Hemoglobin A1c – Case Study

Presentation: The patient is a 46 y/o, 210 pound African-American female who developed type II diabetes three years ago. She is on 12U of NPH insulin each evening after medical nutrition therapy alone was insufficient to control her blood glucose. Over the course of her treatment, her hemoglobin A1c (Hgb.A1c) varied from 5.5 to 6.0%. At the last office visit her Hgb.A1c jumped to 7.5% without change in diet or medications. It was noted on the lab report that this sample was run by a reference lab using a different method because the in house routine immunoassay was down. The reference lab also reported a fetal hemoglobin level of 25%. Her CBC was normal. The discordant Hgb.A1c levels are discussed below.

What is Hgb.A1c? Glycosylation of hemoglobin occurs non-enzymatically during exposure of red blood cells to plasma glucose. Hgb.A1c is formed slowly and irreversibly during the entire 120-day life span of the red cell. Once hemoglobin is glycated, it remains that way until the red cell dies. Hemoglobin A1 is actually composed of three subtypes: A1a, A1b and A1c. Hgb.A1c comprises the predominant glycosylated Hgb.A1 fraction. It is used clinically to reflect the average blood glucose level over a several month period. A small decrease or increase in Hgb.A1c may occur after two - three weeks of a sustained change in blood glucose. The percentage of Hgb.A1c is calculated as the ratio of glycosylated hemoglobin (A1c) divided by the total hemoglobin, times 100.

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\text{Hgb.A1c} = \frac{\text{Hgb.A1c}\%}{\text{Total hemoglobin}} \times 100
\]

Correlation with fasting blood glucose: The Hgb.A1c is a valuable method of monitoring glycemic control in diabetic patients. The level is proportional to the average blood glucose level over the previous four weeks to three months. The American College of Endocrinology recommends values below 6.5% while the American Diabetes Association recommends a level below 7.0%.

Lower than expected levels of Hgb.A1c can be seen in glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease (homozygous) or any condition with shorten red cell survival. The following table correlates Hgb.A1c and fasting glucose levels.

Hgb.A1c Methods: There are several methods to determine HgbA1c in clinical laboratories. The most common method used in clinical labs and physician offices is an immunoassay. The turbidimetric inhibition immunoassay is used at Rex Hospital Laboratory. The assay uses an antibody directed to terminal end of the beta chain of hemoglobin molecule. The antibody is able to detect hemoglobins A, A1, A2, S, C, O and E that have been glycosylated. The total hemoglobin is measured as a separate step. The amount of glycosylated hemoglobin is expressed as a percentage of the total. This method is accurate in sickle cell anemia and hemoglobinopathies C, E and O because the terminal beta-globin chains are identical to hemoglobin A. However, if fetal hemoglobin (Hgb.F) is significantly elevated (more than 10% of the total Hgb), then the Hgb.A1c level will be artificially low. Since Hgb.F is composed of two alpha and two gamma
In the presence of elevated glucose levels, the total half lives, such as albumin, also become glycosylated besides hemoglobin. Several serum proteins with shorter cell lifespan also become glycosylated from these conditions is often small and not clinically relevant.

Increased red cell turnover (shorter red cell lifespan) as seen in hemolytic anemias will result in decreased levels of Hgb.A1c. Beta-thalassemia and acute blood loss will also decrease the Hgb.A1c level because of the shortened red cell lifespan. Other conditions lead to falsely elevated A1c levels. These include alcoholism, lead poisoning, opiate addiction and excessive use of salicylates. The increase from these conditions is often small and not clinically relevant.

Besides hemoglobin, several serum proteins with shorter half lives, such as albumin, also become glycosylated in the presence of elevated glucose levels. The total glycosylated serum proteins and glycosylated serum albumin can provide an estimate of glycemic control over the preceding seven to 14 or 14 to 20 days. However, the clinical utility of this measurement is limited for routine diabetic care.

**Serum fructosamine assay:** The serum fructosamine level also reflects average glucose levels without concern for hemoglobinopathies. The serum fructosamine test is not available at Rex Lab but can be sent to the Mayo Reference Laboratory.

**Discussion of Case:** The most recent Hgb.A1c is elevated and discordant with the previous values. The patient’s glycated fetal hemoglobin was not included in the A1c calculation when measured by immunoassay method in the previous visits. The current elevated level of 7.5% (reference lab method) is a measurement of the total glycosylated hemoglobin. This patient is asymptomatic, has a normal CBC and had no history of a hemoglobinopathy. The finding of a fetal hemoglobin level of 25% was fortuitous. The level of Hgb.A1c of 7.5% is higher than goal for this patient. The reference laboratory method uncovered a hemoglobinopathy that was previously undetected. This level of fetal hemoglobin is consistent with hereditary persistence of fetal hemoglobin. This condition is innocuous and found almost exclusively in African Americans.

Monitoring this patient’s Hgb.A1c requires the reference laboratory method. In general, all patients who are at risk for a hemoglobinopathy should have a hemoglobin electrophoresis at least once during their care to rule out an interfering hemoglobin variant or beta thalassemia. The most common heterozygous hemoglobinopathies i.e. Hgb AS, AC and AE, do not interfere with the standard immunoassay for Hgb.A1c performed at Rex Hospital Laboratory. Hgb.A1c by in a patient with hereditary persistence of fetal hemoglobin can be done at Mayo Reference Laboratory (MML test # 82080) by specific request.

**Clinical sources of error:** Certain clinical states can cause an elevation in fetal hemoglobin. Hemoglobin F elevation can occur in pregnancy during the 23rd to 31st week of gestation. This is a result of selective alteration of erythropoiesis and not from transplacental bleeding from the fetus. Also, many primary hematologic malignancies, anemias and hemoglobinopathies may be associated with increases in fetal hemoglobin.

