## Project summary

The main goal of the project is to investigate different biomolecules involved in lipoprotein metabolism, oxidative stress/antioxidative defense and inflammation in atherosclerosis-related and other diseases. We expect that the results of the proposed studies would improve the understanding of the roles of those molecules as risk factors and markers in several ways. (1) Firstly, the part of our research that is dedicated to the optimization and evaluation of laboratory methods for qualitative and quantitative investigations could provide improved analytical methods, and therefore valid results. (2) We planned to study different biomolecules involved in lipoprotein metabolism, oxidative stress/antioxidative defence and inflammation, as risk factors and biomarkers: standard lipid status parameters, lipoprotein(a), apo(a) size polymorphism, qualitative characteristics and concentrations of LDL and HDL subfractions, plasma markers of cholesterol synthesis (desmosterol, lathosterol) and absorption (campesterol, sitosterol) PCSK9; redox balance markers; activities and concentrations of SOD and PON1and other enzymes important i lipoprotein metabolisim (LCAT and CETP); inflammatory status markers, such as hsCRP, fibrinogen, serum amyloid A (SAA), lipoprotein associated phospholipase A2, pentraxin 3, adhesion molecules, and other markers significant for the diseases under investigation, such as adipokines (leptin, adiponectin, resistin), other cytokines, etc. Using real-time PCR method gene expression and genetic polymorphism is analysed for biomolecules associated with the previously mentioned processes. (3) As cardiovascular, cerebrovascular, renal, pulmonary diseases and cancer result from complex interactions between multiple genes and environmental factors, factor analysis could be a useful tool for defining relationships between variables related to underlying pathophysiological mechanisms. This approach may increase the understanding of the contributory role of the various molecules involved in lipid metabolism, oxidative stress, and inflammation. A better understanding of the mechanisms involved in the apparently complex etiopathogenesis of those diseases could allow not only for earlier diagnosis but also the development of therapeutic agents that can favorably alter the course of the disease before the development of permanent changes. (4) In addition to research focused on clinically significant atherosclerosis, we planned to examine the significance of lipid, oxidative and inflammatory parameters in subclinical disease among asymptomatic individuals, including obese children and young adults at increased risk. We also plan to examine age and gender-related differences in the occurrence, manifestations, and outcomes of atherosclerosis-related diseases, which probably reflect different pathogenetic mechanisms. A special aim of our project dedicated to women’s health was accomplished by investigating the impact of pregnancy on novel cardiovascular risk factors. (5) Targeting towards improved patient care, we have focused on the identification and validation of clinical performances of novel biomarkers which would assist diagnosis or disease monitoring. In order to achieve this goal, we shall evaluate the clinical accuracies of biomarkers associated with lipid, oxidative and inflammatory pathways in the clinical setting. ROC-curve analysis could give insight into the clinical usefulness of the markers and their abilities to discriminate between alternative states of health and enable comparisons of clinical performances of different markers. (6) Finally, it is not enough for a novel biomarker to be clinically useful; it also has to be cost-effective. Therefore, economic evaluation of novel markers was also one of the goals of our investigations. The results of the cost-effectiveness analysis (CEA) could be used as a basis for designing profiles of biochemical and genetic tests that can give sufficient and clinically relevant information at a rational cost. (7) The additional aim of the project was its contribution to the preservation of the body of young scientists and their development: a number of young researchers, students of doctoral studies were included in our project, and their research within the project served as a basis for the preparation of their doctoral dissertations. (8) One of our important goals is to strengthen our international cooperation and to participate in international scientific projects, in order to further improve the quality of the project team research.

Keywords: atherosclerosis; cardiovascular risk; inflammation; LDL and HDL subclasses; oxidative stress; antioxidative defence; gene expression; obesity; ROC-curve; cost- effectiveness analysis.

