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Editorial

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New trends in the long and puzzling history of HbA1c

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The discovery that non-enzymatic glycation of proteins occurred in vivo with an increased intensity in diabetes mellitus has constituted a major step in human biology during the past 50 years. It has led to the identification of numerous post-translational modifications-derived products (PTMDPs), and to the demonstration of their involvement in the complications of various diseases, such as diabetes mellitus, atherosclerosis or chronic renal failure [1]. In the case of hemoglobin, the major glycated species has been defined as HbA1c, characterized by the non-enzymatic binding of glucose to the N-terminal extremities of HbA globin β-chains. HbA1c assay has been recognized for decades as a major tool in the monitoring and follow-up of patients with diabetes mellitus. Recently, its use has been extended to diabetes diagnosis and to other pathological situations [2, 3]. Thus, HbA1c is now recognized as a major biomarker used in clinical practice. However, the long and puzzling history of HbA1c is not yet finished.

Ten manuscripts published in this issue bring interesting additional information to this evolving area of laboratory medicine. Four of them are related to the necessary quality of HbA1c assays and to their current use in clinical practice. The evolution to an extended use of HbA1c assay in patient care has been made possible thanks to the increasing quality of field methods [4] and to the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) – managed international standardization of HbA1c assays which allows traceability to a validated and maintained reference system [5]. It must however be highlighted that, although being accepted at an international level, the implementation of the new reference system, implying especially a change of units and of recommendations, is still inconsistently implemented worldwide. This is well demonstrated in the European survey performed by Pentillä et al. published in this issue [6].

However, although being globally improved, the analytical performances of HbA1c assay methods on data management. Carlsen et al. point out the importance of taking into account the analytical quality of methods, especially measurement bias, when comparing benchmarking results from hospital practitioners or general practitioners [7]. Furthermore, Åsberg et al. highlight the importance of the determination of allowable total error and the consequences of establishing inappropriate thresholds in diabetes diagnosis [8]. In this respect, they confirm that the analytical quality of HbA1c assays is a key point for its optimal use in patient care.

Another topic covered here is the possibility of a substitutive use of HbA1c instead of the oral glucose tolerance test for screening abnormal glucose regulation in specific populations. Wang et al. suggest that HbA1c could constitute an interesting alternative solution in patients undergoing coronary angiography [9], reinforcing conclusions drawn from previous studies showing the interest of HbA1c assay for this indication in other cardiovascular diseases, such as acute ischemic stroke [10].

However, the semiological value of HbA1c is questioned in various clinical situations because of analytical and/or pathophysiological interferences [3]. This is especially the case in the presence of Hb variants, as illustrated here in five papers. This biological occurrence has constituted an important pitfall in clinical practice for many decades, first because the variant (and/or its glycated forms) may interfere with HbA1c determination, and second because it may alter the normal metabolism of hemoglobin or red blood cells (RBCs) [3, 11]. The major analytical issues related to HbA1c measurement in the presence of a variant have been progressively addressed by the development of assay methods that allow the separation of the most common variants and in the majority of cases a reliable HbA1c estimation, e.g., by ion-exchange HPLC, as illustrated in the evaluation study of a new system by Jaisson et al. [12], or by capillary electrophoresis [13, 14]. Besides, automated enzymatic or immunological methods avoid in most cases the interferences of the majority of Hb variants [15, 16]. A comprehensive summary of the currently determined interferences is available at the National Glycohemoglobin Standardization Program (NGSP) website [16].
Thus, most experimental data suggest that the analytical influence of Hb variants on HbA\textsubscript{c} separation and quantification is of limited extent, or is at the least under control. However, this does not mean that all issues related to this subject have been addressed. For example, Ji et al. demonstrate here that \(\beta\)-thalassemia can still lead to misinterpreting HbA\textsubscript{c} results, and highlight the importance of the optimal characterization and knowledge of every method with respect to interferences [17]. Moreover, less common variants may induce unexpected interferences with different impacts on the methods, as recently demonstrated elsewhere by Little et al. [18]. This is also illustrated here by the studies of Camacho Benitez et al. [19] and Bots et al. [20] in this issue. Both of them describe the interference of novel Hb variants (Hb Weesp and Hb Haelen in the first paper, Hb Valme in the second one) on HbA1c quantification by ion exchange HPLC.

Besides, a major recurrent problem is related to the glycation rates and kinetics of Hb variants. A prerequisite for the valid interpretation of HbA\textsubscript{c} results in the presence of an Hb variant is the assumption that glycation rates of HbA and of the variant are comparable. This questioning is not an idle fancy. As a matter of fact, an impaired glycation process has been described in the case of a rare genetic variant, hemoglobin Görwihl [21, 22]. If glycation rates were different, then HbA\textsubscript{c} calculated values would not reflect the actual level of glycemic balance, as the interpretation is scientifically based on HbA glycation criteria only. However, no consistent data about the kinetics of glycation of other Hb species is available so far. Former studies using incubation with radiotopes suggested heterogeneous glycation rates of HbC, HbD, and HbE compared to HbA [23], whereas some other short papers reported contradictory data, especially concerning HbC and HbS [24, 25]. Since then, no robust data have confirmed or infirmed differences in the respective glycation rates between these variants or others and HbA, even though a recent MALDI-TOF mass spectrometry study on in vitro glycated Hbs has suggested increased glycation rates of several Hb variants [26].

In this issue of the journal, Weykamp et al. show very interestingly that the glycation rates of various Hb variants could be close to that of HbA [27]. Using a methodological approach combining measurement of glycated hemoglobin by affinity chromatography, which is supposed to be free from interference of variants and to measure all glycated forms of hemoglobin, and of HbA\textsubscript{c} by capillary electrophoresis, which allows HbA and HbA\textsubscript{c} to be quantified separately and to calculate their specific ratio, the authors have shown that the two methods gave comparable results. It must however be highlighted that this study only brings indirect evidences of comparable glycation rates, and that the direct demonstration of similar in vivo glycation speeds is still lacking. However, waiting for confirmation of the data published here in a larger series of samples using additional analytical approaches, the topic of the glycation kinetics of variants seems to have been partly addressed.

It remains to determine whether the lifespan of RBCs is significantly affected by the presence of the variant, apart from the caricatural situations characterized by a patent hemolysis, such as in the presence of homozygous Hbs or HbC. As a matter of fact, it is well known that HbA\textsubscript{c} value is tightly related to the lifespan of RBCs which is assumed to be 120 days, even though it is not strictly the case in reality. Cohen et al. have clearly demonstrated that the mean age of circulating RBCs ranged from about 40 to 60 days, and that these variations in RBC survival were sufficient to lead to clinically relevant differences in HbA\textsubscript{c} values for a given mean blood glucose [28].

Therefore, some important questions related to HbA\textsubscript{c} metabolism and evaluation are still pending, and HbA\textsubscript{c} and glycated proteins retain unsolved mysteries. Glycation, as well as the incompletely known deglycation processes, may be more complicated than generally assumed. The opinion paper devoted to fructosamine-3-kinase published in this issue by Avermario et al. [29] clearly demonstrates that other still poorly understood mechanisms influence the rate of Hb (and other protein) glycation, which have to be taken into account for optimal result interpretation in patients. Thus, there is still room for new concepts and exciting discoveries in the field of HbA\textsubscript{c} and of the management of patients with diabetes mellitus, which is a stimulating perspective for scientists, clinicians and patients.

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