studies showed higher HbA_{1c} levels in (diabetic as well as non-diabetic) patients with iron deficiency anaemia[24,25]. Cohen et al. concluded from their study that erythrocyte survival varies sufficiently among haematologically normal persons to cause clinically important differences in HbA. The question rises if we should test for anaemia or any other condition that can alter red cell survival before we can diagnose diabetes using the HbA_{1c} level.

In addition, also age and ethnicity are described to have influence on HbA_{1c}, independently of levels of glycaemia. Ziemer et al. found higher HbA_{1c} levels in black persons than in white persons across the full spectrum of glycaemia after adjustments for plasma glucose and other characteristics known to correlate with HbA_{1c} levels[27]. And also subjects of South Asian origin showed to have higher HbA_{1c} levels than white subjects independent of fasting and postprandial glycaemia on OGTT[28].
Pani et al examined whether HbA_{1c} was associated with age in non-diabetic persons. They stated that their results establish clearly that HbA_{1c} increases with age, even after multivariate adjustments for sex, fasting, and 2-hour postload glucose and suggested that non-glycaemic factors may contribute to the relationship of HbA_{1c} with age[29]. Other studies confirm the positive association between age and HbA_{1c} [30,31]. Also these findings of differences in HbA_{1c} in persons of different age and ethnicity makes one cut-off value of HbA_{1c} for diagnosing diabetes highly questionable.
In chapter 2 we described the findings of our study on potential predictors of HbA_{1c} in 8-12 month old non-diabetic infants. We found no association between known risk factors for type 2 diabetes and HbA_{1c}. Also, in the study on determinants of HbA_{1c} in 8-9 year old children described in chapter 3 we found no significant relation between known risk factors for type 2 diabetes and HbA_{1c}. These results suggest that HbA_{1c} may not only reflect the preceding blood glucose levels, but seems to be determined by other factors as well.
The study into determinants of the change in HbA_{1c} between the age of 8 years and the age of 12 years in non-diabetic Dutch children confirms these findings. In this study, described in chapter 4, HbA_{1c} in non-diabetic children appears to be fairly stable over time. The observed lack of association between known risk factors for insulin resistance and HbA_{1c} in this study also suggest that HbA_{1c} in non-diabetic children is relatively unaffected by factors associated with glycaemia and is mainly determined by factors which are fairly constant over time.
Chapter 5 contains the results of a study on genetic and environmental determinants of HbA_{1c} in non-diabetic adults. In this study age, gender, BMI, fasting plasma glucose, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), current smoking and alcohol consumption were independent predictors of HbA_{1c}, together explaining 26.2% of the variance in HbA_{1c}, with fasting plasma glucose only contributing 10.9%. We replicated three of the previously identified SNPs, namely rs1402837, rs4737009 and rs1046896 and the calculated genetic risk scores (GRS) were also independently associated with HbA_{1c}. We found a smaller effect of the "non-glycaemic GRS" in females compared to males and an attenuation of the effect of the GRS of all 12 SNPs with increasing BMI. Also these results suggest that HbA_{1c} is determined by other factors besides preceding glucose levels, and that again raises serious questions about the proposed use of HbA_{1c} for diagnosing diabetes.

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Other disadvantages of using HbA_{1c} for diagnosing diabetes

Two main difficulties regarding the accurate measurement of HbA_{1c} are the large number of variant haemoglobins and glycohaemoglobins, and the fact that HbA_{1c} is not a stand-alone analyte because its quantity is related to the total haemoglobin concentration. As a result of this latter, HbA_{1c} is expressed as a ratio, i.e. HbA_{1c} / total haemoglobin, and this dual measurement causes dual uncertainty in het outcome of the test[32]. As pointed out by Kilpatrick et al.[4], the presence of any abnormal haemoglobin, like in people with sickle cell or haemoglobin C trait, may affect the HbA_{1c} measurement and give misleading results. And despite the standardization of HbA_{1c} measurements[33], the performance of the HbA_{1c} assays will not immediately improve.

Because HbA_{1c} lags changes in blood glucose levels, relying on it will delay diagnoses in many patients. Probably not for patients with type 1 diabetes, usually presenting with classic symptoms, but a delay in the diagnosis of type 2 diabetes will lead to a delay in the treatment and therefore a delay in preventing diabetes complications.

The costs of HbA_{1c} tests are considerably higher compared to those for plasma glucose. Therefore particularly in developing countries inclusion of HbA_{1c} among diabetes diagnostic criteria will increase the economic burden on the health system[34]. And despite the goal of worldwide standardization, it will take years before the accuracy and precision of the HbA_{1c} assay in these countries is comparable with the developed countries.

Conclusion

Finding the best way to diagnose diabetes has been a challenge for many years. The diagnosis based on the development of complications caused by the hyperglycaemia instead of the hyperglycaemia itself is probably most clinical useful, and since HbA_{1c} is firmly established as a measure of the risk for the development of complications, the use of HbA_{1c} for diagnosing diabetes seems to be a logical step. But there are important limitations of the use of an HbA_{1c} level ≥ 6.5% for diagnosing diabetes.

Firstly, evidence suggests that there is a continuous relationship between HbA_{1c} levels and cardiovascular complications, even in the non-diabetic range. A cut point at 6.5% would lead to under treatment of a lot of people at risk for cardiovascular disease.

In addition, other factors besides glycaemia seem to determine HbA_{1c} including e.g. the "glycation gap". But also genetic factors, erythrocyte lifespan, age and ethnicity influence HbA_{1c} independent of plasma glucose levels. Substantial effects of these factors on HbA_{1c} levels, independent of glycaemia,

will have important consequences for the use of HbA_{1c} as diagnostic tool for diabetes. And finally, some practical considerations like variant haemoglobins, assay imprecision and costs, and the delay in diagnosis argue against the use of HbA_{1c} for diagnosing diabetes.

In conclusion, HbA_{1c} offers great potential as a continuous marker for cardiovascular risk, probably even in patients without as well as in patients with diabetes. But, the results presented in this thesis show that the observed variation in HbA_{1c} caused by other factors besides glycaemia makes it unsuitable for dichotomizing and thereby diagnosing diabetes.

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