Limitations of hemoglobin A_{1c} in the management of type 2 diabetes mellitus

Nemin Adam Zhu MD CCFP Sonja Reichert MD MSc CCFP Stewart B. Harris MD MPH FCFP FACPM

Hemoglobin A_{1c} (HbA_{1c}) is a valuable diagnostic and prognostic marker of glycemic control in patients with diabetes that has been validated in clinical trials to correlate with diabetes-related complications.[1-3]

Hemoglobin A_{1c} is hemoglobin that is glycated at the N-terminal valine residue of the β chain of adult hemoglobin.[4] The hemoglobin molecule consists of an iron-containing heme ring and 4 globin chains. The globin chains determine the hemoglobin type. Adult hemoglobin consists of 2 α and 2 β chains and represents 97% of total hemoglobin.[4] Hemoglobinopathies are genetic hemoglobin disorders that either result in the production of an abnormally low quantity of a globin chain (thalassemia) or the production of structurally variant hemoglobin. The most common structural hemoglobin variants include the following: hemoglobin S (also known as *sickle cell hemoglobin*), which is found in 6% to 9% of African Americans; hemoglobin C, which is found in 2.3% of African Americans; and hemoglobin E, which is found in up to 30% of Southeast Asians.[5,6]

As erythrocytes have an average life span of 120 days, HbA_{1c} is a useful measure of glycemic control over the preceding 3 months with an emphasis on the preceding 30 days.[7] However, a number of conditions can affect its reliability.

Case 1

A 58-year-old man presented to the emergency department with a 2-week history of fatigue, polyuria, and polydipsia. Past medical history included sickle cell disease (also known as *hemoglobin SC disease*), with hemoglobin S variant inherited from one parent, and hemoglobin C variant from the other. Investigations

Editor's key points

- Hemoglobin A_{1c} (HbA_{1c}) is a valuable diagnostic and prognostic marker of glycemic control in patients with diabetes. However, there are conditions that can affect the reliability of HbA_{1c} measurement (eg, chronic kidney disease, anemia, and hemoglobinopathy), so it is important to be aware of this blood test's limitations.
- Erythrocytes have an average life span of 120 days. Conditions that prolong the life of erythrocytes or are associated with decreased erythrocyte turnover will lead to increased exposure of cells to glucose and falsely high HbA_{1c} results; conditions that shorten the life of erythrocytes or are associated with increased erythrocyte turnover will lead to reduced exposure of cells to glucose and falsely low HbA_{1c} results.
- The patients presented in this case report had conditions that led to falsely low HbA_{1c} results; however, not all patients with conditions that can affect HbA_{1c} levels will have unreliable test results. Periodic evaluation of self-monitored blood glucose readings is important to ensure concordance with HbA_{1c} results. Discordant results should prompt a venous fasting blood glucose test to ensure meter accuracy. If self-monitored blood glucose readings are accurate, an evaluation of possible conditions that can affect HbA_{1c} is warranted.

Points de repère du rédacteur

- L'hémoglobine A_{1c} (HbA_{1c}) est un précieux marqueur diagnostique et pronostique du contrôle de la glycémie chez les patients diabétiques. Par ailleurs, des problèmes peuvent affecter la fiabilité de la mesure de l'HbA_{1c} (p. ex. néphropathie chronique, anémie, hémoglobinopathie), et il importe donc d'être conscient des limites de ces analyses sanguines.
- La durée moyenne de vie des érythrocytes se situe à 120 jours. Les conditions qui prolongent la vie des érythrocytes ou qui sont associées à une réduction de leur renouvellement entraîneront une augmentation de l'exposition des cellules au glucose, ainsi que des résultats faussement élevés de l'HbA_{1c}; les conditions reliées à une réduction de la durée de vie des érythrocytes ou associées à leur renouvellement plus rapide réduiront l'exposition des cellules au glucose, d'où des mesures faussement basses de l'HbA_{1c}.
- Les patients présentés dans ce rapport de cas avaient des problèmes qui se sont traduits par des résultats faussement bas de l'HbA_{1c}; par contre, tous les patients dont les conditions peuvent affecter les taux d'HbA_{1c} n'auront pas nécessairement des résultats de tests non fiables. Il est important de procéder à une évaluation périodique des lectures de la glycémie automesurée pour s'assurer qu'elles correspondent avec les résultats de la mesure de l'HbA_{1c}. Si les résultats ne concordent pas, il faut procéder à une analyse de la glycémie veineuse à jeun pour assurer l'exactitude des mesures. Si les lectures de la glycémie faites par le patient sont exactes, il y a lieu d'évaluer les éventuelles conditions susceptibles d'affecter l'HbA_{1c}.

