S08 – Laboratorijska koagulacija

S08-1

Faktor XIII – najnovije spoznaje

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Faktor XIII (FXIII) kruži u plazmi kao tetramerički zimogen (pFXIII; FXIII-A2B2). Njegova potencijalno aktivna podjedinica A (FXIII-A) je sintetizirana u stanicama koje potječu iz koštane srži; dakle, prisutan je u trombocitima i monocitima/makrofazima u obliku dimera (cFXIII; FXIII-A2). U plazmi ima viška zaštitne podjedinice B (FXIII-B). Trombin i kalcijevi ioni pretvaraju FXIII u aktivni oblik - transglutaminazu (FXIIIa) u završnoj fazi koagulacijske kaskade. Glavna funkcija FXIIIa je umrežiti lance fibrina i inhibitora plazmina a2 u fibrin glutaminil-lizil izopeptidnim vezama. Na taj način mehanički učvršćuje fibrin i štiti od fibrinolize. FXIII je višefunkcionalni protein koji ja također ključan za održavanje trudnoće i igra važnu ulogu u zacjeljivanju rana i angiogenezi.

Nasljeđen nedostatak FXIII klasificira se kao nedostatak FXIII-A i FXIII-B. Nedostatak FXIII-A je rijedak poremećaj (1:2.000.000), no uzrokuje ozbiljnu hemoragičnu dijatezu. Karakteristično je odgođeno krvarenje iz pupčane vrpce te kod bolesnika koji ne primaju nadomjesnu terapiju dolazi vrlo često do subkutanog, intramuskularnog i intrakranijalnog krvarenja. Usporeno zacjeljivanje rana i spontani pobačaji kod žena također su značajke nedostatka FXIII-A. Puno je češći tip 1 nedostatka (niska aktivnost i antigen) nego tip 2 (niska aktivnost s normalnom ili umjereno sniženom koncentracijom antigena). Nedostatak FXIII-B o kojem se rijetko izvještava ima za rezultat blagu dijatezu. Opisani su različiti oblici ozbiljnog oblika stečenog nedostatka uslijed neutraliziranih i ne-neutraliziranih antitijela na FXIII-A. Značajan broj bolesnika s antitijelima na FXIII boluje od autoimunih bolesti. Danas je dostupan virusno inaktivirani koncentrat pFXIII za liječenje i prevenciju nedostatka FXIII.

Kvantitativna metoda određivanja aktivnosti FXIII treba se rabiti kao metoda probira prvog izbora za dijagnozu nedostatka FXIII. Tradicionalna kvalitativna metoda rastvaranja ugrušaka (engl. *clot solubly assay*) je zastarjela te se ne bi trebala više rabiti kao metoda probira. Kvantitativne metode za FXIII temelje se na dva principa: 1) mjerenje koncentracije amonijaka ispuštenog tijkom reakcije transglutaminaze i 2) primjena testova koji u reakciji koriste obilježene aminske supstrate (inkorporacijski testovi). Prva metoda je lagana za provedbu, brza kinetička metoda, dok je druga metoda osjetljivija i zahtjeva više vremena.

S08 – Laboratory coagulation

S08-1

Factor XIII – the state of the art

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Factor XIII (FXIII) circulates in the plasma as a tetrameric zymogen (pFXIII; FXIII-A2B2). Its potentially active A subunit (FXIII-A) is synthesized in cells of bone marrow origin; it is also present in platelets and monocytes/macrophages in dimeric form (cFXIII; FXIII-A2). In the plasma the protective/carrier B subunit (FXIII-B) is in excess. pFXIII is converted into an active transglutaminase (FXIIIa) by thrombin and Ca2+ in the terminal phase of clotting cascade. The main function of FXIIIa is to cross-link fibrin chains and a2 plasmin inhibitor to fibrin through glutaminyl-lysyl isopeptide bonds. This way it mechanically stabilizes fibrin and protects from fibrinolysis. FXIII, a multifunctional protein, is also essential for maintaining pregnancy and plays an important role in wound healing and angiogenesis.

