

HbA1c as a diagnostic tool in diabetes mellitus

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SUMMARY

Hemoglobin A1c (HbA1c) expresses the ratio between glycated HbA1 and total HbA1 and reflects the average plasma glucose concentration the preceding 2-3 months. It has become the most important marker of chronic hyperglycemia and is closely correlated to late complications of diabetes. The analysis has now been standardized and traceable to an international reference material and reference methods. Standardization of the HbA1c analyses paved the way for it to become the primary tool for diagnosing diabetes. Despite some limitations in which HbA1c is not representative of average glucose concentration, the inclusion of HbA1c as a diagnostic tool will simplify the diagnostics of diabetes compared with the glucose criteria, and hopefully improve the prevention of late complications of diabetes.

CHANGING DIAGNOSTIC CRITERIA OF DIABETES

Chronic hyperglycemia is the hallmark of diabetes mellitus. The diagnosis has until recently been based upon the detection of elevated plasma glucose levels either fasting or two hours after an oral glucose (75 g) tolerance test (OGTT) (1). The aim was initially to identify persons at high risk of developing acute symptoms and signs of hyperglycemia. In 1997 the American Diabetes Association recommended that the diagnostic levels of glucose should be based on the risk of developing late complications of chronic hyperglycemia instead of acute symptoms of hyperglycemia (2). The cut-off levels were determined by epidemiologic studies showing an association between glucose concentrations and retinopathy, which is regarded as the most diabetes specific late complication. The risk of developing retinopathy is characterized by relatively well defined threshold levels of fasting and OGTT glucose concentrations. Consequently, the diagnostic level of fasting plasma glucose was lowered from ≥ 7.8 to ≥ 7.0 mmol/L, whereas the OGTT level at ≥ 11.1 mmol/L remained unchanged. In contrast, the risk of developing chronic hyperglycemia and glucose values above the diagnostic criteria for diabetes shows no threshold, but increases along a continuum of increasing plasma glucose concentrations below the diagnostic levels.

Hemoglobin A1c (HbA1c) is a hemoglobin variant that is formed when glucose binds covalently to the beta-chain of HbA (for references see (3)). The initial Schiff base which is formed is subsequently rearranged to a stable Amadori product. The level of HbA1c closely reflects the average plasma glucose concentration the preceding 2-3 months (4). Approximately 50% of the amount of HbA1c is determined by the average glucose the last preceding month. The association between HbA1c and both glucose concentration and diabetic late complications has been extensively docu-

mented, early by a Norwegian study (5), and later in particular by the DCCT and UKPDS studies of type 1 and type 2 diabetes, respectively (6,7). HbA1c is our most important biomarker for glycemia control in diabetes. Although measurement of HbA1c has become essential for the follow-up of patients with diabetes, diagnostic use of HbA1c has been rejected at several revisions of the diagnostic criteria mainly with referral to the lack of standardization of the analysis. However, the treatment goals for diabetes have been defined in HbA1c units calibrated to the DCCT study. The need for a uniform definition of reference material and reference measurement systems was imminent when the International Federation of Clinical Chemistry (IFCC) defined that HbA1c is the valine glycated N-terminal hexapeptide of the beta-chain of hemoglobin A1 (8). HbA1c is the concentration ratio between the glycated and the total glycated and non-glycated hexapeptide. In addition two reference methods for HbA1c measurements were defined and described. All vendors of HbA1c instruments in Norway have to certify that the results are traceable to the IFCC reference material and method.

Standardization of the HbA1c analysis for the follow-up of patients with diabetes, paved the way for its diagnostic use. Systematic differences between instruments became smaller, and total allowable error in external quality controls became tighter. Total error shrank in the US National Glycohemoglobin Standardization Program (NGSP) from 15% in 2007 to 7% in 2011, which corresponds to the quality of glucose measurements (9). An international Expert Committee revived in 2009 the possibility of diagnostic use of HbA1c in the wake of its standardization (10). The diagnostic cut-off was suggested at $\geq 6.5\%$ (48 mmol/mol) which corresponds to an increased risk of nonproliferative diabetic retinopathy. A report from a World Health Organization (WHO) consultation accepted the diagnostic use of HbA1c at $\geq 6.5\%$ in

Table 1. Diagnostic criteria for diabetes in Norway¹

1. HbA1c \geq 6.5% ²
OR: If HbA1c is regarded inadequate
2. Fasting plasma glucose \geq 7.0 mmol/L
OR:
3. 2-h plasma glucose \geq 11.1 mmol/L after an oral glucose tolerance test
OR:
4. Random plasma glucose \geq 11.1 mmol/L in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.

¹Criteria 1-3 have to be confirmed by repeat testing.

²Analyzed by a laboratory fulfilling the diagnostic quality criteria.

