Post-analytical factors – how should HbA1c results be communicated to clinicians?

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Post-analytical errors in laboratory medicine usually are poorly discussed, but this is certainly one of the issues needing also particular attention, because clinical decisions are expected to be taken on the basis of a laboratory result. In the case of glycated hemoglobin, the main post-analytical factors to be considered are those related to the measurement units and to the interpretation of the result of HbA1c, particularly in relation to the glycometabolic control of the subject. Other source of post-analytical errors, including a longer turnaround time, errors in keyboard entering of the data, failures in the follow-up and documentation of laboratory data, will not be discussed here.

Measurement units

With regard to the units of measurement, in 1998 the European Union introduced a directive on *in vitro* diagnostics (Directive 98/79/EC) requiring that laboratory tests be traceable to a "higher-order method". It has also to be reminded that in 2007 an international consensus statement was signed, saying that "A1c test results should be standardized worldwide, including the reference system and results reporting" (1). So the post-analytical issue was fully addressed at that time. It was also said that "A1c results are to be reported worldwide in IFCC units (mmol/mol; SI units) and derived NGSP units (%)". These statements were reinforced 3 years later but unfortunately these expectations have not been met yet.

Indeed, several countries have shifted to the SI units in various times between 2010 to present (Australia, Check Republic, Finland, Germany, Hungary, Italy, New Zeland, The Netherlands, Serbia, Sweden, UK), some have kept the NGSP % units (Canada and US), and the majority of the rest of the world apparently did not take any official position about. Most of the countries who have chosen to move to the SI units have had a period of dual reporting, where both original and SI units have been used together before switching to the single SI units. Two countries, Japan and Sweden, who did develop in the past their own reference system, decided to change too. Sweden moved to the SI units and Japan had double reporting (NGSP and Japan Diabetes Society units) up to 2013, and then kept the NGSP units only (2). In the US it is very unlikely that the change to SI units will be done, since for many test traditional units are still used. In some cases however (i.e. for glucose) both SI units (mmol/L) and traditional units (mg/dL) are used alternatively or both at the same time, so apparently the situation is at the same confused and conflicting.

Studies are still on the way in order to prove that the new units could improve the outcome of the patients, a topic very difficult to be proven. Indeed, a recent study by Kilpatrick has shown that over 2 years after switching to the SI units did not lead to any marked short-term deterioration in glycemia or a different HbA1c outcome in patients with initial poor glucose control (3). So, it seems that the mayor problem for those countries who did not take a decision yet, especially in the case of less developed countries, efforts should be directed toward adopting higher-quality and standardized methods, possibly meeting the required desirable standards of analytical imprecision and trueness already clearly defined (4). In any case, the decision to move to the SI units should be taken by involving all the stakeholders (diabetologists, family doctors, endocrinologists, pediatricians, nurses, patients associations, head officials of the National and/or Regional health systems, manufacturers of diagnostics) and providing sufficient information. In the countries where this change was done no particular problems came to the light. With regard to the publications, the already mentioned consensus statement (1), editors of journals and other printed material were strongly recommended to require that submitted manuscripts report HbA1c in both SI (IFCC) and NGSP/DCCT units.

Finally, the conversion of the HbA1c data between different units has to be accomplished by using the so-called master equation (NGSP = 0.09148(IFCC) + 2.152 or IFCC = 10.93(NGSP) – 23.50), which is derived by the studies of the IFCC Network of Reference laboratories, which are performed regularly twice a year, and who did prove that this equation is stable over a long period of time (5) [exactly for more than 17 years, according to the last report of the Network Coordinator (Cas Weykamp, personal communication)]. Moreover, comparisons between the NGSP and IFCC networks continue to be conducted twice a year, thus validating the stability and reliability of the networks, and ensuring that results can be converted from DCCT/NGSP units to SI units and vice versa. In order to assist patients and doctors, various facilities have been developed, either by using the Internet community, such the one developed by the NGSP (http://www.ngsp.org/convert1.asp), where a table and a calculator are available, or also the one developed by the UK Diabetes organization (http://www.diabetes.co.uk/HbA1c-units-converter.html). Various applications ("App") are also available by the smart phones.

Two caveats should be always taken into account:

a) Since any value below 2.15 % is zero in SI units (due to the lower specificity of the NGSP method respect to the IFCC reference measurements procedure), various of these facilities either report negative values (with no physiological meaning) or simply do not allow the calculation below a certain threshold (in the case of the UK calculator the values that can be converted should stay in the range 4 to 24 %, in terms of NGSP units).

b) Due to the normal use of just one decimal place to calculate the HbA1c concentration in the NGSP units, and to the rounding automatically performed using the above mentioned calculators, some values in SI units correspond to the same HbA1c values in NGSP units (i.e. 35 and 36 mmol/mol are both equivalent to 5.4 %; 47 and 48 mmol/mol: 6.5 %; 58 and 59 mmol/mol: 7.5 %; 70 and 71 mmol/mol: 8.6 %; just to quote the most frequent values in the physiopathological range of HbA1c).

