Hemoglobin A_{1c}: Standardizacija "zlatnog standarda"

Hemoglobin A_{1c}: Standardization of the "gold standard"

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Sažetak

Hemoglobin A_{1c} (HbA_{1c}) je u proteklih 30 godina primjene postao "zlatnim standardom" u kliničkom praćenju šećerne bolesti. Dobra kontrola glikemije, izražena kroz koncentraciju HbA_{1c} ≤7%, danas je klinički normativ kroz koji se procjenjuje djelotvornost terapije i rizik pojave komplikacija šećerne bolesti i glavna tema komunikacije između dijabetologa i pacijenata.

Raznovrsna i nestandardizirana metodologija, varijabilnost kemijskih entiteta nastalih glikacijom molekule hemoglobina i nepostojanje primarnoga referentnog materijala značajno utječu na pouzdanost primjene HbA_{1c} u kliničkoj praksi. Međunarodna federacija za kliničku kemiju i laboratorijsku medicinu (IFCC) je 2002. godine objavila referentnu metodu za HbA_{1c}. Zajedno s metodom definiran je analit i proizveden primarni, specifični referentni materijal. Međutim, primjena referentne metode onemogućena je značajno nižim rezultatima HbA_{1c} u odnosu na "konvencionalne" metode. Naime, kliničke smjernice i standardi utemeljeni su na rezultatu dugogodišnjih istraživanja, gdje se koristila precizna, ali nedovoljno specifična metodologija. Moguće snižavanje apsolutnih koncentracija HbA_{1c}, koje bi nastalo uvođenjem IFCC-referentnog sustava, predstavlja ozbiljnu prepreku u postizanju i održavanju dobre kontrole šećerne bolesti. Stoga dijabetološka struka energično inzistira na zadržavanju postojećih standarda.

Harmonizacija određivanja HbA_{1c} ključno je pitanje kako individualne skrbi za bolesnike, tako i evaluacije kliničkih istraživanja. Međutim, njena provedba nužno podrazumijeva kombinaciju analitičkih i kliničkih standarda. Stoga je predložen "treći put", odnosno derivacija novog parametra "prosječne glikemije", koji bi sublimirao analitičke prednosti IFCC-referentnog sustava i dragocjene kliničke podatke.

Cilj ovog preglednog članka je prikaz globalnog projekta harmonizacije određivanja HbA_{1c}, te predstavljanje aktivnosti poduzetih u tom smislu u Hrvatskoj.

Ključne riječi: hemoglobin A_{1c}, šećerna bolest, harmonizacija, standardizacija

Abstract

Hemoglobin A_{1c} (HbA_{1c}) has been used as a "gold standard" for clinical management of diabetes mellitus for almost 30 years. A good glycemic control, expressed as HbA_{1c} value ≤7%, today is considered to be a clinical standard for the assessment of both therapeutic efficacy and the risk for development of diabetic complications. It is also a main subject of communication between diabetologists and patients.

Variable and unstandardized methodology, different chemical entities resulting from glycation of the hemoglobin molecule, and a lack of the primary reference material significantly influenced a reliable use of HbA_{1c} in the clinical practice. In 2002, International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has published a reference method for HbA_{1c}. Together with the method, an analyte has been defined, and a primary, specific reference material has been produced. However, the application of the reference method has been dimmed because of the significantly lower HbA_{1c} values, when compared to "conventional" methods. Clinical guidelines and standards have been based on the results of long-term studies where a precise, but insufficiently specific methodology was used. The possibility of lowering absolute HbA_{1c} values, resulting from IFCC reference method implementation, has been recognized as a serious drawback in attaining and maintaining good metabolic control. Therefore, clinical diabetology has insisted vigorously in keeping existing standards.

Harmonization of HbA_{1c} determination is a key issue of both individual patient care and evaluation of clinical research results. However, a combination of both analytical and clinical standards is implicated in pursuing this goal. Thus, a "third way" has been proposed that offers a derivation of the new parameter "mean blood glucose", representing a sublimation of the analytical advantages offered by IFCC-reference system and valuable clinical data.

The aim of this article was to review the global HbA_{1c} harmonization project, and to introduce the respective activities carried out in Croatia.

Key-words: hemoglobin A_{1c}, diabetes mellitus, harmonization, standardization

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Uvod

Šećerna bolest je kronična, progresivna bolest karakterizirana apsolutnim ili relativnim manjkom hormona inzulina. Kronična hiperglikemija koja nastaje zbog manjka inzulina uzrokuje pojavu kasnih mikrovaskularnih komplikacija šećerne bolesti: retinopatiju, neuropatiju i nefropatiju. Također, šećerna bolest je neovisni čimbenik rizika za razvitak makrovaskularnih, prije svega cerebrovaskularnih bolesti (1). Najnoviji pokazatelji govore o visokoj prevalenciji šećerne bolesti u Hrvatskoj (8,9%), uz veliki udio (42%) nedijagnosticirane bolesti u odrasloj populaciji (2). Kompleksna patologija, porast pobola i smrtnosti vezanih za šećernu bolest, te rastući broj oboljelih predstavljaju veliki teret za sustav zdravstvene skrbi, kako globalno, tako i nacionalno. Procjenjuje se da ukupni zdravstveni troškovi bolesnika sa šećernom bolešću dvostruko premašuju troškove osoba bez šećerne bolesti (3). S druge strane, teška invalidnost i gubitak radne sposobnosti koje nastaju zbog kasnih komplikacija šećerne bolesti negativno utječu na kvalitetu života i socijalno blagostanje. Stoga je dragocjeno svako sredstvo koje može poboljšati kontrolu glikemije i usporiti ili spriječiti pojavu kasnih komplikacija, k čemu je i usmjeren stalni razvoj terapijskih mogućnosti. I laboratorijska medicina prepoznala je važnost svoje uloge u ovom području te je, u okviru Međunarodne federacije za kliničku kemiju i laboratorijsku medicinu (engl. *International Federation of Clinical Chemistry,* IFCC) pokrenut program koji, pod naslovom "Globalna kampanja IFCC-a o šećernoj bolesti", objedinjuje niz aktivnosti vezanih uz laboratorijsku dijagnostiku i praćenje šećerne bolesti (4).

