Primjena biološke varijacije – pregledni članak

Application of biological variation – a review

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Sažetak

Ovaj rad sadrži opsežan pregled sastavnica biološke varijacije (BV), tj. intraindividualne (nasumična fluktuacija analita oko osnovne vrijednosti svakog pojedinca) i interindividualne (ukupna varijacija od osnovnih vrijednosti različitih osoba) varijacije, zatim ukratko objašnjava procjenu veličine intraindividualne i interindividualne BV u zdravih i oboljelih ispitanika, podrobno obrađuje osam složenijih primjena procjena BV, te govori o zanimljivim detaljima koji izazivaju najviše rasprava.

Cilj rada je raspraviti kako pomoću današnje tehnologije doći do specifikacija kvalitete dobivenih na temelju BV u zdravih pojedinaca te u kojim se slučajevima koristiti podacima od oboljelih ispitanika.

Konačno, u radu se promiče daljnji razvoj primjene BV, poput upozoravanja liječnika o promjenama u bolesnikovu stanju.

Ključne riječi: biološka varijacija; specifikacije kvalitete; referentna vrijednost promjene; delta provjera; laboratorijski nalaz

Abstract

This paper introduces an extensive revision of the types of components of biological variation (BV), i.e. intraindividual (random fluctuation of analytes around the setting point of each individual) and interindividual (overall variation from the different person's setting point), briefly explains estimation of the magnitude of within- and between subject BV in healthy and non-healthy subjects, details the eight common applications of BV estimates and discusses the most debated points of interests.

The aim is to discuss how quality specifications derived from BV determined in healthy individuals are attainable with current technology and in what cases data from non-healthy subjects should be used.

Finally, the paper promotes further development of BV application, such as notifying doctors about changes in patient status.

Key words: biological variation; quality specifications; reference change value; delta check; laboratory report

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Uvod

Život nije statičko stanje, već stalna fluktuacija sastavnica bioloških tekućina. Biološka varijacija (BV) sastavnica ljudskog tijela koje se ispituju u laboratorijskoj medicini (analita) može se opisati kroz tri različite vrste: varijacija kroz životni vijek, predvidljiva ciklička varijacija koja po prirodi može biti dnevna, mjesečna ili sezonska, te nasumična varijacija. Biološka varijacija koja se zbiva tijekom života osobe povezana je s fiziološkim promjenama koje se odnose na rast, starenje, trudnoću, menopauzu, te druge normalne okolnosti. Pojava predvidljivih cikličkih varijacija uključuje sastavnice na koje utječu vanjski utjecaji kao što su svjetlost - mrak ili godišnje doba, te one čije otpuštanje u krvotok određuju redoviti vremenski razmaci, primjerice otpuštanje različitih hormona podrijetlom iz stanica u krvotok (1). Osim tih vrsti varijacija postoji jedva zamjetljiva varijacija koja utječe na sve analite, sastoji se od nasumične fluktuacije oko osnovne vrijednosti svakog pojedinca, a poznata je kao intraindividualna biološka varijacija. Osnovna vrijednost može se kod svake osobe razlikovati od takvih vrijednosti drugih osoba, a ukupna varijacija koja nastaje iz te razlike poznata je kao interindividualna biološka varijacija (2).

U laboratorijskoj medicini, znanosti koja mjeri sastavnice živih organizama, od presudne je važnosti za pružanje pouzdanih rezultata uzeti u obzir pojam biološke varijacije. Svaki čin mjerenja u sebi sadrži određeni stupanj varijabilnosti. Kod laboratorijskih mjerenja postoji potencijalna varijabilnost u prikupljanju uzoraka, prenošenju i pohrani uzoraka, pripremi reagencija, održavanju instrumenata, vrsti metode i sl., a u medicinskim laboratorijima postoji dodatna i neizbježna varijacija sadržana u biološkom uzorku. Radi dobivanja pouzdanih rezultata sve je izvore varijacije potrebno svesti na najmanju moguću mjeru, a to je moguće kod svih srodnih laboratorijskih procesa osim karakteristične varijacije uzorka. Zato se postavlja pitanje što možemo učiniti? Nužno je savršeno procijeniti sastavnice biološke varijacije (inter- i intraindividualne) te ih adekvatno obraditi tijekom čitavog procesa koji vodi do laboratorijskog nalaza.

Ukratko, procjena veličine intra- i interinvidualne BV iziskuje sljedeći postupak (3):

1. Nekoliko se uzoraka dobiva od nekoliko pojedinaca s poznatim zdravstvenim stanjem.
2. Uzorci se pohranjuju u stabilnim uvjetima do analize.
3. Uzorci se analiziraju i istodobno se određuje analitički CV pomoću kontrolnih materijala ili dvostrukim testiranjem uzorka.
4. Sastavnice varijabilnosti (analitičke, intra- i interindividualne) određuju se primjenom analize varijabilnosti (ANOVA) na dobivenim rezultatima.

