REVIEW ARTICLE

# Hemoglobin $A_{1c}$ for the diagnosis of diabetes

## Practical considerations

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**KEY WORDS**

diagnostic tests, hemoglobin $A_{1c}$, type 2 diabetes

**ABSTRACT**

The International Expert Committee recommends that the diagnosis of diabetes be made if hemoglobin $A_{1c}$ ($HbA_{1c}$) level is ≥6.5% and confirmed with a repeat $HbA_{1c}$ test. The committee recommends against "mixing different methods to diagnose diabetes" because "the tests are not completely concordant: using different tests could easily lead to confusion". Fasting plasma glucose, 2-hour postglucose-load plasma glucose, and oral glucose tolerance tests are recommended for the diagnosis of diabetes only if $HbA_{1c}$ testing is not possible due to unavailability of the assay, patient factors that preclude its interpretation, and during pregnancy. $HbA_{1c}$ testing has the advantages of greater clinical convenience, preanalytic stability, and assay standardization, but when used as the sole diagnostic criterion for diabetes, it has the potential for systematic error. Factors that may not be clinically evident impact $HbA_{1c}$ test results and may systematically raise or lower the value relative to the true level of glycemia. For this reason, $HbA_{1c}$ should be used in combination with plasma glucose determinations for the diagnosis of diabetes. If an $HbA_{1c}$ test result is discordant with the clinical picture or equivocal, plasma glucose testing should be performed. A diagnostic cut-off point of $HbA_{1c}$ ≥6.5% misses a substantial number of people with type 2 diabetes, including some with fasting hyperglycemia, and misses most people with impaired glucose tolerance. Combining the use of $HbA_{1c}$ and plasma glucose measurements for the diagnosis of diabetes offers the benefits of each test and reduces the risk of systematic bias inherent in $HbA_{1c}$ testing alone.

In 1958, Allen et al. recognized that hemoglobin $A_1$ could be separated by cation-exchange chromatography.[1] In 1968, Rahbar first reported that hemoglobin $A_1$ represented a glycated form of hemoglobin that was increased in diabetes,[2] and in 1976 Koenig et al. suggested that because hemoglobin $A_{1c}$ ($HbA_{1c}$) is formed slowly and nonenzymatically at a rate directly proportional to the ambient glucose concentration, it might be a useful indicator of both glucose tolerance and glucose regulation in diabetes.[3,4] Currently, $HbA_{1c}$ is widely accepted as a measure of glycemic control in established diabetes, an indicator of the risk for development of diabetic complications, and a reflection of the quality of diabetes care. In 2008, a committee was appointed by the American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation to consider the use of $HbA_{1c}$ for the diagnosis of diabetes.[5]

Historically, diabetes has been diagnosed on the basis of glucose levels. In the 1950s, Fajans and Conn studied lean, healthy individuals without family histories of diabetes, administered oral glucose loads, and measured glucose levels at time intervals following the glucose loads.[6] Glucose levels were normally distributed, that is, they fell into a symmetrical, unimodal, and bell-shaped distribution characterized by a mean and standard deviation. Diabetes was defined using statistical criteria as glucose levels greater than the mean values plus 2 standard deviations 60, 90, and 120 min after the oral glucose load. In the 1970s, Bennett et al. recognized that in Pima Indians, a population with a high prevalence of diabetes, glucose levels were bimodally distributed, that is, they fell into 2 overlapping bell-shaped distributions.[7] The lower bell-shaped distribution represented glucose levels for the nondiabetic population and the upper bell-shaped distribution represented

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**Received:** September 21, 2009.
**Accepted:** September 21, 2009.
**Conflict of interest:** none declared.
Pol Arch Med Wewn. 2010; 120 (1-2): 37-41

glucose levels for the diabetic population. The National Diabetes Data Group used the antimode of glucose level that optimally distinguished the 2 overlapping distributions to establish fasting and 2-hour postglucose-load glucose criteria for diabetes in 1979.[8] In 1997, the American Diabetes Association examined the cross-sectional relationship between glycemia and diabetic retinopathy to redefine the fasting glucose criterion for diabetes as the level associated with an increased risk of diabetic retinopathy.[9]