Zanimljivo je, međutim, da su ciljevi terapije ove složene bolesti utemeljeni na rezultatu jednostavne laboratorijske pretrage, hemoglobina A_{1c} (HbA_{1c}) (5). Naime, rezultati dugogodišnjih intervencijskih istraživanja, DCCT (engl. *Diabetes Control and Complications Trial*) i UKPDS (engl. *United Kingdom Prospective Diabetes Study*), potvrdili su neprijepornu vezu između razine glikemije, HbA_{1c} i razvitka kasnih komplikacija šećerne bolesti, čime je stvoren temelj za suvremene, globalno prihvaćene terapijske smjernice i preporuke u kojima je kao cilj dobre regulacije glikemije definirana vrijednost $HbA_{1c} \leq 7\%$ (Tablica 1.) (6-9).

Još je zanimljivije da ovaj opće prihvaćeni "zlatni standard", prema kojemu se procjenjuje kako djelotvornost terapije, tako i rizik za razvitak kasnih komplikacija šećerne bolesti, ni nakon gotovo 30 godina primjene nije analitički standardiziran (10, 11). Interpretacija rezultata HbA_{1c} ograničena je, nadalje, i nedovoljno (pre)poznatim biološkim i kliničkim varijacijama (12). Sve ovo predstavlja velik i često podcijenjen problem kod prispodobljivanja individualnih rezultata opće prihvaćenim stručnim smjernicama i preporukama, gdje se predmnijeva nedvojbena interpretacija (13).

Introduction

Diabetes mellitus is a chronic, progressive disease characterized by absolute or relative deficiency of the insulin hormone. Chronic hyperglycemia, which occurs due to insulin deficiency, causes the incidence of subsequent microvascular diabetic complications: retinopathy, neuropathy, and nephropathy. Diabetes is also an independent risk factor for the development of macrovascular, primarily cerebrovascular diseases (1). The most recent indicators point to a high prevalence of diabetes in Croatia (8.9%), with a high proportion (42%) of undiagnosed diabetes in adult population (2). Complex pathology, increased diabetes-related morbidity and mortality, and a growing number of patients are a considerable burden to the healthcare system, both at global and national level. Total healthcare costs for diabetic patients were estimated to be twofold those for non-diabetic individuals (3). On the other hand, severe disability and loss of work ability occurring due to late complications in diabetes exert a negative effect on the quality of life and social welfare. Hence any means to improve glycemia control and retard or prevent the incidence of late complications is valuable, as these are the goals of continuous development of therapeutic potentials. Laboratory medicine has also recognized the importance of its role in this area so that a program entitled "Global IFCC Drive on Diabetes" has been launched by the International Federation of Clinical Chemistry (IFCC) in order to integrate a series of activities related to laboratory diagnosis and follow-up of diabetes mellitus (4).

It is, however, interesting that therapeutic goals of this complex disease are based on results of a simple laboratory test, i.e. hemoglobin A_{1c} (HbA_{1c}) (5). Actually, results of long-term interventional studies DCCT (Diabetes Control and Complications Trial) and UKPDS (United Kingdom Prospective Diabetes Study) confirmed undeniable correlation between glycemic levels, HbA_{1c}, and progression of late complications in diabetes, thus establishing the foundation for modern, globally accepted therapy guidelines and recommendations where the level of $HbA_{1c} \leq 7\%$ is defined as the target of good glycemia regulation (6–9).

It is even more interesting that this generally accepted "gold standard", used to assess both therapy efficiency and the risk for occurrence of late diabetic complications, has not been analytically standardized after almost 30 years of use (10, 11). Interpretation of HbA_{1c} results is further limited by inadequately recognized biological and clinical variations (12). All the above represents a considerable and often underestimated problem occurring during comparison of individual results with widely accepted professional guidelines and recommendations which imply the possibility of definite interpretation (13).

Tablica 1. Korelacija između HbA_{1c} i prosječne glikemije

Table 1. Correlation between HbA_{1c} and mean glycemia

HbA_{1c} (%)	Mean glycemia (mmol/L)
6	7.5
7	9.5
8	11.5
9	13.5
10	15.5
11	17.5
12	19.5

Rezultati naših istraživanja o analitici i kliničkoj primjeni HbA_{1c} na području Hrvatske upućuju na varijabilnost analitičke metodologije i slabu dostupnost i kliničku iskorištenost pretrage (14). Potonje bi se moglo značajno popraviti nakon uvrštavanja HbA_{1c} među opće medicinsko-biokemijske pretrage (15). Međutim, bez poznavanja svih aspekata ovoga kontroverznog područja i postavljanja jasnih kriterija kvalitete kroz redovito provođenje stručnog nadzora, široka primjena pretrage mogla bi donijeti više štete nego koristi, kako za oboljele od šećerne bolesti, tako i za sustav zdravstvene skrbi. Stoga je provođenje edukacije na svim razinama i uspostava programa vanjske kontrole kvalitete u ovom trenutku neodgodiva nužnost, a dobro poznavanje, praćenje i primjena međunarodno prihvaćenih standarda (normi) jedini put prema harmonizaciji određivanja koncentracije HbA_{1c} u Hrvatskoj.

Povijest

Eritrociti zdravog čovjeka sadrže 90% adultnog hemoglobina (HbA) dok ostatak čine produkti alternativne sinteze globina (HbA_2, HbF), te post-translacijskih modifikacija HbA. Još 1958., kromatografijom s kationskim izmjenjivačem, odvojene su tri manje hemoglobinske komponente s jačim negativnim nabojem od hemoglobina A (16). Prema redoslijedu ispiranja s kolone kationskog izmjenjivača, frakcije su nazvane HbA_{1a}, HbA_{1b} i HbA_{1c}. Ovo otkriće povezano je sa šećernom bolesti nekoliko godina kasnije, kad je opisan porast koncentracije "brzih" frakcija hemoglobina u krvi oboljelih od šećerne bolesti (17). Istraživanja koja su potom uslijedila ustanovila su da je HbA_{1c} izravni produkt post-translacijskog vezanja glukoze na molekule hemoglobina i da postoji povezanost između koncentracije HbA_{1c} i prosječne koncentracije glukoze u krvi tijekom prethodna 3 mjeseca, koliki je prosječni životni vijek eritrocita (18, 19). Intrigantna mogućnost da se jednim jedinim mjerenjem dobije objektivni uvid u prosječnu glikemiju tijekom jednog duljeg razdoblja otvorila je

Results of our investigations of HbA_{1c} analysis and clinical application in Croatia pointed to variability in analytical methodology, and poor availability and clinical utilization of the test which could be significantly improved by HbA_{1c} inclusion among general medical biochemistry tests (14, 15). However, without awareness of all aspects of this controversial test issue and definition of clear quality criteria through regular implementation of professional surveillance, broad application of this test could be more harmful than beneficial for both diabetic patients and healthcare system. Hence the implementation of education at all levels and introduction of an extermal quality control program are at the moment an undeferrable necessity, while good knowledge, keeping up-to-date, and application of internationally accepted standards is the only way to harmonize HbA_{1c} determination in Croatia.

