Commentary

HbA_{1c} Standardisation: History, Science and Politics

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Abstract

Significant analytical improvements have occurred since glycated haemoglobin (GHb), measured as total HbA_1, was first used in routine clinical laboratories around 1977. Following the publication of the Diabetes Control and Complications Trial (DCCT) study in 1993 the issue of international standardisation became an important objective for scientists and clinicians. The lack of international standardisation led several countries to develop national standardisation programs. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on Standardisation of HbA_{1c} established a true international reference measurement system for HbA_{1c} and the successful preparation of pure HbA_{1c} calibration material that should lead to further improvements in inter-method and inter-laboratory variability. Reporting of HbA_{1c} has been agreed using the units of mmol/mol (IFCC) and percent (National Glycohemoglobin Standardization Program, NGSP).

History

It is fascinating to consider the analytical improvements that have occurred since glycated haemoglobin (GHb), measured as total HbA_1, was first introduced into clinical laboratories for diabetes monitoring around 1977; at that time methods displayed poor precision, there were no calibrators or material with assayed values for quality control purposes.

Many methods for the measurement of GHb have been developed; these mainly make use of charge or structural differences between the glycated and non-glycated species of haemoglobin.

Methods based on charge difference

From the late 1970s to the mid 1980s the major assays were either electrophoretic or mini-column assays for the chromatographic fraction HbA_1 and later the more specific subfraction HbA_{1c}. The mini-column assays were rapid "short column" variations of the original 1971 Trivelli macro-column procedure that took several days to perform.[1] Fractions were eluted in the order HbA_{1a}, HbA_{1b}, HbF, HbA_{1c}, HbA_0 and HbA_2 with buffers of varying ionic strength.

To improve laboratory comparability the use of multilevel lyophilised bloods with values assigned by manufacturers using their own kit methods to determine the HbA_{1c} values became common. The use of temperature control was shown to achieve better precision than the use of calibrators for correcting temperature variation. Later developments and current HbA_{1c} HPLC assays based on cation exchange incorporate both thermostatted temperature control and the use of calibrators.

Other assays based on charge differences are: agar gel electroendosmosis, agarose gel electrophoresis, isoelectric focusing, and capillary electrophoresis. These methods have been largely discontinued in the clinical laboratory.

Methods based on structural difference

Affinity Separation

In 1981 Mallia et al. described a method that separated GHb based on the binding of the cis-diol groups of the glucose to m-amino phenylboronic acid cross-linked on agarose.[2] GHb binds to the affinity resin, whilst non-glycated haemoglobin does not bind. Quantitation is by spectrophotometry at 415 nm of the glycated and non-glycated fractions.

This method is much less temperature sensitive and does not suffer from analytical interference from HbF and carbamylated haemoglobin, and has long been recognised as the method of choice in patients with haemoglobin variants. Calibration did not exist.

Affinity methods have now been adapted for use on automated chemistry analysers, and affinity gel HPLC is now widely used worldwide. Both adaptations are calibrated using the manufacturers' material.

Immunoassays

In 1991 Dako Diagnostics Ltd. (Ely, UK) marketed the first commercial immunoassay, Novoclone HbA_{1c}, which used enzyme-linked microtitre plates with an antibody specific to the N-terminal eight amino acids of ketoamine HbA_{1c}. The assay was discontinued within a few years. In 1992 the DCA 2000 Point of Care HbA_{1c} analyser was introduced. This manual immunoassay analyser was originally marketed with an eight minute assay programme, and subsequently converted to a six minute assay.[3]

Immunological based assays for HbA_{1c} became more widely used in the mid 1990s and many commercial assays are now available and are targeted against the β N-terminal glycated tetrapeptide or hexapeptide group. Assay design is variable, ranging from immunoturbidimetry to latex enhanced competitive immunoturbidimetry, and operating instructions are available for a large number of chemistry analysers.

Science

Following the introduction of HbA_{1c} methods into routine use, it quickly become apparent that there was a significant difference in the results produced by different laboratories.[4] It was evident that the disparate results obtained were due to the range of methods being used by laboratories and the lack of a primary reference material. Even though the new generation of HbA_{1c} methods now demonstrate a degree of precision that could not be imagined in the mid 1970s, comparison of results from different laboratories would still be at best difficult or more likely impossible if not for standardisation schemes.

