Ispravke Errata

U časopisu Biochemia Medica 2006; 16(Suppl 1) u kojem su objavljeni sažeci s 5. hrvatskog kongresa medicinskih biokemičara u Poreču došlo je do nekoliko pogrešaka. Ispričavamo se autorima i objavljujemo ispravke.

Some mistakes were made in the Supplemet issue of the journal Biochemia Medica 2006; 16(Suppl 1) (abstracts of the 5th Croatian Congress of Medical Biochemists, Poreč, Croatia). We apologize to the authors and bring corrections.

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S7-2a

Genetska analiza multifaktorske bolesti: osteoporoza

Marc Janja
Farmaceutski fakultet Sveučilišta u Ljubljani, Ljubljana, Slovenija

Osteoporoza je bolest u kojoj gubitak čvrstoće u kostima dovodi do lomova uslijed koštane krhkosti. To je česta bolest složene patofiziologije koja uključuje i endogene i okolinske čimbenike. Dokazi polučeni u obiteljskim studijama na blizancima pokazuju kako genetski čimbenici imaju važnu ulogu u regulaciji mineralne gustoće kostiju (BMD), što je glavni fenotip u osteoporozi. Nasljednost BMD procjenjuje se na čak 70–80%. Ovi fenotipovi određeni su utjecajem različitih gena i okolinskih čimbenika. Stoga se osteoporoza smatra poligenom i multifaktorskom oleš ću. Danas je u primjeni nekoliko pristupa za identificiranje gena koji doprinose patogenezi osteoporoze. U početku su studije povezanosti bile uglavnom usredotočene na utvrđivanje gena odgovornih za monogenu koštanu bolest. U novije vrijeme one su okrenute prema identificiranju kromosomnih regija nazvanih lokusi kvantitativnih osobina gena koji reguliraju koštanu masu i skeletnu geometriju. Prednost ovih obiteljskih studija je to što je malo vjerojatno da će polučiti lažno pozitivne rezultate. Međutim, one su manje pogodne za složene bolesti i imaju nisku statističku mogućnost za dokazivanje gena s umjerenim učinkom na BMD. Studije udruženosti čine glavni model u genetici osteoporoze u ljudi. One uključuju identifikaciju polimorfizama pojedinih gena (kandidata) i s njima povezanih alelnih varijanata prema BMD ili prijelomima kostiju u populacijskoj studiji ili studiji s kontrolnom skupinom. Geni kandidati uglavnom se odabiru na osnovi bioloških učinaka na metabolizam kostiju ili aktivnost koštanih stanica. Studije udruženosti gena kandidata relativno se lako provode i mogu otkriti manje

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Genetic analysis of multifactorial disease: osteoporosis

Marc Janja
Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Osteoporosis is a disorder in which the loss of bone strength leads to fragility fractures. It is a common disease with complex pathophysiology involving both endogenous and environmental factors. Evidence from twin family studies indicates that genetic factors play an important role in the regulation of bone mineral density (BMD), which is the main phenotype of osteoporosis. The heritability of BMD has been estimated to up to 70%-80%. These phenotypes are determined by the influence of several genes and environmental factors. Therefore, osteoporosis has been considered a polygenic and multifactorial disease. Several approaches are currently being used to identify the genes that contribute to the pathogenesis of osteoporosis. At the beginning, linkage studies were mostly focused on identification of the genes responsible for monogenic bone disease. Recently, they have been oriented to identification of chromosomal regions called quantitative trait loci which genes regulate bone mass and skeletal geometry. An advantage of these family-based studies is that they are unlikely to give false positive results. However, they are less suitable for complex diseases and have a low statistical power to detect genes with modest effects on BMD. Association studies represent the main model in the genetics of osteoporosis in humans. These involve identification of polymorphisms of particular (candidate) genes and related allelic variants to BMD or bone fractures in a population-based study or case control study. Candidate genes are typically chosen on the basis of their biological effects on bone metabolism or bone cell activity. Candidate gene association studies are relatively easy

