bolesnika. U TB bolesnika 25-OHD je u žena (45,9 ± 26,3; P < 0,005) niži nego u muškaraca (60,7 ± 38,0), ali nema razlike tijekom godine. U 77% (340/441) TB bolesnika postoji manjak 25-OHD, a u 101 iznosi 101,9 ± 31,8 (75-262). Nema razlike između dvije skupina bolesnika niti između spolova za sve. Manjak 25-OHD je u 71% muškaraca (216/304) i 84% (201/239) žena za sve. Manjak D vitamina je nađen u > 75% ovih bolesnika, bez razlike prema osnovnom stanju. U žena je manjak učestaliji i nakon transplantacije su koncentracije niže nego u muškaraca. Doba godine nije utjecalo na 25-OHD.
Zaključak: Veliki udio manjka 25-OHD u žena je nepovoljan je čimbenik nastanka osteoporoze i poremećaja mineralnog metabolizma.

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26.3; P < 0.005) than in men (60.7 ± 38.0), but no difference existed during the year. Deficiency was found in 77% (340/441) KT patients, and in the remaining 101 was 101.9 ± 31.8 (75-262). No difference between sexes was found. In the entire group 25-OHD deficiency was present in 71% men (216/304) and 84% women (201/239). Vitamin D deficiency was found in > 75% patients, without difference regarding diagnosis. In women deficiency was more frequent and after kidney transplantation 25-OHD was less than in men. The seasons did not influence 25-OHD.
Conclusion: Large proportion of 25-OHD deficiency in women is a negative risk factor for osteoporosis and mineral metabolism disorder.

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P08 – Molekularna dijagnostika

P08-1 (Usmeno priopćenje)

Detekcija točkaste mutacije T315I kod bolesnika rezistentnih na terapiju imatinib- mesilatom

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Uvod: Kronična mijeloična leukemija (KML) je mijeloproliferativna bolest karakterizirana nastankom fuzijskog gena BCR-ABL, a posljedično time i fuzijskog proteina bcr-abl. Iako je otkrićem imatinib-mesilata poboljšano liječenje bolesnika s KML, kod manjeg broja bolesnika nakon određenog vremena dolazi do rezistencije na lijek. Jedan od mogućih razloga rezistencije su mutacije u kinaznoj domeni abl. Jedina mutacija rezistentna na danas dostupne tirozin-kinazne inhibitore je točkasta mutacija T315I. Cilj rada bio je detektirati je li prisutnost mutacije T315I uzrok porasta, odnosno visoke razine prijepisa bcr-abl.
Materijali i metode: U istraživanje su bila uključeno 24 bolesnika s KML te neodgovarajućim odgovorom na terapiju imatinib-mesilatom: suboptimalni odgovor na terapiju imalo je 10 bolesnika, 8 bolesnika uopće nije odgovorilo na terapiju, dok je u 6 bolesnika došlo do > 1 log povećanja fuzijskog prijepsa bcr-abl progresivno kroz vrijeme. Napravljen je ASO-PCR prema protokolu Kanga i sur. (Haematologica, 2006; 91:659). Kao pozitivna kontrola, korišten je uzorak DNA bolesnice kod koje je dokazano postojanje mutacije T315I.
Rezultati: Metodom ASO-PCR otkrivena je točkasta mutacija T315I u 4/24 bolesnika (17%). Svi bolesnici kod ko-

P08 – Molecular diagnosis

P08-1 (Oral presentation)

T315I mutation detection in patients resistant to imatinib mesylate therapy

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Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by the presence of BCR-ABL fusion gene and consequently bcr-abl fusion protein. Although the discovery of tyrosine kinase inhibitor (TKI), imatinib mesylate, improved the treatment of CML patients, a proportion of patients develop resistance to drug resulting in increased bcr-abl level. One of possible reasons for resistance are mutations in ABL kinase domain. Some mutations can be overcome by increasing the drug dose or by using the new generation of TKI. The only mutation resistant to currently available TKI is T315I. The aim of this study was to detect if the presence of T315I is the cause for increase or constantly high bcr-abl level.
Materials and methods: Twenty-four CML patients with inadequate response to therapy were included in the study: 10 patients with suboptimal response, 8 patients with failure in response to therapy and 6 patients with > 1 log increase of bcr-abl level over time. ASO-PCR for T315I detection was performed according to Kang et al (Haematologica, 2006; 91:659). Positive control was DNA sample from a patient with confirmed T315I mutation.

