Pitfalls in Hemoglobin A1c Measurement: When Results may be Misleading

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Since the beginning of clinical use in the 1970s, hemoglobin A1c (A1c) has become the standard tool for monitoring glycemic control in patients with diabetes. The role of the A1c test was broadened in 2010, when the American Diabetes Association added A1c as a diagnostic criterion for diabetes. Because of hemoglobin A1c's integral role in diagnosis and treatment, it is important to recognize clinical scenarios and interfering factors that yield false results. The purpose of this review is to describe the A1c measurement, outline clinical scenarios or factors that may yield false results, and describe alternative laboratory biomarkers.

KEY WORDS: diabetes; evaluation; patient centered care; physician decision support.
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CASE

A 62-year-old female with type 2 diabetes mellitus for over 20 years, treated with multiple daily insulin injections, came in for routine follow-up. Her recent medical history is significant for menorrhagia secondary to uterine fibroids. Her typical A1c value is 9.0 %, however, her most recent A1c value was 10.6 %. The patient is quite surprised as she has really focused on managing her diabetes over the past 3 months, and checks her blood glucose before and after meals. Her blood glucose meter download report reveals an average glucose level of 191 mg/dL, which is consistent with an A1c value of 8.3 %. The A1c value does not seem to accurately reflect mean glycemic control in this patient. What could explain the inaccuracy of her A1c value? What other tests could be used?

INTRODUCTION

For decades, hemoglobin A1c (A1c) has remained the standard biomarker for glycemic control. A1c assays became commercially available in 1978, and the American Diabetes Association (ADA) first recommended using A1c in 1988.[1] In 1993, the landmark Diabetes Control and Complications Trial (DCCT) demonstrated its importance as a predictor of diabetes-related outcomes, and the ADA started recommending specific A1c targets in 1994.[2] Other key studies, such as the United Kingdom Prospective Diabetes Study (UKPDS), have consistently demonstrated the correlation between A1c and diabetes complications and have reinforced currently recommended target values.[2,3]

The role of the A1c broadened in 2010 when the ADA added A1c ≥6.5 % as a diagnostic criterion for diabetes, allowing the test to be used for both management and diagnosis.[3] The value of 6.5 % was selected as an optimal cut point for detecting prevalent retinopathy consistent with the definition used for current fasting (≥126 mg/dL) and 2-h (≥200 mg/dL) plasma glucose cut points. Recommending A1c as a diagnostic test was partly based on its advantages over timed glucose tests, including serving as a better index of overall glycemic exposure and risk for long-term complications, offering less biologic variability and preanalytic instability, and being unaffected by acute perturbations in glucose levels due to an acute illness or stress-related event.[4] In addition, because there is no need for fasting or timed samples, the A1c test is more clinically convenient. The clinical convenience of A1c testing has the potential to facilitate screening those at risk and provide the opportunity for early diagnosis and intervention.

Because of A1c's integral role in diagnosis and treatment, it is important to recognize clinical scenarios and interfering factors that yield false results. This review will describe the A1c measurement, outline clinical scenarios or factors that may yield false results, and describe alternative laboratory biomarkers.

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DESCRIPTION OF HEMOGLOBIN A1C

Hemoglobin is the iron-containing oxygen transport metalloprotein in the red blood cells. Hemoglobin's structure consists of a tetramer of two pairs of protein molecules: two α globin chains and two non–α globin chains. The α globin genes are HbA1 and HbA2, whereas the non–α globin genes include β, γ, δ.[5] The normal adult hemoglobin molecule (HbA) consists of two α and two β

chains ($\alpha_2\beta_2$), and makes up about 97 % of most normal human adult hemoglobin.[6] Other minor hemoglobin components may be formed by posttranslational modification of HbA. These include hemoglobins A1a, A1b, and A1c. Of these, A1c is the most abundant minor hemoglobin component. A1c is formed by the chemical condensation of hemoglobin and glucose which are both present in high concentrations in erythrocytes. This process occurs slowly and continuously over the life span of erythrocytes, which is 120 days on average. Furthermore, the rate of A1c formation is directly proportional to the average concentration of glucose within the erythrocyte during its lifespan.[6] Hence, as levels of chronic hyperglycemia increase, so does the formation of A1c. This makes it an excellent marker of overall glycemic control during the time frame of the 120-day lifespan of a normal erythrocyte.