Background

Erythrocytes of a healthy individual contain 90% of adult hemoglobin (HbA) while the remaining percentage are the products of alternative globin synthesis ((HbA_2, HbF) and post-translational HbA modifications. As early as in 1958, three lesser hemoglobin components with the negative charge stronger than that of hemoglobin A were separated by cationic exchange chromatography (16). Based on elution sequence from cationic exchanger, the fractions were termed HbA_{1a}, HbA_{1b} i HbA_{1c}. This discovery was related to diabetes several years later when an increase in concentration of "rapid" hemoglobin fractions was described in blood of diabetic patients (17). Further investigations revealed that HbA_{1c} is a direct product of post-translational glucose binding to hemoglobin molecules, and that there is a correlation between HbA_{1c} level and mean glucose concentration in blood during previous three months, which is the average life-time of erythrocytes (18, 19). The intriguing possibility to gain objective insight into mean glycemia during a prolonged period by

novu dimenziju kontrole i praćenja šećerne bolesti. Paralelni razvoj analitičke metodologije omogućio je uskoro široku primjenu HbA_{1c} u laboratorijskoj i kliničkoj medicini.

Kemija, terminologija i definicije

HbA_{1c} i glikohemoglobin (GHb) su dva različita entiteta koja nastaju glikacijom, odnosno procesom ne-enzimskog kovalentnog vezanja glukoze na slobodne amino-skupine globinskih lanaca (20). Procesu glikacije podložni su svi proteini, a valja ga razlikovati od glikozilacije, koja predstavlja enzimsku fazu u sintezi membranskih i drugih glikoproteina. Stoga je i termin "glikozilirani hemoglobin", (engl. *glycosylated hemoglobin*) koji se dugo koristio u stručnoj literaturi napušten. Početni korak glikacije je kondenzacija slobodne primarne aminokiseline proteinskog lanca s karbonilnom skupinom glukoze, pri čemu nastaje nestabilni međuprodukt, Schiffova baza. Ovisno o koncentraciji glukoze, ona može disocirati ili se pregraditi u stabilni ketoaminski oblik (21). Na molekuli hemoglobina glikaciji podliježu N-terminalne skupine valina, kao i ε-amino skupine lizina α- i β-lanaca. Količina nastalog GHb izravno je razmjerna koncentraciji glukoze kojoj su bili izloženi eritrociti tijekom cirkulacije.

HbA_{1c} je hemoglobin kod kojega je glukoza vezana na N-terminalne skupine valina na jednom ili oba β-globinska lanca, dok je ukupni GHb termin koji označava molekule hemoglobina s glukozom kovalentno vezanom na svim prethodno navedenim amino-skupinama α- i β-lanaca. Iako je HbA_{1c} samo jedna od komponenti ukupnog GHb (sadrži otprilike 60% ukupno vezane glukoze), on se danas koristi kao standardna mjera prosječne glikemije, poglavito radi kliničkih smjernica i preporuka temeljenih na rezultatima velikih intervencijskih studija u kojima se mjerio upravo koncentraciju HbA_{1c} (22).

Metodologija

Suvremene analitičke metode raznovrsne su i mnogobrojne, a temelje se na razlikama fizikalno-kemijskih svojstava između molekula HbA_{1c}/GHb i nemodificiranog hemoglobina (11, 23). Prema načelu određivanja mogu se podijeliti u dvije osnovne skupine. Prva skupina metoda temelji se na razlikama u naboju, a obuhvaća kromatografiju s ionskom izmjenom i elektroforezu. Druga skupina su strukturno-specifične metode: boronatna afinitetna kromatografija i imunokemija. Imunokemijske, kao i metode temeljene na razlikama u naboju mjere HbA_{1c}, dok se afinitetnim metodama određuje ukupni GHb. Svaka od metoda ima određene prednosti i nedostatke, pa njen odabir ovisi o individualnim potrebama i mogućnostima laboratorija. Pritom valja voditi računa o ispunjenju kliničkih i analitičkih kriterija kvalitete.

a single measurment opened up a new dimension of diabetes control and follow-up. Concurrent development of analytical methodology soon enabled wide application of HbA_{1c} in laboratory and clinical medicine.

Chemistry, technology and definitions

HbA_{1c} and glycohemoglobin (GHb) are two distinct entities produced by glycation, i.e. the process of non-enzymic covalent glucose binding to free amino groups of globin chains (20). All proteins are subject to the glycation process which should be differentiated from glycosylation that represents an enzymic phase in the synthesis of membrane and other glycoproteins. On this account the term "glycosylated hemoglobin", which was long used in professional literature, has been abandoned. The initial step in glycation is condensation of free primary amino acid of the protein chain with carbonyl glucose group, with an unstable byproduct, Schiff's base. Depending on glucose concentration, it may dissociate or remodel into stable ketoamine form (21). N-terminal valine groups on α- and β-chains undergo glycation on the hemoglobin molecule, as well as ε-amino lysin groups of α- and β-chains. The amount of produced GHb is directly proportional to the glucose concentration to which erythrocytes are exposed during circulation.

HbA_{1c} is hemoglobin with the glucose bound to N-terminal valine groups on one or both β-globin chains, while total GHb represents hemoglobin molecules with covalently bound glucose in all above mentioned amino groups of α- and β-chains. Although HbA_{1c} is only one of the components of total GHb (it contains approximately 60% of the total bound glucose), it is presently used as a standard measure of mean glycemia, chiefly due to clinical guidelines and recommendations based on results of large-scale interventional studies that involved HbA_{1c} determination (22).

