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Labile glycated hemoglobin: an underestimated laboratory marker of short term glycemia

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Abstract

Objectives: Diabetes mellitus is a major public health problem. Hemoglobin A_{1c} (HbA_{1c}) is a key laboratory parameter in the management of diabetes patients. However, in diabetes monitoring, interpretation of HbA_{1c} results is hampered by the important interindividual variation in red blood cell (RBC) life span. Furthermore, HbA_{1c} only slowly responds to changes in glucose metabolism. Besides HbA_{1c}, there exists a labile HbA_{1c} fraction (l-HbA_{1c}), exhibiting much faster kinetics. As both HbA_{1c} and l-HbA_{1c} are measured by modern standard chromatography, we explored the possibilities of using the l-HbA_{1c} fraction for monitoring glycemia.

Methods: l-HbA_{1c} and HbA_{1c} fractions were simultaneously assayed on a Tosoh G8 analyzer and expressed as %. l-HbA_{1c} results were compared with serum glucose and HbA_{1c}. Concomitantly, RBC distribution width (RDW) was determined on a Sysmex SN analyzer as a marker for erythrocyte life span.

Results: l-HbA_{1c} could be measured with between-run coefficient of variations (CVs) between 2.2 and 2.3%. l-HbA_{1c} correlated with both glycemia ($r=0.80$) and HbA_{1c} results ($r=0.73$). In a multiple regression model ($r^2=0.752$), glycemia and HbA_{1c} were the most determining factors. To a lesser extent, RDW correlated with l-HbA_{1c} ($r=0.158$). Furthermore, the l-HbA_{1c}/HbA_{1c} ratio weakly positively correlated with RDW ($r=0.247$).

Conclusions: L-HbA_{1c} represents an additional marker for monitoring the rapid occurrence of glycemic disorders that escape detection when using only HbA_{1c} and blood

glucose. RDW can be used as an indicator of atypical RBCs life span, in which the l-HbA_{1c} fraction may be helpful.

Keywords: diabetes mellitus; glucose; glycated hemoglobin; labile HbA_{1c} fraction; red cell distribution width (RDW).

Introduction

Diabetes mellitus is a growing worldwide public health problem. Monitoring glycemia plays a key role in diabetes management. In order to assess average glycemia, there are several laboratory parameters [1, 2]. Fructosamine [1] is used for assessing the glycation of plasma proteins. Similarly, glycated albumin [2] has been proposed as a marker reflecting glycemic control status over 2–3 weeks. Serum 1,5-anhydroglucitol (1,5-AG) is a marker, which in contrast with other biomarkers that increase directly with hyperglycemia, shows a decreased plasma concentration. The plasma concentration of 1,5-AG could be a marker of previous (1–2 weeks) exposure to hyperglycemia above the glucose renal threshold, reflecting postprandial hyperglycemic peaks [1].

The most frequently used long-term marker is hemoglobin A_{1c} (HbA_{1c}), a stable compound that allows to assessment of mean glycemia over the last three months preceding the sampling. HbA_{1c} values only slowly respond to changes in glucose metabolism [1]. HbA_{1c} constitutes of an irreversible form of Hb, resulting from a non-enzymatic glycation reaction during the exposure of HbA₀ to glucose. Glycation of Hb is a multi-step process. Besides the HbA_{1c} fraction, there exists a HbA_{1d} aldimine fraction, which is a transient (reversible), exhibiting much faster kinetics [3]. Both HbA_{1c} and the labile HbA_{1d} fractions (labile Hb) are measured by standard chromatography in the routine laboratory. While the HbA_{1c} component has turned out to be globally utilized for monitoring metabolic control, the labile HbA_{1c} fraction (l-HbA_{1c}) has generally been regarded as a disturbing element in clinical analysis. In consequence, literature, regarding the potential clinical utility of the labile HbA_{1c} fraction is scanty [4–8].

The Achilles heel in the interpretation of present-day HbA_{1c} results is the basic assumption that the average life span of erythrocytes, which is 120 days, is a constant [9, 10]. The erythrocyte lifespan is characterized by a relative

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broad distribution: 106 ± 21 days. The mean age of circulating red blood cells (RBCs) may range from 39 to 56 days in diabetic subjects and 38–60 days in nondiabetic controls [9]. This implies that the average exposure time of Hb to glucose is characterized by an important biological variation (coefficient of variation [CV] $\sim 20\%$), which may affect the interpretation of HbA_{1c} results. The biological variation in RBC lifespan exceeds by far the analytical error of modern HbA_{1c} assays [11, 12].

In contrast to HbA_{1c}, l-HbA_{1c} is not affected by the RBC life span to the same extent since it provides information of the recent time interval, which is far away from the RBC life span. This finding is an attractive starting point for further investigating labile HbA_{1c} as a potential diagnostic marker in the context of glycemia monitoring. Since RBC lifespan is difficult to measure in a routine clinical setting, the measurement of red cell distribution width (RDW) provides useful information regarding the age distribution of RBC populations [13].

