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REVIEW

Global standardisation of HbA_{1c}

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Abstract

HbA_{1c} is used for assessing glycaemic control in patients with diabetes. It is also used for treatment goals and as a target for therapeutic intervention. The Direct Control and Complications Trial in the USA showed that HbA_{1c} can be used to predict the risk of complications. Hence, it is important for HbA_{1c} assays to be standardised. The National Glycohemoglobin Standardization Program (NGSP) in the USA was formed in 1996 so that HbA_{1c} results from different laboratories would be comparable to those reported in the DCCT study. There were also HbA_{1c} standardisation programmes in Sweden and Japan. These three standardisation programmes are, in fact, direct comparison methods (DCMs), and yield different HbA_{1c} results. In 1994, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) established a Working Group on Standardisation of HbA_{1c}. This working group has developed a global HbA_{1c} reference system with very much improved intra-assay and inter-assay coefficients of variation. Recommendations have been made to report HbA_{1c} results as IFCC-HbA_{1c} values in SI units (mmol HbA_{1c}/mol Hb) and NGSP-HbA_{1c} (%) as well as estimated average glucose (eAG), once a tight relationship has been shown to exist between eAG and HbA_{1c}.

Key words: Standardisation of HbA1c, estimated average glucose, International Federation of Clinical Chemistry and Laboratory Medicine, National Glycohemoglobin Standardization Program

INTRODUCTION

HbA_{1c} is the gold standard for assessment of glycaemic control in patients with diabetes, reflecting the average blood glucose over the preceding two to three months. It is used for treatment goals and as a target for therapeutic intervention. The Direct Control and Complications Trial (DCCT) in the USA, published in 1993, showed that HbA_{1c} can be used to predict the risk of development of complications in patients with diabetes.[1] Hence, alignment of HbA_{1c} results to that of the DCCT has become clinically important so that HbA_{1c} can be used to predict the risk of various complications. The HbA_{1c} value measured in Malaysia or anywhere in the world should yield similar values to that of the DCCT.

STANDARISATION OF HbA_{1c} METHODS IN USA, SWEDEN AND JAPAN

In 1993, the American Association for Clinical Chemistry (AACC) formed a Subcommittee on Glycohemoglobin Standardization as the methods in existence for the measurement of HbA_{1c} all yielded widely varying results. The National Glycohemoglobin Standardization Program (NGSP) was formed in July 1996 to standardise HbA_{1c} methods so that HbA_{1c} results from different laboratories would be comparable to those reported in the DCCT study.[2] The NGSP standardisation of HbA_{1c} methods has been adopted by many countries including the USA, Canada, Mexico, South America, most European Countries and most Asian Countries. HbA_{1c} standardisation programmes were also established in Japan [Japanese Diabetes Society (JDS) in collaboration with the Japanese Society of Clinical Chemistry (JSCC)][3] and in Sweden (Mono S)[4] in the mid to late 1990s. These three standardisation programmes improved the quality of HbA_{1c} assays in clinical use. The methods used in these standardisation programmes are, however, not primary reference methods but are direct comparison methods (DCMs). These three DCMs yield different results.

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IFCC REFERENCE SYSTEM FOR HbA_{1c}

In 1994, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) established a working group on standardisation of HbA_{1c}. This working group has met regularly since 1995 and has developed a global HbA_{1c} reference system, prepared pure HbA and HbA_{1c} as the primary reference material, developed two reference methods, installed a network of 13 reference laboratories in the USA, Europe and Japan, and prepared secondary reference material.[5,6] HbA_{1c} has been defined as β-N-valine glycated haemoglobin (β-N-(1-deoxy)-fructosyl-haemoglobin), which is the major glycation site of HbA_{1c}. The reference methods are based on two-dimensional peptide mapping after proteolytic cleavage with endoprotease Glu-C, then separation of the resulting glycated and non-glycated N-terminal hexapeptides by reversed-phase high performance liquid chromatography, followed by quantification by mass spectrometry or by capillary electrophoresis (Figure 1).[7] Both methods give identical results.

The precision of the methods used to measure HbA_{1c} was substantially improved using the IFCC standardisation with mean intra-laboratory coefficients of variation (CVs) of 1.0 to 1.2% and inter-laboratory CVs of 1.4 to 1.9%.[8]

Considering the lack of specificity of the DCMs used in the standardisation programmes in USA (NGSP), Japan (JDS/JSCC) and Sweden (Mono S), it is not surprising that the HbA_{1c} results generated by these DCMs are higher than the results produced by the IFCC reference method.

