brozija (36,3%), lijeska (29,1%), dlaka mačke (27,8%), pasji zub (26,5%) i dlaka psa (26,0%). Učestalost pozitivnih sIgE rezultata za ispitivane alergene su slijedom: D. farinae (40,0%), D. pteronyssinus (39,8%), pasji zub (32,2%), ambrozija (32,1%), lijeska (24,6%), dlaka psa (15,5%) i dlaka mačke (14,5%).

Zaključak: Na osnovi dobivenih rezultata može se zaključiti da su najtraženije pretrage sIgE za Dermatophagoides pteronyssinus i Dermatophagoides farinae. Ujedno su to i pretrage s najvećim brojem pozitivnih rezultata za specifični IgE. Da bi pregled najčešće traženih i najčešće pozitivnih alergena bio vjerodostojniji u karlovačkoj regiji, potrebno je obraditi veći broj pacijenata kroz duži vremenski period čime bi se uključio utjecaj raspodjele alergena tijekom godine.

e-pošta: adriana.bokulic@gmail.com

common ragweed (36.3%), hazel (29.1%), cat dander (27.8%), cocksfoot (26.5%) and dog dander (26.0%). The prevalence of positive results is: D. farinae (40.0%), D. pteronyssinus (39.8%), cocksfoot (32.2%), common ragweed (32.1%), hazel (24.6%), dog dander (15.5%) and cat dander (14.5%).

Conclusions: On the basis of this study, it can be concluded that the most frequently requested allergens are D. pteronyssinus and D. farinae. At the same time, D. pteronyssinus and D. farinae are tests with the highest number of positive results. For the overview of the most frequently requested and the most frequently positive allergens to be more reliable, study should include more patients over longer period of time. That way we could include influence of allergens distribution during a year in Karlovac region.

e-mail: adriana.bokulic@gmail.com

P10 – Onkologija i tumorski biljezi

P10-1 (Usmeno priopćenje)

Povezanost citosolnih koncentracije katepsina D i stope preživljenja bolesnica s primarnim rakom dojke

Jelisavac Ćosić S, Kulić A, Kovač Z, Vrbanec D, Sirotković-Skerlev M, Jakić-Razumović J

Klinički bolnički centar Zagreb, Zagreb

Uvod: Katepsin D je lizosomalna aspartat proteaza pojačano izražena u malignom tkivu. Na stanice tumora i strome katepsin D djeluje mitogeno i tako pospješuje invazivnu i metastatsku funkciju tih stanica. Cilj istraživanja je bio utvrditi povezanost koncentracija katepsina D sa stopom preživljenja bolesnica s primarnim rakom dojke. Prognostička značajnost katepsina D je bila dokazana u studiji s 2810 bolesnica ($P < 0,0001$, HR = 1,5). U studiji s 276 bolesnica značajnost je bila prisutna u skupini bolesnica koje nisu primile adjuvantnu terapiju (N = 119, $P = 0,001$, RR = 4,0), a u skupini koja je primila adjuvantnu terapiju nije bilo značajnosti (N = 52, $P > 0,05$, RR = 1,2).

Materijali i metode: U citosolima tkiva tumora 253 bolesnice oboljele od primarnog raka dojke izmjerene su koncentracije katepsina D RIA metodom. Prikupljeni su kliničko-patološki i demografski podatci. Liječnici su bili upoznati s nalazima katepsina D. Podatci o preživljenju su dobiveni od Registra za rak RH. U svrhu utvrđivanja povezanosti koncentracija katepsina D i stope preživljenja bolesnica s primarnim rakom dojke izračunate su univarijatna analiza i razlika Kaplan-Meierovih krivulja. Granična vrijednost je bila 45 pmol/mg proteina.