Standardisation with common calibration was first proposed in 1984 by Peterson et al., who examined factors such as inter-method correlation and reproducibility between laboratories.[5] However, it was only after the publication of the DCCT study in 1993 that the issue of international standardisation of glycated haemoglobin measurements became an important objective for scientists and clinicians.[6] The lack of international standardisation resulted in several countries developing national standardisation programs.

USA: National Glycohemoglobin Standardization Program (NGSP)[7-9]

The NGSP was formed in July 1996 to implement the plan developed by the American Association for Clinical Chemistry Glycohemoglobin Standardisation Subcommittee. The plan was to establish a system of reference laboratories that would support each other in a network that would be used to calibrate and standardise commercial HbA_{1c} methods and analytical systems. The BioRex 70 HPLC System, established in the DCCT Central Laboratory, was chosen as the NGSP "reference standard method". The Missouri DCCT laboratory was established as the Central Primary Reference Laboratory (CPRL) for the NGSP. This laboratory sets the initial calibration for the NGSP Certification Programme as the "set point" as used in the DCCT.

Primary Reference Laboratories (PRLs) were established to support the CPRL, the PRLs using the CPRL BioRex 70 HPLC method and a single haemolysate calibrator prepared by the CPRL with an established target value based on the mean of at least 50 CPRL runs. A number of Secondary Reference Laboratories (SRLs) were established to work directly with manufacturers to assist them in standardising commercial methods for Certification of Traceability to the CPRL BioRex 70 assay. The SRLs use established precise routine automated assays but must have these calibrated against the CPRL calibrator.

The NGSP is responsible for the calibration of HbA_{1c} methods in many parts of the world enabling direct comparison to the DCCT targets. The NGSP has an excellent website and provides up-to-date information on the precision and accuracy of validated commercial methods for HbA_{1c}.[10]

Japanese Standardisation Scheme[11,12]

In 1995 the Japanese Diabetes Society (JDS) in collaboration with the Japan Society of Clinical Chemistry (JSCC) developed a National Standardisation Scheme. Two calibrators (JDS Calibrator Lot 1) were prepared as lyophilised haemolysates. The HbA_{1c} values were assigned as the mean of the two most common high-pressure liquid chromatography (HPLC) analysers, Tosoh (Tokyo, Japan) and Kyoto Daiichi (Kyoto, Japan), both very precise cation exchange methods. At the time, most Japanese laboratories were using one of these methods. These two point calibrators are used for the calibration of all routine HbA_{1c} assays in Japan.

In 2000 a more specific high resolution cation exchange HPLC analyser KO500, developed by the JSCC, was adopted as the calibration method and a second set of National Calibrators (deep frozen blood) called JDS/ JSCC Calibrator Lot 2 were prepared. The HbA_{1c} values of these new calibrators were assigned using the KO500 analyser in four reference laboratories of the JDS /JSCC. To keep consistency with the Japanese Calibrator Lot 1 values, the Calibrator Lot 2 values were adjusted to those of the Calibrator Lot 1.

The JDS/JSCC calibrators are used by approximately 1800 laboratories in Japan. The effect of the calibrators has been a significant improvement in HbA_{1c} results between laboratories and a progressive decrease of mean inter-laboratory variation from values of more than 12% coefficient of variation (CV) to approximately 5%.

Three Japanese reference laboratories are included in the IFCC Reference Laboratory Network. For all IFCC comparative studies that were used to establish the IFCC–JDS Master Equations the KO500 method was calibrated with JDS Calibrator Lot 2.

Swedish Standardisation Scheme[13,14]

The Swedish standardisation scheme uses as its "reference" method the analysis of HbA_{1c} using Mono S HPLC (a strong methylsulfonate cation exchanger on monobeads, Pharmacia LKB Biotechnology, Uppsala, Sweden). Mono S is a relatively specific assay separating HbA_{1c} from all known minor endogenous components except carbamylated haemoglobin and α-chains. The HbF fraction is well separated.

In 1998 common standardisation throughout Sweden was introduced. Five selected Swedish reference laboratories using Mono S HPLC assays assign values to pooled whole blood samples. These laboratories are used for the calibration of all hospital and point of care instruments in Sweden.