Results of the DCCT and UKPDS studies verified the close relationship between glycemic control measured by A1c and the risk for diabetes-related complications. A1c has been widely accepted as the standard used to measure glycemic control over the previous 3 month period and correlates with patients' risk for developing diabetes-related complications.[7] It is important to remember that A1c represents a weighted mean of glucose levels during the preceding 3 month time period. In other words, glucose levels during the most recent 6 week period will have a greater influence on the A1c result compared to levels from the prior 6 weeks.[8] Thus, if the patient has experienced a recent acute change in glycemic control (i.e., treatment with glucocorticoids), the A1c value will be disproportionately affected by the most recent glucose levels. Table 1 shows the correlation between A1c and estimated average glucose.[9] As a general rule, every 1 % change in A1c is associated with an approximate 30 mg/dL change in estimated average glucose.

A1C MEASUREMENT

There are two approaches to measuring A1c. One approach is to separate A1c from other hemoglobin fractions and includes methods such as chromatography and electrophoresis. The other approach targets A1c as an antigen using methods such as immunochemistry.[10] Within this context, the four most commonly used methods to measure A1c are ion-exchange high-performance liquid chromatography (HPLC), boronate affinity HPLC, immunoassay, and enzymatic assays.[11]

Unfortunately, the variety of assays used to measure A1c led to a lack of standardization, which limited the ability to reliably compare reported results across various clinical practices. This lack of standardization resulted in a 1997 International Expert Committee recommendation against using A1c for the diagnosis of diabetes.[4] To address this issue, the National Glycohemoglobin Standardization Program (NGSP) was created in 1996 to standardize A1c results to those of the DCCT and UKPDS trials that established the relationship between A1c and vascular complications. The Central Primary Reference Laboratory of the NGSP network uses the same HPLC method employed in the DCCT and UKPDS studies. This provides consistency and comparability across a variety of clinical laboratories, allowing patients with diabetes to be treated to standardized targets.[2] When measured in an NGSP-certified laboratory, a change in A1c of at least 0.5 % is considered both statistically and clinically significant.[2]

Separately, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) convened a working group in 1995 with the goal of developing a true reference method for A1c. This goal differed from the standardization goal of the NGSP. While NGSP-derived A1c results are reported as a percent, IFCC results are reported using the International System of Units (SI), and are expressed as millimoles of A1c per mole of HbA. NGSP and IFCC values have a linear relationship and use a "master" regression equation for converting values between the two systems. While A1c is typically reported as an NGSP-derived percent in the United States and Canada, many other countries report A1c in IFCC-derived SI units. A consensus statement issued in 2007 by the ADA, the European Association for the Study of Diabetes (EASD), and the International Diabetes Federation (IDF) supports reporting A1c values using both NGSP (%) and IFCC (SI) units.[1] Table 1 shows a comparison between A1c reported in NGSP and IFCC-derived values.[1]

Over the past several years, there has been expanded use of point-of-care (POC) assays to measure A1c. Advantages of POC testing include fingerstick sampling in the provider's office and immediate patient and provider feedback leading to timely adjustments in the treatment regimen. Though use of POC assays has been shown to improve A1c outcomes,[12] there is a lack of evidence from randomized clinical trials.[13] Furthermore, several different POC assays are available and have variable accuracy and precision when compared to NGSP certification criteria.[14] Providers who employ POC A1c testing should make sure the assay's results are comparable to those from an NGSP certified laboratory.

Patients do not need to fast for an A1c test. To measure A1c, a sample of venous whole blood is drawn into a lavender-top tube for analysis. If the sample is not going to be analyzed the same day, there are two options to avoid falsely elevated results

Table 1. Correlations Between Estimated Average Glucose, A1c, and Fructosamine[1,9,45]

Glucose (mg/dl)	A1c (%)	A1c (mmol/mol)	Fructosamine (μmol)
97	5	31	131
126	6	42	203
154	7	53	273
183	8	64	345
212	9	75	417
240	10	86	487
269	11	97	559
298	12	108	631

secondary to in vitro spontaneous glycosylation: the A1c blood sample may be stored at room temperature for up to 2 weeks or the sample can be refrigerated at 2–8 °C for up to 4 weeks.[10]

