jelo, pomoćne tvari), sadržana u imunokemijskoj analizi ima svoj matriks. Sve te komponente, bilo iz biološkog uzorka (lipidi, proteini, ugljikohidrati, sol, voda) ili egzogenih izvora mogu utjecati na ciljni analit čija se koncentracija određuje. Endogene sastavnice uzrokuju interindividualnu i intraindividualnu varijabilnost rezultata. Komponente matriksa imaju mali afinitet vezanja za analit ili antitijelo - obično maskiraju analit ili antitijelo, zbog čega izostaje reakcija vezanja analita s antitijelom.

U budućnosti će se interferencije povećavati u nekim područjima (primjerice kod primjene bioloških lijekova), a u nekim će se područjima smanjivati. Ipak, problemi interferencija u imunokemijskim analizama će biti trajan problem. Stoga stručnjaci laboratorijske medicine, liječnici i stručnjaci koji razvijaju imunokemijske postupke moraju biti svjesni tih problema i smanjivati interferencije na najmanju moguću mjeru.

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4. The matrix effect: Each component of immunoassays (biological sample, buffer, antibody, additive) has its own matrix. All of them, whether coming from biological sample (lipids, proteins, carbohydrates, salt, water), or from exogenous sources, can affect the target analyte to be measured. Endogenous elements cause inter- and intra-individual variability of results. Matrix components have low affinity of binding to analyte or antibody - usually disguise the analyte or the antibody causing the absence of the binding reaction of analyte to antibody.

In the future, in some areas (esspecially associated with implementation of biological drugs) interferences will increase, and in other areas (implementation of high specificity immunoassays) interferences will recede. However, problems of interferences in immunoassays will persist. Therefore, the laboratory medicine experts, the physicians and the experts who develop the immunoassays must be aware of these problems, and to minimize them to a lesser extent.

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S02 – Farmakogenetika

S02-1

Farmakogenetski pristup u optimiziranju terapije

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Farmakogenetika je znanstvena disciplina koja proučava ulogu nasljedstva kod interindividualnih varijacija u odgovoru na lijek. Farmakogenetika pokušava pronaći mogućnost primjene znanja o bolesnikovoj sekvenci DNK u poboljšanju terapije maksimizirajući učinak lijeka, kako bi se lijekovi ciljano davali bolesnicima koji će na njih pozitivno reagirati ili da se izbjegnu neželjene reakcije na lijek. U zadnjih je 10 do 15 godina farmakogenetika privukla pažnju kao disciplina koja bi se mogla primijeniti u bolničkoj skrbi za bolesnika. Dok se u publikacijama još uvijek razrađuje njen klinički utjecaj, znanje potrebno za definiranje i tumačenje istraživanja koja bi procijenilo kliničku korisnost farmakogenetike potaklo je mnoge debate. Primjena farmakogenetike u unapređenju koncepta personalizacije medicine ovisi o sposobnosti kliničkih laboratorija da pruže točne, korisne i pravodobne informacije koje bi kliničarima omogućile propisivanje pravog lijeka (učinkovitost) u pravoj dozi (doziranje), za pravog bolesnika (sigurnosna učinkovitost) i u pravi trenutak.

Citokromi P450 (CYP) su važna skupina enzima u metabolizmu mnogih terapija i endogenih metaboličkih aktiv-

S02 – Pharmacogenetics

S02-1

Pharmacogenetic approach in optimizing therapy

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Pharmacogenetics is the study of the role of inheritance in inter-individual variation in drug response. The ultimate promise of pharmacogenetics is the possibility that knowledge of a patient's DNA sequence might be used to enhance drug therapy to maximize efficacy, to target drugs only to those patients that are likely to respond or to avoid adverse drug reactions. In the past 10 to 15 years pharmacogenetics (PGx) has generated attention as a discipline with potential application to patient care. While the literature is still evolving as to clinical impact, the knowledge needed to define and interpret studies to assess clinical utility has drawn much debate. The application of PGx to enhancing the concept of personalized medicine hinges on the ability of clinical laboratories to provide accurate, timely and useful information, thus enabling the clinicians to administer the right drug (efficacy), at the right dose (dosing), for the right patients (safety efficacy) and at the right time.