Methodology

Current analytical methods are varied and numerous, based on differences in physico-chemical properties between HbA_{1c}/GHb molecules and unmodified hemoglobin (11, 23). Determination methods may be classified into two fundamental groups. The first group of methods is founded on differences in charge, encompassing ion-exchange chromatography and electrophoresis. The second group are structurally specific methods: borate affinity chromatography and immunochemistry. Immunochemical methods and the methods based on differences in charge are used to measure HbA_{1c}, while affinity methods are used to determine total GHb. Each of the methods has some advantages and drawbacks so that the choice depends on individual needs and laboratory possibilities. Fulfilment of clinical and analytical quality criteria should be taken into account during application of these methods.

Klinički zahtjevi:

- Mala individualna varijabilnost.
- Preciznost koja omogućava donošenje kliničke odluke na razini apsolutne razlike u hemoglobinu A_{1c} od 0,35–0,5%.
- Jasna, nedvosmislena interpretacija rezultata za liječnike i bolesnike, temeljena na DCCT i UKPDS kriterijima.

Analitički zahtjevi:

- Precizna, stabilna metodologija određivanja hemoglobina A_{1c}.
- Ukupni koeficijent varijacije <3,0%.
- Stabilne referentne/preporučene vrijednosti koje se mogu ujednačeno interpretirati, neovisno o primijenjenoj metodologiji, vremenu i mjestu određivanja.

Većina suvremenih metoda ispunjava navedene kriterije analitičke i kliničke preciznosti. Međutim, zahtjev za harmoniziranim referentnim/preporučenim vrijednostima, koje bi se mogle nedvosmisleno interpretirati u skladu sa stručnim preporukama, još nije ispunjen. Naime, unatoč odličnoj korelaciji rezultati iz istog uzorka dobiveni različitim metodama značajno se razlikuju, kako zbog različitog analita koji se određuje različitim metodama (GHb *vs*. HbA_{1c}), tako i zbog nedovoljne analitičke specifičnosti metoda temeljenih na razlikama u naboju (24). Dodatni problem predstavljaju i analitičke i biološke interferencije koje mogu značajno utjecati na rezultat. Osim toga, tehnološki napredak omogućio je određivanje HbA_{1c} i izvan laboratorija, u liječničkoj ordinaciji pa čak i u rukama bolesnika, često bez ikakve svijesti o (ne)pouzdanosti tako dobivenih rezultata.
Rezultanta navedenih čimbenika je slaba usporedivost rezultata što je, s obzirom na samu bit kliničke primjene HbA_{1c} - a to je kontinuirano, doživotno praćenje kontrole glikemije, izazvalo ozbiljne implikacije u kvaliteti dijabetološke skrbi i nametnulo potrebu za standardizacijom (25).

Programi standardizacije

Američki program standardizacije određivanja glikohemoglobina (26, 27)

Godine 1996. u SAD je pokrenut program standardizacije određivanja glikohemoglobina (engl. *National Glycohemoglobin Standardization Program,* NGSP), u zajedničkoj organizaciji nacionalnih društava za kliničku kemiju (engl. *American Association for Clinical Chemistry*, AACC) i dijabetologiju (engl. *American Diabetes Association*, ADA). Kao dogovorna metoda usporedbe (engl. *Designated Comparison Method*) uvedena je metoda primijenjena u studijama DCCT i UKPDS (tekućinska kromatografija visoke djelotvornosti na koloni BioRex). Program NGSP omogućuje proizvođačima uspostavu sljedivosti rezultata prema

Clinical requirements:

- Low individual variability.
- Precision that enables making clinical decisions at the level of absolute difference in hemoglobin A_{1c} from 0.35 – 0.5%.
- Clear, unambiguous interpretation of results for physicians and patients based on DCCT and UKPDS criteria.

Analytical requirements:

- Precise, stable methodology for hemoglobin A_{1c} determination.
- Total coefficient of variation <3.0%.
- Stable reference/recommended values that eligible for uniform interpretation independently of the applied methodology, time and place of determination.

Most contemporary methods meet the above criteria of analytical and clinical precision. Nevertheless, the requirement for harmonized reference/recommended values that could be unquestionably interpreted according to professional recommendations has not been met yet. Actually, despite excellent correlation, results obtained from the same sample and by using different methods exhibit significant differences due to both different analyte determined by different methods (GHb vs HbA_{1c}), and insufficient analytical specificity of methods based on differences in charge (24). Additional issues are analytical and biological interferences that may significantly affect the result. Besides, technological advancement enabled HbA_{1c} determination also outside the laboratory, in physician's office or even in patient's hands, often without any awareness of the (un)reliability of thus obtained results.
The consequence of the stated factors is poor comparability of results which, taking into accound the very essence of the clinical application of HbA_{1c} that involves continued, life-long glycemia control, led to serious implications for the quality of diabetologic care and imposed the need for standardization (25).

Standardization programs

American program of standardizing glycohemoglobin determination (26, 27)

In 1996, National Glycohemoglobin Standardization Program (NGSP) was launched in the USA, organized jointly by the American Association of Clinical Chemistry and American Diabetes Association. As a designated comparison method, the method applied in DCCT and UKPDS trials was introduced (high efficiency liquid chromatography on BioRex column). NGSP enables manufacturers to set up result traceability according to DCCT standards via a national reference laboratory. A network of primary and secondary supporting laboratories in the USA and Euro-

standardima DCCT kroz nacionalni referentni laboratorij. Mreža primarnih i sekundarnih potpornih laboratorija u SAD i Europi koristi različite metode (kromatografija, afinitetno vezanje, kapilarna elektroforeza i imunokemija), kalibrirane prema DCCT-sljedivim vrijednostima dobivenim od Nacionalnog referentnog laboratorija. Dodatak programu NGSP je nacionalni program vanjske kontrole kvalitete (engl. *College of American Pathologists, CAP Survey*), temeljen na međulaboratorijskoj razmjeni uzoraka svježe krvi s deklariranim vrjednostima HbA_{1c} prema NGSP-vrijednostima. Programi NGSP i CAP značajno su unaprijedili reproducibilnost određivanja HbA_{1c}, te međulaboratorijsku harmonizaciju rezultata i sljedivost prema DCCT-standardima. Danas preko 98% američkih laboratorija udovoljava propisanim kriterijima reproducibilnosti i slaganja s DCCT-standardom, što je donijelo golemi napredak u kvaliteti dijabetološke skrbi, odnosno primjeni preporučenih ciljeva terapije. Osnovni nedostatak programa NGSP je taj da je dogovorena metoda usporedbe, unatoč iznimnoj reproducibilnosti, nedovoljno specifična da bi bila prihvaćena kao referentna metoda. Tako je, unatoč postignutoj kliničkoj standardizaciji, ostalo otvoreno pitanje analitičke standardizacije.