showed a blood glucose level of 56.3 mmol/L and a pH level of 7.32. There was evidence of hemolytic anemia, with laboratory values revealing a hemoglobin level of 78 g/L, total bilirubin level of 49.1 µmol/L, and lactate dehydrogenase level of 11.44 µkat/L (685 U/L). He was treated with intravenous fluids and insulin, and transitioned to multiple daily injections of insulin. Initial HbA_{1c} testing by high-performance liquid chromatography was not done owing to "interfering, unknown substances." His HbA_{1c} level over the next 2 years was consistently below 6.0%. His multiple daily injections were stopped because of perceived overtreatment. He presented to the emergency department months later with symptomatic hyperglycemia. Self-monitored blood glucose (SMBG) levels were between 18 and 30 mmol/L. His insulin was restarted.

Case 2

A 71-year-old woman with type 2 diabetes taking basal and bolus insulin was assessed in clinic. Past medical history included chronic kidney disease (CKD), nonalcoholic steatohepatitis, and iron deficiency anemia due to chronic gastrointestinal bleeding. She required frequent iron infusions and packed red blood cell transfusions. Recent bloodwork revealed the following values: HbA_{1c} level of 6.0%, hemoglobin level of 75 g/L, reticulocyte count of 175, and ferritin level of 236 pmol/L (105 µg/L). Fasting SMBG levels were 9 to 15 mmol/L, which were inconsistent with the HbA_{1c} value.

Discussion

Hemoglobin A_{1c} is a guideline-recommended test for diagnosis and follow-up in patients with diabetes.[8] There are 2 different methods of measurement. One method, high-performance liquid chromatography, detects the difference in ionic charge of glycated and nonglycated hemoglobin.[9] The second method, immunoassays, uses antibodies to recognize changes in the structure of the glycated molecule.[10]

Although there has been improvement in the accuracy and reliability of measuring HbA_{1c}, the test can be affected by certain conditions.[11-13] Conditions that prolong the life of erythrocytes or are associated with decreased erythrocyte turnover lead to increased exposure of cells to glucose and falsely high HbA_{1c} results[14]; examples include iron deficiency anemia, B12 deficiency anemia, folate deficiency anemia, chronic alcohol use, and asplenia.[14] Conditions that shorten the life of erythrocytes or are associated with increased erythrocyte turnover lead to reduced exposure of cells to glucose and falsely low HbA_{1c} results[14]; examples include acute and chronic blood loss, hemolytic anemia, splenomegaly, and pregnancy.[14]

Hemoglobinopathies can affect HbA_{1c} by altering glycation, interfering with the assay, or causing erythrocytes to be more prone to hemolysis.[4] Therefore, hemoglobinopathies can lead to falsely high or low measurements depending on the variant, the method, and the specific assay used.[14] For example, in β thalassemia there is a high concentration of fetal hemoglobin (HbF), which consists of 2 α and 2 γ chains. Certain immunoassays specifically identify the β chain and will not detect the glycated γ chain that is seen at high levels of HbF, producing a falsely low HbA_{1c} result. This is a known limitation of immunoassays, especially at a HbF level greater than 10%.[15]

The NGSP, previously known as the *National Glycohemoglobin Standardization Program*, provides an overview of various HbA_{1c} assays and possible interference with hemoglobin variants.[16] Our local laboratories (London, Ont) use a test that is not affected by common hemoglobin variants such as hemoglobins S, E, C, and D.[16]

Chronic kidney disease presents a challenge for measuring HbA_{1c}, particularly when the estimated glomerular filtration rate is less than 30 mL/min per 1.73 m^2.[17-20] These patients have uremia and anemia of multifactorial causes (eg, iron deficiency, erythropoietin deficiency, and decrease in erythrocyte survival). Uremia leads to the formation of carbamylated hemoglobin, which can interfere with certain measurement methods.[4] Uremia can also shorten the life span of erythrocytes.[17] In addition, patients with CKD frequently receive erythropoietin to increase erythrocyte production, which can lead to falsely low HbA_{1c} results.[17,18] Therefore, CKD makes HbA_{1c} measurements unreliable.