In particular, cytochrome P450s (CYPs) are an important family of enzymes in the metabolism of many therapeu-

nosti. Ovo će predavanje dati pregled literature i našeg iskustva u određivanju genotipa alela odabranih CYP gena korištenjem nekoliko tehnologija uključujući i test Roche AmpliChip P450 Array.
Čitav niz sakupljenih dokaza pokazuje važnost genotipiziranja CYP, posebno s obzirom na CYP2D6 i CYP2C19. Genotipizacija CYP2D6 dala je jako zanimljive rezultate kod bolesnica s karcinomom dojke liječenih s tamoksifenom, kao i s prolijekovima kao što su analozi kodeina i antiaritmici. CYP2C i njegov polimorfni izooblik CYP2C19 sudjeluju u metabolizmu nekoliko važnih lijekova kao što su triciklični antidepresivi te inhibitori protonske pumpe omeprazol i lanzoprazol. Štoviše, budući da je među svim neželjenim reakcijama na lijekove, terapija varfarinom najčešći uzrok hospitalizacije s medicinskim posljedicama i pratećim troškovima, u ovom ću predavanju dati pregled naših podataka o primjeni farmakogenetičkih informacija u predviđanju doziranja lijeka i uklanjanju poteškoća pri optimiranju antikoagulantne terapije varfarinom.
Prijelaz iz farmakogenetičkih istraživanja u kliničku praksu zahtjeva eksplicitnu analitičku i kliničku procjenu i pomnu analizu ravnoteže dobrobiti i pratećih rizika uključujući i pripadajuće troškove. O ograničenjima korištenja farmakogenetike treba se i dalje raspravljati isto kao i o načinima za nadilaženje tih ograničenja.

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tic agents and endogenous metabolic activities. This presentation will provide a review of the literature and of our personal experience in determining genotypes of alleles of selected CYP genes by using several technologies, including the Roche AmpliChip P450 Array.
A body of evidence demonstrates the importance of CYP genotyping, particularly regarding CYP2D6 and CYP2C19. CYP2D6 genotyping resulted to be very interesting in tamoxifen treated breast-cancer patients, as well as for prodrugs such as codeine opiates and antiarrythmics. CYP2C and its polymorphic isoform CYP2C19 is involved in metabolization of several important drugs including most tricyclic antidepressants, and the proton pump inhibitors omeprazole and lansoprazole. Moreover, being the Warfarin therapy a leading cause of hospitalization among adverse drug reactions, with medical consequences and costs, I will review our data on the application of pharmacogenetic information to predict dosing amounts and to eliminate what has been termed "oversteer" in using warfarin for anticoagulation therapy.
Translation of pharmacogenetic research into clinical practice requires explicit analytical and clinical evaluation, and a careful analysis of the balance of benefits and associated risks, including costs. The barriers to using PGx will be discussed as well as the ways to overcame these barriers.

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S02-2

Od farmakogenetskog genotipa do predviđanja doze lijeka

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Usprkos ubrzanom razvoju farmakogenetike u zadnjih 20-ak godina, svega je nekoliko gena čiji se farmakogenetski polimorfizmi enzima metabolizma lijekova danas rutinski određuju i imaju definiranu kliničku primjenu. Mnoga pitanja priječe put od otkrića novog genetskog polimorfizma do kliničke primjene njegova određivanja. Većinu tih pitanja obuhvaćaju Preporuke za laboratorijsku dijagnostiku i primjenu farmakogenetike u kliničkoj praksi, Američke Nacionalne Akademije za Kliničku Biokemiju – NACB, u svojoj trećoj radnoj verziji iz 2007 godine. Predavanje donosi njihov kratak pregled.
Problemi u farmakogenetskom testiranju. Brojni su primjeri specifičnih problema u farmakogenetici. Zbog velikih razlika u frekvenciji alela između pojedinih etničkih

S02-2

From pharmacogenetic genotype to drug dose prediction

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Despite quick development of pharmacogenetics in the latest 20 years, there are only few genes with pharmacogenetic polymorphisms that are routinely determined, and their clinical usage is clearly defined. Many questions affect the process that start with a new genetic polymorphism discovery and ends with a clinical usage of its determination. Most of these questions are gathered in Guidelines and Recommendations for Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice (2007, 3rd draft) issued by National Academy of Clinical Biochemistry (NACB). This lecture brings a short overview of those guidelines.
Problems in Pharmacogenetic testing. There are many examples of specific problems in pharmacogenetics. Because of great differences in allele frequencies among

skupina, svaki bi laboratorij za sebe trebao testirati njihove frekvencije, te pratiti statistiku trenda. Neke metode (primjerice sekvenciranje) nisu u mogućnosti utvrditi genske promjene poput velikih genomskih insercija ili delecija. Kontrolni DNA materijal trebao bi biti iz obnovljivog, nezavisnog izvora, a pri određivanju bi se trebalo voditi računa o inhibitorima ili interferencijama, koje mogu dovesti do krive interpretacije rezultata testiranja u odnosu na kliničku sliku.

