Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
www.traineecouncil.org

Title: Hemoglobin A_{1c}

Presenter: Ross J. Molinaro, PhD, MT(ASCP), DABCC, FACB

Slide 1: Title Slide

Slide 2: Hemoglobin A_{1c}

Approximately 95% of adult hemoglobin (Hb) is comprised of Hb A in hematologically normal individuals, while Hb A_2 and very low levels of fetal Hb make up the lesser extent of the remaining Hb species. Hb A_{1c} makes up approximately 80% of Hb A_1. While there are various terms for glycated Hb (GHb), "Hb A_{1c}" is the internationally accepted term for reporting all GHb test results.

Slide 3: Formation of Hb A_{1c}

The formation of Hb A_{1c} occurs in humans at a slow rate as a posttranslational modification of Hb throughout the life of the red cell. Hb A_{1c} is formed by the nonenzymatic reaction of glucose with the amino-terminal valine residues of the β-chains of Hb A [Bunn, H. F., Haney, D. N., Gabbay, K. H., and Gallop, P. M. (1975) Biochem. Biopkys. Res. Commun. 67, 103-109]. Glucose binds reversibly to Hb as an aldimine, or Schiff base. This adduct then undergoes an Amadori rearrangement to form a stable ketoamine. The formation of the stable ketoamine is irreversible.

Slide 4: Hb A_{1c} concentrations

The concentration of Hb A_{1c} depends on several factors. The major determining factors are the life span of the red blood cell (RBC) and how long the Hb molecule is exposed to glucose. It is also thought that the permeability of the RBC to glucose influences the amount of glycation. In general, it is accepted that Hb A_{1c} concentrations represent average glucose levels over the preceding 8 to 12 weeks. This is precluded, however, in patients who have underlying anemias, hemolysis, vitamin B12 or folate deficiencies, hemoglobinopathies, or Hb variants, all of which may alter the lifespan of the RBC and therefore the accumulated concentration of Hb A_{1c} in the RBC.

Slide 5: Hb A_{1c} and risk of diabetic complications

It has long been known that increasing amounts of Hb A_{1c} correlate with diabetes complications. Regression models from the Diabetes Complication and Control Trial (DCCT), quantified these relationships. These analyses indicate that the risk associated with any given mean Hb A_{1c} changes as a function of time. The risk of retinopathy progression at any point in time that is associated with any given level of the mean Hb A_{1c} increases with years of follow-up. The analysis shows that as total glycemic exposure increases, retinopathy progression increases. There was a 60% reduction in development or progression of diabetic retinopathy, nephropathy, and neuropathy between the intensively treated group where the mean Hb A_{1c} achieved was approximately 7% and the standard group's mean Hb A_{1c} of 9% over an average of 6.5 years. Furthermore, for any given value of the updated mean Hb A_{1c}, the risk increases with time. It is data from this trial along with others, such as the United Kingdom Prospective Diabetes Study (UKPDS), that ultimately placed the importance of a target Hb A_{1c} value for diabetic patients in efforts to reduce their risk of complications as a result of the disease.

Slide 6: Hb A_{1c} and derived average glucose

Hb A_{1c} is an analyte used for monitoring average glycemia in diabetic patients over time. After the DCCT and UKPDS trials, it was clear there was a direct relationship between Hb A_{1c} and mean blood glucose. Until recently, however, there were not any reliable regression equations available to calculate an estimated average glucose, or eAG. The following table shows the relationship between Hb A_{1c} and eAG in a study sponsored by the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD). The major advantage to reporting Hb A_{1c} as an eAG is that both physicians and patients understand glucose values. Diabetic patients are familiar with dealing with glucose values from their home glucose meters. Of note, the correlation of Hb A_{1c} and eAG is only an estimate. For example, a Hb A_{1c} of 7% equates to an eAG of 154 mg/dL. However, the eAG is anywhere from 123 to 185 mg/dL with 95% confidence. The potential clinical and analytical implications for interpreting eAG in this context are not yet understood. Reporting of the eAG, however, has been endorsed by several clinical groups such as the ADA, EASD, International Diabetes Foundation (IDF) and the American Association for Clinical Chemistry (AACC).