Inherited FXIII deficiencies are classified as FXIII-A and FXIII-B deficiencies. FXIII-A deficiency is a rare (1:2,000,000), but severe hemorrhagic diathesis. Delayed umbilical stump bleeding is characteristic and in non-supplemented patients subcutaneous, intramuscular and intracranial bleeding occurs with relatively high frequency. Impaired wound healing and spontaneous abortion in women also are features of FXIII-A deficiency. Type 1 deficiency (low activity and antigen) is much more frequent than type 2 (low activity with normal or moderately decreased antigen concentration). The rarely reported FXIII-B deficiency results in mild bleeding diathesis. Various forms of severe acquired deficiencies due to neutralizing or non-neutralizing auto-antibody against FXIII-A have been described. A significant portion of patients with an autoantibody against FXIII suffers from autoimmune disease. Virus inactivated pFXIII concentrate is now available for treatment and prophylaxis of FXIII deficiency.

A quantitative FXIII activity assay is to be used as first line (screening) test for the diagnosis of FXIII deficiency. The traditional qualitative clot solubility assay is now obsolete and should not be used as screening test. Quantitative FXIII assays are based on two principles: 1) measurement of ammonia released during the transglutaminase reaction, 2) the incorporation of labeled substrate amine into a glutamine donor substrate protein. The former methods are easy to perform, quick kinetic assays, while the latter ones are more sensitive, but time-consuming labo-

Za klasifikaciju određivanja FXIII-A2B2 i FXIII predlaže se određivanje koncentracije antigena u podjedinici u plazmi i FXIII-A u trombocitima. Mješovita istraživanja i vezane metode koriste se za otkrivanje neutraliziranih i neneutraliziranih autoantitijela na podjedinice FXIII.
Uloga FXIII kod trombotičkih bolesti istražuje se prema dva aspekta: 1) povišena koncentracija FXIII pokazuje se kao čimbenik rizika infarkta miokarda i bolesti perifernih arterija kod žena. 2) tijekom zadnjih desetak godina intenzivno se radilo na istraživanju polimorfizma Val34Leu u genu FXIII-A. Ubrzana je proteolitička aktivacija varijante LEU34 FXIII-A te polimorfizam utječe i na strukturu fibrina. Iako postoje proturječni rezultati, meta-analiza istraživanja o kojima izvještavamo pokazala je zaštitnički učinak polimorfizma Val34Leu u genu FXIII-A protiv venske tromboze i protiv koronarne arterijske bolesti. Interakcije između dva gena te gena i okoline kao inzulinska rezistencija ili koncentracija fibrinogena značajno modificiraju učinak polimorfizma.

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rious methods. For classification determination of FXIII-A2B2 and FXIII subunit antigen in plasma and FXIII-A in platelets are recommended. Mixing studies and binding assays are used to detect neutralizing and non-neutralizing autoantibodies against FXIII subunits.
The involvement of FXIII in thrombotic diseases has been investigated in two aspects. 1) Elevated FXIII level has been shown to be a risk factor of myocardial infarction and peripheral artery disease in women. 2) During the last decade a common polymorphism in the FXIII-A gene, resulting in Val34Leu replacement, has been intensively investigated in this respect. The proteolytic activation of the Leu34 FXIII-A variant is accelerated and the polymorphism also influences the structure of fibrin. Although there are contradictory results, meta-analysis of the reported studies demonstrated the protective effect of FXIII-A Val34Leu polymorphism against venous thrombosis and against coronary artery disease. Gene-gene and gene-environment interactions, like insulin resistance or fibrinogen concentration, considerably modify the effect off the polymorphism.