Laboratorijski nalazi. Preporuka je kako bi rezultati laboratorijskih nalaza trebali jednoznačno predstavljati potvrđenu genetsku strukturu i navoditi samo dokazane karakteristike gena, kako se liječnika ne bi dovodilo u zabunu. Izravno prevođenje genotipa u fenotip može biti opasno, jer fenotipska klasifikacija ne ovisi samo o genotipu, već i o ispitivanom lijeku/supstratu. Laboratorijski nalazi, trebali bi liječniku razjasniti kompleksnu sliku zahvaćenih metaboličkih puteva i testiranih enzima, evaluirati rizik prema inerakcijama i analizirati sadašnji i prošli režim terapije. Alternativno, nalaz bi barem trebao navoditi upozoravajuće primjere interakcija. Laboratorij bi trebao pružati i mogućnost interpretacije nalaza, ali ne preporuča se da laboratorij predviđa i dozu lijeka. Metodologija i ograničenja farmakogenetskog testiranja bi uz analitičku osjetljivost/specifičnost testa trebala biti jasno naznačena na nalazu.

Izbor polimorfizama za testiranje. Polimorfizmi obuhvaćeni testiranjem trebali bi imati definirani klinički utjecaj na funkciju, farmakokinetiku, farmakodinamiku i/ili toksičnost lijeka, no još uvijek nema suglasja o svim kriterijima koje bi taj izbor trebao obuhvatiti.

Ekonomska računica. Sa gledišta financijske isplativosti farmakogenetskog testiranja, preporuka ide u prilog razvoja onih testova koji dovode do ublažavanja posljedica nuspojava, poboljšavaju učinkovitost terapije, ili u konačnici smanjuju cjelokupnu cijenu liječenja. Šire probiranje populacije zasad se ne preporuča.

Primijenjeni modeli. Nekoliko je svijetlih primjera kliničke primjene farmakogenetike. Određivanje enzima CYP2C9 i K-vitamin ovisne reduktaze (VKORC) predstavlja pristup uspješnog procjenjivanja doze u antikoagulantnoj terapiji varfarinom, proizašao iz istraživanja multivarijantnim regresijskim modelima. Sljedeći je primjer određivanje tiopurin metil transferaze (TPMT) u strategiji doziranja onkoloških bolesnika na terapiji azatioprinom. Farmakogenotipizacijski algoritmi enzima CYP2D6 uspješno se primjenjuju i u terapiji nekih psihijatrijskih poremećaja - atomoksetinom, te u endokrinoj terapiji karcinoma dojke - tamoksifenom. Testiranjem enzima UGT1A1, prevenira se pak toksičnost irinotekana u onkologiji.

U zaključku, praćenje terapije komplementarno je farmakogenetskom testiranju i omogućuje izbor lijeka i doze za one lijekove kod kojih je jasna korelacija između genotipa i doziranja.

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individual ethnic groups, every laboratory should perform polymorphism frequencies testing on its own, as well as it should follow their trend statistics. Some methods (e.g. sequencing) are not capable of determining genetic changes like big genomic insertions or deletions. Control material should be derived from the renewable independent source, and while performing the analysis, it should take into account inhibitors or interferences which could lead to misinterpretation of the testing results, when compared to the clinical presentation.

Laboratory results. Recommendation is that laboratory results should unambiguously resemble genetic structure and quote only genetic characteristics that were confirmed, in order not to mislead the physician. Direct "genotype to phenotype" prediction could be dangerous, because phenotype classification does not depend on genotype only, but also on researched drug/substrate. Laboratory results should clarify the clinician a complex picture of metabolic pathways and enzymes affected, evaluate the risk to interactions and analyze the current and previous mode of therapy. Alternatively, the laboratory results should at least cite warning interaction examples. Laboratory should also provide the possibility of interpretation of the findings, but is not recommended for the laboratory to predict drug dose. Methodology and limitations of pharmacogenetic testing should be clearly indicated on the findings, along with the analytical sensitivity/specificity.

