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Research Article

Diabetes mellitus: The long way of standardization of HbA_{1c} to the level of highest metrological order

Standardisierungsprozess der HbA_{1c}-Messungen zur höchsten metrologischen Ebene

Abstract

Glycated haemoglobin (HbA_{1c}) measurements are used in clinical studies and for the management of diabetic patients. Various efforts were made to standardize the HbA_{1c} measurements with consensus standards and standards based on a reference measurement procedure with external calibration. According to ISO 17511 a standard should meet highest accuracy possible, have a defined uncertainty of measurement and the calibration should be traceable to SI units. For HbA_{1c} this has been realized using a LC-ID-MS procedure based on the existing reference measurement procedure.

Keywords: HbA1c reference measurement procedure, LC-IDMS, standardization

Zusammenfassung

Die Bestimmung von (HbA_{1c}) wird in klinischen Studien und bei der Behandlung von Diabetes-Patienten herangezogen. Im Rahmen der Standardisierung des Messverfahrens dieses Analyten wurden Kalibrationssysteme vom Konsensus-Verfahren bis hin zum Referenzmessverfahren mit externer Kalibration entwickelt. Um in der Standardisierung die metrologisch höchste Ebene zu erreichen, wird für das Messergebnis neben Richtigkeit und definierter Messunsicherheit entsprechend dem ISO Standard 17511 die Rückführbarkeit der Kalibrierung auf SI-Einheiten gefordert. Dieses wurde mit der Entwicklung einer LC-ID-MS Methode auf der Basis des bestehenden Referenzmessverfahrens erreicht.

Schlüsselwörter: HbA1c-Referenzmessverfahren, LC-ID-MS, Standardisierung

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HbA_{1c} is a retrospective analyte of the carbohydrate metabolism reflecting the mean blood glucose levels during the last 6 to 8 weeks. Clinical studies revealed a close relation between late diabetic complications and the concentration of glycated proteins in blood. Therefore HbA_{1c} determination is widely used to assess the metabolic status and to monitor the medical treatment of diabetic patients. The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) used HbA_{1c} as one of the main indicators for the quality of diabetes management [1], [2]. HbA_{1c} is meanwhile recommended for screening of diabetes, since HbA_{1c} concentrations in blood are not affected by acute metabolic alterations in the patients.

Standardization of HbA_{1c} determinations affords the establishing and implementation of a reference measurement procedure with the highest accuracy possible. At the end of the 80s a certified reference material of HbA_{1c} was not available and an high performance liquid chromatography (HPLC) peak of HbA_{1c} analysis developed by Goldstein and co-workers [3] became the reference for the calibration of HbA_{1c} measurement.

The HPLC procedure [3] was accepted as a common „voluntary consensus standard" for the HbA_{1c} determination in the DCCT- and UKPDS-studies. Moreover, the procedure was applied as standard protocol for analytical devices for HbA_{1c} measurements. The National Glycohemoglobin Standardization Program (NGSP) became the basis for the calibration of HbA_{1c} measurements in the USA. According to this calibration HbA_{1c} values are reported in