jih je dokazana mutacija imali su visoke vrijednosti razine prijepisa bcr-abl (12,7-38,1%; 24,9-26,1%; 12,5-30,4% prema međunarodnoj ljestvici te stalno pozitivan nalaz kvalitativnog PCR-a kod četvrtog bolesnika). Srednja vrijednost razine fuzijskog prijepisa u trenutku zahtjeva za otkrivanje mutacije u tih bolesnika iznosila je 23,1% te oni nisu reagirali na povećanje terapijske doze imatinib-mesilata.
Zaključak: Ovom metodom moguće je s osjetljivošću 1/1000 otkriti točkastu mutaciju T315I kao jedan od značajnih prognostičkih faktora tijeka bolesti jer je izbor liječenja za nosioce te mutacije transplantacija koštane srži.

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Results: T315I was detected in 4/24 patients (17%). All patients with mutation had high bcr-abl levels (12.7-38.1%; 24.9-26.1%; 12.5-30.4% according to International Scale and permanently positive qualitative PCR for the last patient). Mean bcr-abl level before the mutation request for all 4 patients was 23.1% and the patients did not respond to the increase of therapeutic dose.
Conclusion: ASO-PCR enables, with sensitivity of 1/1000, detection T315I which is one of important prognostic factors for disease course as the treatment of choice for mutation carriers is bone marrow transplantation.

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P08-2 (Usmeno priopćenje)

Genetički polimorfizam tiopurinmetiltransferaze u hrvatskih bolesnika s upalnom bolesti crijeva

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Uvod: Tiopurini su važni lijekovi koji se koriste između ostalog i u liječenju refraktornih ili o steroidima ovisnih bolesnika s upalnom bolesti crijeva. Lijek postaje aktivan metilacijom pomoću enzima tiopurinmetiltransferaze (TPMT). TPMT pokazuje varijabilnu interindividualnu aktivnost povezanu s genetičkim polimorfizmom. Stoga TPMT genotip predstavlja predispoziciju za interindividualne razlike u učinkovitosti lijeka i razvoj nuspojava. Istraživanja su ukazala na potrebu genotipizacije TPMT prije početka terapije tiopurinima. S obzirom na različitu pojavnost polimorfnih TPMT alela u pojedinim populacijama (u bijeloj populaciji oko 11%) cilj ovog istraživanja bio je odrediti učestalost najčešćih polimorfnih varijanti TPMT u naših bolesnika s upalnom bolesti crijeva (UBC).
Bolesnici i metode: Alelne varijante TPMT gena (*2, *3A, *3C) su određene metodama temeljenim na polimeraznoj lančanoj reakciji (PCR-RFLP i PCR u stvarnom vremenu - *Real-time* PCR) u 326 odraslih bolesnika s upalnom bolesti crijeva.
Rezultati: Među bolesnicima uključenim u studiju, 9 je bilo heterozigotnih za polimorfni alel TPMT (2,7%), dok je ostatak imao alele divljeg tipa (*1). Učestalost deficijentnih alela TPMT*2, *3A, i *3C je bila 0,6%, 0,9% i 1,2%.