Selection for polymorphism test. Polymorphisms included in testing should have their clinically impact on function, pharmacokinetics, pharmacodynamics, and/or drug toxicity defined, but there is still no consensus on all the criteria that this choice should include.

Economic calculation. Concerning the financial viability of pharmacogenetic testing, recommendation is in favor of developing tests that lead to the mitigation of side effects, improving the effectiveness of therapy, or ultimately reduce the overall cost of treatment. Wider population screening is not recommended.

Applying the models. There are several examples of clinical applications of pharmacogenetic testing. Determination of enzymes CYP2C9 and vitamin K-dependent reductase (VKORC) is a successful approach derived from multivariate regression models, in estimating the dose in warfarin anticoagulant therapy. The next example is determining thiopurine methyltransferase (TPMT) in the dosing strategy of oncology patients on azathioprin therapy. Pharmacogenetic algorithms of CYP2D6 enzyme are successfully applied in the treatment of certain psychiatric disorders with atomoxetine and the treatment of breast cancer with tamoxifen. Testing UGT1A1 enzyme prevents irinotecan toxicity in oncology.

In conclusion, therapy drug monitoring is complementary with pharmacogenetic testing and it enables selection of drug and dose for those medicines with clear correlation between genotype and dosing.

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S02-3

Farmakogenetika kumarinskih antikoagulanata

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Uvod: Doziranje kumarinskih antikoagulanata predstavlja veliki izazov zbog njihova uskoga terapijskog raspona i značajne varijabilnosti u odnosu doze i učinka lijeka. Ta se varijabilnost djelomično može objasniti polimorfizmom gena CYP2C9 koji kodira glavni metabolički enzim kumarinskih lijekova, te polimorfizmom vitamina K-epoksid-reduktaze (VKOR) čija je uloga redukcija vitamina-K-2,3-epoksida u biološki aktivan vitamin K hidrokinon. Učinak kumarinskih lijekova temelji se na inhibiciji aktivnosti VKOR, a ciljno mjesto djelovanja je protein vitamin-K-reduktaza-kompleks, podjedinica 1 (VKORC1) kodiran istoimenim genom VKORC1. Nosioci kombinacije polimorfizama CYP2C9 i VKORC1 imaju povećani rizik izraženog antikoagulacijskog učinka u usporedbi s osobama koje nisu nosioci polimorfizama ili imaju samo jedan polimorfizam. Bolesnici s varijantnim alelom zahtijevaju značajno niže doze u odnosu na VKORC1-wt (divlji tip). Predloženo je nekoliko postupnika doziranja temeljenih na farmakogenetici navedenih enzima, međutim niti jedan još uvijek nema široku primjenu.
Cilj ovog istraživanja bio je odrediti učestalost genotipova CYP2C9 (CYP2C9*2 i CYP2C9*3) i VKORC1 C1173T, te procijeniti odnos između genskih varijanata CYP2C9 i VKORC1 i primijenjene doze varfarina.
Bolesnici i metode: Bolesnicima s indikacijom primjene varfarina hospitaliziranim na Klinici za unutarnje bolesti Kliničkoga bolničkog centra Zagreb (N = 157) provedena je genotipizacija CYP2C9 i VKORC1. Propisana doza varfarina retrospektivno je povezana s alelnim varijanta enzima. Drugi lijekovi, dob, spol, visina i težina također su bili uzeti u obzir u prilagodbi doze. Genotipizacija CYP2C9 (aleli *2 i *3) je provedena metodom PCR u stvarnom vremenu pomoću TIB MOLBIOL LightMix na instrumentu Roche LightCycler. Genotipizacija VKORC1 C1173T je provedena metodama PCR u stvarnom vremenu i PCR-RFLP.
Rezultati: Udio sporih, intermedijarnih i brzih metabolizatora iznosio je 6%, 61% i 33% s obzirom na CYP2C9 i 13, 49% i 38% za varijante VKORC1. Rezultati su pokazali da oko 76% ispitivanih bolesnika na terapiji varfarinom ima barem jedan „osjetljivi" (CYP2C9*2,*3, VKORC1 1173T) alel i zahtijeva prilagodbu doze. Doziranje varfarina pokazalo