P10 – Oncology and tumor markers

P10-1 (Oral presentation)

Breast cancer cathepsin D expression and survival of patients

Jelisavac Ćosić S, Kulić A, Kovač Z, Vrbanec D, Sirotković-Skerlev M, Jakić-Razumović J

Zagreb University Hospital Center, Zagreb, Croatia

Background: Cathepsin D is lysosomal aspartic protease upregulated in malignant tissue. Its activity is mitogenic on cancer cells and stromal cells and it may promote metastasic and invasive properties of both types of cells. The aim of this investigation was to correlate survival rate of primary breast cancer patients and cathepsin D antigen levels. It was significant prognostic marker in study with 2810 patients ($P < 0.0001$, HR = 1.5). In study with 276 patients it was significant for patients who received no adjuvant therapy (N = 119, $P = 0.001$, RR = 4.0), contrary to group who received adjuvant therapy (N = 52, $P > 0.05$, RR = 1.2).

Materials and methods: Cathepsin D was determined by RIA in tumor tissue cytosols of 253 patients. Conventional clinicopathological and demographic parameters were gathered. The physicians were aware of cathepsin D lab report. Survival follow-up data were provided by National cancer registry office. In order to estimate relationship of survival rate and cathepsin D antigen levels, Kaplan-Meier survival curves and univariate analysis were calculated. Cut-off value was 45 pmol/mg protein.

Rezultati: Medijan vremena praćenja bolesti je bio 90 mjeseci. Univarijatna analiza i razlika Kaplan-Meier-ovih krivulja preživljenja za skupine bolesnica s visokim i niskim koncentracijama katepsina D nisu se statistički značajno razlikovale (P = 0,90, HR = 0,97, (95% CI) = 0,62-1,53). U bolesnica s metastatski negativnim limfnim čvorovima aksile rezultati su slični (P = 0,69, HR = 1,21, (95% CI) = 0,47-3,13).
Zaključak: U našem istraživanju koncentracije katepsin D nisu bile povezani sa stopom preživljenja što može biti rezultat promjene terapije bolesnicama s visokim koncentracijama katepsina D.

e-pošta: jelisavaccosic.sanda@gmail.com

Results: The median follow-up time was 90 months. Univariate analysis and Kaplan-Meier survival curves for groups of patients with high and low cathepsin D antigen levels were not significantly different (P = 0.90, HR = 0.97, (95% CI) = 0.62-1.53). In node negative group of patients the results were similar (P = 0.69, HR = 1.21, (95% CI) = 0.47-3.13).
Conclusions: In our study antigen levels of cathepsin D failed to segregate with the breast cancer patients' survival rates what could be due to altered treatment decision for patients with high cathepsin D levels.

e-mail: jelisavaccosic.sanda@gmail.com

P10-2

Uspoređivanje ukupnog i kompleksnog PSA kod bolesnika s hiperplazijom prostate

Peran N, Slavica V, Meniga D, Pamuković D, Sladić D, Martić A

Opća bolnica Šibenik, Šibenik

Uvod: PSA je glikoprotein kojeg stvaraju epitelne stanice prostate. U serumu postoje 2 glavne forme: cPSA - kompleksni PSA (60-90% tPSA) i fPSA – slobodni PSA (10-30%). Povećanu razinu tPSA osim u karcinomu prostate nalazimo i u benignim bolestima, što smanjuje njegovu specifičnost. Vrijednost tPSA manje od 4,0 ng/mL su normalne; 4,0-10,0 ng/mL granične, dok vrijednosti iznad 10,0 ng/mL mogu ukazati na karcinom prostate. Karcinom je moguć i kod tPSA 2,5-4,0 ng/mL što smanjuje osjetljivost ovog testa. Neke studije pokazuju da glavninu PSA kod pacijenata s karcinomom čini cPSA (85% tPSA) i da njegovo određivanje može povećati specifičnost i osjetljivost u odnosu na tPSA.
Materijali i metode: U ispitivanje je uključeno 146 ispitanika s hiperplazijom prostate, tPSA 2,5-15 ng/mL; 56 ispitanika tPSA 2,5-4,0 ng/mL; 71 ispitanik tPSA 4-10 ng/mL; 19 ispitanika tPSA 10-15,0 ng/mL. TPSA je određivan na Advia Centaur (Siemens) i na Axsym(Abbott) analizatorima, a cPSA na Advia Centaur-u.
Rezultati: Rezultati su prikazani u obliku Spearmanovih korelacijskih koeficijenata i medijana cPSA/tPSA omjera: r = 0,772, cPSA/tPSA omjer = 0,70 u skupini 2,5-4,0 ng/mL; r = 0,874, cPSA/tPSA omjer = 0,78 u skupini 4,0-10,0 ng/mL; r = 0,771, cPSA/tPSA omjer = 0,83 u skupini 10,0-15,0 ng/mL. Usporedbom tPSA na spomenutim aparatima dobiveni su koeficijenti: r = 0,8505 za skupinu 2,5-4,0 ng/mL; r = 0,936 za skupinu 4,0-10,0 ng/mL i r = 0,7901 za skupinu 10,0-15,0 ng/mL.
Zaključak: Rezultati su pokazali postojanje korelacije cPSA i tPSA u svim koncentracijskim područjima, poseb-