P08-2 (Oral presentation)

Genetic polymorphism of thiopurine methyltransferase in croatian inflammatory bowel disease patients

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Introduction: Thiopurines are important drugs used in the treatment of steroid-dependent and refractory inflammatory bowel disease. They exhibit their activity once they are metabolized via methylation by thiopurine methyltransferase (TPMT) enzyme. TPMT displays a range of activities due to genetic polymorphism meaning that frequency of certain TPMT alleles determines interindividual variations. Therefore the likelihood of adverse effect and interindividual differences in therapeutic efficacy may relate to TPMT genotype. It was established in some studies that TPMT testing should be introduced prior to the initiation of thiopurines in therapy. Regarding to different frequences of polymorphic TPMT alleles reported from different populations (in Caucasians 11%) the aim of this study was to evaluate the frequency of the most common TPMT variants in our inflammatory bowel disease patients (IBD).
Patients and methods: The allelic variants of the TPMT gene (*2, *3A,*3C) were analyzed by polymerase chain reaction-based assays (PCR-RFLP and Real-time PCR) in 326 adult IBD patients.
Results: Among the patients enrolled in the study, 9 patients were heterozygous for a variant TPMT allele (2.7%),

Zaključak: TPMT*3C je najučestaliji nefunkcionalni alel TPMT u hrvatskih bolesnika s upalnom bolesti crijeva. S obzirom na nisku pojavnost polimorfnih alela TPMT preporučuje se testirati i kontrolnu skupinu zdravih ispitanika prije konačnih zaključaka o doziranju tiopurinskih lijekova na osnovi farmakogenetičkih podataka u našoj populaciji.

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while the rest had a wild-type allele. Frequencies of deficient alleles TPMT*2, TPMT*3A, and TPMT*3C were 0.6%, 0.9% and 1.2% respectively.
Conclusion: TPMT*3C was the most frequently ocurring nonfunctional TPMT allele in Croatian IBD population. Regarding to relatively lower frequency of TPMT polymorphisms we need further investigation in cohort of healthy controls prior definite conclusion about benefit of pharmacogenetically guided dosing in our population.

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P08-3 (Usmeno priopćenje)

Parcijalna monosomija 4q i parcijalna trisomija 13q: prikaz fenotipa i određivanje kromosomskih točaka loma molekularnim metodama

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Uvod: Fenotipi pacijenata s djelomičnom aneuploidijom često se razlikuju svojim kliničkim manifestacijama ovisno o veličini kromosomske regije uključene u promjenu. Delecije kromosomskih regija 4q31, 4q32 i 4q33-4qter uzrokuju delecija 4q malformacijski sindrom koji uključuje facijalnu dismorfiju, kardiološke poremećaje, defekte udova i zastoj u razvoju. Distalnije 4q delecije, koje uključuje pruge 4q34-q35, nađene su u pacijenata s manje teškim oblikom mentalne retardacije i s manje karakterističnim značajkama sindroma delecija 4q. Parcijalna trisomija 13q (13q14-qter) također je opisana kao karakterističan fenotip koji uključuje tešku mentalnu retardaciju poput one kod trisomije 13. Iako del4q sindrom i dup13q sindrom mogu varirati u svojim fenotipskim značajkama, oni imaju i nekoliko zajedničkih karakteristika. U ovom radu prikazujemo kliničke i molekularne značajke jednogodišnje djevojčice s kariotipom 46,XX,der(4)t(4;13)(q34.1;q14.3)mat, čiji fenotip odražava značajke dvaju različitih sindroma.
Metode: Kromosomske točke lomova i veličina kromosomskih segmenata uključenih u kromosomsku preraspodjelu utvrđeni su metodom fluorescentne in situ hibridizacije (FISH) uporabom proba za specifično molekularno pruganje kromosoma 4 i 13 (MCB4, MCB13), subtelomernih proba za regije 13qter i 4qter te BAC proba za regije 13q21.1 i 13q14.3.

