Review

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HbA_{1c} and biomarkers of diabetes mellitus in *Clinical Chemistry and Laboratory Medicine*: ten years after

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Abstract: Since its discovery in the late 1960s, HbA_{1c} has proven to be a major biomarker of diabetes mellitus survey and diagnosis. Other biomarkers have also been described using classical laboratory methods or more innovative, non-invasive ones. All biomarkers of diabetes, including the historical glucose assay, have well-controlled strengths and limitations, determining their indications in clinical use. They all request high quality preanalytical and analytical methodologies, necessitating a strict evaluation of their performances by external quality control assessment trials. Specific requirements are needed for point-of-care testing technologies. This general overview, which describes how old and new tools of diabetes mellitus biological survey have evolved over the last decade, has been built through the prism of papers published in *Clinical Chemistry and Laboratory Medicine* during this period.

Keywords: biomarkers; *Clinical Chemistry and Laboratory Medicine*; diabetes mellitus; glycation; HbA_{1c}; point-of-care testing.

Introduction

In the first issues (1963–1968) of the journal, which was not yet named *Clinical Chemistry and Laboratory Medicine* (CCLM), only few papers were devoted to diabetes mellitus and its biomarkers. At that time, they dealt with chemical or enzymatic glucose assays, discussing the methods and evaluating the first attempts of automation [1, 2]. Some of them already identified analytical pitfalls related to the type of glass tubes used for the reactions [3]. Clinical aspects were not forgotten, procedures of intravenous glucose tolerance test being discussed [4], and uraemia, which will be addressed later in this review, being already considered a "biochemical problem" [5]. Times have changed, and since this period many manuscripts have covered this field of laboratory medicine. In the special anniversary issue "50 years of CCLM", I had highlighted the milestones of HbA_{1c} history with a special emphasis on the contribution of CCLM [6]. Over the last decade, significant advances have been made regarding HbA_{1c} and more generally diabetes management. This manuscript prepared at the occasion of the sixtieth anniversary of CCLM focuses on the major events linked to HbA_{1c} reported in the journal during this period, broadened to a more general overview on the use of other markers of diabetes mellitus, evaluated either by classical or more innovative (e.g. non-invasive) methods. This review obviously evidences CCLM as a major vector of scientific information related to diabetes mellitus in laboratory medicine and medical practice.

Following the international standardization of HbA_{1c} assays

A major event in history of HbA_{1c} was the description of the reference measurement procedure (RMP) by the IFCC working group on HbA_{1c} standardization published in CCLM in 2002 [7], as described ten years ago in the 50th anniversary issue [6]. Since this period, the standardization of HbA_{1c} assays ensuring traceability to the IFCC-RMP has progressed everywhere internationally. The positive impact of standardization has been noticed in most countries of the world, as described in China, where IFCC initiatives have paved the way for an extended role of HbA_{1c} use across the country [8]. As all manufacturers calibrate their methods against the IFCC-RMP, every laboratory is able to issue HbA_{1c} values aligned with the

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IFCC reference values, which is an indisputable success of the international standardization process. IFCC-RMP is maintained by an international IFCC network of approved laboratories, which guarantees the continuity of the RMP and makes the results worldwide traceable to IFCC-RMP and comparable between laboratories [9]. Modifications of the IFCC-RMP aiming at improving its analytical performances using liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been proposed and discussed [10]. Combined with the improvement of methods by manufacturers, standardization of assays has allowed a continuous increase in quality of results, as developed in the next section, explaining that HbA_{1c} is definitively considered a robust and reliable support for addressing clinical needs.

A pitfall in the expected evolution of HbA_{1c} standardization is the use of new units. In its recommendations, IFCC had asked to report HbA_{1c} values as mmol HbA_{1c}/mol HbA_0 + HbA_{1c}. This expression in mmol/mol, which corresponds to SI units, is well correlated with the conventional expression in percentage of total haemoglobin, used since many years by the National Glycohemoglobin Standardization Program (NGSP) in the United States and internationally [11], as assessed by the master equation established between the two systems after the adoption of IFCC-RMP as international anchor [12]. However, this mode of result reporting is not yet used everywhere in the world and, especially in the clinical community, the expression in percentage is still largely the rule. This is clearly demonstrated by an international survey published in 2015, concluding that the acceptance of the SI units for expression of HbA_{1c} results was slowly spreading throughout Europe, and more slowly outside Europe [13]. No significant evolution has been reported since this time. If one of the ultimate goals of standardization is the use of SI units worldwide [14], much more intensive action plans, especially targeting clinicians and patients, should be conducted for changing habits in clinical settings, but also in the biological community. This is not without consequences. For example, it has been outlined that analytical goals were dependent on the different calibration hierarchies used [15]. Thus, there is still a long way to achieve standardization of practices [16].