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S08-2

Povezanost genotipa i fenotipa u von Willebrandovoj bolesti

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Von Willebrandova bolest (vWb) je najčešći nasljedni poremećaj sa sklonošću krvarenjem koji nastaje uslijed kvantitativnog (tipovi 1 i 3) ili kvalitativnog manjka von Willebrandova faktora (vWF). vW bolest se nasljeđuje autosomno dominantno li recesivno, no žene s blagim oblikom bolesti imaju više simptoma. Objavljena prevalencija vWb u epidemiološkim studijama je do 1% u općoj populaciji iako otprilike 1 u 10.000 osoba ima klinički značajno krvarenje. vWF je veliki glikoprotein koji je bitan u primarnoj hemostazi koja je ovisna o trombocitima, osobito u mikrovaskulaturi. vWF je nositelj prokoagulatnog faktora VIII i štiti ga od proteolitičke degradacije i transportira ga na mjesto ozljede vaskulature. Gen za vWF je lociran na kratkom kraku kromosoma 12 i to 12p13.3.
vW bolest je klasificirana u podskupine: tip 1 - djelomičan manjak vWF, tip 2 - kvalitativni poremećaj i tip 3 - gotovo kompletan nedostatak vWF. Tip 2 se dalje dijeli u četiri kategorije sa specifičnim funkcionalnim defektima: tip 2A

S08-2

von Willebrand disease – link between genotype and phenotype

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Von Willebrand disease (vWd) is the most common inherited bleeding disorder and is due to quantitative (types 1 and 3) or qualitative (type 2) defects of von Willebrand factor (vWF). VW disease is inherited by autosomal dominant or recessive patterns, but women with mild forms are more symptomatic. Reported prevalence of vW disease derived from epidemiological studies of up to 1% in the general population although only approximately 1 in 10,000 individuals has clinically significant bleeding. vW factor is a large glycoprotein which is essential to platelet-dependent primary haemostasis, particularly in the microvasculature where high fluid shear forces are present. vWF also acts as a carrier for procoagulant factor VIII (FVIII) in the circulation, protecting FVIII from proteolytic degradation and transporting it to the site of vascular injury. The VWF gene is located on the short arm of chromosome 12 at 12 p13.3. VWD is classified into three main sub-types: Type 1 – a partial quantitative deficiency of

pokazuje selektivni gubitak vrlo visokih multimera vWF (HMW) i povezano sniženu interakciju trombocita i vWF. Tip 2B je povezan s povećanom afinitetom vWF za trombocitni glikoprotein Ib; tip 2M je povezan uzorkom multimera kao u normalne osobe no postoji selektivno snižena interakcija vWF i trombocita. Veliki je broj otkrivenih mutacija i karakteriziran u vW bolesti.
Početna dijagnoza vW bolesti zasnovana je na kliničkoj i fenotipskoj informaciji. Genetsko ispitivanje ima ulogu u diferencijalnoj dijagnozi bolesti, ispitivanju obitelji i savjetovanju što je od osobite vrijednosti u tipu 3 vW bolesti. Genetsko ispitivanje ima dodatnu ulogu u diferencijalnoj dijagnozi razlikujući vW bolest tip 2 N i blage hemofilije.
Identifikacija i genetska osnova poremećaja informira nas o izboru liječenja i pojašnjava mehanizam pojedinih podtipova vW bolesti. Očito je da genetsko testiranje ima ograničenu vrijednost u slučajevima gdje fenotip jasno otkriva podtip vW bolesti.
Karakterizacija fenotipa i identifikacija mutacija u genu vWF u bolesnika s vW bolesti doprinosi razumijevanju genetike i biokemije vWF i vW bolesti.