The RDW is a parameter, which reflects the degree of heterogeneity of erythrocyte volume (conventionally known as anisocytosis). Computationally, RDW is the CV of the mean corpuscular volume (MCV). Higher RDW values, therefore, reflect greater heterogeneity in MCV, which is usually caused by perturbation in erythrocyte maturation or degradation [13].

As we want to improve the diagnostic information provided by our HbA_{1c} chromatographic testing, we wanted to explore in the present study the diagnostic value of reporting l-HbA_{1c} as a marker of medium-term glucose regulation.

Materials and methods

Analyses were carried out on fresh samples at the laboratory of Clinical Chemistry of the Ghent University Hospital. Samples were obtained over a period of approximately one year (January–December 2021). Eleven thousand five hundred and twelve samples (5,873 males and 5,639 females, age: 47.5 ± 13.0 years) from outpatients ($n=7,567$), patients admitted at the emergency department ($n=914$), inpatients ($n=2,097$) of the Ghent University Hospital, and samples externally referred to our lab ($n=934$) were included in the study. No data were excluded from the analysis.

HbA_{1c} percentage was assayed in K₂EDTA blood using ion-exchange chromatography on a TOSOH G8 Analyzer based on separations with a TSKgel G8 Variant HSi Column. In this instrument, the Hb constituents are separated, and the detection is carried out at dual wavelengths at 415 nm and 510 nm. In this method, HbA_{1c} is assayed with high precision (less than 2% CVs) [12]. Validation of the chromatograms was executed using the manufacturer's software.

Analytical imprecision of l-HbA_{1c} was calculated from a representative period ($n=30$, single measurement/run) of routine internal quality control measurements on the Tosoh G8 Analyzer using a single lot of the HbA_{1c} Control Level 1 and 2 (Tosoh) material.

Serum glucose was assayed using a hexokinase method on an Architect c16000 chemistry analyzer (Abbott Diagnostics, Dallas, US) [13]. RDW was determined in K₂EDTA blood on a Sysmex XN analyzer (Sysmex; Kobe, Japan) [14].

Data were analyzed using MedCalc version 15.5 (MedCalc Software, Mariakerke, Belgium). Values are expressed as mean $\pm$ standard deviation (for normally distributed groups). The normality of data distribution was evaluated by the Kolmogorov–Smirnov test. In search for correlations between variables, the Pearson correlation test was used. Multivariate logistic regression was performed to assess the additive diagnostic value of labile Hb. Multiple regression analysis was performed, using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) with labile fraction as the dependent variable and potentially confounding factors. A p -value <0.05 was considered *a priori* to be statistically significant.

Results

Baseline characteristics and biochemical parameters in the patient group are summarized in Table 1. The l-HbA_{1c} showed a between-run CV of 2.3% (level 1, mean l-HbA_{1c} = 1.43%) and 2.2% (level 2, mean l-HbA_{1c} = 1.8%), respectively. Figure 1 shows the correlation between the l-HbA_{1c} and the HbA_{1c} fractions: $y(\text{l-HbA}_{1c}, \%) = 0.512 + 0.307x(\text{HbA}_{1c}, \%)$; $n=11,601$, $r=0.73$; $p<0.001$. The l-HbA_{1c} fraction also correlated well with serum glucose concentration: $y(\text{l-HbA}_{1c} \text{ fraction}, \%) = 0.115x(\text{serum glucose, mmol/L}) + 1.655$; $n=11,601$, $r=0.80$; $p<0.0001$, Figure 2. The obtained regression equations were similar in in- and outpatients. The l-HbA_{1c} fraction did only very weakly correlate with RDW: $y(\text{l-HbA}_{1c} \text{ fraction}, \%) = 4.88\text{RDW} + 188.8$; $r=0.158$; $p<0.001$. The l-HbA_{1c}/HbA_{1c} ratio showed a moderate correlation with the RDW: $y(\text{l-HbA}_{1c}/\text{HbA}_{1c} \text{ ratio}) = 0.2843 + 0.00764\text{RDW}(\%)$, $r=0.247$, $p<0.0001$ (Figure 3).

In a multiple regression model (depicted in Table 2), a determination coefficient of 0.752 was calculated with the

Table 1: Baseline characteristics and biochemical parameters of subjects enrolled in this study.

Parameter	
Age, years	47.5 ± 13
Gender, M/F	5,871/5,637
HbA _{1c} , %	6.60 ± 1.39
Labile Hb, %	2.53 ± 0.58
Red cell distribution width, %	13.8 ± 1.9
Origin of patients	
Outpatients, n (%)	7,567 (65.7%)
Emergency department, n (%)	914 (7.9%)
Inpatients, n (%)	2,097 (18.2%)
Externally referred, n (%)	934 (8.1%)

HbA_{1c}, hemoglobin A_{1c}. Data are presented as mean $\pm$ standard deviation.