Interestingly, significant racial and ethnic differences seem to exist in A1c readings for a given average glucose value. For example, Caucasians have been reported to have an absolute A1c reading approximately 0.1 % to 0.4 % lower for the same average glucose levels when compared to other ethnicities such as Hispanics, Blacks, or Asians. The reasons for these differences remain unclear.[15]

FALSELY ELEVATED A1C

Any condition that prolongs the life of the erythrocyte or is associated with decreased red cell turnover exposes the cell to glucose for a longer period of time, resulting in higher A1c levels. Iron deficiency anemia is a commonly reported condition associated with falsely elevated A1c. Studies in patients with and without diabetes have demonstrated that treatment of iron deficiency anemia lowers A1c,[16–18] although the exact mechanism remains unclear.[19] Other conditions associated with decreased red cell turnover are also associated with falsely elevated A1c including vitamin B-12 and folate deficiency anemias, and asplenia.[20,21]

There are conflicting reports regarding the effects of recent red blood cell transfusion on A1c. Traditionally, the perception has been that exposure of red cells to the high glucose concentrations of the storage medium results in a falsely elevated A1c in the transfused patient. However, more recent data suggest that a dilutional effect from the significant volume of red cells transfused from a patient without diabetes can result in a falsely decreased A1c.[22] Until these conflicting reports are further clarified, A1c results in a recently transfused patient should be considered uninterpretable.

Severe hypertriglyceridemia (concentrations >1,750 mg/dL),[23] severe hyperbilirubinemia (concentrations >20 mg/dL), and uremia may also falsely elevate A1c.[10] When a laboratory panel containing one of these results includes an A1c measurement, the clinician should consider a notation indicating that the A1c may be unreliable in order to avoid misinterpreting the result when referencing it in the future.

Several medications and substances have also been reported to falsely elevate A1c including lead poisoning[2], chronic ingestion of alcohol, salicylates, and opioids.[24–28] Ingestion of vitamin C may increase A1c when measured by electrophoresis, but may decrease levels when measured by chromatography. This may be due to inhibition of glycosylation via a competitive mechanism.[29] Although this effect was seen with ingestion of 1 gram of vitamin C daily for 3 months, a dose commonly ingested by many patients, this study was conducted in patients without diabetes, and the clinical relevance is unclear.

Table 2 summarizes conditions associated with falsely elevated A1c.

In summary, when evaluating a patient with diabetes who has one of the conditions described above (most commonly iron-, vitamin B-12-, or folate deficiency- anemias), the clinician should consider that the A1c result may be falsely elevated and not reflective of the patient's true level of average glycemia. Prior to making any therapeutic changes, the clinician could ask the patient to monitor capillary glucose readings before and 2 h after meals for several days and evaluate if the self-monitored readings are consistent with the expected A1c level. Additionally, the clinician could measure an alternative index of glycemic control (see discussion below "Alternatives to using A1C"). Both approaches serve to avoid potentially inappropriate treatment intensification and minimize the risk for hypoglycemia.

FALSELY LOWERED A1C

Similarly, any condition that shortens the life of the erythrocyte or is associated with increased red cell turnover shortens the exposure of the cell to glucose, resulting in lower A1c levels. Conditions such as acute and chronic blood loss, hemolytic anemia, and splenomegaly can all cause falsely lowered A1c results.[19]

Patients with end-stage renal disease generally have falsely low A1c values. This is primarily due to the associated chronic anemia with decreased red cell survival.[30] However, the complex interplay of other contributing factors, including erythropoietin therapy and the presence of uremia, can have differing effects which further influence A1c.[31] Overall, in patients with end-stage renal disease, A1c tends to underestimate patients' average glycemia and the clinician should consider using an alternative index of glycemic control.

A1c may not be a true reflection of glycemia during pregnancy primarily because of both the decreased life span of the red blood cell from about 120 days to about 90 days as well as increased erythropoietin production.[32] A1c values decline during pregnancy by 12–16 weeks of gestation with a further decrease that plateaus by gestational weeks 20–24.[33,34] A1c levels may start to rise again in the third trimester.[35] Because A1c values are generally falsely low during pregnancy, A1c should not be used for diagnosing gestational diabetes. Instead, an oral glucose tolerance test should be used for screening and diagnosis, and glucose management during pregnancy should be primarily determined using self-monitored blood glucose.