P10-2

Comparasion of total and complexer PSA in patients with prostatic hyperplasia

Peran N, Slavica V, Meniga D, Pamuković D, Sladić D, Martić A

Šibenik General hospital, Šibenik, Croatia

Introduction: Prostate Specific Antigen is a glycoprotein produced in the prostate. PSA exist in the blood in two forms: cPSA and fPSA. Increased serum PSA is found in prostate cancer, however it also increases in various benign diseases so tPSA has low specificity. Cases of CaP have been found when PSA was 2.5-4.0 ng/mL which suggest that tPSA has low sensivity. Some studies have shown that majority of tPSA in cancer patients is cPSA (about 85% of tPSA), and have demostrated that cPSA offers improved specifity and sensitivity over tPSA.
Materials and metods: 146 patients with prostatic hyperplasia and tPSA concentrations 2.5-15 ng/mL were included in the study: 56 had tPSA 2.5-4.0 ng/mL; 71 had tPSA 4.0-10 ng/mL, and 19 had tPSA 10-15 ng/mL. Total PSA was measured with both the Advia Centaur (Siemens) and Axsim (Abbott) analyzers; cPSA was measured with the Advia Centaur system.
Results: The results were presented using Spearmans correlation coeffcients and median cPSA/tPSA ratio: r = 0.772 and cPSA/tPSA = 0.70 in group 2.5-4 ng/mL; r = 0.874 and cPSA/tPSA = 0.78 in group 4.0-10.0 ng/mL; r = 0.771 and cPSA/tPSA = 0.83 in group 10.0-15.0 ng/mL. Comparison of tPSA measured with both analyzers shows a high rate of correlation in all group.
Conclusions: The results have shown the existance of corelation betwen cPSA and tPSA in all concentration ranges, especially in range 4.0-10.0 ng/mL. The biggest cPSA/tPSA ratio is in the group of 10.0-15.0 ng/mL, where is the

no u skupini od 4,0-10,0 ng/mL. Najveći omjer cPSA/tPSA je nađen u skupini od 10,0-15,0 ng/mL gdje je mogućnost prisustva karcinoma najveća. ADVIA Centaur PSA(Siemens) test pokazuje visoki stupanj korelacije s Total PSA (Abbott) testom u svim koncentracijskim područjima.

e-pošta: nena.peran1@gmail.com

P11 – Toksikologija i TDM

P11-1

Utjecaj ekstrakcije na GC/MS analizu lijekova

Mandić S[1], Nestić M[2], Mandić D[3], Horvat V[1], Debeljak Ž[1], Majetić-Cetina N[1]

[1]Odjel za kliničku laboratorijsku dijagnostiku, Klinička bolnica Osijek, Osijek
[2]Zavod za sudsku medicinu i kriminalistiku Medicinskog fakulteta Sveučilišta u Zagrebu, Zagreb
[3]Zavod za javno zdravstvo Osječko-baranjske županije, Osijek