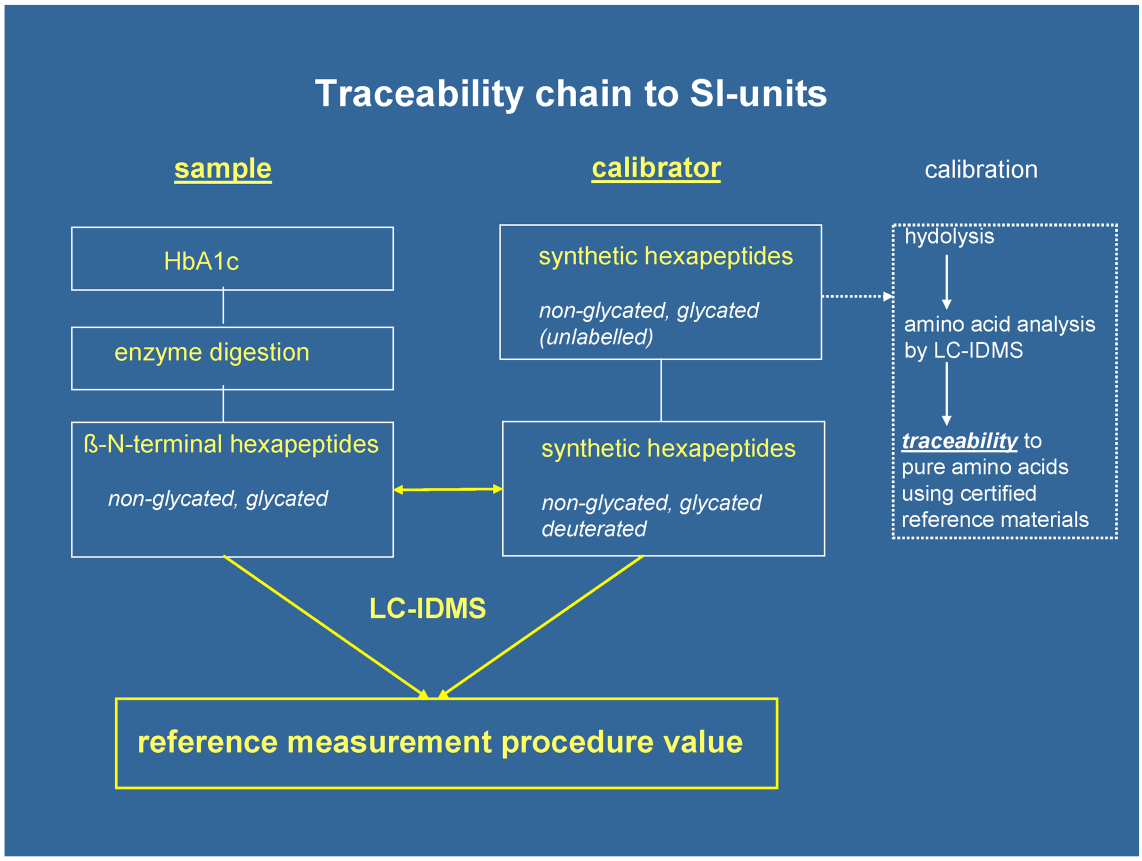

Figure 1: Traceability chain of HbA_{1c} measurements to SI units. The calibration of the reference peptides is based on certified amino acid concentrations.

% of blood haemoglobin concentration. Other national standardization activities were performed in Japan and Sweden. Further investigations showed that the separation of HbA_{1c} from other proteins by HPLC was incomplete and the specificity was not appropriate for international standardization [4].

At the end of the 90s, the International Federation of Clinical Chemistry (IFCC) had established a Working Group to develop an international reference measurement procedure for HbA_{1c} traceable to a standard of higher metrological order. Chromatographically purified HbA_0 and HbA_{1c} preparations from human blood were used as calibrators [5]. A reference measurement procedure was developed using these calibrators.

The principle of the IFCC reference measurement procedure was the proteolytic treatment of the blood sample with endoproteinase Glu-C cleaving glycated or non-glycated hexapeptides from the N-terminal β-chains of the haemoglobin. The hexapeptides were analyzed by HPLC-MS or by HPLC-capillary electrophoresis. The ratio of the glycated and non-glycated N-terminal hexapeptides of the haemoglobin β-chain expressed in percent was defined as the IFCC unit. The decision created considerable confusion, since from now on there were two different calibrations with the same unit on the market.

The results between the laboratories of the Working Group were regularly monitored by intercomparison surveys. The quality of measurements, expressed in terms of inaccuracy and imprecision, was improved during the measurement campaigns. From the collected data of the IFCC Working Group a “master equation” was developed to convert values from IFCC units into NGSP units and vice versa.

Since 2007, laboratories in Europe, Australia, and Japan calibrated their analytical systems for HbA_{1c} according to the IFCC reference measurement procedure and started to report their results in mmol/mol to avoid confusions [6]; US laboratories remained with NGSP calibration and express HbA_{1c} values in NGSP % .

The effort of the IFCC Working Group resulted in a remarkable improvement with respect to the NGSP consensus calibration. However, the key problem of the IFCC reference measurement procedure revealed to be the quality of HbA_{1c} and HbA_0 calibrator preparations and their traceability to the SI standard. During the inter-comparison studies a more robust LC-MS reference measurement procedure improving the reproducibility of measurement was established [7], [8].

In a recent study the traceability of HbA_{1c}, measurement results to SI units has been achieved by isotope-dilution mass spectrometry, using glycated and non-glycated β-N-terminal hexapeptides of defined purity and stated uncertainty of measurement as calibrators and deuterated β-N-terminal hexapeptides as internal standards [9]. The traceability is accomplished by hydrolysis of the unlabelled hexapeptide standards and the determination of the amino acid concentration by LC-ID-MS calibrated with certified amino acid standards [10] (Figure 1).