Supplements and medications associated with falsely lowered A1c include vitamin E, Ribavirin, and interferon-alpha. Vitamin E, at doses of 600–1200 mg per day, can reduce protein glycation,[10,36] whereas Ribavirin and interferon-alpha can cause a reversible hemolytic anemia.[37,38]

Table 2 summarizes conditions associated with falsely lowered A1c.

Table 2. Conditions Associated with Falsely Elevated or Lowered A1c

Condition	Effect on A1c	Comments
Anemias associated with decreased red cell turnover	False Increase	I.e., iron deficiency, vitamin B-12, folate deficiency anemias
Asplenia	False Increase	Increased erythrocyte lifespan
Uremia	False Increase	Formation and detection of carbamyl-hemoglobin
Severe hypertriglyceridemia	False Increase	When level >1,750 mg/dL
Severe hyperbilirubinemia	False Increase	When level >20 mg/dL
Chronic alcohol consumption	False Increase	Formation of acetaldehyde-HbA1 compound
Chronic salicylate ingestion	False Increase	Mechanism uncertain, may interfere with assay
Chronic opioid ingestion	False Increase	Mechanism uncertain
Lead poisoning	False Increase	Mechanism uncertain
Anemia from acute or chronic blood loss	False Decrease	Includes hemolytic anemia
Splenomegaly	False Decrease	Decreased erythrocyte lifespan
Pregnancy*	False Decrease	Decreased erythrocyte lifespan
Vitamin E ingestion	False Decrease	Reduced glycation
Ribavirin and interferon-alpha	False Decrease	Possibly due to hemolytic anemia
Red blood cell transfusion†	False Increase or False Decrease	High glucose concentration in storage medium (False Increase) Dilutional effect (False Decrease)
Hemoglobin variants	False Increase or False Decrease	Depends on method and assay used A1c generally reliable for heterozygous variants, but not homozygous variants (See Table 3)
Vitamin C ingestion	False Increase or False Decrease	May increase A1c when measured by electrophoresis May decrease levels when measured by chromatography due to competitive inhibition of glycosylation

**Expect falsely low A1c values through the 2nd trimester, but may rise during the 3rd trimester*
†Typically reported to falsely elevate A1c, but may also result in false decrease

In summary, when evaluating a patient with diabetes and one of the conditions described above (most common are renal failure and pregnancy), the clinician should consider that the A1c result may be falsely lowered and not reflective of the patient's true level of average glycemia. In this scenario, the clinician may miss the opportunity to make a change in therapy placing the patient at risk for prolonged, untreated hyperglycemia with accelerated development of diabetes-related complications. As discussed previously, the clinician could evaluate the patient's capillary glucose readings or measure an alternative index of glycemic control.

HEMOGLOBIN VARIANTS

Hemoglobin variants can make interpretation of A1c quite challenging as these patients can have either falsely elevated or falsely lowered A1c. The most common variants worldwide are hemoglobin S and hemoglobin C. Overestimation or underestimation of A1c for many hemoglobin variants will differ depending on both the type of method and the specific assay used. Therefore, for patients with hemoglobin variants, it is important for the clinician to know the laboratory's method for measuring A1c.[24,39,40] In some cases, it may be preferable to use an alternative method of measuring glycemic control instead of A1c (see discussion below). In general, A1c measurement is not reliable in patients with homozygous hemoglobin variants (i.e., HbS or HbC), whereas A1c measurement can be used in patients with heterozygous hemoglobin variants (i.e., HbAS, HbAC) as long as an appropriate assay is used.[41] Table 3 summarizes the effects of some common hemoglobin variants on A1c measurements from commonly used methods. The presence of a hemoglobin variant may be suspected in a variety of situations including a discordance between the patient's self-monitoring glucose measurements and the A1c value, an A1c result >15 %, a markedly different A1c result compared to the previous value when a different method is used to measure A1c, or the presence of anemia with abnormal red cell indices on a complete blood count.[42]

ALTERNATIVES TO USING A1C

In situations where A1c may not accurately reflect glycemic control, using an alternative index is desirable. Such potential indices include fructosamine, glycated albumin, 1,5-anhydroglucitrol (1,5-AG), and continuous glucose monitoring (CGM).