Evaluation of performances and quality assessment of HbA_{1c} assays

The overall quality of routine HbA_{1c} assays has continued to improve during the last decade, as reported in many evaluation papers published in CCLM. They described analytical performances consistent with the standard requirements, and demonstrated that most of the classical interferences in HbA_{1c} measurements (labile HbA_{1c}, carbamylated Hb) had been eliminated. These papers dealt with the most recent methods implemented in the market, including capillary electrophoresis [17–19], automated immunological [20] or enzymatic [21] methods, affinity chromatography [22] as well as new versions of cation exchange high performance liquid chromatography (HPLC) devices [23, 24]. However, the occurrence of an unexpected interference must not be forgotten in some particular situations, including the change of kit on a same HPLC device [25] or in case of a specific interference with a given method, as reported for aspirin in HPLC [26]. The well-known analytical interference in HbA_{1c} measurements related to the presence of Hb variants, as assessed by the abundant literature devoted to this topic since many years, will be treated in specific section of the paper. In addition, some of these papers have evaluated and verified the ability of new field methods to align with the IFCC referent measurement system [27, 28], thus participating in the optimal efficiency of HbA_{1c} measurement in laboratory strategies [29].

An important challenge in the global management of the "HbA_{1c} system" is to define the best modalities of evaluation of performance characteristics and quality criteria of methods. After initial evaluations of the methods made upon their bringing to market, additional information has to be regularly obtained based on the daily performances of HbA_{1c} assays in field laboratories by external quality assessment (EQA) trials. Among them, several projects at national [30] or international levels, like $EurA_{1c}$ [31], have confirmed the continuous global improvement of methods, constituting a goad for manufacturers to increase the level of the performances of their methods. Besides, clinical laboratory professionals can use this information for selecting high performance techniques and using them according to laboratory good practices and manufacturers' recommendations, in order to reach stricter and stricter analytical goals as recommended by EQA providers [32, 33]. Indeed, the analytical quality of HbA_{1c} methods is key for interpreting results at wide levels (e.g. national) for benchmarking purpose [34], and it was advocated that method performance criteria should be clearly defined when tendering for HbA_{1c} analyzers [35].

Different models of result exploitation and representation have been proposed to exploit data issued from EQA trials and debated in the journal [36, 37]. Among them, the Sigma-metrics approach proposed by the IFCC Committee on Education in the use of Biomarkers of

Diabetes (C-EUBD) is now widely used in publications and reports from EQA providers [38]. These studies showed that most commercial assays provided values close to the IFCC reference values, demonstrating that the trueness issue had been satisfactorily addressed with the global standardization of HbA_{1c} assays and the traceability to the IFCC reference measurement procedure. The major differences between methods, constituting room for improvement, were related to imprecision.

However, it turned out, when interpreting the results of these quality control trials, that a key factor for the adequate quality assessment of methods was the commutability of control materials. Indeed, this general matter of concern, regularly developed in the journal [39, 40], has been especially demonstrated for HbA_{1c}. In a large operation involving several clinical laboratories, reference laboratories and manufacturers, it has been confirmed that the quality control material matrix (i.e. fresh whole blood or lyophilized) had a significant impact on the results of some methods [41]. This point must be considered in the design of new EQA trials and carefully considered upon result interpretation [41, 42]. The necessity to use fresh patient samples for evaluating new devices under real clinical conditions has also been outlined [43]. However, this important topic must be systematically considered in all its aspects, since if native, unprocessed, matrixes reproduce at best the behaviour of patient samples, their handling is much more complicated than that of processed samples and needs a thorough evaluation of feasibility, as underlined during the first attempts to use this type of materials [44]. The development of commutable certified reference materials usable for method validation or quality control purpose has been addressed in a manuscript describing the production of commutable whole blood reference materials with certified values established by liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS) [45].

Point-of-care testing (POCT) of HbA_{1c}

A specific topic is the determination of HbA_{1c} in clinical units or doctor offices by POCT. This is a unique situation for a POCT analysis, because in general POCT is primarily used in emergency departments and intensive care units for issuing immediate results. In the case of diabetes, HbA_{1c} assayed by POCT is of interest for clinicians since it provides a useful information for feeding the discussion with the patients during the consultation, in the global frame of the educational approach of diabetes treatment. In a cross-sectional study, it has been shown that accurate HbA_{1c} POCT results available during consultation improved diabetes care at general practitioner offices [46].