Global Standardisation

A common feature of these national programs is the absence of internationally recognised and accepted reference materials and measurement procedures to assure the accuracy and comparability of HbA_{1c} measurements at a global level. To address these shortcomings, and to achieve a uniform international standardisation of HbA_{1c} measurements, the IFCC established a Working Group on HbA_{1c} Standardisation (WG-HbA_{1c}) with the aim to develop a complete reference measurement system based on the concepts of metrological traceability. In addition to reference methods and materials, essential elements of a comprehensive reference measurement system include the definition of the measurand (including the unit) in regards to the intended clinical use and the individuation of reference laboratories that possibly collaborate in a network.[15,16] For this project, the decision was made to define HbA_{1c} as haemoglobin molecules having a stable adduct of glucose to the N-terminal valine of the haemoglobin β chain (βN-1-deoxyfructosyl-haemoglobin). The rationale was that this quantity is biochemically well characterised, is the major form of HbA_{1c} in human blood, and most of the commercial HbA_{1c} tests claim to measure only this form. Two equivalent reference methods specifically measuring this hexapeptide were then developed, with a combination of HPLC and electron-spray mass spectrometry (MS) or, alternatively, a two dimensional approach using HPLC and capillary electrophoresis (CE) with UV detection.[17] The WG-HbA_{1c} was also successful in preparing primary reference materials (purified HbA_0 and HbA_{1c}) to calibrate the reference procedures.[18] In 2001, the IFCC reference methods were unanimously accepted by the National Societies of Clinical Chemistry following a ballot and published as approved IFCC reference methods.[17] In the meantime, a network of laboratories was established, using either the HPLC-MS or the HPLC-CE option.

Politics

It soon became apparent that there were significant differences between the HbA_{1c} values of the IFCC Network laboratories and each of the three national networks' designated comparison methods (DCMs), and also significant differences between each of the DCMs. But, in each individual case the relationship between each DCM and the IFCC was linear.

The relationships of each DCM with the IFCC method have been stable and reproducible over several years, and are described by the following master equations:[19]

- NGSP HbA_{1c} = 0.915 (IFCC HbA_{1c}) + 2.15
- JDS/JSCC HbA_{1c} = 0.927 (IFCC HbA_{1c}) + 1.73
- Swedish HbA_{1c} = 0.989 (IFCC HbA_{1c}) + 0.88

Table. Suggested units and target values for HbA_{1c} when measured with methods traceable to the IFCC reference system.[23] A comparison with the current figures is also given. Reprinted with permission from Walter de Gruyter.

	Current[a]	IFCC traceable methods
Reference interval (non-diabetics)	4–6%	20–42 mmol/mol
Target for treatment in diabetics[b]	<7%	<53 mmol/mol
Change of therapy in diabetics[b]	>8%	>64 mmol/mol

[a] refer to methods aligned to the U.S. National Glycohemoglobin Standardization Program.
[b] as recommended by American Diabetes Association.

The joint IFCC Committee on Nomenclature, Properties and Units and IUPAC (International Union of Pure and Applied Chemistry) Subcommittee on Nomenclature, Properties and Units (C-NPU) proposes "mmol/mol" be used as the unit of measurement for HbA_{1c}; this represents the SI unit for this analyte.[20] This option, i.e. the use of a completely different unit (mmol/mol instead of percentage), will avoid confusion when recalculating old HbA_{1c} targets to the new IFCC standardised values if clinical laboratories wish to implement HbA_{1c} results traceable to the IFCC reference system (Table). Other advantages of this approach may include a positive impact of changing of scale of reported HbA_{1c} results allowing clinicians and diabetic patients to better understand the analyte changes and increased potential for future use of HbA_{1c} as a diagnostic tool.[21] Currently clinicians and patients may perceive HbA_{1c} small changes as unimportant, although they are linked to large health effects.

The impact of both changes proposed by IFCC would be to significantly change the numeric results provided to clinicians. To discuss a joint approach an initial meeting was held in London in January 2004 between the International Diabetes Federation (IDF), European Association for the Study of Diabetes (EASD), the American Diabetes Association (ADA), the IFCC WG-HbA_{1c} and a representative of the NGSP.[22] This meeting suggested a way forward was to report HbA_{1c} in terms of "average plasma glucose", and a study (Mean Blood Glucose Study) was organised to investigate this relationship.