Fructosamine measurement has been commercially available since the 1980s, and refers to the product formed by the nonenzymatic reaction of a sugar and a protein (in this case glucose and albumin). Because the half life of albumin is much shorter than that of an erythrocyte (about 20 days vs. about 120 days), fructosamine reflects a much shorter period of glycemic control compared to A1c, typically the preceding 2 to 3 weeks.[43,44] Therefore, it provides a better assessment of recent changes in glycemic control.

However, fructosamine measurement also has limitations. Since the assay depends on albumin concentrations, patients with hypoproteinemia/hypoalbuminemia such as nephrotic syndrome or severe liver disease may have falsely

Table 3. Interference from Common Hemoglobin Variants on A1c Measurements from Commonly Used Methods[24]

Method	Interference from HbC	Interference from HbS	Interference from HbE	Interference from HbD
Abbott Architect/Aeroset	Yes	Yes	*	*
Arkray ADAMS A1c HA-8180V (Menarini)	No	No	A1c not quantified	A1c not quantified
Axis-Shield Afinion	No	No	No	No
Bayer A1cNOW	Yes	Yes	No	No
Beckman AU system	Yes	Yes	No	No
Beckman Synchron System	No	No	No	No
Bio-Rad D-10 (A1c program)	No	No	No	No
Bio-Rad Variant II NU	–	–	No	No
Bio-Rad Variant II Turbo	No	No	Yes	Yes
Bio-Rad Variant II Turbo 2.0	No	No	No/Yes (conflicting reports)	No
Bio-Rad in2it	Yes	No	Yes	No
Ortho-Clinical Vitros	No	No	No	No
Roche Cobas Integra Gen.2	No	No	No	No
Roche/Hitachi (Tina Quant II)	No	No	No	No
Sebia Capillarys 2 Flex Piercing	No	No	No	No
Siemens Advia HbA1c (original version)	Yes	Yes	*	*
Siemens Advia A1c (new version)	No	No	*	*
Siemens DCA 2000	No	No	No	No
Siemens Dimension	No	No	No	No
Tosoh G7	Yes	No	Yes	No
Tosoh G8	No	No	Yes	No
Trinity (Primus) HPLC (affinity)	No	No	No	No

* *Generally assumed there is no interference*
Adapted with permission from: HbA1c assay interferences. National Glycohemoglobin Standardization Program Web site. Available at: http://www.ngsp.org/interf.asp. Updated August, 2012. Accessed November 8, 2012

low levels. It is unclear whether or not the assay should be corrected for serum protein or albumin concentrations. Other reported interferences include uremia, lipemia, and ascorbic acid.[44] Because of these limitations, fructosamine is not considered a replacement for A1c. Its utility as a measure of glycemic control is reserved for when A1c may be inaccurate, or to assess recent changes in glycemic control. Table 1 shows the correlation between estimated average glucose, A1c, and fructosamine.[9,45]

The term fructosamine encompasses all glycated proteins. Glycated albumin is an example of a fructosamine also used as a measure of glycemic control. Glycated albumin is typically reported as a percentage of total albumin, and also reflects glycemic control over the preceding 2 to 3 weeks. Its use has been particularly advocated in patients with end stage renal disease where A1c typically underestimates glycemic control, and glycated albumin would provide a more accurate assessment of recent glycemia.[30,46,47]

Plasma 1,5-anhydroglucitol (1,5-AG) is a naturally occurring dietary polyol that has been proposed as a measure of glycemic control. Renal reabsorption of 1,5-AG is competitively inhibited by glucose. Hence, there is a negative correlation between 1,5-AG levels and glycemic control. Plasma 1,5-AG is a marker of short-term glycemic control, typically reflecting glucose values over the preceding 48 h to 2 weeks. The test may be useful to detect post-prandial hyperglycemia and glycemic variability, especially in the presence of target A1c. This test is affected by renal

