Case Study

What Clinical Laboratorians Should Do in Response to Extremely Low Hemoglobin A1c Results

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ABSTRACT

Extremely low hemoglobin A1c (HbA1c) results below reference range are rare, and the causes and clinical implications associated with low HbA1c results are not well understood among clinical laboratorians. A case of extremely low HbA1c results was reported, in which liver cirrhosis, subacute hemorrhage and recent transfusion all contributed to the low result. This case illustrates when HbA1c should not be used as a clinically relevant diabetes marker. However, low or extremely low HbA1c (<5.0% or <4.0%) may occur in apparently healthy individuals. When this occurs, it is an independent risk factor associated with liver diseases, hospitalization, and all-cause mortality. From the clinical laboratory perspective, the clinical cause of extremely low HbA1c should be determined, and suggestions of different test utilization or increased health surveillance should be given to care providers.

Keywords: hemoglobin A1c, diabetes, liver disease, mortality

Clinical History

The patient was a 52-year old Caucasian woman with a history of alcoholic liver cirrhosis and repeated bouts of encephalopathy secondary to liver cirrhosis. She had one such episode 2 weeks prior, and fell and hit her head. She did not report any untoward effects afterwards. However, the patient felt progressively more lethargic and went to a community hospital near her home to seek care. A computed tomography scan revealed 2 areas of subacute hemorrhage in the right subinsular region. She was transferred to the medical center facility and admitted to neuro intensive care unit (ICU).

Abbreviations

DCCT, Diabetes Control and Complications Trial; HbA1c, hemoglobin A1c; HPLC, high performance liquid chromatography; ICU, intensive care unit; NGSP, National Glycohemoglobin Standardization Program; NHANES, National Health and Nutrition Examination Survey; POCT, point of care testing; RBC, red blood cell

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Table 1 lists her lab values upon admission and discharge, respectively. Two units of fresh frozen plasma and 1 unit of red blood cells (RBCs) were transfused soon after admission. An HbA1c was ordered on the same day of admission, and the result from an ion-exchange high performance liquid chromatography (HPLC) instrument (Bio-Rad D-10, Bio-Rad, Hercules, CA) was 2.8%. The result was duplicated upon repeating the test. No interference or hemoglobin variant peak was identified on the chromatogram. The reportable range for the HbA1c test is 3.3 to 18.8%.

Discussion

As recommended by the American Diabetes Association, HbA1c is a biomarker for diagnosing and monitoring diabetes mellitus and prediabetes in today's standard of care.[1] Also referred to as glycated hemoglobin, HbA1c provides an assessment of the average glycemic status of an individual over the course of 2 to 3 months. Diagnostic cut-offs for HbA1c are well established, and HbA1c level correlates well with the development of diabetes complications. HbA1c measurement does not require patients to be fasting, has greater preanalytical stability, and is less affected by acute physiological perturbations, and

Table 1. Lab Values of the Patient Upon Admission and Discharge

Test	Result Upon Admission	Result Upon Discharge	Reference Range
Glucose	111 H	100 H	65-99 mg/dL
Total protein	5.0 L	5.6 L	6.3-8.3 g/dL
Albumin	2.6 L	2.6 L	3.5-5.0 g/dL
Alkaline phosphatase	139 H	177 H	35-104 U/L
ALT	22	23	5-50 U/L
AST	36 H	59 H	10-35 U/L
Total bilirubin	13.8 H	13.4 H	0-1.2 mg/dL
Direct bilirubin	5.3 H	4.2 H	0-0.3 mg/dL
Ammonia	133 H	N/A	11-51 μmol/L
Prothrombin time	31.1 H	21.6 H	12.0-15.0 sec
INR	3.0	1.9	
Partial thromboplastin time	45.0 H	38.7 H	23.0-36.0 sec
Platelet function analysis EPI closure time	>186 H	N/A	92-184 sec
Platelet function analysis ADP closure time	>300 H	N/A	62-108 sec
WBC	5.35	3.81 L	4.50-11.00 k/μL
RBC	1.85 L	2.40 L	4.20-5.50 m/μL
Hemoglobin	6.8 LL	8.6 L	12.0-16.0 g/dL
Hematocrit	22.2 L	26.9 L	37.0-47.0%
MCV	120.0 H	112.1 H	82.0-100.0 fL
MCH	36.8 H	35.8 H	27.0-34.0 pg
MCHC	30.6 L	32	31.0-37.0 g/dL
RDW-SD	82.1 H	106.1 H	37.0-55.0 fL
MPV	9.8	9.9	8.8-13.2 fL
Platelet count	84 L	72 L	150-400 k/μL
Nucleated RBC	0.40	0.00	/100 WBC
Neutrophils	62.9	70.0 H	39.0-69.0%
Lymphocytes	18.3 L	13.6 L	25.0-45.0%
Monocytes	12.7 H	11.5 H	0.0-10.0%
Eosinophils	3.7	2.6	0.0-5.0%
Basophils	0.7	1.3 H	0.0-1.0%
Immature glanulocytes	1.7 H	1.0	0.0-1.0%