Radi osiguranja pouzdanosti procjena BV kod nekoliko je predanalitičkih čimbenika potrebna pomna kontrola. Ako

Introduction

Life is not a static condition, but a continuous fluctuation of the components in biological fluids. The biological variation (BV) of the human body components examined in laboratory medicine (analytes) can be described as of three types, namely, variation over the span of life, predictable cyclical variation that can be daily, monthly or seasonal in nature, and random variation. The biological variation occurring over a person's lifetime is related with the physiological changes inherent to growth, aging, pregnancy, menopause, and other normal circumstances. Predictable cyclical variation occurs in components that are affected by outside influences such as light-dark or a season of the year and those whose release to the circulation is dictated by regular time intervals, such as the release of various hormones of cellular origin to the circulating blood (1). Apart from these types of variations, there is a subtle variation affecting all analytes which consists of random fluctuation around the setting point of each individual, known as the within-subject or intraindividual biological variation. Each person's setting point may be different from another's, and the overall variation resulting from this difference is known as between-subject or interindividual biological variation (2).

In laboratory medicine, a science that measures constituents from living organisms, it is essential to take into consideration the biological variation concept to provide reliable results. All acts of measuring have some degree of inherent variability. In laboratory measurements there is a potential variability in sample collection, sample transport and storage, preparation of reagents, maintenance of instruments, type of method, etc. and in medical laboratories there is additional and inevitable variation inherent to the biological sample. To obtain reliable results all the sources of variation should be minimized and this is possible for all related laboratory processes except the inherent variation of the sample. So what can we do? We have to perfectly estimate the components of biological variation (within-subject and between-subject) and appropriately manage them during the entire process leading to the laboratory report.

Briefly, estimation of the magnitude of within- and between subject BV requires the following procedure (3):

1. A number of samples are obtained from a number of individuals whose health status is known.
2. Samples are stored in stable conditions until analysis.
3. Samples are analyzed and simultaneously, analytical CV is determined by control materials or duplicate sample testing.
4. The components of variance (analytical, within-subject and between-subject) are determined by analysis of variance (ANOVA) of obtained results.

To assure the reliability of BV estimates, several preanalytical factors require close control. If the subjects stu-

su ispitanici zdravi pojedinci, tada trebaju zadržati normalan način življenja tijekom prikupljanja uzoraka. Uvjeti za uzimanje krvi strogo su definirani te se primjenjuju kod svih ispitanika. Prikupljanje uzoraka treba provoditi zdravstveno-laboratorijski tehničar u isto doba dana te uz isto vrijeme podveze žile i to nakon što se ispitanik odmorio nekoliko minuta. Isti se uvjeti primjenjuju kod procjene BV u osoba koje nisu zdrave (npr. osoba s nekom bolešću, primatelja presatka) uz dodatni zahtjev da te osobe budu u stabilnom stanju u okviru svog oboljenja (4,5).

Kad su ti uvjeti ispunjeni, rezultati procjena BV u odraslih osoba su slični bez obzira na razlike među drugim uključenim čimbenicima. S tehničkog stajališta, vremenski razmak za uzorkovanje ne smije biti kraći od jednog dana međutim, osim ovog ograničenja, duljina tog intervala je irelevantna (dnevni, tjedni, mjesečni itd.) (6). Broj ispitanika i uzoraka nisu od izrazitog značenja, no može se preporučiti uključivanje barem 10 ispitanika i 5 uzoraka. Analitička metoda, instrument i reagensi koji se koriste u studiji nemaju nikakav učinak na procjene BV. Čimbenici povezani s ispitanikom poput spola, dobi, rase i mjesta boravka nisu uzrokom promjena u rezultatima (2,7). Sva ova navedena razmatranja dokazana su u nekoliko studija u kojima se BV određivala za brojne analite (8-10). Sve dosad dostupne procjene BV (za oko 320 analita) prikupljene su u bazu podataka koja se obnavlja svake dvije godine (11,12). Takve su informacije već na raspolaganju za laboratorijsku uporabu za mnoge uobičajene pretrage. Unatoč tome, u tom području ima još dosta posla, jer postoji na stotine analita za koje BV još nije određena te buduće aktivnosti treba usredotočiti na te pretrage.