Interpretation of an HbA1c result

The principal use of HbA1c is certainly the one related to the assessment of glycemic control in diabetic patients. Indeed, from the Diabetes Complication and Control Trial (DCCT) (6) and the United Kingdom Prevention Diabetes Study (UKPDS) (7) we have learn that a reduction in HbA1c level caused a decrease in the incidence of the complications of diabetes mellitus, mostly retinopathy, with optimal reduction achieved at an HbA1c value of 7% (53 mmol/mol). Based on these studies, HbA1c testing may be used to monitor the effectiveness of therapy or patient compliance (8). From these studies a first target of 7.0 % (53 mmol/mol) for individuals with Type 1 or Type 2 diabetes to reduce microvascular and macrovascular complications was established by the American Diabetes Association (ADA), followed later on by the Canadian Diabetes Association (CDA). Other thresholds were recommended by the CDA guidelines, as follows:

a) A target HbA1c of 6.5 % (48 mmol/mol) in individuals with Type 2 diabetes to reduce the risk of nephropathy, taking into account the possible risk of hypoglycemia.

b) A target HbA1c value of 8.5 % (69 mmol/mol) in children under 5, or of 8.0% (64 mmol/mol) in children 6 to 12 years old and of ≤7.0 % (≤53 mmol/mol) in children 13 to 18 years of age.

c) A target HbA1c value of ≤7.0 % (≤53 mmol/mol) in pre-pregnancy.

d) A target HbA1c value of ≤6.0 % (≤42 mmol/mol; or within the reference range), in pregnancy.

In 2008 the results of an international trial aimed to calculate HbA1c-derived average glucose values calculated from the HbA1c results (ADAG) was published (9). This trial confirmed that for an average increase in HbA1c of 1 % (10 mmol/mol) a mean worsening of plasma glucose was about 29 mg/dL (1.6 mmol/L), but the reporting of an estimated average glucose (eAG) calculated from the HbA1c value was not included among the consensus statements above mentioned, due to a set of limitations in the ADAG study.

Another potential use of HbA1c is for the diagnosis of diabetes. The threshold to this end (6.5 %, or 48

mmol/mol) was derived from an accurate analysis of the third National Health and Nutrition Examination Study (NHANES III). This recommendation has been then adopted by ADA and other Associations, and finally in 2010 by the World Health Organization. A number of papers have been published since then, with controversial opinions about the sensitivity of HbA1c for detecting diabetes. At present, not enough data are available to support the use of HbA1c for the screening of diabetes, and some National Associations (i.e. in UK and in Germany) have proposed different flowcharts where HbA1c is measured in association with fasting plasma glucose or with oral glucose tolerance test (OGTT) to diagnose diabetes in high-risk individuals.

Finally, HbA1c has been found to be the best predictor of 10 year fatal and non-fatal cardiovascular events and all-cause mortality, compared to fasting and 2 h post prandial glucose, in individuals between 50 and 75 years of age without diabetes (10). Another study, the North American Atherosclerosis Risk in Communities (ARIC) study, proved that in non-diabetics HbA1c was a better predictor of diabetes and cardiovascular disease compared to fasting glucose.

About reporting

As far as I know, there is no consensus on how HbA1c should be reported after a blood exam, since almost every laboratory has its own format and tradition. According to what is recommended in most of the classical textbooks of laboratory medicine, it is a normal praxis to present, together with the numerical result and measurement units of the observed HbA1c value, the reference intervals. In my opinion such a practice should be abandoned, since, as for other analytes such as total cholesterol, it is now more important to report a desirable level, or some target values.

A possible example for reporting the result of a determination of glycated hemoglobin in human blood, could then be the following: b-glycated hemoglobin (HbA1c): 38 mmol/mol (desirable value: <39 mmol/mol; cut-off for the diagnosis of diabetes: >47 mmol/mol; therapeutic target: <53 mmol/mol).

Since most of the methods are now standardized, I do not believe that the type of the method used (i.e. HPLC or immunochemistry) should be reported any more.

Conclusions

Long time has passed since the discovery of HbA1c and its introduction in the laboratory practice related to the management of diabetes mellitus. Great improvements have been achieved on the analytical side, but still a lot of work has to be done to achieve a world-wide standardization of this important laboratory test with regard to the post-analytical phase. I believe that much effort should be now pushed at the level of international associations (such as IFCC, EFLM or WHO) in order to promote the creation, the diffusion and finally the application of other ad-hoc consensus documents or guidelines.

References

1. *Consensus Committee. Consensus statement on the worldwide standardization of the hemoglobin A1c measurement: the American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. Diabetes Care 2007;30:2399–2400.*
2. *Sacks DB. Measurement of hemoglobin A1c. A new twist on the path to harmony. Diabetes Care 2012;35:2674-80.*
3. *Kilpatrick ES, Rigby AS, Atkin SL, Barth JH. Glycemic control in the 12 months following a change to SI hemoglobin A1c reporting units. Clin Chem 2013;59:1457-60.*
4. *Braga F, Dolci A, Montagnana M, Pagani F, Paleari R, Guidi GC, at al. Revaluation of biological variation of glycated hemoglobin (HbA1c) using an accurately designed protocol and an assay traceable to the IFCC reference system. Clin Chim Acta 2011;412:1412-6.*
5. *Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem 2008;54:240–8.*
6. *The Diabetes Control and Complications Trial research group. The effect of intensive treatment of diabetes on the development and progression of long-term complica-*

tions in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–86.

7. *U.K. Prospective Diabetes effect of intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (ULPDS33) U.K. Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837–53.*
8. *Higgins T. HbA1c - An analyte of increasing importance. Clin Biochem 2012;45:1038-45.*
9. *Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1c assay into estimated average glucose values. Diab Care 2008;31: 1473– 8.*
10. *Van't Riet E, Rijkelijkhuizen JM, Alssema M, Nijpels G, Stenouwer CDA, Heine RJ, et al. HbA1c is an independent predictor of nonfatal cardiovascular disease in a Caucasian population without diabetes: a 10 year follow up of the Hoorn Study. Eur J Cardiovasc Prev Rehabil 2012;19:23–31.*