hence is more convenient to use in clinical practice than other diabetes tests such as fasting glucose or glucose tolerance test. Diabetes is known as a disease that benefits from prevention, and early detection and treatment, and is a comorbidity of other diseases and surgeries. Therefore, the ordering and monitoring of HbA1c is highly encouraged in both primary and specialty care settings. It has been incorporated in the physician quality reporting system.

HbA1c is formed from the posttranslational addition of glucose to the N-terminal valine of the β chain of hemoglobin A through an Amadori rearrangement. Its level is therefore directly proportional to 2 factors: the average concentration of blood glucose and the lifetime of the RBCs. In order to use HbA1c as a valid biomarker for blood glucose burden with the established reference ranges and cut-offs, the assumption is that the lifetime of the RBCs is relatively constant (approximately 120 days). When the assumption is invalid due to physiological or pathological reasons, the use of HbA1c as a diagnostic or prognostic marker for diabetes is not valid. These include the conditions that may either shorten or prolong the RBC life span. **Table 2** lists various physiological or pathological conditions in which the HbA1c level is not a reliable indicator of long-term glycemic status.

Table 2. Physiological or Pathological Conditions in Which the HbA1c Level is not a Reliable Indicator of Long-Term Glycemic Status, Using Established Reference Ranges and Cut-Offs

RBC Lifespan ↓, HbA1c ↓	Increased production (high altitude, pregnancy, erythropoietin use)
	Hemorrhage
	Chronic bleeding
	Hemolytic anemia
	Chronic kidney failure
	Liver cirrhosis, chronic hepatitis, ribavirin use
	Alcoholic liver disease
	Folic acid deficiency
	Hemoglobinopathies (eg, thalassemia major)
	Spherocytosis
	Aplastic anemia
RBC Lifespan ↑, HbA1c ↑	Iron deficiency anemia (due to increased malondialdehyde)
	Infection/tumor-related anemia (due to decreased erythropoietin production)
RBC Transfusion, HbA1c ↑ or ↓	Increased glucose concentration in the storage medium
	Dilutional effect

Laboratory Role in Diagnosis

Clinical testing of HbA1c should be conducted using a National Glycohemoglobin Standardization Program (NGSP)-certified method traceable to the Diabetes Control and Complications Trial (DCCT) reference method. Five major methodologies exist for HbA1c measurement in the clinical laboratories: enzymatic assay, immunoassay, ion-exchange HPLC, boronate affinity, and capillary electrophoresis.[2] The analytical sensitivity, specificity and susceptibility to interference vary between different

methods and vendors. However, through the efforts of NGSP, the results between different certified platforms are usually comparable. There is also wide availability of waived point of care testing (POCT) HbA1c devices, commonly based on immunoassay or boronate affinity. Although these devices may be NGSP-certified, the precision, accuracy, and susceptibility to interferences and hemoglobin variants, as well as the end-user proficiency, can be problematic.[3,4] Currently there is lack of evidence to prove the clinical benefits of POCT HbA1c in outcomes, costs or use of clinical resources.[5]

In conditions where diabetes diagnosis or glycemic control needs to be evaluated, and HbA1c is not a valid marker, alternative methods including fasting glucose, oral glucose tolerance test, or continuous glucose monitoring can be used. Fructosamine, glycated albumin, or 1,5-anhydroglucitol may also be used. However, no clear diagnostic cut-off or prognostic significance has been established for these latter markers.