In recent years, the use of fasting and postglucose-load glucose criteria for the diagnosis of diabetes have been faulted because of their inconvenience, the fact that they require patients to be seen in the morning after an overnight fast, and their susceptibility to modification by short-term lifestyle changes that patients may make in preparation for doctor visits.[10] Glucose measurements are also susceptible to preanalytic and analytic errors and they exhibit substantial intra-individual biologic variation.[9] Improperly collected and handled blood samples are susceptible to in-vitro glycolysis. Failure to collect blood samples in sodium fluoride containing tubes, failure to collect blood samples on ice rather than at room temperature, and failure to separate the plasma promptly may all result in decreases in plasma glucose levels. Bias in the standardization of the glucose assay and differences in instrumentation may also introduce error into the measurement of glucose.[11,12] In addition, intra-individual biologic variability in fasting (6% coefficient of variation) and 2-hour postglucose-load glucose levels (17% coefficient of variation) may make glucose a less than optimal diagnostic tool.[13] Unlike preanalytic and analytic error, which will predictably lower or raise measured glucose values relative to true glucose values, the biologic variability is likely to be at random, that is, equally likely to lower or raise the measured glucose value relative to the true glucose value.

$HbA_{1c}$ has the advantages of not requiring an overnight fast or a morning blood draw. In addition, it is familiar and generally available to clinicians in developed countries. It is less likely than glucose to be affected by short-term lifestyle changes. $HbA_{1c}$ is relatively stable at room temperature and shows less intra-individual biologic variability (4% coefficient of variation) than fasting or postglucose-load glucose levels.[13] In addition, the precision and accuracy of the $HbA_{1c}$ assay have benefited from an international effort to improve assay standardization.[14]

Despite its greater clinical convenience, preanalytic stability, biologic stability, and assay standardization, $HbA_{1c}$ has an important limitation. $HbA_{1c}$ suffers from the potential for systematic error – analytic bias that consistently lowers or raises the measured value relative to the true value. This becomes a major problem when $HbA_{1c}$ is used as the only diagnostic criterion for diabetes as it will result in some individuals having persistently high or low measured $HbA_{1c}$ values relative to the true $HbA_{1c}$ value. Although perhaps not immediately obvious, this is less of a problem when $HbA_{1c}$ is used as a measure of glycemic control in established diabetes. In that role, $HbA_{1c}$ is routinely compared with multiple self-monitored blood glucose values and inconsistencies are readily identified.

Any condition that decreases mean erythrocyte age will lower $HbA_{1c}$ test results independent of glycemia and regardless of the assay method used.[15] Examples of such conditions include hemolytic anemia and recovery from acute blood loss. Similarly, any condition that increases erythrocyte age such as prior splenectomy or aplastic anemia will increase $HbA_{1c}$ test results independent of glycemia.

Structural hemoglobinopathies and thalassemia syndromes may also impact $HbA_{1c}$ test results, with the impact depending on the pathologic processes involved and the assay method employed. HbS-trait, which affects approximately 8% of African Americans; HbC-trait, which affects approximately 3% of African Americans; and HbE-trait, which affects 10% and in some areas even more than 50% of Southeast Asians, are all reported to affect some $HbA_{1c}$ assay methods.[16] Elevated hemoglobin F, which is associated with thalassemia syndromes, also affects some assay methods.[16]

Uremia, hyperbilirubinemia, hypertriglyceridemia, chronic alcoholism, chronic ingestion of salicylates, vitamin C ingestion, and opiate addiction have also been reported to interfere with some assay methods, falsely increasing results.[16] In some assays, vitamin C and vitamin E ingestion have also been reported to falsely lower $HbA_{1c}$ results.[16] Iron deficiency, which affects up to 20% of menstruating women[17] and many pregnant women, has been reported to increase $HbA_{1c}$ test results by altering the structure of the hemoglobin molecule and making it easier to glycate.[18]