IFCC: Radna grupa za standardizaciju određivanja HbA_{1c} (28, 29)

Međunarodna federacija za kliničku kemiju (engl. *International Federation of Clinical Chemistry*, IFCC) utemeljila je 1995. godine radnu grupu za standardizaciju određivanja HbA_{1c}. U okviru ovog programa definiran je analit i načinjen je primarni referentni materijal kao smjesa kromatografski pročišćenog HbA_{1c} i HbA_0 u liofiliziranom obliku, te je razvijena i objavljena referentna metoda. Ona se temelji na kromatografskom razdvajanju i identifikaciji β-N-terminalnih heksapeptida HbA_{1c} i HbA_0, dobivenih djelovanjem enzima endoproteinaze Glu-C na molekule intaktnog hemoglobina. Zanimljivo je da su razvijena dva identično djelotvorna sustava identifikacije peptidnih fragmenata, i to masena spektrometrija i kapilarna elektroforeza, pa se može govoriti o dvije jednako vrijedne referentne metode, podjednako specifične za HbA_{1c} i neosjetljive na interferencije abnormalnih hemoglobina (HbS, HbC) i post-translacijski promijenjenih molekula (acetilirani i karbamilirani hemoglobini). Metode su linearne u širokom rasponu klinički relevantnih vrijednosti (2,5–11%) i pokazale su dobru unutar- i među-laboratorijsku reproducibilnost (CV=1,5%). IFCC program je uspostavio i mrežu referentnih laboratorija (7 europskih, 2 u Japanu i 1 u SAD), koji sudjeluju u dvije godišnje usporedne studije, s ciljem evaluacije stabilnosti i uzajamne sljedivosti novih serija kalibratora i kontrola, uz istodobnu kontrolu laboratorijske kvalitete prema kriterijima mreže. Jednom godišnje analizira se 8 *pool* (tj. skupnih) uzoraka pune krvi u laboratorijima IFCC-mreže, koji se ustupaju proizvođačima za kalibraciju

pe use different methods (chromatography, affinity binding, capillary electrophoresis, and immunochemistry), calibrated according to DCCT traceable values obtained from the National Reference Laboratory. An addition to NGSP is a national external quality control program (CAP Survey; College of American Pathologists), based on interlaboratory exchange of fresh blood samples with HbA_{1c} values declared according to NGSP values. NGSP and CAP Survey have considerably promoted reproducibility of HbA_{1c} determination and interlaboratory harmonization of results and traceability according to DCCT standards. Presently, over 98% of American laboratories meet the prescribed criteria of reproducibility and compliance with DCCT standard, the fact which has resulted in vast progress in the quality of diabetologic care, i.e. application of recommended therapy targets. The basic disadvantage of NGSP is that the designated comparison method, despite exceptional reproducibility, is insufficiently specific to be accepted as a reference method. Thus, despite the attained clinical standardization, the question of analytical standardization has remained unsolved.

IFCC: Working Group on Standardization of HbA_{1c} Determination (28, 29)

International Federation of Clinical Chemistry (IFCC) established in 1995 a Working Group for Standardization of HbA_{1c} Determination. The analyte was defined as part of this initiative, primary reference material was prepared as a mixture of chromatographically purified HbA_{1c} and HbA_0 in lyophilized form, and a reference method was developed and published. It is based on chromatographic separation and identification of β-N-terminal hexapeptides of HbA_{1c} i HbA_0 obtained by the action of endoproteinase Glu-C enzyme on intact hemoglobin molecules. It is interesting that two equally efficient systems of identification of peptide fragments were developed, i.e. mass spectrometry and capillary electrophoresis or, actually, two equally valuable reference methods equally specific for HbA_{1c} and insensitive to interferences of abnormal hemoglobins (HbS, HbC) and of post-translationally altered molecules (acetylated and carbamylated hemoglobins). The methods are linear for a broad range of clinically relevant values (2.5%–11%) and showed good intra- and interlaboratory reproducibility (CV=1.5%). IFCC program also established a network of reference laboratories (7 European, 2 in Japan and 1 in the USA) which participate in two annual comparison studies with a view to evaluating the stability and mutual traceability of new series of calibrators and control samples, with concurrent laboratory quality control according to the network criteria. Once a year, 8 pool samples of whole blood are analyzed in IFCC network laboratories and handed over to manufacturers for calibration of commercial methods. Surveillance program for manufacturers has also been anticipated.

Tablica 2 Korelacija između NGSP/DCCT i IFCC vrijednosti HbA_{1c}

Table 2 Correlation between NGSP/DCCT and IFCC values of HbA_{1c}

HbA1c (%)		Δ
NGSP/DCCT	IFCC	
4	2.1	1.9
5	3.2	1.8
6	4.3	1.7
7	5.4	1.6
8	6.4	1.6
9	7.5	1.5
10	8.6	1.4
11	9.7	1.3
12	10.7	1.3

komercijalnih metoda. Predviđen je i nadzorni program za proizvođače.

Unatoč neprijepornoj analitičkoj superiornosti, primjenu IFCC-referentnog sustava onemogućila je činjenica da su rezultati HbA_{1c} značajno niži od NGSP-DCCT-sljedivih vrijednosti (Tablica 2.).

Budući da su međunarodne preporuke dobre metaboličke regulacije, kao i referentne vrijednosti za nedijabetičku populaciju utemeljene na studijama DCCT i UKPDS, relevantne dijabetološke organizacije (engl. *International Diabetes Federation*, IDF; ADA, *European Diabetes Study Group*, EASD; *International Society for Pediatric and Adolescent Diabetology,* ISPAD) suglasile su se u zaključku da bi nagla promjena koju bi izazvao prijelaz na IFCC-sustav standardizacije proizvela nepotrebnu konfuziju i mogla imati nesagledive kliničke, psihološke pa i financijske implikacije, tim više jer IFCC-sustav nije validiran niti u jednoj kliničkoj studiji. Stoga bi za primjenu IFCC-referentnog sustava bilo nužno postići široki konsenzus kliničke i laboratorijske medicine, i to na temelju rezultata kliničkih studija. Ovim se zaključkom upućuje na izniman oprez prilikom doslovne primjene propisa Europske Unije, koja je od 2003. godine obvezala sve proizvođače koji distribuiraju svoje reagense na njenom području, na "sljedivost" prema IFCC-referentnoj metodi (22, 23, 30).