U svakodnevnoj laboratorijskoj praksi BV nalazi osam glavnih primjena uz svoju iskoristivost u epidemiološkim studijama:

1. Postavljanje specifikacija kvalitete za analitički rad.
2. Vrednovanje kliničke važnosti promjena u uzastopnim rezultatima pojedinca.
3. Procjena korisnosti populacijski utemeljenih referentnih vrijednosti.
4. Određivanje optimalnog uzorka (npr. plazma, serum, 24-satna mokraća, prva jutarnja mokraća) za analizu specifičnog analita.
5. Odabir najbolje pretrage među nekoliko testova za specifičnu kliničku svrhu (npr. dijagnoza, praćenje).
6. Odabir najinformativnijih jedinica za svaki analit kod izvještavanja o rezultatima.
7. Određivanje broja analiza potrebnih za homeostatske osnovne vrijednosti pojedinca.
8. Validacija novih postupaka u laboratoriju.

died are healthy individuals, they should maintain their normal lifestyle during the time of sample collection. The conditions for blood extraction are strictly defined and applied to all subjects. Sample collection should be performed by the same phlebotomist, at the same time of day, and with the same tourniquet time, after the subject has rested for some minutes. These same conditions are applied when estimating BV for non-healthy persons (e.g. patients with disease, transplant recipients) with the additional requirement that they be in a stable condition within their non-healthy status (4,5).

When these conditions are fulfilled, the results of BV estimates in adults are similar regardless of differences in other intervening factors. From technical viewpoint, the sampling interval cannot be less than one day, but apart from this restriction the length of the interval is irrelevant (daily, weekly, monthly, etc.) (6). The number of subjects studied and samples taken is not highly important, but it may be recommended that at least 10 subjects and 5 samples be used. The analytical method, instrument and reagents used in the study make no difference in BV estimates. The factors inherent to the subject, such as sex, age, race, geographical place of residence, do not produce changes in results (2,7). These considerations have been manifested in several studies in which BV was estimated for numerous analytes (8-10). All the currently available estimates of BV (around 320 analytes) have been compiled in a database which is updated every two years (11,12). Hence, for many common tests, this information is already available for laboratory use. Nonetheless, much work in this line remains as there are hundreds of constituents for which BV has not yet been estimated. Future work should focus on these specific tests.

In daily laboratory practice, BV has eight main applications, apart from being useful in epidemiological studies:

1. Setting quality specifications for analytical performance.
2. Evaluating the clinical significance of changes in consecutive results from an individual.
3. Assessing the usefulness of population-based reference values.
4. Determining which sample (e.g. plasma, serum, 24-urine, first-morning urine) is optimal for analyzing a specific constituent.
5. Selecting the best test among several for a specific clinical purpose (eg, diagnosis, monitoring).
6. Selecting the most informative units of expression for each analyte for reporting results.
7. Determining the number of analyses needed to establish an individual's homeostatic set point.
8. Validating new procedures in a laboratory.

Postavljanje specifikacija kvalitete za analitički rad

Određivanje specifikacija kvalitete utemeljenih na intra- i interindividualnoj BV potvrđeno je širom svijeta kao druga hijerarhijska strategija koja je dogovorena na konferenciji u Stockholmu jer udovoljava općim potrebama dijagnosticiranja i praćenja (13). Jednostavno rečeno, za pojedinca se u svrhu dijagnoze traži jedna pretraga te se rezultat uspoređuje s populacijski utemeljenim referentnim intervalom ili graničnom vrijednošću; u tom je slučaju za laboratorije ključno održati razdiobu rezultata unutar prosjeka te stoga treba na najmanju mjeru svesti analitičko odstupanje (engl. *bias*). U svrhu praćenja izdaju se uzastopni zahtjevi za pretragom, a glavni izvor laboratorijske varijacije koju treba svesti na najmanju moguću mjeru je analitička nepreciznost. Stoga specifikacije kvalitete, korištene kao granice koje se mogu tolerirati za nepreciznost i analitičko odstupanje, imaju jasno praktično opravdanje i iskoristivost.

Usto, dokazano je da ako se odstupanje (također nazvano sustavnom pogreškom) u laboratorijima koji rade s istom populacijom održi ispod 1/4 zbroja intra- i interindividualne BV, onda se mogu koristiti zajednički referentni intervali (14); vrijednost tog zbroja smatra se *poželjnim* analitičkim odstupanjem. Kod praćenja bolesnika na rezultat može utjecati slučajna pogreška (engl. *hazard error*) sa najviše 11% (15,16); rezultirajuća vrijednost smatra se *poželjnom* nepreciznošću. Kod analita s izrazitom homeostatskom regulacijom i vrlo niskim vrijednostima BV (poput natrija, klorida, albumina) opće prihvaćeno je da se može primjenjivati minimum specifikacija kvalitete koje su utemeljene na većoj frakciji BV; s druge strane, kod slabo reguliranih analita s visokom biološkom varijabilnosti (poput analita u mokraći, triglicerida, nekoliko enzima itd.) mogu se koristiti optimalne specifikacije kvalitete dobivene iz frakcija koje su manje od spomenutih (17).