Race and ethnicity may also impact $HbA_{1c}$ independent of glycemia. A large international study designed to define the relationship between $HbA_{1c}$ and mean blood glucose levels suggested that the relationship between $HbA_{1c}$ and mean blood glucose was different for African Americans compared to Caucasians such that for any given mean glucose level, African Americans tended to have higher $HbA_{1c}$ levels.[19] We have also described $HbA_{1c}$ by race and ethnicity among patients with both impaired glucose tolerance enrolled in DPP (Diabetes Prevention Program) and in patients with recent-onset, drug-naïve type 2 diabetes enrolled in ADOPT (A Diabetes Outcome Progression Trial).[20,21] We found that $HbA_{1c}$ levels were significantly higher in African Americans, Hispanics, Asians, and other races and ethnicities compared to whites before and after adjusting for factors that differed among groups and might affect glycemia.

When interferences with $HbA_{1c}$ are recognized, alternative forms of testing may be employed to assess glycemia. Unfortunately, factors affecting

the accuracy of $HbA_{1c}$ measurement may not be recognized clinically, especially if $HbA_{1c}$ is assessed without measuring glucose.

The International Expert Committee recommends that the diagnosis of diabetes be made if the $HbA_{1c}$ level, measured using clinical laboratory equipment and not point-of-care instruments, is ≥6.5% and confirmed with a repeat $HbA_{1c}$ test.[5] The International Expert Committee further states that "mixing different methods to diagnose diabetes should be avoided because the 3 tests are not completely concordant; using different tests could easily lead to confusion".[5] If, however, $HbA_{1c}$ testing is not possible due to unavailability of the assay, patient factors that preclude interpretation, and during pregnancy when changes in red cell turnover make the assay problematic, previously recommended fasting, 2-hour postglucose-load glucose, and oral glucose tolerance tests and criteria should be used.[5]

Clearly, $HbA_{1c}$ should not be used in patients with factors known to affect the validity of the assay, and the laboratory methodology used to test $HbA_{1c}$ should be appropriate to the population being screened. Thus, for example, in a population with a high prevalence of a specific hemoglobinopathy, the method chosen to assay $HbA_{1c}$ should not be affected by that hemoglobinopathy. $HbA_{1c}$ should be employed to diagnose diabetes when it is convenient and available. When, for example, a patient is seen in the afternoon for an acute illness in a hospital-based clinic and is determined to be at risk of undiagnosed diabetes, $HbA_{1c}$ testing is appropriate. Conversely, if a patient is being seen in the morning for a scheduled periodic health appraisal and is already fasting for a lipid assessment, then fasting glucose testing is perfectly appropriate.

We believe that if $HbA_{1c}$ is used to test for diabetes, it should be used in combination with plasma glucose. This is especially true if the $HbA_{1c}$ result is inconsistent with the clinical symptoms or signs or if the measured $HbA_{1c}$ is close to the diagnostic threshold. If an $HbA_{1c}$ test result is equivocal, for example, between 6% and 6.5%, it should be confirmed with a plasma glucose test (fasting plasma glucose, 2-hour postglucose-load plasma glucose, or oral glucose tolerance test). Studies have suggested that the 2-hour postglucose-load glucose level is a more sensitive test for the diagnosis of diabetes than either the fasting glucose or the $HbA_{1c}$ level.[22,23] Indeed, our work demonstrates that a diagnostic cut-off point for $HbA_{1c}$ of ≥6.5% misses a substantial number of patients with type 2 diabetes, including some with fasting hyperglycemia, and misses most patients with impaired glucose tolerance.[23] Several studies have shown that intensive lifestyle modification or metformin can delay or prevent the development of diabetes in people with impaired glucose tolerance.[24] Combining the use of $HbA_{1c}$ and glucose tests for the diagnosis of diabetes offers the benefits of each test and reduces the risk of systematic bias inherent in $HbA_{1c}$ testing alone in patients with unrecognized factors resulting in persistently low $HbA_{1c}$ levels.