S02-3

Pharmacogenetics of coumarin anticoagulants

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Introduction: Dosing of the coumarin type anticoagulants presents a challenging task due to their narrow therapeutic range and a large variability in dose-response relationship. This variability can partly be explained by polymorphisms of the CYP2C9 gene which encodes the main metabolizing enzyme of coumarins, and by polymorphisms of vitamin K epoxide reductase (VKOR). Coumarins act by inhibiting VKOR activity, their target having been identified as the protein vitamin K reductase complex subunit 1 (VKORC1) which is encoded by the homonymous gene VKORC1. Carriers of a combination of CYP2C9 polymorphism and VKORC1 polymorphism had an increased risk of severe overanticoagulation compared to subjects with no polymorphism or only one polymorphism. Patients with VKORC1 polymorphism required significantly lower doses than VKORC1 wild-type patients. Empirical dose tables based on pharmacogenetics of these enzymes have been proposed, but none of them has yet been widely accepted. The aim of this study was to assess the frequencies of CYP2C9 (CYP2C9*2 and CYP2C9*3 alleles) and the VKORC1 C1173T genotype and to determine the relationship between genetic variations of CYP2C9 and VKORC1 and warfarin dose.
Patients and methods: Patients with an indication for warfarin hospitalized at the Department of Internal Medicine, University Hospital Center Zagreb (N = 157) were genotyped for CYP2C9 and VKORC1 polymorphisms. Prescribed dose on discharge was retrospectively linked to the enzyme variants. Concomitant medications and age were also taken into account when adjusting the required warfarin dose. The genotyping of CYP2C9 (alleles *2 and *3) was performed by Real time PCR method, using TIB MOLBIOL LightMix in Roche LightCycler instrument. The genotyping of VKORC1 C1173T was performed by Real time PCR method in LightCycler Fast Start DNA Master plus HybProbe master mix, and by PCR-RFLP method.
Results: Slow, intermediate and fast metabolizers accounted for 6%, 61% and 33% regarding CYP2C9, and for 13%, 49% and 38 % regarding VKORC1 variants, res-

je značajnu korelaciju s alelnim varijantama VKORC1 (P < 0,001) i CYP2C9 (P = 0,04). Bolesnici s visokom aktivnosti VKORC1 i/ili CYP2C9 trebali su statistički značajno više doze varfarina (6,5 ± 1,38 mg) u odnosu na bolesnike s intermedijarnom (2,48 ± 1,62 mg; P < 0,001) i niskom aktivnosti VKORC1 i CYP2C9 (0,87 ± 0,88 mg; P = 0,03).
Zaključak: Polimorfne varijante VKORC1 i CYP2C9 imaju značajan utjecaj na doziranje varfarina i mogu poslužiti kliničarima kao objektivan parametar u procjeni odgovora na kumarinske antikoagulante.

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pectively. Our findings show that ~76% of hospitalized patients on warfarin treatment have at least one "sensitive" (CYP2C9*2,*3, VKORC1 1173T) allele. Warfarin dosage on discharge showed correlation with VKORC1 (P < 0.001) and CYP2C9 allele variants (P = 0.041). Patients with VKORC1 and/or CYP2C9 high activity alleles required statistically higher warfarin doses (6.5 ± 1.38 mg) compared to patients with intermediate (2.48 ± 1.62 mg; P < 0.001) and low activity alleles (0.87 ± 0.88 mg; P < 0.03).
Conclusion: VKORC1 and CYP2C9 variants have significant influence on the definition of warfarin dose and could serve clinicians as important genetic markers for coumarin type drug response.