P08-3 (Oral presentation)

Partial monosomy 4q and partial trisomy 13q: phenotype and molecular mapping of the breakpoints

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Introduction: Phenotype in patients with segmental aneuploidy often vary in their clinical manifestation depending on the size of the chromosomal region involved. Deletions of chromosome regions 4q31, 4q32, and 4q33-4qter lead to a distinctive malformation syndrome (deletion 4q syndrome) of facial dysmorphism, cardiac and limb defects and developmental delay. More distal 4q deletions involving bands q34-q35 have been found in patients presenting with less characteristic features and less severe mental retardation. Partial trisomy 13q (13q14-qter) has also been shown to cause a characteristic phenotype associated with severe mental deficiency resembling that of trisomy 13. Although del4q and dup13q syndromes vary in their phenotypes, they have also several features in common. Herein presented are the clinical and molecular findings in a 1-year-old female with a karyotype 46,XX,der(4)t(4;13)(q34.1;q14.3)mat, whose phenotype exhibits the features of the two distinctive syndromes.
Methods: The chromosome breakpoints and the size of segments involved in the rearrangement were determined by FISH applying chromosome 4 and 13 specific molecular banding (MCB), subtelomeric probes for 13qter and 4qter, and BACs mapped in 13q21.1 and 13q14.3, respectively.

Rezultati: Primjenjujući shemu 169 array-mapiranih MCB knjižnica prema Weise i sur., 2008, utvrđeno je da duplikacija kromosomske regije 13q uključuje 73,95 Mb dok delecija kromosoma 4 uključuje 14,6 Mb.
Zaključak: Budući prikazi slučajeva koji će biti detaljno klinički i molekularno opisani, doprinijet će detaljnijoj karakterizaciji delecija 4q sindroma te će ukazati na regije kromosoma 13 odgovorne za razvoj trisomija 13 sindroma.

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Results: According to the labeling scheme for all 169 array-mapped MCB libraries (Weise *et al*., 2008) it was estimated that the duplication encompassed 73.95 Mb of 13q, and the deletion of chromosome 4 14.6 Mb. The report of further cases with very good clinical and molecular characterization will add to a more precise description of the deletion 4q syndrome and will pinpoint the critical region on chromosome 13 responsible for trisomy 13.

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P08-4 (Usmeno priopćenje)

Rano otkrivanje promjena genoma tehnikom I - FISH u bolesnika sa staničnom atipijom sedimenta urina

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Uvod: Karcinom mokraćnog mjehura jedan je od vodećih karcinoma u muškoj populaciji. Oko 90.000 slučajeva dijagnosticira se u Europi i 260.000 u svijetu. Visoko osjetljivim, specifičnim i ne invazivnim tehnikama molekularne biologije kao što je interfazni FISH (I-FISH) moguće je detektirati promjene genoma u stanicama sedimenta urina te tako razlikovati malignu atipiju stanica od benigne atipije. Cilj je bio ispitati i dokazati prisutnost promjena genoma u atipičnim stanicama sedimenta urina. Tehnikom I - FISH detektirane su numeričke i strukturne promjene (CEP3, CEP7, CEP 17 i LSI 9p21) u uzorcima urina s dokazanom staničnom atipijom.
Materijali i metode: UroVysion test omogućava istovremeno otkrivanje više različitih promjena u genomu jedne atipične stanice. Analizirano je 270 uzoraka urina u bolesnika kod kojih je bila prisutna stanična atipija.
Rezultati: Pozitivan nalaz nađen je u 113 (42%) bolesnika. U 24 (21%) bolesnika bile su prisutne numeričke i strukturne promjene, u 83 (74%) samo numeričke, a u 6 (5%) samo strukturne promjene.
Zaključak: Tehnikom I-FISH moguće je detektirati i pratiti poznate nam promjene genoma u stanicama tumora mokraćnog mjehura i na taj način razlikovati benignu od maligne stanične atipije.