To progress discussion relating to the new reference method to standardise the HbA_{1c} results, along with the anticipated documentation that the assay does indeed indicate average blood glucose, a further meeting was held in Milan, Italy on 4 May 2007, at which a consensus agreement emerged. The following statements have been approved by the ADA, EASD, IDF and IFCC:[23]

1. HbA_{1c} test results should be standardised worldwide, including the reference system and results reporting.
2. The new IFCC reference system for HbA_{1c} represents the only valid anchor to implement standardisation of the measurement.
3. HbA_{1c} results are to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation.
4. If the ongoing "average plasma glucose study" fulfils its *a priori* specified criteria, an HbA_{1c} derived average glucose (ADAG) value calculated from the HbA_{1c} result will also be reported as an interpretation of the HbA_{1c} results.
5. Glycaemic goals appearing in clinical guidelines should be expressed in IFCC units, derived NGSP units and as ADAG.

The committee states that all the organisations agreeing with this consensus statement propose that these recommendations be implemented globally as soon as possible.

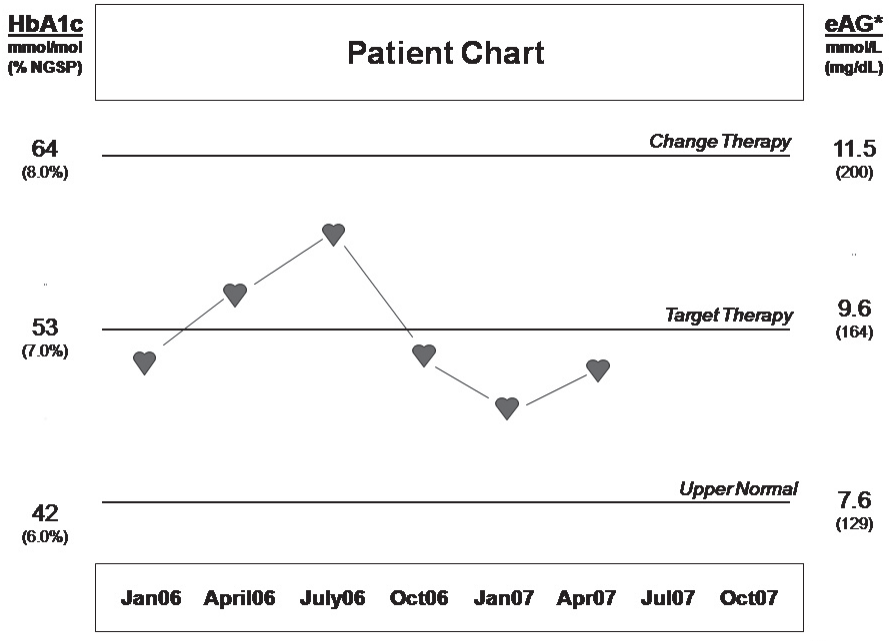

Figure. Example of a patient chart showing IFCC HbA_{1c}, NGSP HbA_{1c} and estimated average glucose (eAG).* Data taken from DCCT outcome while ongoing study results are awaited.

We now have full agreement that the IFCC reference measurement system is the anchor for global standardisation of HbA_{1c} measurements and the values should be reported in mmol/mol and NGSP values (%) should be derived and also reported.

In September this year the results of the Mean Blood Glucose Study were presented during the EASD meeting and several clinical societies supported the reporting of an "estimated average glucose" (eAG).

One approach to the reporting of eAG is as a "comment" to the HbA_{1c} results. An example of how these results can be combined is shown in the Figure.

This new initiative to express test results in scientifically correct units along with a clinically relevant interpretation of those results is not an uncommon practice (e.g. creatinine and estimated GFR). Consequently, clinicians will have the opportunity to convey the concept of chronic glycaemia in terms and units most suitable to the patients under their care.

The challenge now is to implement these recommendations globally; this will involve clinicians, biochemists, external quality assessment organisers, patient groups and manufacturers undertaking a large scale educational program. It is vital that all relevant parties are aware of the implications and benefits of these changes. When adopted we can be sure of true global standardisation of HbA_{1c}.

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