## Sažetak projekta OI 175035

Osnovni cilj projekta je ispitivanje različitih biomolekula uključenih u metabolizam lipoproteina, oksidativni stres/antioksidativnu zaštitu i inflamaciju u bolestima povezanim sa aterosklerozom i drugim oboljenjima. Rezultati ovih istraživanja doprinose boljem razumevanju uloge ispitivanih molekula kao faktora rizika, i to na više načina. (1) Pre svega, deo naših istraživanja koji je posvećen optimizaciji i validaciji laboratorijskih metoda za kvantitativna i kvalitativna ispitivanja, usmeren je na poboljšanje analitičkih metoda i obezbeđenje validnost rezultata daljih istraživanja. (2) Istraživanja obuhvataju analizu različitih biomolekula koji učestvuju u metabolizmu lipoproteina, oksidativnom stresu/antioksidativnoj zaštiti i inflamaciji. Ova istraživanja obuhvataju pored standardnih parametara lipidnog statusa i novije parametre: lipoprotein (a), polimorfizam veličine apo(a), ispitivanje kvalitativnih i kvantititativnih svojstava LDL i HDL subfrakcija, određivanje plazmatskih markera sinteze (desmosterola i latosterola) i apsorpcije holesterola (kampesterola i sitosterola), PCSK9; parametre redoks-statusa, enzimskih aktivnosti i koncentracija SOD i PON1i drugih enzima koji sudeluju u metabolizmu lipoproteina LCAT i CETP; markere inflamacije, kao što su hsCRP, fibrinogen, serumski amiloid a (SAA), lipoproteinska fosfolipaza A2, pentraksin 3, adhezioni molekuli, i druge markere od značaja za ispitivane bolesti, kao što su adipokini (leptin, adiponektin, rezistin), drugi citokini, itd. Primenom real-time PCR metode ispituje se genska ekspresija i genski polimorfizam ispitivanih biomolekula povezanih sa napred navedenim procesima (3) Obzirom da kardiovaskularne, cerebrovaskularne, bubrežne, plućne bolesti i kancer nastaju kao rezultat složenih interakcija između različitih gena i faktora sredine, faktorska analiza može poslužiti kao koristan alat za razjašnjavanje veza između ispitivanih varijabli, grupisanih u klastere zavisno od patofizioloških mehanizama sa kojima su povezane. Ovakav pristup olakšava razjašnjavanje uloga ispitivanih biomolekula u metabolizmu lipida, oksidativnom stresu i inflamaciji. Bolje poznavanje mehanizama koji učestvuju u složenoj etiopatogenezi ispitivanih bolesti može da omogući ne samo raniju dijagnozu, već i razvoj lekova koji bi mogli da se primene pre nego što dođe do ireverzibilnih promena. (4) Pored istraživanja usmerenih na klinički značajnu aterosklerozu, planirano je i ispitivanje značaja lipidnih, oksidativnih i inflamatornih parametara u subkliničkoj fazi bolesti, kod asimptomatskih ispitanika, uključujući gojaznu decu i mlade sa povećanim rizikom. Naša istraživanja su takođe usmerena i na specifične karakteristike zavisne od pola i starosti koje se odnose na pojavu, manifestacije i ishod ateroskleroze i drugih bolesti, a verovatno proizilaze iz različitih patogenetskih mehanizama. Poseban cilj našeg projekta, posvećen zdravlju žena, je ispitivanje uticaja trudnoće na novije kardiovaskularne faktore rizika. (5) U cilju unapređenja zdravstvene zaštite, deo naših istraživanja usmeren je na validaciju kliničkih performansi novijih biomarkera koji bi mogli da se primene u dijagnostici i praćenju toka bolesti i terapije. Ispitivanje kliničke tačnosti lipidnih, oksidativnih i inflamatornih markera ROC-analizom omogućuje realnu procenu njihovih sposobnosti da diskriminišu između alternativnih stanja zdravlja, kao i međusobno poređenje performansi različitih markera. (6) Konačno, pored dobrih kliničkih performansi, potrebno je da primena novih biomarkera bude i ekonomski isplativa. Na osnovu rezultata dobijenih cost-effectiveness analizom (CEA), moguće je definisati profile biohemijskih i genetičkih testova koji mogu da obezbede klinički relevantne informacije uz racionalan trošak. (7) Dodatan cilj našeg projekta je i doprinos očuvanju i stvaranju naučnog podmlatka: u tom cilju, u rad na projektu uključili smo i mlade istraživače, čije je učešće u istraživanjima u okviru projekta bilo u funkciji izrade njihovih doktorskih disertacija. (8) Jedan od naših značajnih ciljeva je dalje jačanje uspostavljene međunarodne saradnje i učešće tima u međunarodnim naučnim projektima, što bi svakako doprinelo podizanju kvaliteta naučnog rada projektnog tima.

Ključne reči: ateroskleroza; kardiovaskularni rizik; inflamacija; sufrakcije LDL i HDL; oksidativni stres; antioksidativna zaštita, genska ekspresija; gojaznost; ROC-kriva; cost-effectiveness analiza

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