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P08-4 (Oral presentation)

Early detection of genomic changes with I – FISH in patients with atipical cells in urine

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Introduction: Bladder cancer is one of the most prominent cancers in men. About 90,000 cases are newly diagnosed in Europe and 260,000 worldwide. With highly sensitive, specific and non invasive tehniques of molecular biology, like I-FISH, it is possible to detect genomic changes i urine cells and in that way differenciate malignant and benignant cells.
Materials and methods: UroVysion test detects aneuploidy of chromosomes 3, 7, 17 and loss of thew 9p21 locus via fluorescence in situ hybridization (FISH). A total of 270 urine specimens were analysed.
Results: 113 (42%) speciments werw pozitiv. In 24 (21%) samples we found numerical and structural changes, in 83 (74%) only numerical changes and in 6 (5%) only structural changes.
Conclusion: I-FISH allows to detect genomic changes in bladder cancer cells and to differenciate between malignant and benignant cells.

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P08-5

Mutacija gena FLT3 (FLT3/ITD) u akutnoj mijeloičnoj leukemiji (AML)

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Uvod: FLT3 gen kodira FLT3 receptor s tirozin-kinaznom aktivnošću koji sudjeluje u proliferaciji i diferencijaciji hematopoetskih stanica. Mutacije FLT3 receptora u obliku intronskog tandemskog udvajanja (ITD) u blizini jukstamembranske ili točkaste mutacije aspartanske kiseline 835 unutar kinazne domene (KD), uzrokuju njegovu aktivaciju. Varijacije ITD u duljini produkata umnažanja povezane su s nepovoljnim kliničkim značajkama i prognozom u odraslih bolesnika s *de novo* AML.
Cilj: Utvrditi postojanje FLT3/ITD mutacije u odraslih bolesnika s *de novo* AML metodom obrnute transkripcije – lančane reakcije polimeraze (RT-PCR) (Noguera NI i sur.).
Materijali i metode: Iz 20 uzoraka (14 uzoraka koštane srži i 6 venske krvi) odraslih bolesnika s *de novo* AML (11 muškaraca, 9 žena, medijan starosti 62 godine i raspona 36- 82 godina) su izolirane mononuklearne stanice. Za otkrivanje FLT3/ITD mutacije je korištena metoda Noguere i sur. uz početnice za ITD na eksonima 14 i 15. Nakon elektroforeze na agaroznom gelu produkt umnažanja divljeg tipa (FLT3/WT) ima veličinu od 130 parova baza (pb), dok je u pozitivnih uzoraka (FLT3/ITD+) prisutan i dodatni produkt umnažanja veći od 130 pb.
Rezultati: U negativnih uzoraka (FLT3/WT) dobiven je produkt umnažanja veličine 130 pb. U pozitivnih uzoraka (FLT3/ITD+) se uz produkt umnažanja veličine 130 pb nalazi i dodatni produkt umnažanja veći od 130 pb. 20% odraslih bolesnika s *de novo* AML (4/20) bilo je FLT3/ITD+ (3/14 uzoraka koštane srži; 21% i 1/6 venske krvi; 17%).
Zaključak: RT-PCR i elektroforeza na agaroznom gelu korisna je metoda probiranja FLT3/ITD u odraslih bolesnika s *de novo* AML. U daljnjem istraživanju je, poradi prognoze bolesti, potrebno odrediti točnu veličinu dodatnog produkta umnažanja.

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P08-5

FLT3 gene internal tandem duplication (FLT3/ITD) in acute myeloid leukemia (AML)