2011 (11), but did not define a subgroup of high-risk individuals based on HbA1c levels such as suggested by the International Expert Committee (HbA1c 6.0-6.4%) (10) and the American Diabetes Association (HbA1c 5.7-6.4%) (1). The Norwegian Directorate of Health endorsed the WHO report in September 2012, and modified it so that HbA1c is the recommended diagnostic analysis for diabetes. Glucose measurements (fasting or OGTT) should be used when HbA1c is unreliable as a measure of the level of glycemia. The current diagnostic criteria for diabetes in Norway are listed in Table 1.

DIAGNOSTIC CONSIDERATIONS

For correct diagnostic use of HbA1c it is essential that the result is 1) representative of the patient's glycemia, 2) within acceptable limits of deviation from the "true value", and 3) correctly interpreted according to the diagnostic criteria.

Although there is a strong and positive correlation between average plasma glucose concentration and HbA1c levels, large individual differences exist (for references see (1,10,12,13). A rapid and stable change in plasma glucose level requires up to 30 days for a new steady state level of HbA1c (14). HbA1c is unsuitable to diagnose rapidly progressing type 1 diabetes. Conditions affecting the glycation rate of hemoglobin differ between individuals. The life span of erythrocytes markedly influences HbA1c levels within each red blood cell and the average HbA1c level of the individual. HbA1c levels higher than expected based on average glucose concentrations are expected when the average erythrocyte age is increased. This can be seen either caused by reduced erythrocyte synthesis such as iron deficiency anemia, vitamin B12 deficiency, chronic kidney disease, and bone marrow suppression or by reduced hemolysis after splenectomy. A lower HbA1c than expected is observed in conditions with increased red blood cell synthesis such as recent iron substitution in iron deficiency anemia or treatment with erythropoietin. Increased hemolysis will reduce the average erythrocyte age and subsequently result in lower HbA1c levels than expected. Among the ten genetic loci associated with HbA1c level in Caucasians, three are in loci associated

with fasting glucose levels, whereas seven are in genes related to erythrocyte physiology (15). A decision to use HbA1c diagnostically must also consider the probability that the patient has hemoglobin variants that may interfere with the analysis. A list of variants and their influence on HbA1c measurements is presented by the NGSP website (<http://www.ngsp.org/interf.asp>). Gestational diabetes has its own glucose based diagnostic criteria and has not included HbA1c.

HbA1c reference values of native samples are determined by certified laboratories. The WHO report did not specify quality criteria for diagnostic use of HbA1c, but stated that the analysis must be traceable to the IFCC standardization and that the laboratory has to participate in external quality assessment programs (16). In Norway all instruments are calibrated according to the IFCC reference material. The results are converted from IFCC format (mmol/mol) to the NGSP level (%), which is similar to DCCT level, by the formula (8):

$$\text{NGSP (\%)} = 0.0915 \cdot \text{IFCC (mmol/mol)} + 2.15$$

The external quality assessment program in Norway is organized by NOKLUS both for laboratories in hospitals and in primary care. The native control sample which is distributed to the laboratories is given a reference value for HbA1c by a collaborating reference laboratory. The total allowable error at the diagnostic level of 6.5% is set to 7%. This means that for a reported HbA1c value to be acceptable it has to be between 6.0-7.0% when the reference value of the sample is 6.5%. A total allowable error of 7% is in accordance with the NGSP requirements. In addition the laboratory has to document an internal quality control coefficient of variation (CV) \leq 2%. The external quality requirement will be fulfilled by more than 90% of the participating Norwegian laboratories (personal communication). The limit of the internal quality control CV is quite strict, but is based on reported desirable quality performance of HbA1c (9). It remains to be seen how these requirements will influence the laboratories willingness and efforts to obtain diagnostic HbA1c quality.

Diabetes can be diagnosed if HbA1c, fasting plasma glucose or the OGTT result is above the cut-off level in two independent tests (1). HbA1c is the preferred