učinke. Nedostatci uključuju mogućnost lažno pozitivnih rezultata zbog čimbenika koji mogu izazvati zabunu te populacijsko uslojavanje. Ispituje se udruženost mnogih gena kandidata s fenotipom osteoporoze: gen receptora vitamina D, geni estrogenskih receptora, gen kolagena tip I a1, gen TGF beta, gen IL-6, geni BMP, gen LRP 5 itd. Treći pristup u genetici osteoporoze su šire studije genoma. Tu se rabi tehnologija *microarray*, a može se odjednom analizirati do 500.000 biljega u jednom uzorku DNA. Veći nedostatak ovoga pristupa je visoka cijena. Studije u genetici osteoporoze imaju važne implikacije za kliničku praksu. Geni koji reguliraju BMD i krhkost kostiju potencijalno su važni kao ciljevi za nove lijekove, kao dijagnostički biljezi za procjenu pojedinačnog rizika, te kao sredstvo za identifikaciju osoba koje ne će odgovoriti na liječenje u farmakogenetskim studijama.

E-mail: janja.marc@ffa.uni-lj.si

to perform and can be powered to detect small effects. Disadvantages are the possibility of false positive results due to confounding factors and population stratification. There are a number of candidate genes studied in association with osteoporosis phenotype: vitamin D receptor gene, estrogen receptor genes, collagen type I a1 gene, TGF beta gene, IL-6 gene, BMP genes, LRP 5 gene, etc. The third approach in the genetics of osteoporosis refers to the genome-wide studies. They use microarray technology and are able to analyze up to 500,000 markers in one DNA sample at once. The high price is an important disadvantage of this approach. The studies in the genetics of osteoporosis have important implications for clinical practice. The genes that regulate BMD and bone fragility are potentially important as targets for new drugs, as diagnostic markers for assessment of individual risk, and as a tool for identification of treatment nonresponders in pharmacogenetic studies.

E-mail: janja.marc@ffa.uni-lj.si

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P1-3

Kronična mijeloična leukemija – važnost praćenja terapije: prikaz slučaja

Radić-Antolic M, Zadro R, Davidović-Mrsić S, Sertić D, Labar B

Klinički bolnički centar Zagreb, Zagreb, Hrvatska

U listopadu 2000. godine 49-godišnjoj bolesnici dijagnosticirana je kronična mijeloična leukemija: uz normalan broj eritrocita i trombocita u perifernoj krvi imala je 215x10^9/L leukocita te povećanu jetru i slezenu; u koštanoj srži utvrđena je izrazita proliferacija bijele loze te dokazana translokacija t(9;22)(q34;q11) (Philadelphia kromosom) i fuzijski gen BCR-ABL. Odmah je započeta terapija hidroksiurejom i interferonom alfa te je postignuta potpuna hematološka remisija bez molekularne remisije. Nakon 2 godine citogenetičkom je analizom dokazano 67% Ph pozitivnih interfaznih jezgara (Ph+I.J.), kod bolesnice se pojavio recidiv te je započeta terapija imatinib-mesilatom u dozi od 400 mg/dan. Nakon 6 mjeseci terapije postignuta je potpuna citogenetička remisija (FISH negativan na prisutnost Ph+I.J.), ali je RT-PCR bio pozitivan na fuzijski gen BCR-ABL. Kvantitativnim PCR utvrđen je mali broj kopija fuzijskog gena (BCR-ABL/ABL<0,01) s tendencijom opadanja u slijedećih 10 mjeseci te je postignuta i djelomična molekularna remisija. Nastavljena je terapija imatinibom

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Chronic myelogenous leukemia – therapy monitoring: case report

Radić-Antolic M, Zadro R, Davidović-Mrsić S, Sertić D, Labar B

Zagreb University Hospital Center, Zagreb, Croatia

A 49-year-old female patient was diagnosed with chronic myelogenous leukemia in October 2000: hepatosplenomegaly was present along with increased white blood cell count (215x10^9/L) but normal red blood cell count and platelet count; the presence of the Philadelphia chromosome (translocation t(9;22)(q34;q11)) and BCR-ABL fusion gene was confirmed along with distinct leukocyte proliferation in the bone marrow. Initial therapy consisted of hydroxyurea and interferon alpha (Intron A), and the patient achieved complete hematologic response but no molecular response. Two years later, bone marrow cytogenetic analyses showed 67% of Ph positive interphase cells. The patient relapsed and imatinib 400 mg/ day was started. Six months later, complete cytogenetic remission was achieved (FISH was negative for Ph positive interphase cells) but qualitative PCR was positive for BCR-ABL. The BCR-ABL/ABL ratio was low and decreasing in the next 10 months, and partial molecular response was achieved. Imatinib was continued at 400 mg/day. Twenty-three