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Introduction: The FLT3 gene encodes a tyrosine kinase receptor that regulates proliferation and differentiation of hematopoietic stem cells. Mutation of the FLT3 receptor, either by internal tandem duplication of the juxtamembrane domain (ITD) or by point mutation of the aspartic acid 835 of the kinase domain (KD), causes constant activation of the FLT3 receptor. Although FLT3/ITD is the most common mutation in adult *de novo* AML, they are variable in length and therefore associated with a poor AML prognosis.
Aim: To indicate the FLT3/ITD presence by reverse-transcriptase-polymerase chain reaction (RT-PCR) (Noguera NI et al.) in adult *de novo* AML patients.
Materials and methods: From 20 samples (14 bone marrows and 6 peripheral bloods) of adult *de novo* AML patients (11 men and 9 woman, median age of 62 years; range 36– 82 years) mononuclear cells were isolated. RT-PCR according to Noguera NI et al. with specific forward and reverse FLT3 primers on exons 14 and 15 and electrophoresis on agarose gel were used for indicating the FLT3/ITD presence. Negative cases (FLT3/WT) had one expected product of 130 base pairs (bp), whereas all positive cases had additional amplicon longer than the expected product of 130 bp.
Results: Negative cases (FLT3/WT) had only one expected product of 130 bp. All positive cases appeared on agarose gel as one additional amplicon longer than the expected product of 130 bp. 20% (4/20) of adult de novo AML patients were FLT3/ITD+ (3/14 bone marrows; 21% and 1/6 peripheral blood; 17%).
Conclusion: RT-PCR and electrophoresis on agarose gel is adequate as a screening method for FLT3/ITD presence in adult *de novo* AML patients. In the future their variability in length should be investigated because of AML prognosis.

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P08-6

Povezanost polimorfizama inzulinskog receptora (IR) i supstrata 1 inzulinskog receptora (IRS-1) s karcinomom prostate

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Uvod: Tijekom posljednjih godina nekoliko gena inzulinskog signalnog puta se povezuje s karcinomom prostate (KP). Dva od tih gena kandidata su: gen za inzulinski receptor (IR) i za supstrat 1 inzulinskog receptora (IRS-1). Oba gena su uključeni u procese stanične proliferacije, razvoja i rasta tkiva. Cilj ovog istraživanja bio je ispitati povezanost polimorfizama IR 1058 C/T i IRS-1 G972R s karcinomom prostate. Također smo željeli ispitati moguću povezanost navedenih polimorfizama sa stadijem karcinoma procijenjenom prema Gleasonovom bodovanju.
Materijali i metode: Ispitali smo 119 bolesnika s KP, a u kontrolnu skupinu smo uključili 61 bolesnika s benignom hiperplazijom prostate (BHP). Bolesnici s KP podijeljeni su u podskupine s većim (Gleason stupnjevi 7-9) i manjim (Gleason stupnjevi 4-6) stupnjem malignosti. Polimorfizmi IR 1058 C/T i IRS-1 G972R genotipizirani su PCR-RFLP metodom.
Rezultati: Nije pronađena razlika u učestalosti genotipova (P = 0,794) niti alela (P = 0,621) IRS-1 G972R polimorfizma između KP i BHP skupina. Međutim, pronađena je značajna razlika u razdiobi genotipova i alela IR 1058 C/T polimorfizma. Nosioci polimorfnog alela (C/T+T/T) su bili učestaliji u skupini bolesnika s BHP nego u KP (54,10% prema 37,82%); P = 0,040; OR (95% CI) = 0,52 (0,28-0,96). Nije pronađena razlika u distribuciji IRS-1 niti IR polimorfizma u podskupinama prema Gleason bodovanju.
Zaključak: Pokazala se povezanost polimorfizma IR 1058 C/T s karcinomom prostate, dok za polimorfizam IRS-1 G972R povezanost nije dokazana. Niti jedan istraživani polimorfizam nije povezan sa stupnjem malignosti karcinoma prostate.

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P08-6

Insulin receptor (IR) and insulin receptor substrate 1 (IRS-1) polymorphisms and prostate cancer