Table 1: Overview of HbA_{1c} standardization efforts

Implemented in		Literature	Traceability to SI
1993	Consensus standard of HbA_{1c} measurements (NGSP) UKPDS/DCCT-studies	[1], [2]	no
2002	IFCC reference measurement procedure	[5]	no
2006/2008	Improved IFCC reference measurement procedure	[7], [8]	no
2010	LC-ID-MS procedure based on the IFCC reference measurement procedure	[11]	yes

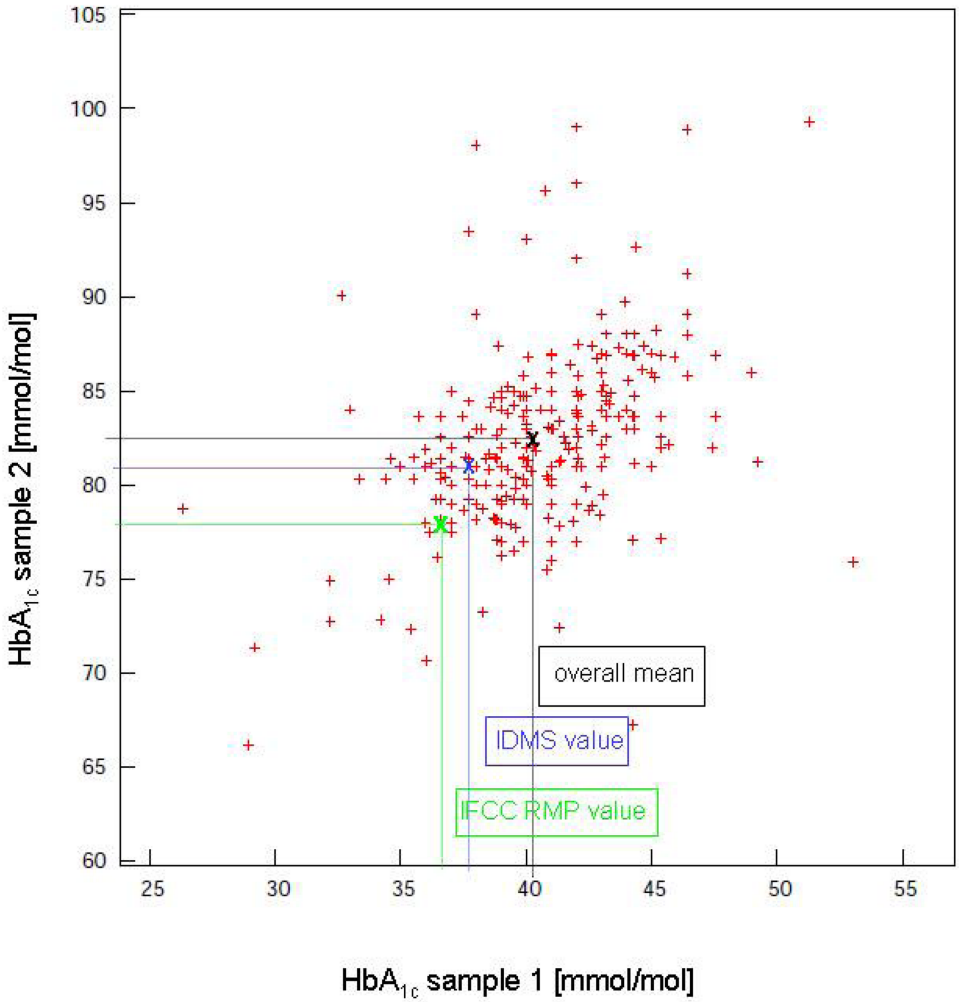

Figure 2: Results of an external quality assessment scheme run by INSTAND e.V. for HbA_{1c}

The Youden-plot demonstrates the measurement results of two samples (sample 1 and sample 2) from 325 routine laboratories (red cross), their overall mean (black cross, 40.4 and 82.5 mmol/mol, respectively), and the results obtained by the IFCC reference measurement procedure (IFCC RMP value, green cross, 36.6 and 78.4 mmol/mol, respectively), and by the IDMS procedure (IDMS value, blue cross, 37.9 and 81.2 mmol/mol, respectively).

In a recent article Little et al. [11] claim the development of HbA_{1c} standardization "from chaos to order" during the last 15 years with the NGSP and IFCC standardization efforts and discuss problems arising from the implementation of the IFCC Reference System. They state that "there are different interpretations of 'traceability'". According to ISO Standard 17511 the traceability of measurement is realized through an "unbroken chain of comparison" using a reference measurement procedure of highest metrological order and, if possible, a reference material of highest metrological level which is traceable to SI units [12]. With this ISO standard the "traceability of measurement" is obviously defined (Table 1).

The new reference measurement procedure is appropriate to set target values in external quality assessment schemes.

In Figure 2 the dispersion of routine values around the different target values is demonstrated in a recent external quality assessment scheme (EQAS) for HbA_{1c}. The three possible target values (consensus value, IFCC reference measurement procedure value, and IDMS value) are given in this scheme. The new calibration using the LC-ID-MS procedure leads to slightly higher HbA_{1c} levels than the recent calibration according to the IFCC reference measurement procedure.

In summary, a new standardization of HbA_{1c} measurement has been established at the highest level of accuracy, with stated uncertainty of measurement and with traceability to SI units following the IFCC reference measurement protocol using LC-ID-MS as procedure of highest metrological order.

Notes

Competing interests

The authors declare that they have no competing interests.

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