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S02-4

Uloga polimorfizama receptora sulfonilureje (SUR-1) u regulaciji šećerne bolesti tipa 2

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Šećerna je bolest tipa 2 postala jedan od vodećih javnozdravstvenih problema, kako u svijetu, tako i u Hrvatskoj. Terapijski pristup u prvoj fazi uključuje samo prilagodbu načina života. Ukoliko se na taj način ne postigne odgovarajuća regulacija, prelazi se na terapiju oralnim lijekovima za regulaciju hiperglikemije (OHL), a potom i na inzulin.
Preparati sulfonilureje su OHL koji se već dugo upotrebljavaju u liječenju šećerne bolesti. Mehanizam djelovanja derivata sulfonilureje uključuje poticanje izlučivanja inzulina iz beta-Langerhansovih otočića gušterače. Derivati sulfonilureje vežu se za receptor sulfonilureje (SUR-1) koji je funkcionalna jedinica ATP-ovisnog kalijevog kanala (KATP). Druga komponenta kanala je Kir6.2, unutrašnji ionski kanal koji oblikuje membransku poru. Vezanje sulfonilureje na receptor uzrokuje zatvaranje kalijevog kanala, otvaranje kalcijevog kanala ovisnog o naponu, porast unutarstanične koncentracije kalcija i stimulaciju otpuštanja inzulina iz sekretornih granula egzocitozom. Zatvaranje kalijevog kanala nije potaknuto samo sulfonilurejama, već i stvaranjem ATP-a u metabolizmu glukoze, što ukazuje na značajnu ulogu *SUR-1* i *Kir6.2* gena, ne samo kod terapije derivatima sulfonilureje, već i kod dijabetičara općenito.
U terapiji šećerne bolesti vrlo je česta kombinirana terapija korištenjem više različitih vrsta lijekova. Naime, iako

S02-4

Sulfonylurea receptor-1 (SUR-1) polymorphisms in regulation of type 2 diabetes

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Type 2 diabetes has become one of the leading health issues worldwide. In the beginning, therapeutic approach includes only lifestyle modifications. If this approach doesn't yield desired metabolic control, oral hypoglycaemic agents are introduced and sometimes even insulin therapy is required.
Sulfonylureas are hypoglycaemic agents used for diabetes treatment since the 1950's. Mechanism of action includes increased insulin release from beta cells in pancreas. They bind to sulfonylurea receptor-1 (SUR-1), which is a functional subunit of the ATP-sensitive potassium channel (KATP) located in pancreatic beta cells in islets of Langerhans. The other component of potassium channel is Kir6.2, inwardly rectifying ion channel forming a pore, encoded by gene KCNJ11. Sulfonylureas bind to SUR-1 subunit of potassium channel causing its closure, opening of voltage-gated Ca2+ channels, increase of intracellular Ca2+ concentration and stimulation of insulin release from secretory granules by exocytosis. Closure of KATP is not initiated only by sulfonylurea action, but also by ATP production in glucose metabolism, indicated significant role of SUR-1 and Kir6.2, not only in diabetics on sulfonylurea therapy, but in diabetes in general.
In therapy of type 2 diabetes, combined approach is very common. In most of the patients sulfonylurea thera-

kod većine bolesnika standardna terapija preparatima sulfonilureje postiže očekivane rezultate u regulaciji šećerne bolesti, kod jednog je dijela bolesnika taj učinak znatno ograničen. U tom se slučaju prvo pristupa kombiniranoj terapiji s nekoliko OHL-a, kombinaciji OHL-a i inzulina te naposljetku terapiji samo inzulinom. Prema rezultatima velikog prospektivnog istraživanja UK Prospective Diabetes Study (UKPDS) koje je trajalo 20 godina, svake godine 5-7% dijabetičara liječenih sulfonilurejom prelazi na inzulinsku terapiju uslijed neuspjeha liječenja.
Geni koji kodiraju sintezu SUR-1 i Kir6.2 proteina smješteni su na lokusu 11p15.1. Gen za SUR-1 (*ABCC8*, engl. *ATP-binding cassette, sub-family C (CFTR/MRP), member 8*) sastoji se od 39 eksona i proteže se oko 100 kpb u duljinu. Kir6.2 gen (*KCNJ11*, engl. *potassium inwardly-rectifying channel, subfamily J, member 11*) se sastoji od samo jednog eksona.
Polimorfizmi ovih gena dovode do povećane vjerojatnosti otvaranja kalijevog kanala, smanjene osjetljivosti kanala na inhibitorno djelovanje ATP-a i povećane granične vrijednosti za otpuštanje inzulina. Ovi mehanizmi mogu dovesti do promijenjenog odgovora na terapiju sulfonilurejom te do lošije kontrole šećerne bolesti, što se može kvantificirati mjerenjem biokemijskih i antropometrijskih pokazatelja koji su u korelaciji sa stupnjem regulacije.
U ovom će izlaganju biti prikazani dosadašnji rezultati proučavanja povezanosti SUR-1 i KCNJ11 polimorfizama sa fenotipovima povezanim sa šećernom bolesti tipa 2 kod bolesnika na terapiji sufonilurejom.