od 400 mg/dan. Dvadeset i tri mjeseca od početka terapije imatinibom FISH analizom pronađeno je 2% Ph+I.J., a kvantitativnim PCR porast omjera BCR-ABL/ABL 5 puta. Bolesnica je tada još uvijek bila u hematološkoj remisiji. U slijedećih 6 mjeseci prekinuta je terapija zbog nedostatka lijeka, što je vjerojatno uzrokovalo još veći porast Ph+I.J. (13%) te porast omjera BCR-ABL/ABL 20 puta. S obzirom na gubitak potpune citogenetičke remisije i porast broja kopija fuzijskog gena, uzrok treba tražiti u pojavi mutacija koje su u većini slučajeva uzrok rezistencije na terapiju imatinibom. Dio mutacija može se svladati povećanjem doze lijeka, što je u slučaju ove bolesnice i učinjeno povećanjem doze imatiniba na 600 mg/dan. Već nakon 4 mjeseca od povišenja doze smanjio se postotak Ph+I.J., ali je još uvijek bio povišen omjer BCR-ABL/ABL. U slijedećoj kontroli, nakon 5 mjeseci, zabilježen je pad Ph+I.J. (5%), a i omjer BCR-ABL/ABL je pao na vrijednosti ispod 0,01. Zadnja kontrola u svibnju 2006. pokazuje ponovni ulazak u potpunu citogenetičku remisiju s još uvijek pozitivnim RT-PCR na BCR-ABL. Prikazom ovoga slučaja pokazana je važnost praćenja terapije kvantitativnim PCR čija je dijagnostička osjetljivost između analize FISH i RT-PCR, a upravo je taj dio praćenja terapije najosjetljiviji. Osobito je to važno kod bolesnika koji ne postignu potpunu molekularnu remisiju, tj. ostaju pozitivni u RT-PCR, jer svaki porast broja kopija može značiti ponovnu pojavu bolesti.

E-mail: mradicantolic@net.hr

months of the introduction of imatinib therapy, 2% of Ph positive interphase cells were found and BCR-ABL/ABL ratio increased five-fold but the patient was still in hematologic remission. During the following 6 months, imatinib therapy was discontinued, which was the probable cause of increase in the number of Ph positive interphase cells (13%) and BCR-ABL/ABL ratio (20x). The loss of complete cytogenetic remission, the increase in BCR-ABL copy number and resistance to therapy are the most common results of a mutation event in the BCR-ABL kinase domain. Increased drug concentrations can overcome some of these mutations so imatinib was increased to 600 mg/day. Four months later, evaluation showed a decrease in Ph positive interphase cells (5%) but the BCR-ABL/ABL ratio remained high. The same regimen of imatinib was continued, five months later bone marrow cytogenetics revealed 4% of Ph positive interphase cells and BCR-ABL/ABL ratio below 0.01. The last follow-up in May 2006 showed complete cytogenetic response but still detectable BCR-ABL fusion gene. This case report indicates the importance of the most sensitive part of therapy monitoring by quantitative PCR with diagnostic sensitivity between FISH analysis and qualitative PCR. This is extremely important for patients without complete molecular remission (qualitative PCR positive for BCR-ABL) where the increase in BCR-ABL copy number can predict disease progression.

E-mail: mradicantolic@net.hr

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Analitička validacija testa Seradyn-Innofluor za određivanje koncentracije everolimusa

Šurina B[1], Knotek M[2], Cvrlje-Čolić V[2], Katičić M[2], Prskalo M[2], Preden-Kereković V[1], Flegar-Meštrić Z[1]

[1]Zavod za kliničku kemiju, KB Merkur, Zagreb, Hrvatska
[2]Klinika za unutarnje bolesti, KB Merkur, Zagreb, Hrvatska

Jedan od preduvjeta za čim bolji ishod transplantiranog bolesnika svakako je izabrati optimalnu individualnu kombinaciju imunosupresivne terapije. Everolimus je novi antiproliferativni imunosupresivni lijek koji se rabi u transplantaciji solidnih organa. On je dio imunosupresivnih protokola, obično u kombinaciji s inhibitorom kalcineurina ili s mikofenolatom, uz istodobnu primjenu steroida ili bez nje. Terapijsko praćenje everolimusa rezultira smanjenom incidencijom akutnog odbacivanja i manjom in-