Vrednovanje kliničke važnosti promjena u uzastopnim rezultatima pojedinca

Vrednovanje kliničke važnosti promjena u dva uzastopna rezultata iste osobe podrazumijeva uzimanje u obzir i analitičkih i fizioloških izvora varijacije (uz pretpostavku da su predanalitički izvori varijacije minimalizirani). Brojčana vrijednost koja označava medicinski značajne promjene između dva rezultata, klasično nazvana „presudna razlika" (3) čiji je današnji naziv Referentna vrijednost promjene (engl. *Reference change value*, RCV) (18), potječe iz formule:

$$RCV = k \times \sqrt{2} \times \sqrt{CV_A^2 + CV_I^2}\ ,$$

s time da je k = 1,65 za jednosmjerni test i rizik vjerojatnosti α od 95%, dok CV_A predstavlja analitički, a CV_I intraindividualni koeficijent varijacije.

Setting quality specifications for analytical performance

Setting quality specifications based on within- and between subject BV has been recognized to be the second hierarchical strategy agreed worldwide at the Stockholm conference because it satisfies the general needs of diagnosis and monitoring (13). In a simple way, a single test from a subject is requested for diagnostic purposes and the result is compared with the population-based reference interval or a cutoff value; in this case, it is essential for laboratories to maintain the distribution of results well centered and, consequently, analytical bias has to be minimized. For monitoring or follow-up purposes, consecutive requests for a test are made and the main source of laboratory variation to be minimized is analytic imprecision. So quality specifications used as tolerance limits for imprecision and bias have clear practical utility.

Additionally, it has been demonstrated that if bias (also called systematic error) of laboratories working with the same population is maintained below ¼ of the sum of within- plus between subject BV, common reference intervals can be shared (14); the resulting value of this sum is considered to be the *desirable* bias. In monitoring, hazard error can affect the result only by 11% (15,16); the resulting value of this fraction is considered to be the *desirable* imprecision. For analytes with strong homeostatic regulation and very low BV values (such as sodium, chloride, albumin), it has been accepted that *minimum* quality specifications can be used which are based on a larger fraction of BV; on the other hand, for poorly regulated analytes with high BV values (such as urine analytes, triglycerides, several enzymes, etc.), *optimum* quality specifications derived from smaller fractions than those mentioned could be used (17).

Evaluating the clinical significance of changes in consecutive results from an individual

Evaluating the clinical significance of changes in two consecutive results from an individual implies taking into consideration both the analytical and the physiological sources of variation (assuming that preanalytical sources of variation are minimized). The numerical value that delineates medically significant changes between two results, classically named "critical difference" (3) and today called Reference Change Value (RCV) (18), comes from the formula:

$$RCV = k \times \sqrt{2} \times \sqrt{CV_A^2 + CV_I^2}\ ,$$

with k = 1.65 for a one tail test and a probability risk α of 95%, and CV_A and CV_I the analytical and the within-subject (or intraindividual) coefficients of variation, respectively.

Neki se autori koriste neznatno drugačijom formulom u kojoj je dio sa zagradama zamijenjen s CV_{I+A} (19); unatoč tome, rezultati dobiveni pomoću obje formule gotovo su istovjetni.

Za laboratorije koji žele postići poželjnu specifikaciju kvalitete, formula ovisi isključivo o CV_I, s time da je vrijednost RCV ista za sve laboratorije (iako je određena za svaki analit) (20). Time se podrazumijeva shvaćanje transverzalne RCV za otkrivanje promjena u zdravstvenom stanju, što predstavlja bolji pristup od populacijski utemeljenog referentnog intervala s obzirom da se kod većine analita zapaža izrazita karakterističnost, odnosno individualnost (intraindividualna BV niža od interindividualne BV) (11,12).