Globalni projekt harmonizacije (31, 32)

Razrješenje svojevrsne pat-pozicije između dva sustava standardizacije povjereno je radnoj grupi za HbA_{1c} (engl. *Working Group of the HbA_{1c} Assay*), koja je, pod predsjedanjem predstavnika međunarodne dijabetološke federacije (engl. *International Diabetes Federation*, IDF) održala inicijalni sastanak u Londonu početkom 2004. godine. Na tom su sastanku predstavnici ADA, EASD, IDF, IFCC i NGSP razmotrili aktualno stanje određivanja HbA_{1c}, mogućnosti

Despite unquestionable analytical superiority, application of IFCC reference system was rendered impossible by the fact that HbA_{1c} results are significantly lower than NGSP-DCCT traceable values (Table 2).

Since international recommendations on good metabolic regulation and reference values for nondiabetic population are both based on DCCT and UKPDS studies, relevant diabetologic organizations (IDF, International Diabetes Federation; ADA; EASD, European Diabetes Study Group; ISPAD, International Society for Pediatric and Adolescent Diabetology) have agreed in a commom conclusion that an abrupt change due to transition to IFCC standardization system would produce unnecessary confusion and might have incalculable clinical, psychological and even financial implications, the more so since IFCC system has not been validated in any clinical study. Therefore, it would be necessary to achieve a broad consensus of clinical and laboratory medicine for application of the IFCC reference system, and this should certainly be achieved on the basis of results of clinical studies. This conclusion indicates the need for extraordinary caution in literal application of regulations by the European Union which, starting from year 2003, committed all manufacturers distributing their reagents in its area to traceability according to the IFCC reference method (22, 23, 30).

Global harmonization project (31, 32)

The task to break a kind of a deadlock between the two systems was conferred to the Working Group of the HbA_{1c} Assay which held its first meeting in London at the beginning of 2004 under the chairmanship of the International Diabetologic Federation (IDF). Representatives of ADA, EASD, IDF, IFCC and NGSP discussed the current status of HbA_{1c} determination, the possibilities created by the development of IFCC reference method, and recommended

koje su se otvorile razvitkom referentne metode IFCC-a, te preporučili način i opseg njene provedbe. Zaključeno je sljedeće:

1. Referentna metoda IFCC-a usvaja se kao globalni standard za kalibraciju svih komercijalnih metoda.
2. Referentna metoda IFCC-a usvaja se kao temelj "međunarodnoga certifikacijskog procesa" u postojećim mrežama referentnih laboratorija.
3. Rezultati HbA_{1c} do daljnjega se izražavaju u DCCT-ekvivalentima.

   Napomena: Statističkom evaluacijom usporednih rezultata iz sustava standardizacije NGSP i IFCC dobivena je tzv. "master-formula" [NGSP = (0,915× IFCC)+2,15] kojom se IFCC-rezultati mogu pretvoriti u klinički smislene vrijednosti, usporedive s DCCT-utemeljenim preporukama, a NGSP može kao temelj primijeniti referentni sustav višeg reda (29). Master-formula nije predviđena za uporabu u individualnim laboratorijima, već može poslužiti proizvođačima da bi rezultate metoda, standardiziranih prema IFCC-sustavu (u skladu s propisima EU) mogli prevesti u klinički prihvatljive vrijednosti.
4. Pokreće se veliki projekt redefiniranja HbA_{1c}, u smislu uspostave novoga izvedenog parametra, čiji radni naslov je "prosječna glikemija" (engl. *mean blood glucose*, MBG).

   Članovi radne grupe artikulirali su kroz ovaj zaključak odavno uočenu potrebu da se udovolji potrebi podizanja osjetljivosti testa u svijesti oboljelih od šećerne bolesti. Naime, dokazano je teško uvjeriti bolesnike da razmjerno mala promjena u rezultatu (npr. sa 7% na 9%) može imati veliki, u ovom slučaju poguban utjecaj po njihovo zdravlje (33). Taj se problem dodatno zaoštrava mogućim prijelazom na IFCC-sustav, u kojem bi već 5% HbA_{1c} značilo lošu regulaciju glikemije (34). S druge strane, nedavno izvješće upućuje na mogućnost pogrješnog klasificiranja značajnog broja bolesnika (do 30%) primjenom "master-formule" u preračunavanju rezultata s IFCC- u DCCT-ekvivalente, što dodatno podupire ideju o izvedbi novog parametra prosječne glikemije (35). Temelj ovoj inicijativi pružila je matematička definicija veze između HbA_{1c} i prosječne glikemije, do koje se došlo retrospektivnom analizom profila glikemije u 7 točaka učinjenih u okviru studije DCCT (36):

   $$\text{prosječna glikemija (PG)} = 1{,}84 \times \text{IFCC } HbA_{1c}$$

   Uz uvjet da se uočena korelacija potvrdi u prospektivnim studijama, otvara se mogućnost redefiniranja mjere prosječne glikemije pretvaranjem HbA_{1c} u vrijednost analognu koncentraciji glukoze, s konačnim ciljem uspostave izravnoga i prepoznatljivog parametra koji bi bio podjednako prihvatljiv i bolesnicima i zdravstve-

the manner and scope of its implementation. The following conclusions were reached:

1. IFCC reference method was adopted as a global standard for calibration of all commercial methods.
2. IFCC reference method was adopted as the basis for international certification process in existing networks of reference laboratories.
3. Until further notice, HbA_{1c} results were to be expressed in DCCT equivalents.

   Note: Statistical evaluation of comparative results from NGSP and IFCC standardization systems yielded the so-called master formula [NGSP = (0.915 × IFCC) + 2.15] which allows the transfer of IFCC results into clinically sensible values comparable to DCCT based recommendations, with the possibility for NGSP to apply a higher order system as a basis (29). The master formula was not designed as a basis for application in individual laboratories but it may allow manufacturers to transfer results of the methods, standardized according to the IFCC system (in accordance with EU regulations), into clinically acceptable values.
4. A large-scale project of redefining HbA_{1c} was launched with the view to setting up a newly derived, tentatively termed mean blood glucose.