Uvod: U kliničkoj i forenzičkoj toksikologiji često postoji potreba dokazivanja prisutnosti određenih lijekova. Koncentracije tih lijekova mogu biti različite, od vrlo niskih do vrlo visokih. Priprema uzorka za analizu, u smislu izbora metode ekstrakcije, otapala i uvjeta analize na GC/MS-u uvelike može utjecati na to koji će se lijekovi i u kojim koncentracijama moći odrediti.
Materijali i metode: Tri različita koncentracijska nivoa komercijalne serumske kontrole Abbott Immunoassay MCC (Bio Rad) koja sadrže 23 različita lijeka pripremili smo korištenjem tri najčešće metode ekstrakcije u kliničkoj i forenzičkoj toksikologiji: tekuća-tekuća ekstrakcija pomoću Toxi-Tubes A (Varian), tekuća-tekuća ekstrakcija pomoću Chem Elut (Varian) i ekstrakcija na čvrstom nosaču na Amberlite XAD-2 adsorbensima (Sigma Aldrich). Svi ekstrakti su otopljeni u kloroformu i analizirani na GC/MS (Agilent).
Rezultati: Prikazane metode ekstrakcije pokazale su različite rezultate, kvalitativno i kvantitativno. Najbolje rezultate pri najnižim koncentracijama pokazao je Toxi-Tubes A sistem (dokazano je 18 lijekova). Pri srednjim i visokim koncentracijama najbolje rezultate dao je XAD-2 (dokazano je 22 lijeka), ali samo kod ekstrakcije s 5 mL otapala, dok su kod ekstrakcije s 1 mL otapala najbolje rezultate dali Toxi-Tubes A i Chem Elut sistem (dokazano je 19 lijekova pri srednjim odnosno 20 lijekova pri visokim koncentracijama).
Zaključak: Od prikazanih metoda prividno najbolje rezultate pokazala je metoda ekstrakcije na XAD-2 adsorbensu sa 5 mL otapala, no kada se uzmu u obzir i drugi parametri kao što su utrošeno vrijeme, kemikalije i složenost postupka, najbolje rezultate dala je ekstrakcija sa Toxi-Tubes A sistemom.

e-pošta: mandic.sanja@gmail.com

high occurrence of cancer. ADVIA Centaur PSA (Siemens) showes high rate of corelation in determination of Total PSA with Axsym (Abbott) in all concentration ranges.

e-mail: nena.peran1@gmail.com

P11 – Toxicology and TDM

P11-1

Influence of extraction on GC/MS analysis of drugs

Mandić S[1], Nestić M[2], Mandić D[3], Horvat V[1], Debeljak Ž[1], Majetić-Cetina N[1]

[1]Department for Clinical Laboratory Diagnostics, Osijek University Hospital, Osijek, Croatia
[2]Department of Forensic Medicine and Criminology, School of Medicine, University of Zagreb, Zagreb, Croatia
[3]Institute of Public Health of Osijek-Baranya County, Osijek, Croatia

Introduction: In clinical and forensic toxicology there is often need for confirmation of certain types of drugs. Their concentrations can vary, from very low to very high levels. Sample preparation, in terms of choice of extraction technique, solvent and chromatographic conditions of analysis can have great influence (qualitative and quantitative) on results.
Materials and methods: We have prepared three different concentration levels of commercial serum control Abbott Immunoassay MCC (Bio Rad), which comprises 23 different drugs, with three most common extraction techniques in forensic and clinical toxicology: liquid-liquid extraction with Toxi-Tubes A (Varian), liquid-liquid extraction with Chem Elut (Varian) and solid phase extraction on Amberlite XAD-2 (Sigma Aldrich). All extracts were reconstituted in chloroform and analyzed in GC/MS (Agilent).
Results: Those three extraction methods showed different results, both qualitatively and quantitatively. Best results at low concentrations levels showed Toxi-Tubes A system (18 drugs confirmed). At medium and high concentrations levels best results were obtained with XAD-2 (22 drugs confirmed), but only when we used 5 mL of solvent. With 1 mL of solvent, best results were obtained with Toxi-Tubes A and Chem Elut systems (19 drugs at medium and 20 drugs at high concentration levels confirmed).
Conclusion: Best results amongst displayed methods showed XAD-2, but when we take into consideration other parameters such as time consumed, amount of chemicals and complexity of procedure, best results were obtained with TOXI-TUBES A system.

e-mail: mandic.sanja@gmail.com