Procjena korisnosti populacijski utemeljenih referentnih vrijednosti

Procjena korisnosti populacijski utemeljenih referentnih vrijednosti vidljiva je kroz omjer intraindividualne i interindividualne BV, što se naziva „pokazateljem individualnosti". Kad je taj pokazatelj niži od 1, što je uobičajeno za većinu do danas prikupljenih analita, dva uzastopna rezultata istog ispitanika mogu biti izvan RCV, no prilično unutar populacijski utemeljenog referentnog intervala. U tom je slučaju tumačenje rezultata pretrage sigurnije ako je pretraga zatražena barem dvaput te je razlika dvaju rezultata za istu pretragu viša od odgovarajuće RCV. Posljedica toga jest da usporedba rezultata jedne pretrage s populacijski utemeljenim referentnim intervalom predstavlja zadovoljavajuću praksu samo za analite s pokazateljem individualnosti većim od 1. Najvišu osjetljivost u te svrhe imaju pokazatelji individualnosti koji su jednaki ili niži od 0,6 te jednaki ili veći od 1,4 (3).

Određivanje optimalnog uzorka za analizu specifičnog analita

Zaključak o tome koja je vrsta uzorka (plazma, serum, 24-satna mokraća, prva jutarnja mokraća) optimalna za analizu specifičnog analita može se dobiti na temelju procjena intraindividualne biološke varijacije. Uzorak s nižim CV_W je najbolji, jer je izvor varijabilnosti svojstven tjelesnoj tekućini sveden na najmanju moguću mjeru. Primjerice, CV_I kreatinina u serumu je 5,3%, a u 24-satnoj mokraći 16% te se za praćenje bubrežnog poremećaja radije odabire kreatinin u serumu. Kod većine su analita serum ili plazma bolji nego uzorci mokraće zbog jače fiziološke regulacije sastojaka krvi u usporedbi s homeostazom mokraće.

Odabir najbolje pretrage za specifičnu kliničku svrhu

O odabiru najbolje pretrage između nekoliko njih za specifičnu kliničku svrhu (npr. dijagnoza, praćenje) također je

Some authors use a slightly different formula that substitutes the parenthesis section by CV_{I+A} (19); nevertheless, the results obtained with the two formulas are practically identical.

For laboratories reaching desirable quality specification, the formula depends exclusively on the CV_I, and then the RCV value is the same for all laboratories (although explicit for each analyte) (20). This would imply a transversal RCV concept for detecting changes in health status, which is a better approach than the population-based reference interval since the majority of analytes have strong individuality (within-subject BV lower than between-subject BV) (11,12).

Assessing the usefulness of population-based reference values

Assessing the usefulness of population-based reference values can be performed by determining the ratio of within-subject- to the between-subject BV, which is named "index of individuality". When this index is lower than 1, which is usual for the majority of analytes compiled up-to-date, two consecutive results from a subject may be outside the RCV but well within the population-based reference interval. In this case, interpretation of a test result is more certain to be correct if at least two requests have been made and the difference between the two results for the same test is higher than the corresponding RCV. As a consequence, comparison of the result of a single test with the population-based reference interval is a satisfactory practice only for analytes with the index of individuality higher than 1. The maximum sensitivity for these purposes is possible with individuality indexes equal or lower than 0.6 and equal or higher than 1.4 (3).

Determining optimal sample for analyzing a specific constituent

Determining which sample type (plasma, serum, 24-urine, first-morning urine) is optimal for analyzing a specific constituent) may be based on within-subject biological variation estimates. The sample with lower CV_W is the best one because the source of variability inherent to the body fluid is minimized. For example, serum creatinine CV_I is 5.3% and 24 hour urine CV_I is 16% so that serum creatinine is preferred for the follow up of renal disorder. For the majority of analytes, serum or plasma are better than urine samples because of the stronger physiological regulation of blood components, compared with urine homeostasis.

Selecting the best test for a specific clinical purpose

On the selection of the best test among several ones for a specific clinical purpose (e.g. diagnosis, monitoring)

moguće odlučiti korištenjem procjena BV. Za dijagnozu je bolje upotrijebiti jednu pretragu i usporediti rezultat s populacijski utemeljenim referentnim intervalom ili graničnom vrijednošću, nego koristiti serijske pretrage za isti analit. Za otkrivanje, primjerice, bubrežnog poremećaja teorijski se mogu zatražiti različite pretrage. U tablici 1 su prikazani CV_I, CV_G, pokazatelj individualnosti (II) i RCV za različite analite (tablica 1).

can also be decided using BV estimates. For diagnosis, it is better to use a single test and compare the result with a population-based reference interval or a cut-off value than using serial tests for the same analyte. For example, various tests can be (in theory) ordered to detect a renal disorder. In the following table, the CV_I, CV_G, individuality index (II) and RCV for various renal related analytes are shown (table 1).