Issuing reliable HbA_{1c} results by POCT implies a high quality of devices and their appropriate use by clinicians. Generally, POCT assays, in the same way as laboratory methods, have globally improved over the last years, as demonstrated in the EQA trials mentioned above. However, if some analysers behave correctly and provide reliable results [47], other ones show borderline or unacceptable performances [47, 48]. A meta-analysis published in 2017 evidenced a substantial variability in bias within devices, emphasizing that devices with a significant bias compared to laboratory methods could influence decision-making [49]. As an additional factor, lot-to-lot variation and inter-device differences may contribute to a poorer analytical performance of some POCT devices used in a given clinical setting [50]. The interest of HbA_{1c} determination by POCT is thus undisputable, but quality criteria and a full knowledge about the performances by end-users is absolutely necessary. It is essential that laboratory professionals but also clinicians are aware of these data and choose the most reliable equipment.

Other aspects related to blood sampling outside the laboratory have also been addressed in the journal. HbA_{1c} determination from dry blood samples has been discussed as well as the possibility to use a volumetric absorptive micro-sampling (VAMS). This latter method which collects a fixed volume of blood has proven to be acceptable for patients and to give good results in the laboratory, especially if VAMS samples are stored in a liquid medium (wet absorptive micro-sampling) at home. It is clear that performances of such systems are better when performed in a laboratory setting than after home sampling by end-users, but education of patients is likely to improve the quality of the information provided [51–53]. Incidentally, another paper has questioned the validity of the interchangeability of venous and capillary blood values in case of intense oxidative stress [54].

Influence of Hb variants on HbA_{1c} results

The influence of Hb variants on HbA_{1c} results is not a trivial issue, as underlined in a large study reporting the high number of Hb variants discovered during routine HbA_{1c} measurements in China [55]. Doubts have often been expressed about the reliability of HbA_{1c} values in these

situations [56]. It seems however that, currently, the interference of the most common Hb variants is relatively limited, or at least well described and controlled by most methods [57]. Several reports about evaluations of new methods based on different principles have confirmed this trend [17–19, 22, 24]. In some cases, however, Hb variants may affect HbA_{1c} measurements, especially when performed by ion-exchange chromatography, as evidenced in many reports [58–65]. In other cases, unusual variants were detected using HPLC and/or capillary electrophoresis without interfering with HbA_{1c} quantification, confirming that separative methods provide a useful additional information [66, 67].

Although being "just" a letter, a very important paper has been published in 2015 [68], that addressed a very old and recurrent question related to the speed of glycation of various Hb variants. Indeed, differences in glycation rates could be an additional factor of confusion when interpreting HbA_{1c} results obtained by methods taking variants into account for calculations, which underlines the added value of the information provided by separative methods. By contrast, an identical glycation rate, provided there are no alterations of red blood cell (RBC) lifespan, could allow to calculate HbA_{1c} values even in the presence of Hb variants. Very interestingly, the results of this study performed in a large number of samples strongly suggested, admittedly indirectly, that glycation rates of variants S, C, D and E were the same as that of HbA. This major finding proves highly helpful for the interpretation of results. In the case of Hb G and J, no definitive conclusions could be made. Another paper suggested that HbH had also a glycation rate comparable to that of HbA [69].

In all cases, the interpretation of HbA_{1c} results must be cautious in the presence of Hb variants, and every laboratory should be aware about the performances and limitations of the techniques used. Most common variants found in Europe generally do not interfere with HbA_{1c} determination and provide values close to those obtained by the IFCC reference method [70], but it was outlined that others found especially in Asian countries like Korea could induce more important biases [71]. In two papers, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been proposed as a choice method to overcome the interference of Hb variants. In addition, the authors proposed that evaluation of alpha chain glycation, based on MALDI-TOF MS results, could be clinically valuable being free of interference from alpha globin variants [72, 73].