Slide 7: Methods of measurement

Based on recent College of American Pathologists (CAP) survey data, approximately 29 Hb A_{1c} methods from 10 manufacturers are currently in use in clinical laboratories. Most methods can be classified into one of two groups according to assay principle. The first group uses methods that measure Hb A_{1c} on the basis of charge differences between glycated and nonglycated components, which includes cation-exchange chromatography. The second group uses methods that separate components on the basis of structural differences. Examples include boronate affinity chromatography and immunoassay. Most charge-based and immunoassay methods quantify Hb A_{1c}, which is defined as Hb A with glucose attached to the N-terminal valine of one or both Hb A chains. Boronate affinity methods detect not only Hb A_{1c} but also other amino group-glucose adducts such as glucose-lysine adducts and glucose-chain N-terminal valine adducts. It should also be mentioned that Hb A_{1c} point of care analyzers are available. However, they are not sufficiently accurate or precise enough at this time to be used for diagnosis of diabetes and should not be used for this purpose.

Data from the CAP Glycohemoglobin Proficiency Testing survey shows the distributions of methodologies used for measuring Hb A_{1c}. The ADA recommends that all laboratories performing testing participate in the CAP accuracy based proficiency testing survey for Hb A_{1c}, which uses fresh whole blood samples.

Slide 8: Standardization efforts

It was noticed shortly after the DCCT and UKPDS trials that Hb A_{1c} results reported in clinical laboratories for the same blood sample could differ considerably among methods. Therefore, standardization efforts were established on both the national and international levels. In 1996, the NGSP was initiated to standardize test results among laboratories to DCCT equivalent values. In 1997, the International Federation of Clinical Chemistry (IFCC) formed a committee to develop a higher-order reference method and reference materials for Hb A_{1c} analysis. Reference HPLC mass spectrometry and HPLC capillary electrophoresis methods were approved in 2001 [Jeppsson J-O, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mauri P, Mosca A, Paroni R, Thienpont L, Umemoto M, Weykamp CW. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002; 40: 78-89]. Although the clinical values obtained with assays standardized with the reference IFCC method correlate well (r = 0.999) with NGSP values, the absolute Hb A_{1c} values reported differ by 1.5%–2.0% Hb A_{1c}. The relationship between the NGSP network and IFCC networks was evaluated and a master equation was developed to document this relationship (NGSP = [0.9148 * IFCC] + 2.152). In 2007, the IFCC recommended that IFCC Hb A_{1c} be expressed as mmol Hb A_{1c}/mol Hb. With these new units, the master equation changed (NGSP = [0.09148 * IFCC] + 2.152), thereby avoiding any confusion between NGSP and IFCC results.

The NGSP and CAP have tightened the criteria for manufacturer certification in efforts to further decrease measurement variability. Because of this, emphasis has been placed on manufacturers to improve the accuracy and precision of Hb A_{1c} assays. Almost all Hb A_{1c} methods in clinical laboratories in the United States are certified by the NGSP. These standardization efforts have helped to lead the ADA and the World Health Organization (WHO) to include Hb A_{1c} as the preferred test for the diagnosis of diabetes. For more details, please see the Pearl of Laboratory Medicine on the diagnosis of diabetes.

Slide 9: Advantages

In addition to the standardization efforts of Hb A_{1c} measurement, a main advantage to Hb A_{1c} testing as a measure of average glycemia is that the results are not affected significantly by acute fluctuations in blood glucose concentrations, such as those occurring with illness or after meals. The main advantage to Hb A_{1c} testing for diagnosis is that the results do not require fasting.

Slide 10: Limitations

Several Hb variants as well as carbamylated Hb may interfere with some assay methods (independently of any effects due to shortened RBC survival). As shown by this slide's hypothetical HPLC analyses, depending on the particular Hb variant and assay method, Hb A_{1c} results can be either falsely increased or falsely decreased. For more information on method specific interferences of Hb variants, visit the NGSP website. Given that interferences are method specific, product instructions from the manufacturer should be reviewed before the Hb A_{1c} assay method is used. In addition, when selecting

an assay method, a laboratory should consider characteristics of the patient population served (a high prevalence of Hb variants).

If altered RBC turnover interferes with the relationship between mean blood glucose and Hb A_{1c} values, or if a suitable assay method is not available for interfering Hb variants, alternative non-Hb-based methods for assessing average glycemia, such as the fructosamine assay, may be useful.

Slide 11: Hb A_{1c} Pearls

There are several key points to remember about Hb A_{1c}. Hb A_{1c} is formed from a nonenzymatic reaction between glucose and free N-terminal valine groups on the β-chain of the Hb A molecule. Its concentrations depend on the amount of glucose exposure to RBC's over the life span of the RBC. Increasing Hb A_{1c} levels correlate with diabetic complications, such as retinopathy as was demonstrated in the DCCT and UKPDS trials. National and international standardization efforts together have paved the way towards accurate and precise Hb A_{1c} measurement. The majority of assays used to measure Hb A_{1c} currently are dependent on either charge or structure. Each of these methodologies has both advantages and limitations which should be understood before implementation in the clinical laboratory.

Slide 12: References