Table 4. Main Characteristics of Alternative Indices of Glycemic Control

	Fructosamine	Glycated albumin	1,5-AG	CGM
Timeframe of reflected average glycemia	2–3 weeks	2–3 weeks	48 h to 2 weeks	3–7 days
Mechanism	Measures non-enzymatic reaction of glucose and serum protein (albumin)	Measures non-enzymatic reaction of glucose and serum protein (albumin)	Renal reabsorption competitively inhibited by glucose (negative correlation with average glycemia)	Measures interstitial glucose levels semi-continuously
Conditions where may be most useful	Assessment of recent changes in average glycemia	End stage renal disease	Evaluation of post prandial glucose control	Evaluation of glycemic variability
Conditions where results may be unreliable	Hypoproteinemia (including cirrhosis, nephrotic syndrome) Uremia Lipemia	Hypoproteinemia (including cirrhosis, nephrotic syndrome) Uremia Lipemia	Renal failure Pregnancy Chronic liver disease	During periods of high glycemic variability Ingestion of: acetaminophen, ascorbic acid

1,5-AG 1,5-anhydroglucitrol; CGM continuous glucose monitoring

hemodynamics and may not be a reliable indicator of glycemia in renal failure (false high), pregnancy (false low), or chronic severe liver disease. In 2003, the FDA approved an automated, commercially available assay (GlycoMark™) to measure 1,5-AG levels.[48–50] Some insurance plans may not cover Glycomark ™.

Another method used to reflect glycemia is continuous glucose monitoring (CGM). CGM sensors measure interstitial glucose levels on a semi-continuous basis, and reflect glycemic fluctuations while the device is worn. Three CGM devices are currently FDA approved for use in the United States (Medtronic CGM™, and Dexcom SEVEN PLUS™ and G4 PLATINUM™), and patients can wear their accompanying disposable sensors for 3, 7, and 7 day periods respectively. Data obtained from wearing a CGM sensor for up to 5 days has been shown to correlate well with A1c.[51] CGM readings may be inaccurate during periods of high glycemic variability, as well as after ingestion of acetaminophen or ascorbic acid.[52,53] Recent guidelines recommend clinicians consider using daily CGM in essentially all adult patients with type 1 diabetes, and intermittent CGM in any adult patient with diabetes and suspected nocturnal hypoglycemia, dawn phenomenon, postprandial hyperglycemia, hypoglycemic unawareness, or when significant therapeutic changes are made such as initiating or intensifying insulin.[54] Insurance plans reimburse charges for insertion and interpretation, particularly in patients with type 1 diabetes and in patients with type 2 diabetes treated with insulin.

Although frucotsamine, glycated albumin, 1,5-AG, and CGM have been shown to correlate with mean glucose levels,[48] it is important to remember that only A1c has been validated as a measure of both long-term glycemic control and risk for development of complications.[3] Therefore, A1c remains the gold standard to measure average glycemic control. The other methods described should only be used in situations where A1c results may be inaccurate or misleading, or to supplement A1c data. Table 4 summarizes the main characteristics of alternative indices of glycemic control.

CONCLUSION

A1c remains the gold standard to monitor glycemic control in patients with diabetes, and is also used as a diagnostic criterion. Because of standardized measurement, clinicians can successfully use A1c in the vast majority of patients to diagnose diabetes, and to monitor glycemic control and risk for developing complications. However, it is important to keep in mind that there are a variety of clinical scenarios that may result in falsely elevated or falsely lowered A1c values. In these situations, it may be preferable to use an alternative measure of glycemic control, such as fructosamine, glycated albumin, 1,5-AG, or CGM. These tests may also be used to complement A1c, as they reflect a more recent period of glycemia. Ultimately, however, it is important to remember that only A1c has been validated as a predictor of diabetes-related outcomes, and it is still recommended as the primary measure of glycemic control. It is incumbent on the clinician to know when A1c results should be questioned, such as when the A1c value is discordant from the patient's self-monitoring blood glucose values, or when there has been an acute change in glycemia such as recent treatment with glucocorticoids.

CASE CONCLUSION

The patient had a fructosamine and 1,5-AG assay performed, as well as a hemoglobin level. The result of the fructosamine test was 353 μmol/L (reference range 190–270), and the result of the 1,5-AG test was 5.5 mcg/mL (reference range 7.3–36.6), both consistent with her average glucose values and not her A1c. Hemoglobin concentration was found to be very low (7.5 g/dL), and subsequent evaluation revealed severe iron deficiency anemia. The patient is being evaluated and treated for her anemia and uterine fibroids. Until resolution, blood glucose monitoring, fructosamine, and 1,5-AG will be used to monitor her glycemic control.

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