Figure 1: Correlation between labile Hb form and HbA_{1c}: y (labile Hb, %) = $0.512 + 0.307x$ (HbA_{1c}, %); $n=11,512$, $r=0.73$; $p<0.001$. The gray area represents the 95% confidence zone.

Figure 2: Correlation between labile Hb and serum glucose concentration: y (labile Hb, %) = $1.655 + 0.115x$ (serum glucose, mmol/L); $n=11,512$, $r=0.80$; $p<0.001$. The gray area represents the 95% confidence zone.

Figure 3: Labile Hb/HbA_{1c} ratio according to red cell distribution width (RDW): y (labile Hb/HbA_{1c} ratio) = $0.285 + 0.00757RDW$ (%), $n=11,512$, $r^2=0.061$, $p<0.0001$. The outer lines represent the 95% confidence zone.

labile Hb fraction as the dependent variable. Both serum glucose ($t=83.23$) and HbA_{1c} ($t=60.04$) were shown to be the major determinants of the labile fraction, followed by the RDW ($t=25.12$).

Table 2: Summary of multiple regression analysis.

	Variable	β	SE	p-Value
Labile HbA _{1c} , $r=0.752$	Constant	0.29		
	Glucose, mmol/L	0.0788	0.0027	<0.0001
	HbA _{1c} , %	0.1625	0.0027	<0.0001
	RDW, %	0.041	0.0016	<0.0001

HbA_{1c}, hemoglobin A_{1c}; RDW, red cell distribution width; SE, standard error.

Discussion

The present study shows that both serum glucose and HbA_{1c} show a comparable correlation coefficient with HbA_{1c}. These findings are in agreement with the view of l-HbA_{1c}, being a marker of recent average glucose exposure (the last couple of days) [15].

By using common clinical laboratory technology at no extra cost, l-HbA_{1c} represents a reliable method for monitoring the rapid occurrence of glycemic disorders that escape detection when using only blood glucose concentration and HbA_{1c} [3].

RDW reflects the degree of heterogeneity of erythrocyte volume, which provides useful information regarding the age of the erythrocyte population. The observed correlation of RDW on the results reflects the effect of variation in RBC life span. An increased RDW mirrors a profound deregulation of erythrocyte homeostasis involving both impaired erythropoiesis and abnormal RBC survival, which may be attributed to a variety of underlying metabolic abnormalities [16].

Although the exact mechanisms that underlie the association of RDW are unknown, systemic factors that alter erythrocyte homeostasis, such as inflammation and oxidative stress, likely play a role. Inflammation might contribute to increased RDW levels by not only impairing iron metabolism, but also by inhibiting the production of or response to erythropoietin or by shortening RBC survival [17]. In conditions associated with an atypical RBC life span (e.g., chronic hemolysis, blood transfusions, some hemoglobinopathies), labile Hb may be used as an additional check in the interpretation of HbA_{1c} results. On the other hand, the labile Hb fraction is less determined by the variation in RBC life span [3]. In contrast to serum/plasma glucose, which is characterized by an important intra-individual variation [18] and suffers from important pre-analytical issues, l-HbA_{1c} integrates the recent fluctuations in plasma glucose concentrations.

As the area under the curve on the chromatogram generated by the l-HbA_{1c} fraction is typically only about 38%

of the HbA_{1c} signal, the relative error for l-HbA_{1c} is somewhat higher as compared to the one for HbA_{1c} [19], but in the same magnitude as that of fructosamine [20]. In contrast to HbA_{1c} [21], l-HbA_{1c} have not been standardized, which is a drawback in the interchangeability of l-HbA_{1c} test results. It should be noted that l-HbA_{1c} can only be quantified when using a chromatographic or electrophoretic HbA_{1c} assay and not when using an immunochemical assay.

l-HbA_{1c} has many advantages as an additional diagnostic test since it reflects the glycemia in the hours preceding the sampling. At no extra cost (when using a chromatographic or electrophoretic HbA_{1c} method), l-HbA_{1c} can be used as a biomarker for predicting response to diabetes treatment. Using the obtained l-HbA_{1c}/HbA_{1c} regression equation, a predicted HbA_{1c} value can be calculated based on the l-HbA_{1c} value, assuming a steady-state of a patient's actual metabolic regulation. This would allow the clinician to predict the eventual effect of a therapy change in three to four months (the erythrocyte life span). This can be of help to improve the quality of monitoring diabetic patients and, if necessary, to adjust their treatments.

The l-HbA_{1c} may also be useful to shed light on the ongoing scientific debate regarding the “glycation gap”. The glycation gap (G-gap) refers to the potential deviation of glycated HbA_{1c} away from the other indirect estimate of blood glucose attainment such that it might read substantially lower or higher than expected [22].

The l-HbA_{1c} fraction no longer should be regarded as a disturbing element in chromatographic or electrophoretic HbA_{1c} assays. The l-HbA_{1c} has a potential adjunctive role for glycemic assessment. Future studies will be needed to establish the exact position of l-HbA_{1c} in the landscape of diabetes diagnostics.

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