Clinical challenges of HbA_{1c}

It has been well demonstrated and acknowledged in literature that HbA_{1c} was a major tool in the clinical management of patients with diabetes mellitus, the regular follow-up with HbA_{1c} measurements improving the quality of glycaemic control and decreasing the development of long-term complications. Besides, HbA_{1c} evaluation has been extended to diabetes diagnosis and recommended in many countries for this purpose. An identical recommendation has been endorsed by World Health Organization [74, 75]. As the clinical interest of this "gold standard" parameter had no more to be demonstrated, most papers published in the journal in the last ten years were rather related to its mode of use, which still deserves attention. For example, it was underlined that monitoring frequency had to be carefully considered in order to achieve commonly recommended HbA_{1c} targets, this being a major challenge for healthcare systems [76]. The validation of HbA_{1c} as a new test for diagnosis of diabetes besides blood glucose has reinforced the needs for quality requirements of HbA_{1c} assays [74, 75], analytical bias and imprecision being both key factors for the predictive value of the test [77].

Besides, new clinical information drawn from HbA_{1c} measurements has been mentioned in the journal. For example, in a large cohort of Chinese patients, it was shown that testing for HbA_{1c} in addition to oral glucose tolerance test (OGTT) during screening helped to identify patients with early beta cell function impairment [78]. Its use in the diagnosis of gestational diabetes mellitus as rule-in test [79] in association with other standard well controlled diagnostic tests [80] was also proposed. Beside diabetes mellitus, HbA_{1c} proved to be a relevant biomarker in populations with cardiovascular troubles. A 2017 meta-analysis indicated that elevated HbA_{1c} was an indicator of mortality risk in patients with ST-segment elevation myocardial infarction, suggesting the need for a more intensive management of glycaemic control in these patients [81]. HbA_{1c} was also found to add significant information for detecting diabetes but also prediabetes when compared to OGTT in patients undergoing coronary angiography, thus constituting a possible alternative in diagnosis [82].

HbA_{1c} thus proved to be an irreplaceable tool in diabetes mellitus but also in other clinical situations. However, whereas most analytical challenges of HbA_{1c} assays have been addressed, clinical challenges remain for HbA_{1c} test use. Indeed, HbA_{1c} results are not interpretable in a

variety of specific situations, thus necessitating to adopt alternative approaches.

The first major challenge is the complex interpretation of HbA_{1c} values in clinical situations characterized by alterations of RBC turnover. The semiological value of HbA_{1c} is dependent by nature on unaltered Hb metabolism and RBC lifespan, the measured value being the result of normal Hb metabolism during the whole lifespan of RBC in the circulation, generally estimated at 120 days, although physiologically varying between 110 and 130 days. In medical practice, many pathological states or therapeutic interventions may modify RBC turnover or Hb metabolism. This is the case of anemia, haemolysis (whatever the cause), troubles of iron metabolism, transfusions and/or administration of drugs (e.g. erythropoietin treatment). These pitfalls have been extensively reviewed in literature [6], and pending analytical aspects have been developed above.

Another cause of HbA_{1c} value alteration, less highlighted in the literature, is the competition between nonenzymatic post-translational modifications. This is especially the case for carbamylation during chronic kidney disease (CKD). Carbamylation is characterized by the binding of another adduct, isocyanic acid, to proteins, including Hb. Isocyanic acid is a by-product generated by the spontaneous dissociation of urea, the intensity of this process being increased in CKD because of hyperuremia. Like glycation, carbamylation is able to alter protein structure and functions, and its involvement in the pathophysiology of CKD and other chronic diseases like atherosclerosis has been demonstrated [83]. An association between circulating carbamylated proteins and coronary artery disease has been described in this journal [84]. Isocyanic acid is able to compete with glucose to bind to Hb amino groups, and especially to the N-terminal valine residue of beta chains, leading to the formation of carbamylated Hb (cHb). For long, cHb has represented a concern for HbA_{1c} measurement, but the evolution of methods has almost completely annihilated this interference. However, cHb formation is still a concern from a pathophysiological point of view. Using animal models of diabetes and renal insufficiency, it has been demonstrated that carbamylation was a competitor of glycation for protein modification *in vivo*, and especially for HbA_{1c} formation [85]. An inverse relationship could be found between HbA_{1c} and cHb values, suggesting that HbA_{1c} values had to be very carefully interpreted in CKD patients, not only because of troubles of Hb metabolism due to anaemia and/or treatments by erythropoietin, but also of this phenomenon of competition. Of note, competition occurs at a systemic level and applies to all circulating proteins, affecting fructosamine and glycated albumin assays described in the next section, and more generally all body proteins [86].

Other nonenzymatic glycation-derived products

Whereas HbA_{1c} is the most popular product derived from nonenzymatic glycation in clinical practice, the evaluation of other Amadori products has been proposed in the management of diabetes mellitus, but these biomarkers are more sparsely used in clinical practice, depending on medical habits and on regions in the world.