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VWF, type 2 – a qualitative abnormality of VWF, and type 3 – a virtually complete absence of VWF. Type 2 is further divided into four sub-categories with specific functional defects: type 2A shows a selective loss of high molecular weight (HMW) vWF multimers and an associated decrease in platelet-vWF interaction; Type 2B is associated with and increased affinity of VWF for platelet glycoprotein Ib; type 2M is associated with a VWF multimers pattern that is similar to normal, but with reduced vWF-platelet interaction. An enormous diversity of mutations has been characterized in VWD.
The initial diagnosis of vW disease is based on clinical and phenotypic information. Genetic analysis may provide information to confirm or support the initial diagnosis and additionally to permit family studies and counseling which are of particular value in type 3 vWD. Genetic investigation also plays a role in differential diagnosis. Genetic investigation also plays a role in differential diagnosis, distinguishing vWD type 2N and mild hemophilia.
Identification of the genetic basis of the disorder may inform treatment choices and improve our understanding of the mechanism underlying different vWD subtypes. It is apparent that genetic testing is likely to have limited clinical utility in cases where the phenotype clearly reveals the VWD sub-type.
Characterization of the phenotype and identification of mutations in VWF gene in patients with vW disease contribute of understanding of the genetics and biochemistry of VWF and VWD.

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S08-3

Hemostatski sustav kod djece

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Značajke hemostatskog sustava kod djece nisu bile u potpunosti poznate dugi niz godina ponajprije zbog tehničkih poteškoća s uzorkovanjem u male djece te potrebe za relativno velikom količinom uzorka krvi za analizu. Najveći doprinos razumijevanju hemostatskog sustava kod djece može se pripisati Maureen Andrew, koja je prva objavila referentne intervale 30-tak hemostatskih parametara za djecu prema dobi, zasebno za nedonoščad, zdravu novorođenčad te djecu 1-16 godina starosti. Spoznaja o različitim vrijednostima velikog broja hemostatskih parametara između djece i odraslih, kao i o njihovoj promjeni

S08-3

Hemostatic system in children

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For many years, understanding and characterization of hemostatic system in children has lagged behind those of adults, primarily due to technical difficulties in blood sampling in small children and the requirement of large amounts of blood volume. The greatest contribution in this field has been done by Maureen Andrew who first published age-dependent reference intervals for premature infants, healthy full-term newborns, and children 1-16 years of age. The knowledge that the levels of multiple hemostatic parameters differ between children and

tijekom djetinjstva postavilo je temelj koncepta razvojne hemostaze.

Hemostaza je dinamičan proces koji započinje in-utero. Fetalna jetra započinje sintezu fibrinogena u šestom gestacijskom tjednu, dok se zgrušavanje krvi i fibrinolitička aktivnost mogu opaziti u jedanaestom gestacijskom tjednu. Fiziološke koncentracije koagulacijskih proteina postupno rastu tijekom fetalnog i neonatalnog života. Molekularne strukture većine fetalnih koagulacijskih proteina su istovjetne zrelim strukturama, osim fibrinogena i plazminogena koje sadrže veći udjel sijalinske kiseline i von Willebrandovog faktora (VWF) koji se sastoji od multimera izrazito velike molekularne mase. Promjena fetalnog VWF u adultni oblik odvija se tijekom prvih tjedana postnatalno.

U novorođenčadi plazmatske koncentracije faktora ovisnih o vitaminu K (II, VII, IX, X) i kontaktnih faktora (XI, XII prekalikreina, kininogena velike molekularne mase) su snižene i iznose približno 50% vrijednosti u odraslih. Postupan se porast koncentracija odvija tijekom prvih 6 mjeseci života te, iako dostiže približno 80% vrijednosti u odraslih, ostaju snižene tijekom djetinjstva. Plazmatske vrijednosti gotovo svih inhibitora zgrušavanja su snižene u odnosu na odrasle te iznose približno 20% za inhibitor puta tkivnog faktora, 35% za protein C (PC) i protein S (PS), te 50% vrijednosti za antitrombin i heparinski kofaktor II. Iako se vrijednosti odraslih dostižu tijekom 6 mjeseci života, koncentracije PC ostaju snižene tijekom djetinjstva sve do mladenačke dobi. Nadalje, u novorođenčadi se PS nalazi isključivo u slobodnom, aktivnom obliku zbog nedostatka veznog proteina C4b.

U novorođenčadi su plazmatske koncentracije samo malog broja faktora zgrušavanja (V, VIII, XIII i fibrinogena) približno slične vrijednostima u odraslih. Za razliku od toga, vrijednosti VWF su povišene tijekom prva 2 mjeseca života, dok su vrijednosti alfa 2 makroglobulina približno dvaput više u odnosu na vrijednosti u odraslih te ostaju povišene tijekom djetinjstva.