Tablica 1. Podaci o BV analita u dijagnostici i praćenju bubrežnih poremećaja

Table 1. BV-derived data for analytes in renal disorders

Analyte	CVI (%)	CVG (%)	II	RCV (%)
Serum creatinine	5.3	14.2	0.4	13.3
Serum cystatin C	4.6	13.0	0.4	14.2
Urine creatinine, output	11.0	23.0	0.5	34.1
Serum urea	12.3	18.3	0.7	38.1
Urine urea	17.4	25.4	0.7	53.9

Pretraga s visokom osjetljivošću za dijagnozu je ureja u serumu i to zbog najvišeg II. U slučaju praćenja bolesnika s bubrežnim poremećajem cistatin C zbog niže RCV ima najveću osjetljivost za otkrivanje promjene u zdravstvenom stanju.

The test with high sensitivity for diagnosis is serum urea because of the highest II. In the case of the follow up of patients with a renal disorder, serum cystatin C has the maximum sensitivity to detect a change in health status because of its low RCV.

Odabir najinformativnijih jedinica kod izvještavanja o rezultatima

O odabiru najinformativnije jedinice za izražavanje rezultata može se također zaključiti na temelju procjena BV kod analita izraženih u različitim jedinicama. Kod većine sastojaka mokraće rezultati se mogu izraziti kao koncentracija, količina i omjer u odnosu na kreatinin; dodatno se mogu koristiti četiri vrste uzorka mokraće: 24-satna, prva jutarnja, slučajni uzorak te 2-satni uzorak. Primjeri za kalcij i kreatinin pokazuju da 24-satna mokraća predstavlja uzorak s najnižom intraindividualnom varijacijom i to još nižom ako je analit izražen kao izlučena količina. To važi za sve analite u mokraći prikupljene u našoj bazi podataka (tablica 2).

Selecting the most informative units of expression for reporting results

Selecting the most informative units of expression for reporting results can also be made on the basis of BV estimates of analytes, expressed in different units. For the majority of urine constituents, results can be expressed in concentration, output and ratio versus creatinine. Additionally, four types of urine samples can be used: 24h, first morning, random, and 2 hours. Examples for calcium and creatinine illustrate that 24 hour urine is the sample with lower within-subject variation and is even better if expressed in output units. This is valid for all urine analytes compiled in our database (table 2).

Određivanje broja analiza potrebnih za homeostatske osnovne vrijednosti pojedinca

Određivanje broja analiza potrebnih za utvrđivanje biološke osnovne vrijednosti pojedinca vrlo je dobro objašnjeno u radovima Frasera (3,21), gdje je najvažniji zaključak da za većinu sastavnica ljudskog tijela nije dovolj-

Determining the number of analyses needed to establish an individual's homeostatic set point

Determining the number of analyses needed to establish an individual's biological set point is very well explained in Fraser's articles (3,21) where the most important conclusion is that, for the majority of human body compo-

Tablica 2. Procjene BV za analite u mokraći

Table 2. BV estimates for urine analytes

Urine calcium	**CV_I (%)**	**CV_G (%)**
Concentration, first morning	44	38
Concentration, 24h	28	37
Concentration, random	39	35
Output, 24h	27	38
Calcium/creatinine ratio, 24h	26	30
Calcium/creatinine ratio, 2h	41	64
Urine creatinine	**CV_I (%)**	**CV_G (%)**
Concentration, first morning	31	29
Concentration, 24h	24	25
Concentration, random	36	32
Output, 24h	11	23

CV_I - within-subject biological variation; CV_G - between subject biological variation

no jedno određivanje da bi se ustanovila homeostatska osnovna vrijednost. Ta je činjenica obično zaboravljena u već postojećim smjernicama za dijagnozu, gdje se općenito zagovara samo jedna pretraga. Stoga je u buduće prepravke tih smjernica potrebno uključiti laboratorijske stručnjake kako bi se osigurala zadovoljavajuća razina laboratorijske zdravstvene skrbi.

Validacija novih postupaka u laboratoriju

Validacija novih postupaka u laboratoriju predstavlja uobičajenu praksu, kao i specifičan zahtjev kod provedbe sustava upravljanja kvalitetom prema ISO 15189 (22) ili ISO 9001 (23). U toj je aktivnosti za svaki postupak potrebna specifikacija kvalitete za koju se već mnogo godina ističe da bi trebala biti dobivena na temelju biološke varijacije (12,24-26).

Cilj

Usredotočiti se na glavne točke rasprave naznačene u mnogim prezentacijama podataka o BV od skupine autora ovog članka tijekom posljednjih 10 godina (na koji se način mogu pomoću današnje tehnologije postići specifikacije kvalitete dobivene temeljem BV u zdravih osoba, te u kojim se slučajevima treba koristiti podacima od ispitanika koji nisu zdravi).