The oldest one is the fructosamine assay, which corresponds to the determination of all glycated plasma proteins, using a colorimetric test based on the reduction of nitrotetrazolium blue to formazan by fructosamine (or cetoamine) groups [87], partly through generation of superoxide anion [88]. This test, which has been modified and improved after its first description, is easy to perform and relatively cheap, but presents the drawback of being non-specific. Due to the half-life of plasma proteins, especially albumin which is the major contributing protein in this test due to its abundance, the period explored by fructosamine assay before blood collection is 2–3 weeks instead of 4–8 weeks in the case of HbA_{1c}. In a more restricted practice (i.e. forensics), fructosamine assay in vitreous humor can be an additional useful marker of antemortem glycaemic conditions, in addition to HbA_{1c} in blood [89]. Glycated albumin measurement has more recently been proposed and is currently used in some countries, especially in Asia, but less frequently in Europe [74]. This assay determines the ratio of glycated albumin (measured by an enzymatic method) to total albumin (measured by a colorimetric method). This more expensive technique is also more specific than fructosamine assay, the period covered by the test being the same. In both cases, troubles of protein metabolism (e.g. thyroid dysfunctions) may lead to incorrect results. Besides, both tests suffer from the lack of standardization and of determination of validated decision limits, although some recent papers have started to establish reference values or clinical limits in disease [90, 91]. Correspondences between fructosamine and glycated albumin values and clinical thresholds used

for fasting plasma glucose and HbA_{1c} have also been proposed [92]. However, until now, fructosamine and glycated albumin assays are used in most cases as surrogates for HbA_{1c} assays when the interpretation of HbA_{1c} results is impeded by interferences, for example in case of Hb variants [93].

Another interest in the determination of these markers has been evidenced by discrepancies between HbA_{1c} results and those of these other markers of glycaemic balance, having given rise to the notion of glycation gap [94]. Beyond its biological curiosity, glycation gap has an intrinsic value, since it is associated with differences in the development of complications in patients with diabetes. It could be explained by different processes of deglycation by specific enzymes, especially fructosamine-3-kinase (FN3K) [95]. Such a hypothesis has been explored in different papers published in the journal, investigating the link between FN3K genetic variability and diabetes complications, and suggesting the interest of its determination in management of patients [96, 97]. Besides, a simple colorimetric method allowing to determine the FN3K activity has also been described [98]. These different tools used in large patient populations could contribute to better understand this phenomenon of glycation gap and its consequences in terms of diabetes complications, and to precise the clinical utility of glycation gap, recently recommended in patients with coronary artery disease preferentially to HbA_{1c} [99].

More incidentally, another marker of short-term glycaemic control (not derived from glycation), serum 1,5 anhydroglucitol, has been suggested for monitoring diabetes [100], but relatively few studies have highlighted this alternative marker in the journal during the last decade, except as complement of HbA_{1c} for better assessing glycaemic variability [101], or for use in specific situations like early steps of gestational diabetes [100].

Beside Amadori products, advanced glycation end-products (AGEs), which are terminal products of the glycation reactions, may also be evaluated in patients with diabetes mellitus. AGEs are considered pathophysiological agents involved in the development of complications of diabetes and other chronic diseases, as extensively reported in literature [102]. Besides, some of them, especially pentosidine and carboxymethyllysine, may be assayed as biomarkers in blood of patients. For example, it has been reported in this journal that circulating pentosidine concentrations were correlated with the severity of coronary artery disease [103]. They can also be used to assess the quality of glycemic balance even at early steps of type 1 diabetes onset in children [104]. However, these adducts are formed mainly on long-lived proteins like extracellular matrix proteins, and their instantaneous measurement in blood may not be representative of their accumulation in the organism. For that reason, non-invasive devices have been developed to evaluate AGE content in skin [105]. These methods are based on the fluorescence properties of several AGEs, especially pentosidine. Skin autofluorescence, which is correlated with fluorescent AGE content, may thus be used for evaluating the degree of skin protein modifications in different pathologies including diabetes mellitus and chronic renal diseases [106]. An interesting concept is that the measurement of these products in skin may be a reflect of all metabolic stresses accumulated by the organism with time and could constitute a "metabolic memory" [107]. This concept may be applied to other clinical contexts than diabetes mellitus. For example, it has been shown, in this journal, that skin autofluorescence was correlated with frailty in elderly subjects [108].