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Analytical validation of Seradyn-Innofluor assay for everolimus measurement

Šurina B[1], Knotek M[2], Cvrlje-Čolić V[2], Katičić M[2], Prskalo M[2], Preden-Kereković V[1], Flegar-Meštrić Z[1]

[1]Department of Clinical Chemistry, Merkur University Hospital, Zagreb, Croatia
[2]University Department of Medicine, Merkur University Hospital, Zagreb, Croatia

Individualized optimal combination of immunosuppressive therapy is one of the necessary prerequisites for the best possible outcome of transplant patients. Everolimus is a novel antiproliferative immunosuppressant used in solid organ transplantation. It is a part of immunosuppressive protocols, usually in combination with calcineurine inhibitor, or with micophenolate, with or without simultaneous

cidencijom nuspojava u primatelja solidnih organa liječenih everolimusom (1). Zbog kraćeg poluživota everolimusa lakše se postiže ciljni učinak i ujednačeniji terapijski raspon lijeka. Stoga smo za potrebe bolesnika u kojih je učinjena transplantacija bubrega u našoj ustanovi evaluirali test Seradyn-Innofluor za everolimus, koji je zasad jedini dostupan na tržištu. Test Seradyn-Innofluor za određivanje koncentracije everolimusa koncipiran je na načelu imunokemijskog određivanja uz ekscitaciju fluorofora polariziranim svjetlom (metoda FPIA). Sva određivanja učinjena su na analizatoru TDX tvrtke Abbott. Kratka analitička validacija testa obuhvaćala je: nepreciznost u seriji, nepreciznost iz dana u dan, netočnost, osjetljivost i linearnost. Kao terapijsko područje testa navodi se koncentracija od 3-8 μg/L (2). Nepreciznost u seriji za nisko, srednje i visoko koncentracijsko područje (n=10) iznosila je: 7,2%, 7,9% i 3,4%. Pripadajuća netočnost za nisko, srednje i visoko koncentracijsko područje, izražena na komercijalnim kontrolnim uzorcima, iznosila je 2,5%, 10,8% i 6,1%. Nepreciznost iz dana u dan u navedenim kontrolnim uzorcima testa (nisko, srednje, visoko, n=10) iznosila je 18,5%, 17,5% i 18,5%. Dilucijom uzoraka dokazali smo donju granicu osjetljivosti od 2 μg/L i linearnost od 40 μg/L. Dobivena je zadovoljavajuća analitička validacija testa. Ističemo nešto lošiju nepreciznost iz dana u dan, KVa=18,5%, što smatramo nedostatkom testa.

E-mail: branka.surina@hdmb.hr

steroid administration. Therapeutic drug monitoring of everolimus results in a decreased incidence of acute rejection and lower incidence of side effects in solid organ recipients treated with this immunosuppressive drug (1). The shorter half-life of everolimus facilitates achieving the target effect and a more homogeneous therapeutic scope of the drug. Therefore, for the needs of patients who had kidney transplantation performed at our Hospital, we evaluated the everolimus Seradyn-Innofluor assay, which is currently the sole such assay available on the market. The Seradyn-Innofluor assay for measuring the everolimus concentration is based on the principle of immunochemical determination with fluorophore excitation with polarized light (FPIA method). All determinations were performed on an Abbott TDx analyzer. Short analytical validation of the assay included the following: imprecision in series, day to day imprecision, inaccuracy, sensitivity, and linearity. The concentration range between 3 and 8 μg/L was set as a therapeutic scope of the assay (2). Within-series imprecision for the low, medium and high concentration range (n=10) was 7.2%, 7.9% and 3.4%, respectively. Inaccuracy for the low, medium and high concentration range, expressed on the commercial control samples, was 2.5%, 10.8% and 6.1%, respectively. Day-to-day imprecision in the samples stated above (low, medium and high, n=10) was 18.5%, 17.5% and 18.5%, respectively. Using sample dilution we demonstrated minimum sensitivity level of 2 μg/L and linearity of 40 μg/L. Satisfactory analytical assay validation was obtained. We point out a somewhat poorer day-to-day imprecision rate, KVa=18.5%, which we consider to be a limitation of the assay.

E-mail: branka.surina@hdmb.hr