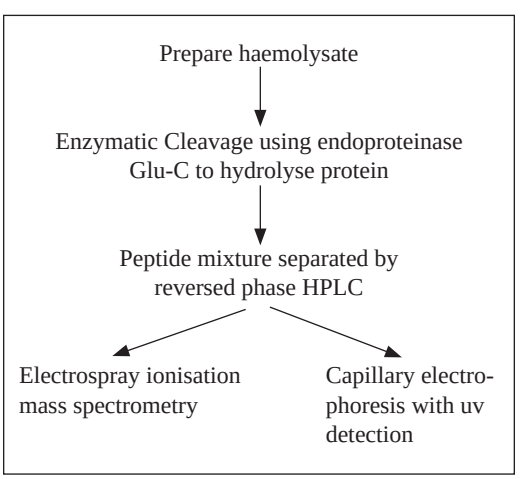

FIG. 1: IFCC-HbA_{1c} reference method

Relationship between the IFCC reference method and the direct comparison methods

Correlations between the IFCC reference method and the DCMs have yielded the following master equations[8] which have been found to be very robust:

1. NGSP-HbA_{1c} = 0.915(IFCC-HbA_{1c}) + 2.15% (r^2 = 0.998)
2. JDS/JSCC-HbA_{1c} = 0.927(IFCC-HbA_{1c}) + 1.73% (r^2 = 0.997)
3. Swedish-HbA_{1c} = 0.989(IFCC-HbA_{1c}) + 0.88% (r^2 = 0.996)

Reasons for implementing the IFCC reference method

The In-Vitro Diagnostic (IVD) Directive concerning medical devices in Europe states that diagnostic manufacturers must guarantee the traceability of their routine measurements to reference methods and materials of higher metrological order. This implies that manufacturers must calibrate using IFCC methodology, being the reference method of higher metrological order. A second reason for change is to improve globally the quality of HbA_{1c} assays.

Concerns by clinicians over the reporting of IFCC-HbA_{1c}

IFCC-HbA_{1c} values are lower than the NGSP-HbA_{1c} values by about 2%. This has caused considerable disquiet amongst diabetes specialists who are very concerned that reporting of the lower IFCC-HbA_{1c} values may lead to misinterpretation of the degree of glycaemic control and, thus, confuse both doctors and patients. The learning curve for both doctors and patients might be as long as three years. During this period there will be a worsening of glycaemic control resulting in adverse clinical outcomes for patients. Whilst the biochemist may wish to report IFCC-HbA_{1c} values, diabetes specialists prefer the reporting of IFCC-HbA_{1c} values as mean blood glucose (MBG) since this would be readily understood by both doctors and patients. The main concern which the IFCC and many biochemists have regarding the reporting of MBG is that an additional error would be introduced which could be substantial if there is considerable scatter about the linear regression between MBG and IFCC-HbA_{1c}.

RECOMMENDATIONS MADE REGARDING THE REPORTING OF HbA$_{1c}$

A meeting was held in London on 20 January 2004 amongst the International Diabetes Federation (IDF), European Society for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) with the Chairman of the IFCC-HbA$_{1c}$ working group and a representative of NGSP.[9] This International Diabetes Working Group, led by the IDF, agreed that the IFCC Reference Method should become the global reference standard and that all manufacturers should calibrate to the new method. DCCT-aligned (NGSP) HbA$_{1c}$ values would continue to be reported in the interim period. It was accepted at that meeting that MBG would be reported instead of HbA$_{1c}$, provided that an international study to be conducted prospectively showed that there was a tight correlation between IFCC-HbA$_{1c}$ values and MBG. Public and professional education programmes about the new reporting units and system should be planned.

The IFCC, IDF, EASD and ADA met in Milan, Italy on 4 May 2007, chaired by the President of IFCC, Dr Jocelyn Hicks, and agreed the following.[10,11]

1. HbA$_{1c}$ results would be reported worldwide in IFCC units (mmol HbA$_{1c}$/mol Hb), instead of % HbA$_{1c}$, in order to avoid reporting the lower HbA$_{1c}$ values which may be confusing to both clinicians and patients, and derived NGSP units (%) using the IFCC-NGSP master equation.
2. If the ongoing "average plasma glucose study" fulfills its specified criteria, an A1C-derived average glucose (ADAG) value calculated from the HbA$_{1c}$ result will also be reported.
3. Glycaemic goals appearing in clinical practice guidelines should be expressed in IFCC units, derived NGSP units and ADAG.

In September 2007, the interim results of the ADAG Study were presented at the EASD meeting in Amsterdam and several clinical societies supported the reporting of an estimated average glucose (eAG) instead of MBG or ADAG.

A meeting was held on 12 December 2007 in Milan, Italy between the IFCC and diagnostic companies. It was agreed at this meeting that all manufacturers should implement worldwide traceability to IFCC reference system for HbA$_{1c}$. The deadline for implementing the IFCC reference system for all instruments in current use is 31 Dec 2009. The name of the test in laboratory reports and clinical setting would be HbA$_{1c}$ and not, as used in the United States, A1C. All new instruments sold after 1 Jan 2011 would report HbA1c in both SI (mmol/mol – no decimals) and NGSP-derived units (percentage – one decimal place). The implementation of estimated average glucose (eAG) would be discussed after the ADAG clinical trial was published.

The following is a summary of a United Kingdom press release in April 2008 by the Association for Clinical Biochemistry and Diabetes UK regarding the reporting of HbA$_{1c}$.

