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Pearls of Laboratory Medicine
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TITLE: Alternative Markers of Glycemia

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Hello, my name is Khushbu Patel. I am a Clinical Chemistry fellow at Washington University School of Medicine. Welcome to this Pearl of Laboratory Medicine on "Alternative Markers of Glycemia."

Slide 2:
In patients with diabetes, plasma glucose concentrations can fluctuate from day to day. Measurement of glycated proteins provides an integrated plasma glucose concentration over an extended time and is a good indicator of glycemic control. The advantages it offers over fasting glucose measurements is that it does not require fasting and it is not subject to wide fluctuations. The current gold standard that is used for diagnosis of diabetes and risk assessment of microvascular complications is a glycated form of hemoglobin that is referred to as hemoglobin A_{1c}. For details on hemoglobin A_{1c}, please refer to the Pearl of Laboratory Medicine entitled "Hemoglobin A_{1c}" by Dr. Ross Molinaro (www.traineecouncil.org). This Pearl will focus on the alternative markers of glycemia: fructosamine, glycated albumin, and 1,5-anhydroglucitol (1,5-AG).

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Fructosamines are compounds that result from the glycation of primary amines. Fructosamine is a ketoamine formed from joining of glucose to amino groups of proteins through a nonenzymatic mechanism involving a labile Schiff base intermediate and the Amadori rearrangement to form a stable ketoamine. This process is similar to the formation of hemoglobin A_{1c}. The reaction is slow, nonreversible, and is proportional to the amount of glucose in circulation.

Glycated albumin refers to the ketoamine formed when glucose conjugates to albumin, the most abundant serum protein. It is thought that measurement of glycated serum proteins is mainly a measure of glycated albumin. However, the assays for measuring glycated albumin and fructosamine are different.

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The assay commonly used to measure fructosamine is the nitroblue tetrazolium colormetric procedure. The test relies on the ability of the Amadori compound (glycated) to reduce nitroblue tetrazolium (NBT) to yield a highly colored formazan dye. The change in absorbance is measured spectrophotometrically (530 nm). Because the assay is colorimetric, high concentrations of hemoglobin (>100 mg/dL) and bilirubin (> 4mg/dL) interfere with the assay. Reducing substances in the blood such as superoxide dismutase from red blood cells can interfere with the NBT method for fructosamine. A newer method (marketed by Diazyme as glycated serum proteins) uses Fructosaminase™ to oxidize the Amadori product to yield hydrogen peroxide, which is measured colorimetrically.

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The assay to measure glycated albumin involves calculating a ratio of glycated albumin to total albumin and can be measured in both serum and plasma. Like hemoglobin A_{1c}, it is expressed as a percent. Several methods can be used to quantify glycated albumin including enzymatic assays, HPLC, affinity chromatography, colorimetry, and immunoassays. Affinity chromatography is the most commonly used assay. The boronic acid reacts with the cis-diol groups of glucose bound to hemoglobin to form a reversible five-member ring complex. This is similar to the boronate affinity chromatography assays used to measure hemoglobin A_{1c}.

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Compared to hemoglobin $A_{1c,}$ glycated albumin and fructosamine reflect a shorter time frame of glycemic control. Hemoglobin in red blood cells (RBCs) has a lifespan of 120 days; therefore, hemoglobin A_{1c} measurements represent integrated glucose concentrations over the preceding 2-3 months. The circulating half-life for fructosamine and glycated albumin is approximately 20 days; therefore, the concentration of these proteins reflects glucose control over a period of 2-3 weeks.

Hemoglobin A_{1c} is affected by altered RBC lifespan, hemoglobin variants, and blood transfusions. Anemia and hemolysis result in lower hemoglobin A_{1c}; whereas, polycythemia results in increased hemoglobin A_{1c}.

Gross changes in serum protein concentrations and half-life will affect fructosamine and glycated albumin measurements. Results would be invalid in patients with nephrotic syndrome, cirrhosis, dysproteinemias, or after rapid changes in acute phase reactants. In patients with nephrotic syndrome, hyperthyroidism, and glucocorticoid excess, values will be misleadingly low. Additionally, fructosamine and glycated albumin values will be higher in patients with liver cirrhosis and hypothyroidism.

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A major barrier to the use of these alternate assays is a lack of evidence linking fructosamine and glycated albumin to microvascular outcomes. A recent study by Selvin and colleagues (Selvin et al., Lancet Diabetes Endorinol 2014) has attempted to address this issue. The objective of this study was to characterize associations between these alternative markers with incident diabetes, retinopathy, and chronic kidney disease in a community-based cohort of more than 10,000 participants. The study found that prediction of incident chronic kidney disease (CKD) and retinopathy by fructosamine and glycated albumin was comparable to hemoglobin A_{1c}.