Pitanja za raspravu

Specifikacije kvalitete dobivene su na temelju podataka o biološkoj varijaciji u zdravih ispitanika te na temelju najboljeg uzorka (24-satna mokraća, ukupna izlučena količina). Premda tu praksu nekoliko autora smatra prilično

nents, a single determination is not sufficient to establish the homeostatic set point. This is usually forgotten in the already existing guidelines for diagnosis where, in general, a single test is advocated. Thus, there is a need to include laboratory professionals in future revisions of these guidelines to assure satisfactory laboratory-related healthcare.

Validating new procedures in a laboratory

Validating new procedures in a laboratory is a common practice and also a specific requirement when implementing a quality management system, according to ISO 15189 (22) or ISO 9001 (23). In this activity, a quality specification for each procedure is needed and it should be derived from biological variation, as has been promoted since many years ago (12, 24-26).

Aim

To focus on main points of discussion debated in numerous presentations of data on BV made by the authors in the past 10 years (how quality specifications derived from BV in healthy subjects are attainable with current technology and in what cases data from non-healthy subjects should be used).

Points of discussion

Quality specifications have been derived from data on biological variation in healthy subjects and based on the best sample (24 hour urine, expressed in output units). Although several authors judge this practice quite idealistic

idealističkom za analite s vrlo niskim CV_I kao što su natrij, albumin ili kloridi, Slika 1. prikazuje da oko 10% laboratorija koji sudjeluju u vanjskom programu osiguranja kvalitete Španjolskog društva za kliničku biokemiju i molekularnu patologiju (SEQC) postižu specifikaciju za ukupnu pogrešku na temelju BV za albumin, 20% za HDL-kolesterol, 50% za natrij i klorid i 70% za kalcij. Ta činjenica dokazuje da su specifikacije zasnovane na biologiji realistične i mogu se danas postići u rutinskim laboratorijima.

Drugo se pitanje bavi analitima koji se gotovo isključivo testiraju kod ispitanika koji nisu zdravi, kao što je HbA_{1C} koji se koristi za praćenje bolesnika sa šećernom bolesti. Ranije smo u ovom tekstu spomenuli da bi u svrhu praćenja bolesnika laboratorij trebao smanjiti svoju analitičku nepreciznost ispod polovine intraindividualne BV. To je teško kod HbA_{1C} s CV_I koji iznosi 1,9% i poželjnim CV_A od 1,0% (12).

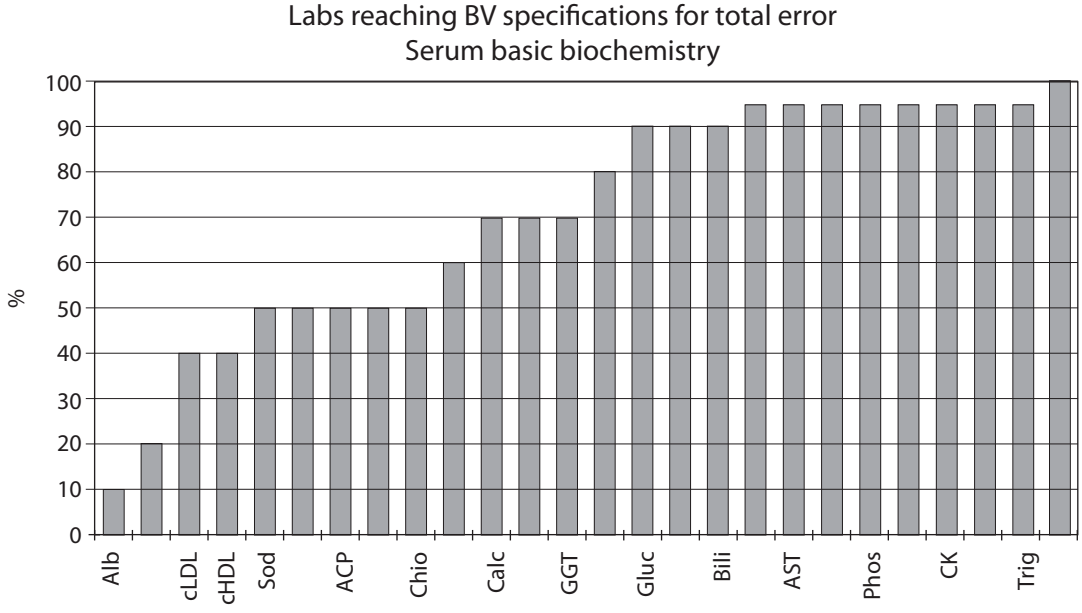
U takvoj specifičnoj situaciji od velikog je interesa osvrnuti se na BV u bolesnika sa šećernom bolesti kod kojih je procijenjeno da CV_I iznosi 4,3% za HbA_{1C}, a posljedično poželjan CV_A 2,1% (27,28) koji se može lako postići rutinskim HPLC metodama. Štoviše, primjenom najviše razine kvalitete prema Stockholmskoj konferenciji o konsenzusu, međunarodna anketa upućena na ime 2538 kliničara koji prate dijabetičke bolesnike u 7 zemalja dokazala je da je traženi medicinski raspon za CV_A od 2,2 do 9,1% (ovisno o zemlji) za niske vrijednosti HbA_{1C} (29).