Alternative biomarkers to HbA_{1c} include fructosamine, glycated albumin, and plasma 1,5-anhydroglucitol levels. However, only HbA_{1c} has been validated in randomized controlled trials to predict diabetes-related complications.[5,14] The widespread use of alternative biomarkers is not established and is not currently recommended by Canadian guidelines.[21]

Patient 1 had sickle cell disease (hemoglobin SC disease) causing hemolytic anemia, which led to a falsely low HbA_{1c} result. Whether the more common sickle cell trait (also known as *hemoglobin AS*) affects HbA_{1c} is inconclusive. A retrospective cohort study of 4620 African Americans found that sickle cell trait significantly lowered HbA_{1c} results compared with individuals without sickle cell trait (5.72% vs 6.01% [mean HbA_{1c} difference of -0.29%; 95% CI -0.35% to -0.23%]).[22] However, 2 older studies did not find an effect of sickle cell trait on HbA_{1c}.[23,24]

Patient 2 had considerable anemia due to chronic gastrointestinal bleeding and CKD, which led to a falsely low HbA_{1c} result. However, iron deficiency leads to reduced erythrocyte production and reduced erythrocyte turnover. This allows more time for glycation to occur and reveals a falsely high HbA_{1c} result. A systematic review found falsely high HbA_{1c} values in iron deficiency anemia.[25] The discrepancy was estimated to be less than 0.5%. However, some studies looking at HbA_{1c} values after treatment of

iron deficiency anemia demonstrated a significant reduction in HbA_{1c} values of 1.2% ($P<.001$).[25]

Not every patient with a condition that can affect HbA_{1c} levels will have unreliable test results. However, family physicians should be aware of the possible limitations of the test. Periodic evaluation of SMBG readings is important to ensure concordance with HbA_{1c} measurements. Discordant results should prompt a venous fasting blood glucose test to ensure meter accuracy.[7] If SMBG readings are accurate, an evaluation of possible conditions that can affect HbA_{1c} is warranted.

Self-monitored blood glucose remains a valuable tool in diabetes management. Instructing patients to complete SMBG readings as dictated by the severity of their diabetes or medication regimen is crucial, especially when HbA_{1c} measurement is unreliable, in order to evaluate glycemic control. More advanced monitoring options such as continuous glucose monitoring or flash glucose monitoring can also be considered.[26]

Conclusion

Hemoglobin A_{1c} is a marker of glycemic control and has been correlated with diabetes-related complications. However, there are conditions that can affect the reliability of HbA_{1c} measurements, which might also affect clinical decision making. Health care providers should be aware of the limitations of this blood test.

Dr Zhu is a recent graduate of the Chronic Disease Management Enhanced Skills program in the Schulich School of Medicine and Dentistry at the University of Western Ontario in London and currently works with the Primary Care Diabetes Support Program at St Joseph's Health Care London. **Dr Reichert** is Assistant Professor in the Department of Family Medicine at the Schulich School of Medicine and Dentistry at the University of Western Ontario. **Dr Harris** is Professor in the Department of Family Medicine, Diabetes Canada Chair in Diabetes Management, and Ian McWhinney Chair of Family Medicine Studies at the Schulich School of Medicine and Dentistry at the University of Western Ontario.