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py accomplishes desired metabolic control, however in a number of patients that effect is very limited. In that case, sulfonylurea is combined with insulin, and in more severe cases only insulin therapy is indicated. According to the results of large 20-years prospective study (UK Prospective Diabetes Study - UKPDS), each year there is a failure of sulfonylurea therapy and introduction of insulin in 5-7% of type 2 diabetics.
The genes encoding SUR-1 and Kir6.2 are located on the chromosome 11p15.1. Gene for the SUR-1 (ABCC8, ATP-binding cassette, sub-family C (CFTR/MRP), member 8) consists of 39 exons, and spans 100 kbp in length. Gene for the other subunit Kir6.2 (KCNJ11, potassium inwardly-rectifying channel, subfamily J, member 11) consists of only one exon.
Polymorphisms of these genes can lead to increased probability of opening of potassium channel, decreased sensitivity on ATP inhibition and increased cut-off for insulin release. These mechanisms can lead to modulated response to sulfonylurea therapy and poor metabolic control of type 2 diabetes. This can be quantified by measuring biochemical and anthropometric parameters that are in correlation with degree of metabolic control.
This lecture will present results of our research on association of SUR-1 and KCNJ11 polymorphisms with diabetes associated phenotypes in patients on sulfonylurea therapy.

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S02-5

Utjecaj farmakogenetičkih varijacija na koncentracije antiepileptika u serumu

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Uvod: Danas se u liječenju epilepsije koristi velik broj antiepileptika. Zbog značajnih interindividualnih razlika u koncentracijama i učinkovitosti lijeka, terapijsko praćenje je često i nužno. Uz kliničke i vanjske čimbenike, genetičke se predispozicije također drže važnima u individualizaciji terapije i zauzimaju istaknuto mjesto u stvaranju algoritama za odabir najprikladnijeg lijeka i doze za svakog pojedinog bolesnika. Među farmakogenetičkim biljezima

S02-5

Impact of pharmacogenetic variations on antiepileptic serum level

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Introduction: A large number of antiepileptics is used today to treat epilepsy. Therapeutic monitoring of this disorder is frequent and necessary due to considerable interindividual differences in drug concentrations and efficacy. Along with clinical and exogenous factors, genetic predispositions have been recognized as an important factor in therapy individualization and they take a promi-

polimorfni transportni proteini, odgovorni za prijenos lijekova preko različitih barijera, nametnuli su se kao važni čimbenici varijabilnosti i bioraspoloživosti različitih antiepileptika. Transportni proteini koji imaju značajnu ulogu u farmakokinetici nalaze se u epitelnim membranama probavnog sustava, bubrega i jetre. Pokazalo se da ABC-transporteri koji kodiraju transportne proteine P-glikoprotein (ABCB1) i protein multirezistencije 2 (ABCC2) utječu na koncentracije lijekova - supstrata.

Cilj ovog istraživanja bio je ispitati utjecaj polimorfnih varijanti gena ABCB1 (C3435T, C1236T, G2677T/A) i ABCC2 (C24T, G1249A) na koncentracije antiepileptika u serumu. Ispitana je ovisnost koncentracija lamotrigina u plazmi bolesnika na mono- i politerapiji (karbamazepin, okskarbazepin, levetiracetam, fenitoin, fenobarbiton, topiramat, valproat) i navedenih genskih varijanti. Kao kovarijante u statističkoj obradi uključeni su dob, spol, visina, težina, jetreni i bubrežni biokemijski parametri.

Bolesnici i metode: 122 bolesnika s epilepsijom, u dobi od 18-70 godina, podijeljeni su u skupine prema terapiji: monoterapija lamotriginom (N = 25), lamotrigin i lijekovi induktori (N = 60), lamotrigin i inhibitori (N = 19) i skupina koja uz lamotrigin uzima i induktor i inhibitor (N = 18). Genotipizacija ABCB1 provedena je metodama PCR u stvarnom vremenu i PCR-RFLP, a ABCC2 metodom PCR-RFLP. Terapijsko određivanje koncentracije antiepileptika provedeno je metodom HPLC s diode array detektorom i imunokemijskim metodama.