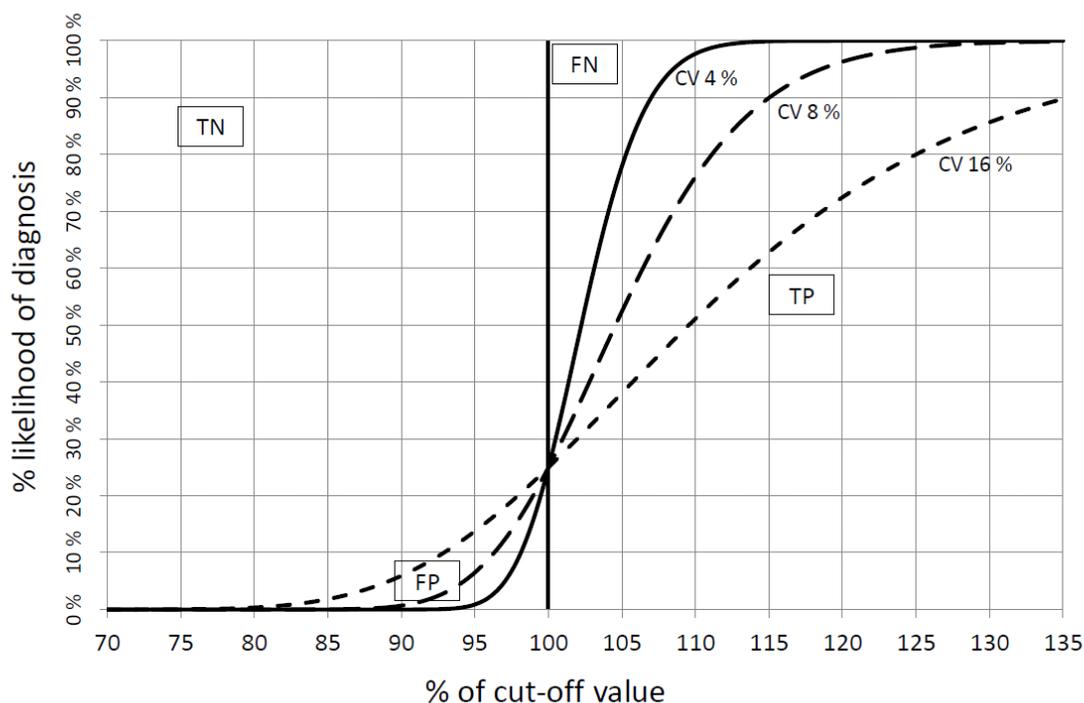


Figure 1. Per cent likelihood of diabetes diagnosis in individuals with a true value for the diagnostic test expressed as per cent of the diagnostic-cut-off value at different coefficients of variation (CVs). The likelihood is estimated based on two consecutive tests equal to or above the cut-off level of the same test in the same individual using CVs of 4%, 8%, and 16% representative of HbA1c, fasting plasma glucose, and 2-h glucose after OGTT, respectively. The model assumes that the tests are performed without bias. TN = true negatives, FP = false positives, FN = false negatives, and TP = true positives.

test. An elevated test result has to be confirmed by a second test of the same substance above the cut-off level normally a few weeks later, but the time between the two tests has not been specified. If for example both HbA1c and fasting plasma glucose are above the cut-off level, the patient has diabetes because two independent tests are elevated. If fasting plasma glucose is above the cut-off, and HbA1c below, fasting plasma glucose should be controlled and confirmed elevated before a diagnosis can be established. If the patient has symptoms or signs of diabetes, one test result above the cut-off is sufficient.

It has been argued that the implementation of HbA1c among the diagnostic tests will disturb the epidemiologic data of diabetes. When screening a population without diabetes with HbA1c, fasting plasma glucose and OGTT, it is generally observed that the fraction with OGTT results above the diagnostic threshold will be largest, elevated fasting plasma glucose comes second, whereas the fraction with HbA1c above the cut-off is the smallest (17). It has been claimed that HbA1c will detect fewer patients with diabetes than the glucose criteria. This is based on the assumption that an elevated result will be equally reproduced at the control test, which is required for the diagnosis, regardless of method employed. However, the total coefficient of variation (CV) including biological and analytical variation within individuals is much higher for OGTT compared with fasting plasma glucose

which is intermediate and HbA1c with the lowest CV of approximately 4% (18-20). An elevated HbA1c level is more likely to be reproduced and confirm a diagnosis of diabetes compared with an elevated OGTT result. When the true value of an individual is 100% of the threshold value, the likelihood of having two consecutive tests equal to or above the cut-off is 25% independent of the CVs when systematic bias is excluded (Figure 1). The likelihood increases to almost 100% when the true value is 110% of the cut-off value for a test with a total CV of 4%, which is typical performance of HbA1c. This corresponds to a HbA1c level of 7.2%. For fasting plasma glucose the CV is typically at 8%. A true glucose level 10% higher than the cut-off (7.7 mmol/L) results in a likelihood of diagnosing diabetes at approximately 75%. The likelihood is reduced to 50% when the individual true concentration of plasma glucose after OGTT (typical CV 16%) is 110% of the cut-off (12.2 mmol/L). True fasting plasma glucose level has to be at least 8.7 mmol/L in order to obtain a similar level of diabetes diagnostic likelihood as a true HbA1c value of 7.2% due to the differences in CVs.

CONCLUSION

International standardization of the HbA1c analysis has made it suitable not only for the follow-up of patients with diabetes, but also for diagnostic purpose.

Although the interpretation of HbA1c as a biomarker of average plasma glucose has some limitations in particular related to erythrocyte turnover and metabolism, HbA1c has several advantages and requires no fasting or time-consuming testing and is stable and more conveniently handled than plasma glucose. Using HbA1c as the primary diagnostic criterion should make it easier to detect diabetes at an early stage. Early identi-

fication of patients with diabetes should be encouraged as it is fundamental for successful prevention of late complications of diabetes.

ACKNOWLEDGEMENT

The discussion and estimation of diagnostic likelihood in collaboration with Daniel Sachse is greatly appreciated.

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