   Members of the Working Group expressed in this conclusion the long observed necessity to meet the need to increase test sensitivity in the minds of diabetic patients. In fact, it has been demonstrated that patients are hard to convince that a relatively slight change in result (e.g., from 7% to 9%) may have vast, in this case disastrous, effect on their health (33). This problem is additionally aggravated by possible transfer to the IFCC system where even 5% HbA_{1c} would imply poor glycemia regulation; in a preliminary trial by Swedish authors, this has to most patients been shown to be an unreasonably low limit that eventually led to poorer metabolic control (34). On the other hand, a recent report has pointed to the possibility of erroneous classification of a significant number of patients (up to 30%) by applying the master formula to convert IFCC results into DCCT equivalents, which additionally supports the idea of preparing a new parameter of mean glycemia (35). Foundation for this initiative was mathematical definition of correlation between HbA_{1c} and mean glycemia which was arrived at by retrospective analysis of 7-point glycemic profile performed within the DCCT study (36):

   $$\text{mean glycemia (MG)} = 1.84 \times \text{IFCC } HbA_{1c}$$

   Provided that the observed correlation is confirmed in prospective studies, there opens up a possibility to redefine mean glycemia measures by converting HbA_{1c} into a value analogous to glucose concentration, with

nim stručnjacima. U obrazloženju ovog prijedloga navedene su prednosti poput jasne revizije testa, kojom se isključuje mogućnost pogrješne interpretacije, bolje prihvatljivosti i razumljivosti za bolesnike kroz širenje ljestvice klinički značajnih vrijednosti i moguće buduće primjene testa u dijagnostici dijabetesa.

5. Održavanje programa edukacije zdravstvenih stručnjaka i bolesnika o novom parametru.

Problem ove koncepcije je što dosadašnji podatci o vezi između HbA_{1c} i prosječne glikemije upućuju na veliku disperziju vrijednosti (37). Stoga planirana istraživanja moraju prije svega rasvijetliti pravu prirodu veze između ova dva parametra i rizika za razvitak dijabetičkih komplikacija, i to u svim klinički relevantnim skupinama (zdrave osobe, trudnice, dijabetičari različite etničke pripadnosti). Ne manje važno je i istraživanje mogućih bioloških i farmakoloških interferencija. Multicentrično istraživanje čiji protokol je usvojen od strane radne grupe u lipnju 2005. godine, a koja će, predviđa se, biti završena do 2007. godine trebala bi dati odgovor na sva otvorena pitanja i ponuditi temelj za definitivnu globalnu harmonizaciju HbA_{1c} i/ili iz njega izvedenog parametra prosječne glikemije (38, 39).

Gdje smo mi?

Laboratorijsko određivanje HbA_{1c} u Hrvatskoj prisutno je već 25 godina (40). Tijekom tog razdoblja uvodile su se i rigorozno evaluirale različite analitičke metode, u skladu s globalnim razvojem područja (41–43). Međutim, na nacionalnom planu je izostala odgovarajuća podrška struke u smislu praćenja i unaprjeđenja kvalitete, te osiguranja uvjeta za primjenu međunarodnih standarda.

Rezultati istraživanja provedenog početkom 2005. godine pokazali su slabu dostupnost pretrage na području Hrvatske (radi se u samo 27 laboratorija), te šesterostruko manji broj određivanja u odnosu na preporučene potrebe za postojeću dijabetičku populaciju, što predstavlja pogoršanje u odnosu na pokazatelje iz 1999. godine (44, 14). Metodologija je danas razmjerno ujednačena, s imunoturbidimetrijskim metodama zastupljenim u čak 92% laboratorija, ali postoji veliki rasap u referentnim intervalima koji su se kretali u rasponu od <5,7% do <7%. Također, zabrinjavajući je podatak da se u 4 laboratorija (15%) rezultati izdaju u obliku IFCC-ekvivalenata. Sudionici istraživanja iskazali su gotovo jednoglasno zanimanje za sudjelovanjem u programu vanjske kontrole kvalitete, koji u vrijeme provođenja ankete još nije bio organiziran.

Uvrštavanjem HbA_{1c} među opće medicinsko-biokemijske pretrage ostvaren je formalni preduvjet za širu primjenu pretrage u sustavu primarne zdravstvene zaštite, što predviđa i "Hrvatski model zaštite oboljelih od šećerne bolesti" (45). Međutim, sva otvorena pitanja koja opterećuju analitiku i kliničku primjenu HbA_{1c} ostala su bez odgovora. Početkom 2004. godine, na inicijativu Referentnog cen-

the final aim of setting up a direct and recognizable parameter that would be equally acceptable to both patients and healthcare professionals. This proposal was argumented by stating advantages like clear test revision which excludes the possibility of wrong interpretation, better acceptability and comprehensibility for patients through expanded scale of clinically significant values and potential future test utilization in diagnosis of diabetes.

5. Implementation of the program of educating medical professionals and patients on the new parameter.

The problem in this concept is that past information on the association between HbA_{1c} and mean glycemia indicate high value dispersion (37). Therefore, the studies planned must, above all, elucidate the true character of correlation between these two parameters and the risk for developing diabetic complications in all clinically relevant groups (healthy individuals, pregnant women, diabetic patients of various ethnic background). Of no lesser importance is also the study of potential biological and pharmacological interferences. A multicentric study, with the protocol adopted by the Working Group in June 2005 and an anticipated completion in 2007, should provide answers to all overt issues and the foundation for definitive global harmonization of HbA_{1c} and/or HbA_{1c}-derived parameter of mean glycemia (38, 39).

Where are we?

Laboratory determination of HbA_{1c} has been present in Croatia for 25 years (40). During this period, various analytical methods have been introduced and rigorously evaluated in keeping with global advancement in the field (41–43). However, adequate professional support has been absent at the national level in terms of monitoring and promoting quality and provision of conditions for application of international standards.

The results of a study conducted at the beginning of 2005 demonstrated poor test availability in Croatia (it is performed in only 27 laboratories), and six-fold lower number of determinations compared to recommended requirements for existing diabetic population, which is a decline compared to indicators from year 1999 (44, 14). The methodology is presently rather uniform, with immunoturbidimetric methods applied in as many as 92% of laboratories, but there is a large distribution of reference values that ranged from <5.7% to <7%. Also, it is rather concerning that results in four laboratories (15%) are issued in the form of IFCC equivalents. Study participants almost unanimously expressed their interest in participating in the external quality control program which was still not organized at the time of the survey.