Rezultati: Dokazana je statistički značajna korelacija koncentracije lamotrigina u ovisnosti o: dodatnim lijekovima ($P < 0,001$), ALT, dobi i težini ($P < 0,01$). Statistička analiza pokazala je graničnu korelaciju između koncentracije lamotrigina i varijante C24T gena ABCC2 ($P = 0,074$; CC+CT: TT, 12,2: 22,8 µmol/L). Za genotip 1249GG i 1249AA gena ABCC2 srednje vrijednosti koncentracija lamotrigina iznosile su 11,6 i 18,9 µmol/L. Analiza genotipa ABCB1 također ukazuje na tendenciju korelacije polimorfizma C1236T s koncentracijom lamotrigina (CC: TT, 14,8: 11,1 µmol/L) i polimorfizma C3435T (CC: TT, 13,3: 10,4 µmol/L); niska učestalost genotipa TT je ograničavajući čimbenik za statističku analizu.

Zaključak: Preliminarni rezultati ukazuju da polimorfizmi ABCB1 i ABCC2 mogu utjecati na biodostupnost antiepileptika i mogu se ubrojiti u interindividualne farmakokinetske varijabilnosti. Zbog proturječnih rezultata u literaturi i još nejasne uloge transportnih proteina ABC u farmakokinetici antiepileptika držimo da je potrebno daljnje istraživanje na većem broju ispitanika u svrhu uključivanja ovih farmakogenetičkih pokazatelja za algoritme doziranja antiepilepsijskih lijekova.

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nent place in developing algorithms for selection of the most appropriate drug and dose for each patient. Among pharmacogenetic markers, polymorphic transport proteins, which are responsible for drug transport across various barriers, offered themselves as significant factors of variability and bioavailability of different antiepileptics. Transporter proteins that play important role in pharmacokinetics are located in intestinal, renal and hepatic epithelial membranes. ABC transporters such as P-glycoprotein (ABCB1) and multidrug resistance-associated protein 2 (ABCC2) affect bioavailability of their substrate drugs.

Aims of the study were to evaluate the impact of polymorphisms in the ABCB1 (C3435T, C1236T, G2677T/A) and ABCC2 (C24T, G1249A) on antiepileptic drug disposition. We therefore correlated plasma levels of lamotrigine in mono- and polytherapy (carbamazepine, oxcarbazepine, levetiracetame, phenytoin, phenobarbiton, topiramate, valproate) with gene variants. As covariates, age, gender, height, weight, liver and renal biochemical parameters were included.

Patients and methods: 122 epileptic patients, from 18-70 years old, were stratified into lamotrigine monotherapy group (N = 25), a group receiving lamotrigine plus inductors (N = 60), inhibitors (N = 19) or both (N = 18). ABCB1 genotyping (C1236T, C3435T, G2677T/A) was performed by Real-time PCR and PCR-RFLP; ABCC2 (G1249A, C24T) by PCR-RFLP methods. Therapeutic drug monitoring was performed by HPLC with diode array detector and immunoassay.

Results: We found significant differences in lamotrigine concentrations depending on: add-on therapy ($P < 0.001$); ALT, age and weight ($P < 0.01$). Statistical analysis showed borderline correlation between lamotrigine concentrations and C24T variant ABCC2 gene ($P = 0.074$; CC+CT: TT, 12.2: 22.8 µmol/L). 1249GG and 1249AA carriers of the ABCC2 gene had mean lamotrigine concentration of 11.6 and 18.9 µmol/L, respectively. Mutation on ABCB1 gene also revealed tendency toward correlation of C1236T genotypes with lamotrigine concentrations (CC: TT, 14.8: 11.1 µmol/L) and for C3435T (CC: TT, 13.3: 10.4 µmol/L), but the number of TT genotype carriers was a limiting factor for statistical significance.

Conclusions: Preliminary data are suggestive and point that ABCB1 and ABCC2 polymorphisms may influence AED disposition and may account for interindividual pharmacokinetic variability. Due to controversial results reported in literature and still indefinite role of ABC transport proteins in the pharmacokinetics of antiepileptics, we believe that further investigation is necessary on a larger number of patients in order to include these pharmacogenetic indicators in dosing algorithms for antiepileptic drugs.

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