Clearly, such an approach introduces some diagnostic uncertainty but medicine is full of uncertainty and physicians are well trained to deal with it. The recommendation to use both $HbA_{1c}$ and glucose tests to diagnose diabetes is consistent with current diagnostic criteria: a plasma glucose-dependent diagnosis must now be confirmed by a second plasma glucose measurement unless there is unequivocal, symptomatic hyperglycemia. Using both $HbA_{1c}$ and a glucose test is no more burdensome than the current requirements and indeed, both tests may be performed on the same day, establishing a diagnosis without the need for retesting on a separate day.

**Acknowledgments** This work was supported by the Michigan Diabetes Research and Training Center funded by DK020 572 from the National Institute of Diabetes and Digestive and Kidney Diseases.

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# Hemoglobina glikowana $A_{1c}$ w diagnostyce cukrzycy

## Uwagi praktyczne

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**SŁOWA KLUCZOWE**

cukrzyca typu 2, hemoglobina glikolowana, testy diagnostyczne

**STRESZCZENIE**

The International Expert Committee zaleca rozpoznawanie cukrzycy, gdy w dwóch pomiarach stwierdza się wartość hemoglobiny glikowanej ($HbA_{1c}$) ≥6,5%. Jednoczesne stosowanie kilku rekomendowanych dotychczas różnych metod diagnostycznych może bowiem prowadzić do niejednoznacznych wniosków. Wykonywanie wcześniej rekomendowanych badań, takich jak pomiar glikemii na czczo czy doustny test obciążenia glukozą, jest wskazane tylko w przypadku braku dostępności pomiarów $HbA_{1c}$, obecności czynników uniemożliwiających interpretację pomiaru $HbA_{1c}$ oraz u kobiet w ciąży. Zaletami pomiarów $HbA_{1c}$ są większa wygoda, standardyzacja badań, mniejsze ryzyko błędów przedlaboratoryjnych niż w przypadku pomiarów glikemii. Jednakże używanie pomiarów $HbA_{1c}$ jako jedynej metody diagnostycznej wiąże się z większym prawdopodobieństwem wystąpienia błędów systematycznych, gdyż wyniki pomiarów $HbA_{1c}$ mogą być fałszywie zaniżane lub zawyżane przez wiele (często nierozpoznanych) czynników. Dlatego nadal istnieje uzasadnienie dla oceny poziomów $HbA_{1c}$ w kontekście pomiarów glikemii w diagnostyce cukrzycy. Badanie glikemii zaleca się szczególnie wtedy, gdy wyniki $HbA_{1c}$ są sprzeczne z obrazem klinicznym albo niejednoznaczne. Przyjęcie diagnostycznego punktu odcięcia dla $HbA_{1c}$ ≥6,5% może prowadzić do niepełnej wykrywalności cukrzycy typu 2. Ponadto poziom ten nie pozwoli na wykrycie większości przypadków nieprawidłowej tolerancji glukozy oraz nieprawidłowej glikemii na czczo. Stosowanie w diagnostyce cukrzycy dwóch metod – pomiaru $HbA_{1c}$ oraz oznaczania poziomu glukozy w osoczu – pozwala łączyć zalety obu testów i zredukować ryzyko błędu systematycznego wynikającego z pomiaru tylko $HbA_{1c}$.

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**Praca wpłynęła:** 21.09.2009.
**Przyjęta do druku:** 21.09.2009.
**Nie zgłoszono sprzeczności interesów.**
Pol Arch Med Wewn. 2010;
120 (1-2): 37-41
Tłumaczył lek. Łukasz Strzeszyński