1. HbA$_{1c}$ should be standardised using the IFCC Reference Measurement procedure.
2. An extensive education programme should be developed urgently for all healthcare professionals and people with diabetes to help the understanding and interpretation of the new IFCC units.
3. HbA$_{1c}$ results should be reported in both IFCC units (mmol/mol) and derived NGSP units (%).
4. There is currently insufficient experimental evidence to support the introduction of eAG as the study was conducted on a predominantly white population.
5. Further research into the use of eAG in children, different ethnic groups and pregnancy is required.

A1C-DERIVED AVERAGE GLUCOSE STUDY

The ADAG study[12] was published in August 2008. The study had recruited 507 subjects from ten international centres. There had initially been eleven centres but one centre withdrew from the study. The study populations comprised 268 subjects with type 1 diabetes, 159 subjects with type 2 diabetes and 80 non-diabetic subjects. HbA$_{1c}$ levels obtained at the end of three months and measured in a central laboratory were compared with the average glucose levels during the previous three months. The average glucose was calculated by combining weighted results from at least two days of continuous glucose monitoring performed four times, with seven-point daily self-monitoring of capillary glucose performed at least three days per week. Approximately 2,700 glucose values were obtained by each subject over three months. A

TABLE 1. Estimated Average Glucose (eAG).

NGSP-HbA_{1c} (%)	mg/dL*	mmol/L†
5	97 (76–120)	5.4 (4.2–6.7)
6	126 (100–152)	7.0 (5.5–8.5)
7	154 (123–185)	8.6 (6.8–10.3)
8	183 (147–217)	10.2 (8.1–12.1)
9	212 (170–249)	11.8 (9.4–13.9)
10	240 (193–282)	13.4 (10.7–15.7)
11	269 (217–314)	14.9 (12.0–17.5)
12	298 (240–347)	16.5 (13.3–19.3)

Data in parentheses are 95% confidence intervals
*Linear regression eAG (mg/dL) = 28.7 x HbA_{1c} – 46.7
†Linear regression eAG (mmol/L) = 1.59 x HbA_{1c} – 2.59

TABLE 2. NGSP-HbA_{1c} values and corresponding IFCC-HbA_{1c} and eAG values.

NGSP-HbA_{1c} (%)	IFCC-HbA_{1c} (mmol/mol)	eAG (mmol/L)
6.0	42	7.0
7.0	53	8.6
8.0	64	10.2

reasonably good linear regression was obtained between the eAG and HbA_{1c} (r^2 = 0.84, P = 0.0001). Table 1 shows the corresponding eAG concentrations in both mg/dL and mmol/L for any given NGSP-HbA_{1c} value. Table 2 shows the corresponding IFCC-HbA_{1c} values in SI units (mmol HbA_{1c}/mol Hb) and eAG concentrations for any given NGSP-HbA_{1c} value.

CONCLUSION

There is now general agreement that we should begin to report HbA_{1c} values in both SI units (mmol/mol) and derived NGSP units (%). Whilst the ADA also favours the reporting of eAG based on the findings of the ADAG study, this should perhaps await further studies assessing the relationship between eAG and HbA_{1c} in different ethnic groups, children and in pregnancy.

REFERENCES

1. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin dependent diabetes mellitus. N Eng J Med 1993; 329:977-86.
2. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL, Sacks DB, Goldstein DE. The National Glycohemoglobin Standardization Program: a five-year progress report. Clin Chem 2001; 46:1985-92.
3. Shima K, Endo J, Oimomi M, et al. Interlaboratory differences in GHb measurement in Japan, the fifth report of the GHb standardization committee, the Japan Diabetes Society. J Japan Diab Soc 1998; 41:317-23.
4. Mosca A, Paleari R. Standardisation schemes for haemoglobin A_{1c} determination. In: John WG, editor. Monitoring Glycaemic Control in the Diabetic Patient. London: Hartcourt Health Communication, Mosby International Limited; 2001. p. 137-50.
5. Finke A, Kobold U, Hoelzel W, Weykamp C, Jeppsson J-O, Miedema K. Preparation of a candidate primary reference material for the international standardisation of HbA_{1c} determinations. Clin Chem Lab Med 1998; 36:299-308.
6. Hoelzel W, Miedema K. Development of a reference system for the international standardisation of HbA_{1c}/glycohemoglobin determinations. Int Fed Clin Chem 1996; 9:62-7.
7. Jeppsson J-O, Kobold U, Barr J, et al. Approved IFCC reference method for the measurement of

HbA_{1c} in human blood. Clin Chem Lab Med 2002; 40:78-89.

8. Hoelzel W, Weycamp C, Jeppsson J-O, et al. IFCC Reference System for measurement of haemoglobin A_{1c} in human blood and the National Standardisation Schemes in the United States, Japan and Sweden: A Method Comparison Study. Clin Chem 2004; 50:166-74.
9. Report of the ADA / EASD / IDF Working Group of the HbA_{1c} Assay. Diabetologia 2004; 47/5:R53-54.
10. Panteghini M, John WG. Implementation of haemoglobin A_{1c} results traceable to the IFCC reference system: the way forward. Clin Chem Lab Med 2007; 45:942-4.
11. Consensus statement on the worldwide standardisation of the HbA_{1c} measurement. Diabetologia 2007; 50:2042-3.
12. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. Diabetes Care 2008; 31:1473-8.