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Background: In recent years, a few genes in the insulin signalling pathway have been associated with prostate cancer (PC) susceptibility. Two such gene candidates are the insulin receptor (IR) and the insulin receptor substrate 1 (IRS-1) genes. Both, the IR and IRS-1 are involved in appropriate cell proliferation, tissue development and growth. The aim of this study was to examine the association of IR 1058 C/T and IRS-1 G972R polymorphisms with PC. We also aimed to examine possible association with cancer severity assessed by Gleason score.
Materials and methods: We have studied 119 patients with PC and 61 patients with benign prostate hyperplasia (BPH) as a control group. Patients with PC were divided into subgroups of higher (Gleason score 7-9) and lower (Gleason score 4-6) malignancies. The genotyping of two polymorphisms (IR 1058 C/T and IRS-1 G972R) was performed by the PCR-RFLP method.
Results: There was no difference in genotype (P = 0.794) nor allelic (P = 0.621) frequency of the IRS-1 G972R polymorphism between PC and BPH patient groups. However, there was a significant difference in IR 1058 C/T polymorphism genotype and allelic distribution. Carriers of the polymorphic allele (C/T+T/T) were more frequent in a group of BPH, than in the PC group (54.10% vs. 37.82%); P = 0.040; OR (95% CI) = 0.52 (0.28-0.96). No difference was found in IRS-1 and IR polymorphism distribution in subgroups according to Gleason score.
Conclusion: IR 1058 C/T polymorphism seems to be associated with prostate cancer, whereas IRS-1 G972R is not. Neither polymorphism is associated with the degree of prostate cancer malignancy.

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P08-7

Mutacije onkogena bcr-abl u imatinibom liječenoj kroničnoj mijeloičnoj leukemiji

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Uvod: Ciljana terapija malom molekulom STI571 (imatinib), inhibitorom tirozin kinze onkoproteina bcr-abl pokazuje značajan terapijski učinak u kroničnoj mijeloičnoj leukemiji (KML). Ipak, unatoč ovoj činjenici dio bolesnika razvija terapijsku rezistenciju.
Ispitanici i metode: Od 34 bolesnika na terapiji imatinibom u Kliničkoj bolnici Dubrava, petoro bolesnika pokazuje kvanitativnim PCR-om (TaqMan) konstantno mjerljive razine molekularnog biljega. Ove bolesnike smo testirali na prisutnost tri češće točkaste mutacije (Thr315Ile, Phe311Leu and Met351Thr) u abl dijelu bcr-abl onkogena metodom ASO-DNA-PCR (osjetljivosti 1/10.000 stanica).
Rezultati: Nismo našli mutaciju Thr315Ile, dok je mutaciju Met351Th imalo svih petoro pacijenata, dvoje od njih i prije početka liječenja. Jedan je bolesnik dobio mutaciju Phe311Leu u tijeku terapije.
Zaključak: Povišena proporcija mutacija u tijeku liječenja sugerira klonalnu evoluciju bolesti. Također iz ovih podataka zaključujemo da pojava mutacija u abl dijelu bcr-abl onkogena nije vezana samo za faze ubrzanja ili progresije bolesti, već da u nekim slučajevima mutacije mogu postojati i u kroničnoj fazi bolesti te prije liječenja imatinibom.

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P08-7

BCR-ABL oncogene mutations in imatinib treated chronic myeloid leukemia

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Introduction: Targeting the tyrosine kinase activity of BCR-ABL represents promising therapeutic strategy in chronic myeloid leukemia (CML). Despite remarkable efficiency of the tyrosine kinase inhibitor STI571 (imatinb), resistance in some patients has been observed.
Patients and methods: Out of 34 imatinib treated CML patients at University Hospital Dubrava, five of them were showing consistently high levels of BCR-ABL transcripts monitored by real-time PCR (TaqMan technology). We screened those patients for Thr315Ile, Phe311Leu and Met351Thr point mutations using allele-specific oligonucleotide (ASO)-DNA-PCR (with sensitivity of 1/10,000 cells).
Results: Thr315Ile mutations was not detected. All patients showed Met351Thr mutation and in two of them it was present prior to imatinib therapy. One patient acquired Phe311Leu mutation during imatinib therapy.
Conclusion: Increased proportion of mutated sequences during treatment detected by ASO-PCR suggests clonal evolution of mutated cells. Our data imply that in CML patients treated with imatinib, ABL mutations are not restricted to the accelerated phase of the disease, and that, in some cases, mutations occur in chronic phase of the disease and prior to STI571 therapy.