Competing interests
None declared

Correspondence
Dr Nemin Adam Zhu; e-mail **nemin.zhu@sjhc.london.on.ca**

References

1. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-Year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008;359(15):1577-89. Epub 2008 Sep 10.
2. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000;321(7258):405-12.
3. Nathan DM; DCCT/EDIC Research Group. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study at 30 years: overview. *Diabetes Care* 2014;37(1):9-16.
4. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem* 2001;47(2):153-63. Erratum in: *Clin Chem* 2001;47(6):1141.
5. Smaldone A. Glycemic control and hemoglobinopathy: when A1C may not be reliable. *Diabetes Spectr* 2008;21(1):46-9.
6. National Institute of Diabetes and Digestive and Kidney Diseases [website]. *Sickle cell trait & other hemoglobinopathies & diabetes (for providers).* Bethesda, MD: National Institute of Diabetes and Digestive and Kidney Diseases; 2014. Available from: **www.niddk.nih.gov/health-information/diagnostic-tests/sickle-cell-trait-hemoglobinopathies-diabetes.** Accessed 2019 Jan 4.
7. Berard LD, Siemens R, Woo V; Diabetes Canada Clinical Practice Guidelines Expert Committee. Monitoring glycemic control. Diabetes Canada 2018 clinical practice guidelines for the prevention and management of diabetes in Canada. *Can J Diabetes* 2018;42:S47-53.
8. Diabetes Canada Clinical Practice Guidelines Expert Committee. Diabetes Canada 2018 clinical practice guidelines for the prevention and management of diabetes in Canada. *Can J Diabetes* 2018;42(Suppl 1):S1-325.
9. Sacks DB. Hemoglobin variants and hemoglobin A1c analysis: problem solved? *Clin Chem* 2003;49(8):1245-7.
10. Rhea JM, Koch D, Ritchie J, Singh HV, Young AN, Burgess T, et al. Unintended reporting of misleading Hb A(1c) values when using assays incapable of detecting hemoglobin variants. *Arch Pathol Lab Med* 2013;137(12):1788-91.
11. Little RR, Wiedmeyer HM, England JD, Wilke AL, Rohlfing CL, Wians FH Jr, et al. Interlaboratory standardization of measurements of glycohemoglobins. *Clin Chem* 1992;38(12):2472-8.
12. Sacks DB. Measurement of hemoglobin A(1c): a new twist on the path to harmony. *Diabetes Care* 2012;35(12):2674-80.
13. Tran HA, Silva D, Petrovsky N. Case study: potential pitfalls of using hemoglobin A1c as the sole measure of glycemic control. *Clin Diabetes* 2004;22(3):141-3.
14. Radin M. Pitfalls in hemoglobin A1c measurement: when results may be misleading. *J Gen Intern Med* 2014;29(2):388-94. Epub 2013 Sep 4.
15. Sabath DE. Case study: artifactually low hemoglobin A_{1c} in a patient with high hemoglobin F. *Clin Diabetes* 2000;18(4):179-84.
16. NGSP [website]. *Factors that interfere with HbA1c test results.* Bethesda, MD: NGSP; 2010. Available from: **www.ngsp.org/factors.asp**. Accessed 2019 Jan 4.
17. Wright L, Hirsch I. The challenge of the use of glycemic biomarkers in diabetes: reflecting on hemoglobin A1C, 1,5-anhydroglucitol, and the glycated proteins fructosamine and glycated albumin. *Diabetes Spectr* 2012;25(3):141-8.
18. Ng JM, Cooke M, Bhandari S, Atkin SL, Kilpatrick ES. The effect of iron and erythropoietin treatment on the A1C of patients with diabetes and chronic kidney disease. *Diabetes Care* 2010;33(11):2310-3. Epub 2010 Aug 26.
19. Harada K, Sumida K, Yamaguchi Y, Akai Y. Relationship between the accuracy of glycemic markers and the chronic kidney disease stage in patients with type 2 diabetes mellitus. *Clin Nephrol* 2014;82(2):107-14.
20. Jung M, Warren B, Grams M, Kwong YD, Shafi T, Coresh J, et al. Performance of non-traditional hyperglycemia biomarkers by chronic kidney disease status in older adults with diabetes: results from the Atherosclerosis Risk in Communities Study. *J Diabetes* 2018;10(4):276-85. Epub 2017 Dec 21.
21. Imran SA, Agarwal G, Bajaj HS, Ross S; Diabetes Canada Clinical Practice Guidelines Expert Committee. Targets for glycemic control. *Can J Diabetes* 2018;42(Suppl 1):S42-6.
22. Lacy M, Wellenius G, Sumner A, Correa A, Carnethon MR, Liem RI, et al. Association of sickle cell trait with hemoglobin A1c in African Americans. *JAMA* 2017;317(5):507-15.
23. Bleyer AJ, Vidya S, Sujata L, Russell GB, Akinnifesi D, Hire D, et al. The impact of sickle cell trait on glycated haemoglobin in diabetes mellitus. *Diabet Med* 2010;27(9):1012-6.
24. Sumner AE, Thoreson CK, O'Connor MY, Ricks M, Chung ST, Tulloch-Reid MK, et al. Detection of abnormal glucose tolerance in Africans is improved by combining A1c with fasting glucose: the Africans in America Study. *Diabetes Care* 2015;38(2):213-9.
25. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. *Diabetologia* 2015;58(7):1409-21. Epub 2015 May 21.
26. Beck RW, Connor CG, Mullen DM, Wesley DM, Bergenstal RM. The fallacy of average: how using HbA_{1c} alone to assess glycemic control can be misleading. *Diabetes Care* 2017;40(8):994-9.

This article has been peer reviewed.
Cet article a fait l'objet d'une révision par des pairs.
Can Fam Physician 2020;66:112-4