The serum fructosamine assay: The serum fructosamine level also reflects average glucose levels without concern for hemoglobinopathies. The serum fructosamine test is not available at Rex Lab but can be sent to the Mayo Reference Laboratory.

**HPLC method:** The HPLC (high performance liquid chromatography) method measures Hgb.A1c by ion exchange. The method utilizes the difference in molecular weight and charge to separate hemoglobins on a column. The HPLC method is able to distinguish between glycated and nonglycated fetal hemoglobin and hemoglobin A. The HPLC method is not affected by the presence of high levels of Hgb.F. Accordingly, HPLC is the most accurate method to measure Hgb.A1c in patients with very high Hgb.F (>30%). However, HPLC is more technically difficult to perform and requires specialized equipment. Furthermore, patients with sickle cell anemia (SS), homozygous CC or EE have no hemoglobin A and HPLC cannot determine Hgb.A1c levels. Most reference labs no longer use HPLC to measure Hgb. A1C.

**Mayo Reference Laboratory:** Rex Hospital’s reference lab uses a similar but different immunoassay that provides accurate results for the most common hemoglobinopathies. This includes hereditary persistence of fetal hemoglobin provided the level of hemoglobin F is less than 30%. The hemoglobin A1c test is #82080 and requires one lavender-top (EDTA) tube. *(An interesting side bar regarding this test is that hemoglobin Raleigh interferes with the A1c result. Dr. William Dunlap described the first patient with hemoglobin Raleigh in the mid 1970’s. He stills sees the patient today.)*

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**Stephen V. Chiavetta, MD**

**References:**

7. Personal communication with Dr. William Dunlap.
Comparison of Immunohistochemical (IHC) Estrogen and Progesterone results with the Genomic Health Oncotype Dx RT-PCR method.

The Genomic Health Oncotype DX Breast Cancer Assay is a 21 gene based reverse transcriptase polymerase chain reaction (RT-PCR) method that initially was utilized for testing estrogen receptor positive, node negative breast cancer patients to determine risk of recurrence and responsiveness to therapy. This testing has subsequently expanded to postmenopausal node positive and estrogen receptor positive patients. Beginning February 1, 2008, Oncotype DX reports have included estrogen and progesterone receptor scores based upon RT-PCR evaluation for estrogen and progesterone receptor RNA genetic expression. Similar to the original Oncotype DX breast cancer assay, the test involves RNA extraction from formalin-fixed paraffin-embedded tumor tissue. An estrogen receptor score of ≥ 6.5 indicates a positive result, whereas a progesterone score of ≥ 5.5 is considered positive.

Since February 2008 we have compared the findings from Genomic Health Oncotype DX Breast cancer assay with the immunohistochemical (IHC) Dako Assay performed at Rex Hospital. To date, a total of 55 cases were available for review. Fifty three of fifty five (96.4%) estrogen receptor results correlated, whereas fifty one of fifty five (92.7%) progesterone results agreed. The two discordant estrogen receptor results involved two borderline or “low positive” estrogen receptor IHC staining with negative Genomic Health RT-PCR. The progesterone receptor results for both cases were negative by both methods. The progesterone receptor IHC results for the three cases were strongly positive for estrogen receptor assay by both methods. The fourth discordant progesterone receptor result involved a case of both intraductal and invasive ductal carcinoma. The estrogen receptor assays were positive in both assays, but the progesterone receptor IHC result was negative and the Genomic Health RT-PCR test was positive. A closer look at the case demonstrated a negative progesterone receptor IHC result in the invasive ductal carcinoma component of the tumor, whereas the intraductal carcinoma was positive. This case illustrates the value of morphologic confirmation by the IHC method compared to a molecular method, which measures receptor status as a tissue extract of the paraffin section.

With any two laboratory methods or measurements, absolute agreement is highly unlikely. In this case, there is good correlation between the Rex and Genomic Health Oncotype estrogen and progesterone receptor evaluation. The findings are similar to other reportable comparisons between IHC and the Genomic Health RT-PCR methods (Figure 1). 1-3

John P. Sorge, MD

References:

Prostate Cancer Awareness at Rex Senior Center

September was National Prostate Cancer Awareness Month, and 372 men were screened for prostate cancer on Saturday, Sept. 27 as part of Rex Healthcare’s annual celebration. The screening event took place at the Rex Senior Health Center and included opportunities for men and their families to find life-saving health information, and to enjoy live music and food in a festival-like atmosphere.

“We de-emphasize the cold, clinical aspect of the screening and turn it into a community event,” said Dr. Leroy Darkes, medical director of the Senior Health Center. “It creates a comfortable and supportive environment for everyone involved.”

Now in its eleventh year of free prostate screenings, the Rex Senior Health Center has detected at least one case of prostate cancer each year. In 2007, Rex Healthcare screened 342 men.

A prostate specific antigen (PSA) blood test was included as part of the screening. All participants have a tube of blood drawn by one of our volunteer phlebotomists and the Rex Laboratory analyses and reports the data to the Point of Care Testing Department who determines whether the PSA value is normal or abnormal based on the gentleman’s age and race. DuWayne Engman then uses the data to generate letters and envelopes that will be mailed to each gentleman. These letters include the PSA value, its interpretation (normal or abnormal) and the DRE findings recorded by the physician on the day of the screening.

I would like to especially thank Sonia Atkinson and DuWayne Engman who served as my co-chairs for the event and special thanks to all the laboratory volunteers who worked very hard to make the event a success:

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