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P08-8

Standardizacija kvantitativnog praćenja fuzijskog prijepisa bcr-abl prema međunarodnoj ljestvici

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Uvod: Najčešći oblik praćenja tijeka kronične mijeloične leukemije bila je tehnika kvalitativnog PCR-a, no pojavom PCR-a u stvarnom vremenu omogućeno je kvantitativno praćenje prijepisa bcr-abl. S obzirom da je rezultat ovisan o odabiru sondi, „kućepaziteljskog gena" te o upotrijebljenim standardima, na prijedlog European Leukemia Net, podprojekt "Molecular monitoring" pokrenuta je standardizacija kako bi se mogli uspoređivati rezultati dobiveni u različitim ustanovama.
Materijali i metode: Bilo je potrebno pripremiti izolate RNA 30 uzoraka određenog raspona prijepisa bcr-abl (0,001-10%), kvantificirati ih metodom koja se rutinski koristi u Kliničkom zavodu za laboratorijsku dijagnostiku (KZLD), te iste uzorke poslati u referentni centar Sveučilišne klinike u Mannheimu, u svrhu dobivanja faktora pretvorbe i izražavanja rezultata prema međunarodnoj ljestvici (IS). Postupak je započet primitkom 4 uzorka različitih razrjeđenja prijepisa bcr-abl iz referentnog centra u kojima je izvršena kvantifikacija u svrhu dobivanja preliminarnog faktora pretvorbe. Slijedio je postupak dobivanja konačnog faktora pretvorbe u koji je bilo uključeno 30 uzoraka bolesnika. Iz svakog je uzorka pripremljeno 6 alikvota s 2 x 10^7 leukocita. Iz 3 alikvota napravljena je kvantifikacija prijepisa bcr-abl metodom koja se rutinski koristi u laboratoriju. Preostala 3 alikvota zajedno s rezultatima poslana su u referentni centar gdje je isti postupak ponovljen.
Rezultati: Usporedbom rezultata iz oba centra dobiven je preliminarni faktor pretvorbe 0,7830, a potom i konačni faktor pretvorbe 0,3911, kojim se rezultat dobiven u KZLD pretvara u rezultat prema IS.
Zaključak: Standardizacijom je omogućeno izražavanje rezultata prema IS te usporedivost rezultata s ostalim ustanovama. Na temelju praćenja dinamike u promjeni količine prijepisa bcr-abl moguće je lakše pratiti tijek bolesti i kvalitetnije odabrati način liječenja bolesnika.

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Standardization of molecular monitoring of bcr-abl fusion transcript according to International Scale

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Introduction: The most frequent method for monitoring therapy in chronic myeloid leukemia patients was qualitative PCR. Development of quantitative real-time PCR enables nowadays relative bcr-abl quantification. The result depends on the probe and housekeeping gene selection as well as in the standards used. For this reason the subproject "Molecular Monitoring" within the European Leukemia Net intends to standardize the results obtained in different laboratories.
Materials and methods:The aim of the study was to prepare 30 RNA samples that homogenously cover a range of > 3 logs between 0.001 and 10% bcr-abl, to perform the quantification by the method routinely used in laboratory and to send the same samples to the reference laboratory of the Mannheim University Hospital in order to obtain conversion factor for expression of results according to International Scale (IS). The procedure started with the reception of 4 lysates with different dilutions of bcr-abl positive WBC in healthy donors WBC from reference laboratory.
Results: Quantification of bcr-abl was performed by the method routinely used in laboratory and the results sent to reference laboratory revealed the preliminary conversion factor of 0.7830. Next, 30 RNA samples were prepared in 6 aliquots, 2 x 10^7 WBC each. Bcr-abl quantification was performed in 3 aliquots. Other 3 aliquots together with the obtained results were sent to the reference laboratory where the same procedure was repeated. Comparison of results from both centers revealed the final conversion factor of 0.3911 that generates the results according to IS.
Conclusion: Standardization enables the expression of results according to IS and comparability of results within institutions. Monitoring of dynamics of the change in bcr-abl fusion transcript quantity enables proper disease monitoring and decision in treatment options.

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