By incorporating HbA_{1c} among general medical biochemistry tests, formal prerequisite was realized for broad test application in primary health care, which is also an-

tra za šećernu bolest RH pokrenut je program standardizacije određivanja HbA_{1c}, koji nastoji urediti ovu osjetljivu problematiku na nacionalnoj razini kroz postizanje sljedećih ciljeva (44):

1. Stvoriti temelj stručnog nadzora, i to uspostavom posebnog modula za HbA_{1c} u okviru programa vanjske kontrole kvalitete Hrvatskog društva medicinskih biokemičara i obveze sudjelovanja u istom za sve laboratorije u kojima se određuje HbA_{1c}.
2. Kroz različite forme stručne izobrazbe (tečajevi, radionice, publikacije) osigurati trajan protok informacija važnih za laboratorijske stručnjake koji se bave određivanjem HbA_{1c}.
3. Podatci dobiveni kroz istraživanje postojećeg stanja na području određivanja HbA_{1c} u Hrvatskoj glede analitičke metodologije i kliničke iskoristivosti pretrage trebaju se koristiti ne samo u edukacijske svrhe, već i kao argument za pregovore kod ugovaranja ove pretrage na razini primarne zdravstvene zaštite, i to odmah po uspostavi mehanizama trajne edukacije i stručnog nadzora opisanih u prethodnim točkama.
4. Osigurati strogu primjenu međunarodno prihvaćenih standarda za određivanje koncentracije HbA_{1c}, i to kroz sustav dokumenata Hrvatske komore medicinskih biokemičara koji se odnose na standarde laboratorijske prakse u Hrvatskoj.

Ciljevi nacionalnog programa standardizacije HbA_{1c} već se ostvaruju. Definirane su preporuke za postupke određivanja u medicinsko-biokemijskim laboratorijima (46), proveden je program trajnog usavršavanja (47), pokrenut je i (pre)dugo nedostajući program vanjske procjene kakvoće (Modul 8 programa vanjske kontrole HDMB).

Zaključak

Promjene laboratorijskih metoda i/ili referentnih intervala uobičajena su praksa i uglavnom ne izazivaju poremećaje u kvaliteti zdravstvene skrbi. Međutim, HbA_{1c} je specifična iznimka, jer ga koriste ne samo zdravstveni stručnjaci, već i bolesnici i njihove obitelji kao moćno sredstvo suradnje u postizanju terapijskih ciljeva (48). Premda je analitička harmonizacija, odnosno ujednačavanje rezultata primjenom egzaktne referentne metodologije i čistoga referentnog materijala nužna, njena provedba mora biti usklađena s prihvaćenim kliničkim standardima i smjernicama, temeljenim na preciznoj, ali nedovoljno specifičnoj analitičkoj metodologiji. Laboratorijska medicina je u slučaju HbA_{1c} morala uvažiti činjenicu da laboratoriji, radne grupe za standardizaciju i metrološke ustanove nisu same sebi svrha, već služe ostvarenju ciljeva specifične grane kliničke medicine, u ovom slučaju dijabetologije.

Globalni projekt harmonizacije određivanja HbA_{1c} danas je svakako ključni zadatak kliničkih i laboratorijskih struka

ticipated by the "Croatian Model of Medical Protection of Diabetic Patients" (45). However, all open questions that encumber analysis and clinical application of HbA_{1c} remained without answers. At the beginning of 2004, a program of standardization of HbA_{1c} determination was launched upon the initiative of the Reference Center for Diabetes of the Republic of Croatia in an attempt to regulate this sensitive problem area at the national level by attainment of the following goals (44):

1. Establish the basis for professional surveillance by setting up a specific module for HbA_{1c} within the framework of the external quality control program by the Croatian Society of Medical Biochemists and by the obligation for all laboratories that determine HbA_{1c} to participate.
2. Ensure continuous information flow through various types of professional education (courses, workshops, publications) as it is important for laboratory experts engaged in HbA_{1c} determination.
3. The data acquired by investigating existing conditions of HbA_{1c} determination in Croatia regarding analytical methodology and clinical utilization of the test should be used not only for educational purposes but also as an argument in negoations during contracting this test at the primary health care level immediately after setting up the mechanisms of continuous education and professional surveillance described in previous sections.
4. Ensure rigorous application of internationally accepted standards for HbA_{1c} determination through a system of documents by the Croatian Chamber of Medical Biochemists related to laboratory practice standards in Croatia.

The objectives of the national program for HbA_{1c} determination are already in the process of implementation. Recommendations have been defined for determination procedures in medical biochemistry laboratories (46), continuous education program was implemented (47), and a (too) long missing external quality control program has been launched (Module 8 of the external quality control program by the Croatian Society of Medical Biochemists).

Conclusion

Changes in laboratory methods and/or reference intervals represent a common practice and mostly do not cause disturbances in healthcare quality. However, HbA_{1c} is a specific exception as it is not used only by medical professionals but also by both patients and their families as a powerful means of collaboration in achieving therapeutic goals (48). Although analytical harmonization, i.e. standardization of results by applying precise reference methodology and defined reference material, is necessa-

uključenih u dijabetološku skrb. U njega su uključene sve relevantne međunarodne ustanove, što s jedne strane upućuje na važnost rješavanja ovog problema, a s druge strane daje jamstvo da će se ostvariti postavljeni cilj, a to je uspostava jedinstvene mjere i jedinstvenog parametra koji bi s najvećom pouzdanošću pružio objektivni uvid u kontrolu glikemije. Medicinsko-biokemijska struka u Hrvatskoj i na ovom, kao i na drugim područjima, ima punu odgovornost za kontinuirano praćenje, usvajanje i primjenu međunarodnih standarda.

ry, its implementation must be consistent with clinical standards and guidelines based on precise yet insufficiently specific analytical methodology. In case of HbA_{1c}, laboratory medicine should have acknowledged the fact that laboratories, working groups for standardization and metrologic institutions do not exist as an end in themselves but serve to accomplish the goals of a specific branch of clinical medicine, in this case diabetology.

The global project of harmonization of HbA_{1c} determination is presently indeed a major task for clinical and laboratory professions involved in diabetologic care. All relevant international institutions are involved in execution of this task, which, on one hand, indicates the importance of solving this issue and, on the other, provides a guarantee that the goal will be realized, i.e. that a unique parameter and measure will be established that will, with highest reliability, provide objective insight into glycemia control. Medical and biochemical profession in Croatia has in this, as well as in other fields, full responsibility for continuous follow-up, adoption and application of international standards.

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