Previous studies indicated that HbA1c is not an accurate diabetes marker in patients with advanced liver diseases.[6,7] In a study by Lahousen et al,[7] 40% of study subjects with liver cirrhosis and 20% with hepatic fibrosis were found to have HbA1c levels below 4.5% using either ion-exchange HPLC or immunoassay. In subjects with hepatic fibrosis treated with ribavirin, 50% of patients had HbA1c levels below 4.5%. Liver is the major body organ involved in glucose and insulin metabolism, as well as erythropoiesis. Liver cirrhosis or fibrosis, caused by either alcohol abuse or hepatitis viruses, leads to disturbances in these important functions. Liver cirrhosis may promote insulin resistance and impair insulin secretion, thus leading to diabetes mellitus. Ribavirin was widely used in combination with interferon to treat viral hepatitis before the second-generation direct-acting antivirals were available. One of the major side effects of ribavirin is reversible hemolytic anemia. Alcoholic liver cirrhosis is also often associated with vitamin deficiency, which contributes to megaloblastic and sideroblastic anemia. Both procoagulant and anticoagulant factor levels are decreased in liver cirrhosis. Clinically there is often increased bleeding risk, as evidenced in the case patient. Low HbA1c levels in advanced liver diseases may be attributed to impaired erythropoiesis, decreased protein synthesis, and/or decreased RBC survival. On the other hand, diabetic patients may present with liver steatosis or cirrhosis, making glycemic control monitoring in these situations a necessity. Since fructosamine or glycated albumin level is also impacted by protein synthesis function of the liver, blood glucose concentration is the best test for monitoring glycemic status in diabetic patients with advanced liver disease.

As diabetes mellitus diagnosis and complications are correlated with high HbA1c levels above reference range, not much clinical emphasis has been placed on extremely low HbA1c levels below reference range. However, in the Atherosclerosis Risk in Communities Study population, evidence shows that low HbA1c level ($<$5%) is a poor prognostic factor and is associated with increased risk of all-cause mortality and cancer death, despite multiple positive features such as young age, lower body mass index, and lower prevalence of hypercholesterolemia and coronary artery diseases in this subpopulation.[8] A similar study reported that extremely low HbA1c levels ($<$4%) are associated with increased risk of all-cause mortality after adjustment for age, race, ethnicity, and sex in the National Health and Nutrition Examination Survey (NHANES) III population.[9] Extremely low HbA1c levels $<$4% is also associated with abnormal liver enzymes and liver steatosis in a large NHANES cohort without diabetes.[10] A J-shaped association between HbA1c and abnormal liver enzymes, hepatic steatosis, risk of liver disease hospitalization, or all-cause mortality has been identified in multiple studies,[8-10] with the nadir of the curve at approximately 4.7 to 5.5%. Both lower and higher HbA1c levels outside of this range are associated with increased risks. This suggests extremely low HbA1c may also have clinical significance. The cause of extremely low HbA1c should be determined, and increased health surveillance should be conducted in apparently healthy individuals with low HbA1c levels. This is particularly important considering the emphasis on disease prevention and population health.

Patient Follow-Up

In the case presented above, multiple factors made HbA1c an unsuitable marker for determining the patient's glycemic status. First, the patient had a history as well as active ongoing liver cirrhosis, as evidenced by the abnormal liver enzyme ratios (AST:ALT = 1.6-2.6, which is strongly correlated with alcoholic liver cirrhosis). Secondly, she also

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had 2 areas of subacute hemorrhage in the brain, presumably due to her fall 2 weeks prior. These conditions suggest not only was erythropoiesis impaired, but also the lifespan of the RBCs was shortened. The protein synthesis function of her liver was also impaired, as evidenced by low total protein and albumin levels. All would lead to low HbA1c levels. Finally, the patient also received RBC transfusion early in the hospital stay, which further complicated the interpretation of the HbA1c result. Therefore, the HbA1c result was not reported to the ordering physician. Instead, a comment was attached to the order and a call was made to the ordering physician explaining that HbA1c should not be used as a glycemic indicator for this patient. The patient did not present strong clinical indicators for diabetes during this encounter. However, with her active liver disease, monitoring glycemic status consistently for possible future diabetes should be an important part of her care plan. For this purpose, fasting glucose or oral glucose tolerance test should be used.

The patient's clinical status gradually improved. Her coagulopathy improved but did not normalize. She was discharged after 3 days of hospital stay. **LM**

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