for analytes with very low CV_I, such as sodium, albumin or chloride, Figure 1 shows that around 10% of the laboratories participating in the external quality assurance program of the Spanish Society of Clinical Biochemistry and Molecular Pathology (SEQC) attain the specification for total error derived from BV for albumin, 20% for HDL cholesterol, 50% for sodium and chloride and 70% for calcium. This fact demonstrates that specifications based on biology are realistic and can be reached for routine laboratories today.

Another point deals with analytes that are almost exclusively tested in non-healthy subjects, such as HbA_{1C} used for monitoring diabetic patients. It has been mentioned above that laboratory should reduce its analytic imprecision below half of the within-subject BV for monitoring and follow-up purposes. This is difficult for HbA_{1C}, with a CV_I of 1.9% and a desirable CV_A of 1.0% (12).

In this particular situation it is of great interest to look at BV in diabetic patients in whom a CV_I of 4.3% has been estimated for HbA_{1C} and a consequent desired CV_A of 2.1% (27,28) that is easily attainable with HPLC routine methods.

Moreover, by applying the highest quality level of the Stockholm consensus conference, an international enquiry addressed to 2538 clinicians that monitor diabetic patients in 7 countries has evidenced that medical requirement for CV_A ranges from 2.2 to 9.1% (depending on the country) for low HbA_{1C} values (29).



Slika 1. Laboratoriji postižu specifikacije kvalitete za ukupnu pogrešku

Figure 1. Labs reaching BV specifications for total error serum basic biochemistry

Budućnost (Zaključci)

Prema našem mišljenju najvažniji napredak u ulozi medicinskog laboratorija jest *informiranje liječnika o promjenama u bolesnikovu stanju*. Tu bi praksu trebalo u skoroj budućnosti primijeniti u svim laboratorijima, jer su svi neophodni elementi za to danas već dobro poznati. Ta se aktivnost temelji na vrijednostima RCV koje su jasno definirane u uvjetima zdravlja te mnogim situacijama kad je riječ o bolesti (18,25,26,30), kao i na statističkoj vjerojatnosti za definiranje značajne promjene koju je odlično objasnio Fraser (13,30). Laboratorijski bi informacijski sustav trebao moći:

- prepoznati vjerojatnu dijagnozu svakog bolesnika;
- identificirati ciljani analit za dijagnosticiranu patologiju;
- primijeniti RCV za ciljani analit na svaki par uzastopnih rezultata istog bolesnika;
- označiti drugi nalaz unaprijed dogovorenim znakom nakon brojčanog rezultata ciljanog analita.

Ta će praksa biti korisnija, nego širom svijeta korištene oznake za usporedbu svakog rezultata pretrage s odgovarajućim populacijski utemeljenim referentnim intervalom zato što, kao što je u ovom tekstu već spomenuto, većina analita ima izrazitu individualnost i usporedba rezultata pretrage bolesnika s prethodnom vrijednošću treba u budućnosti zamijeniti klasičan referentni interval.
Autori se nadaju da će ovaj kratak pregled biti koristan za poboljšanje kvalitete zdravstvene skrbi.

Future (Conclusions)

In our opinion, the most important advance of the role of a medical laboratory is to *notify doctors about changes in patient status*. This practice should be applied to all laboratories in the near future because all the necessary elements are already well known today. This activity is based on the RCV values clearly defined for healthy and many non-healthy situations (18,25,26,30) and on the statistical probability to define a significant change, excellently explained by Fraser (13,30). The laboratory information system should be able to:

- recognize the presumptive diagnosis of each patient;
- identify the target analyte for pathology;
- apply the RCV for the target analyte to each pair of consecutive results from the same patient;
- flag the second report with a predefined signal after the numeric result of the target analyte.

This practice will be more useful than the worldwide used flags for comparing each test result with the corresponding population-based reference interval because, as has been mentioned above, most analytes have strong individuality and comparison of a test result from a patient with the previous value will replace the classic reference interval.
The authors hope this short review will be useful for improving the quality of healthcare.

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