Other non-invasive methods have been proposed for evaluating AGEs in skin, using physical approaches. Raman and near-infrared spectroscopies may evidence specific molecular signatures of individual AGEs, which can be theoretically quantified [109, 110]. However, these promising non-invasive procedures are not still in current practice due to their limitations for quantification purposes, and must be evaluated in view of their clinical effectiveness compared to the accepted standard protocols [102]. Non-invasive strategies involving other components may be developed in the future after further evaluation, like ^{13}C-glucose breath tests for detecting metabolic syndrome in adolescents as typical event of type 2 diabetes onset [111], or the use of animals, like diabetes alert dogs for detection of hypo or hyperglycaemia, as discussed in details in a recent opinion paper [112].

Glucose has not been forgotten

Although being an historical laboratory test and one of the most assayed parameters in routine practice, in clinical laboratories but also by POCT, at bedside by professionals or at home by patients themselves, glucose measurement remains an important matter of attention inside the clinical biology community. Performances of glucose assays are regularly evaluated by national EQA surveys in most countries [113], allowing to continuously guarantee the best performances for this well-described and standardized assay. Evaluations of new POCT devices

have regularly been reported in *CCLM*, concluding to their overall good analytical quality [114–117], provided interferences are well characterized, as documented for example after intravenous administration of high doses of vitamin C [118].

However, over the last years, several evidence-based studies have confirmed and reinforced the well-admitted notion that preanalytical conditions had a major impact on glucose values, leading to discussions about the best conditions for avoiding to issue incorrect results due to defects in this initial phase of the biological test. Especially, it was evidenced that the rate of glucose degradation by glycolysis enzymes differed significantly according to the additives present in the tubes [119]. When collected in lithium-heparin blood test tubes, decrease in glucose concentrations during the first 2 h was shown to be dependent on various parameters related to blood cells, confirming the necessity of early tube centrifugation and plasma separation [120, 121]. Although discrepant opinions were expressed [122], if has been acknowledged that citrate-containing tubes were able to preserve at best glucose after sampling [123, 124]. When combined with other components like sodium fluoride and EDTA, citrate buffer was described to allow a long-term stability of glucose values until 96 h [125]. Gel barrier tubes constitute also an alternative for favouring a good conservation [126]. Interestingly, the use of tubes containing liquid rather than lyophilized additives was suggested to be a factor of result alteration due to the change of total volume, especially if tubes were incompletely filled [127]. However, changes from classical conditions of blood collection to more efficient "anti-glycolysis" ones are not without consequences from a clinical point of view. They have a significant impact on the clinical targets determined for diabetes diagnosis and survey, and thus on the validity of applicable guidelines. Thus, they may generate a situation of confusion, mentioned and discussed since the early 2010s [128, 129], making it necessary to carefully describe preanalytical conditions for any update of guidelines, as underlined in the case of gestational diabetes in the journal [130–132] and elsewhere [133].

Other challenges are emerging regarding glucose determinations, especially following the implementation of continuous glucose monitoring technologies, which use minimally invasive sensors and constitute major tools for assessing glycaemic control, especially in type 1 diabetes patients, where instantaneous blood glucose fluctuations have to be carefully monitored [134, 135]. However, as glucose is measured in compartments other than blood, values may be different, and a complete process of standardization has to be set up, which is a mission of a specific IFCC scientific division working group [136].

Conclusions

HbA_{1c} has now a long history in laboratory medicine, and has proven to be robust enough to face all types of challenges, related to complex analytical aspects, international standardization process and consensual clinical use. Indisputably a major biomarker of diabetes mellitus in spite of some well described limitations, HbA_{1c} must continue to be evaluated by clinical laboratories according to the strictest quality rules [137], in order to be the basis of all screening, diagnostic and monitoring strategies in diabetes. This underlines the importance of the maintenance of the international IFCC network of reference laboratories, and the necessity for all laboratories to fully exert their responsibilities in terms of quality and performances [75]. However, HbA_{1c} has to evolve and to co-exist with other biomarkers of diabetes, evaluated either by conventional laboratory methods or by innovative, sometimes non-invasive, approaches. The panel of biological tests and medical devices able to participate in the evaluation of glycaemic control and diabetes complications is continuously enriched by new approaches, providing complementary information, every biomarker or external device having its specific indications.

The whole history of HbA_{1c} and diabetes biomarkers can be followed through *Clinical Chemistry and Laboratory Medicine* editorials, original clinical and biological studies, evaluation reports, opinion papers, letters. In the next years, the journal will continue to tell this history and to be the natural repository for gathering and analyzing new information in the field, and the right place for discussions to foster innovative strategies in patient care.

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