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In patients with reduced red blood cell life span such as hemolytic anemia and hemoglobin H disease, hemoglobin A_{1c} can be misleadingly low. Additionally, there are several hemoglobin variants that can cause falsely low or high hemoglobin A_{1c} measurements. There are over 1,000 hemoglobin variants identified and approximately 1% of diabetic patients in the US carry a variant. Therefore, fructosamine and glycated albumin can be used as alternatives to hemoglobin A_{1c} for assessing glycemic control in patients with altered red blood cell turnover and hemoglobin variants.

Unlike hemoglobin A_{1c}, the assays for fructosamine and glycated albumin are not standardized. Different labs utilizing different assays will report different reference intervals; therefore, absolute values are difficult to interpret and these assays are more useful when comparing changes over time within a patient.

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1,5-anhydroglucitol (1,5-AG) is a 6-carbon monosaccharide originating in the diet and structurally similar to glucose. It is not metabolized and is excreted by the kidneys. Its reabsorption in the renal tubules is competitively inhibited by glucose. As serum glucose concentrations surpass the renal threshold (180 mg/dl), 1,5-AG is excreted in the urine, leading to its rapid reduction in the serum. Thus, poor glycemic control is associated with low rather than high serum concentrations. 1,5-AG reflects glucose concentrations over a 1 to 2 week time period. Studies have shown that upon strict glycemic control, 1,5-AG concentrations recover at 0.3 ug/dL/day.

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The utility of 1,5-AG in diabetes was first discovered in 1975 and it received FDA approval in 2003 for short-term glycemic monitoring. In the US, it is currently marketed under the trade name GlycoMark™. The assay is a 2-step enzymatic method. In the first step, glucose is converted to glucose 6-phosphate by glucokinase allowing only 1,5-AG to react in the second step.1,5-AG reacts with pyranoze oxidase (PROD), which oxidizes the hydroxyl group on the 2nd position. Hydrogen peroxide generated from this reaction is detected using peroxidase that generates a color.

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Selvin and colleagues examined associations between 1,5-AG and microvascular complications in a recent study (Selvin et al., Clin Chem 2014). The study included 10,000 participants from a community-based cohort. Baseline 1,5-AG measurements were determined and the participants were followed for approximately 20 years for incident diabetes, retinopathy, and chronic kidney disease (CKD). The study found that low 1,5-AG concentrations were associated with increased risk for retinopathy and CKD. However, when incorporating 1,5-AG into predictive models that included traditional risk factors and hemoglobin A_{1c}, no meaningful improvement in the c-statistic for diabetes, retinopathy, or CKD was observed.

A limitation of the study was that it relied on a single measurement of 1,5-AG that may result in misclassification bias.

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To summarize, 1,5-AG reflects glycemic state over the preceding 1-2 weeks. It has poor sensitivity at high glucose concentrations since it is inversely dependent on serum glucose concentrations. Therefore, it may have limited utility for screening and diagnosis of diabetes. It does not add much incremental predictive value for diabetic complications in conjunction with other risk factors and hemoglobin A_{1c}.

Additionally, the kinetics of 1,5-AG are regulated by tubular reabsorption. The Selvin study excluded patients with decreased baseline GFR. It remains uncertain how 1,5-AG might be useful in patients with CKD not related to diabetes.

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There are several key points to remember about alternative markers of glycemia. Like hemoglobin A_{1c}, fructosamine is formed from a non-enzymatic reaction between glucose and serum proteins. Glycated albumin assays specifically measure glycation of albumin, the most abundant serum protein. While hemoglobin A_{1c} reflects glycemic status over 2-3 months, fructosamine and glycated albumin reflect glycemic state over the preceding 2-3 weeks due to shorter half-life of serum proteins compared to hemoglobin. Glycated albumin and fructosamine assays have not benefited from standardization efforts as hemoglobin A_{1c}. Although studies have shown that these markers can accurately predict risk of developing microvascular complications, currently, there are no guidelines for treatment goals using these alternative markers.

1,5-AG is a marker of glycemic excursion, which is defined as wide fluctuations between pre- and post-prandial glucose concentrations. It reflects glycemic state over the preceding 1-2 weeks. It is unmetabolized and reabsorbed in the kidney; however, its reabsorption is competitively inhibited by glucose. Therefore, high serum glucose concentrations lead to decreased 1,5-AG concentrations. It adds limited value in risk assessment over standard measures such as risk factors and percent hemoglobin A_{1c}. Further studies are required to determine whether this marker can be useful in predicting the effects of glycemic excursion on diabetic complications.

Slide 14: References

Slide 15: Disclosures

Slide 16: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on “Alternative Markers of Glycemia.”