Fibrinolitički sustav u djece je razvojan proces sličan sustavu zgrušavanja. Vrijednosti plazminogena su snižene na 50% vrijednosti, a vrijednosti inhibitora plazmina na 80% vrijednosti u odraslih sve do 6 mjeseci života. Plazmatske koncentracije antigene komponente tkivnog aktivatora plazminogena značajno su povišene neposredno nakon rođenja, progresivno se smanjuju nakon prvog dana života te ostaju snižene tijekom djetinjstva za približno 50% u odnosu na odrasle. Za razliku od toga, plazmatske koncentracije antigene komponente inhibitora aktivatora plazminogena 1 značajno su povišene nakon rođenja te ostaju povišene tijekom djetinjstva. Nezrelost hemostatskog sustava ima za posljedicu smanjeno stvaranje trombina kao i smanjenu fibrinolitičku aktivnost tijekom djetinjstva u odnosu na odrasle.

adults, and vary over the pediatric age range, has led to introduction of the.concept of developmental hemostasis.

Hemostasis is a dynamic process which begins in-utero. Fetal liver begins to synthesize fibrinogen at 5.5 weeks of gestation whereas blood clotting and fibrinolytic activity can be detected at 11 weeks of gestation. Physiologic concentrations of coagulation proteins gradually increase during fetal and neonatal life. The molecular structure of the majority of fetal coagulation proteins is identical to adult structures, except fibrinogen and plasminogen which contain increased amounts of sialic acid and von Willebrand factor (VWF) which is made up of ultra-large molecular weight multimers and changes to the adult plasma form postnatally.

In newborns, plasma concentration of vitamin K-dependent proteins (II, VII, IX, X) and contact factors (XI, XII prekallikrein, high molecular weight kininogen) are approximately 50% of adult values, increase gradually during the first 6 months of life to about 80% of adult normal values, but remain decreased throughout childhood. Furthermore plasma levels of almost all coagulation inhibitors are reduced, from approximately 20% for tissue factor pathway inhibitor, 35% for protein C (PC) and protein S (PS) to 50% of adult levels for antithrombin and heparin cofactor II. Although adult levels are usually reached by 6 months of age, plasma concentrations of PC remain decreased through childhood until adolescence. In addition PS circulates in newborns only in its free active form due to the absence of C4b binding protein.

Plasma concentrations of coagulation factors V, VIII, XIII and fibrinogen are close to adult values. Moreover, the levels of VWF are increased through the first 2 months of life, while the levels of alpha 2 macroglobulin that are nearly 2-fold higher than adult values, remain increased through childhood.

Fibrinolytic system in children is an evolving age-dependent process similar to coagulation system. Plasminogen levels are decreased to 50% and plasmin inhibitor to 80% of adult values until 6 months of age. Plasma concentrations of tissue-type plasminogen activator antigen are significantly increased at birth, progressively decrease after the first day of life, and remain decreased at approximately 50% of adult values throughout childhood. On the contrary, concentrations of plasminogen activator inhibitor-1 antigen at birth and throughout childhood are significantly increased compared to adult values. Therefore, the immaturity of hemostatic system in newborns results in decreased thrombin generation and hipofibrinolytic activity during childhood.

Since hemostatic system in children is profoundly different compared to adults, the knowledge of developmental hemostasis and applying age-dependent reference ran-

S obzirom na značajne razlike hemostatskog sustava kod djece u odnosu na odrasle, za ispravno postavljanje dijagnoze hemostatskih poremećaja kod djece te optimalnu prevenciju i liječenje, neophodna je uz poznavanje značajki razvojne hemostaze, primjena referentnih intervala prema dobi.

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ges are essential for diagnosis, optimal prevention and treatment of hemostatic disorders in children.

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S08-4

Procjena kvalitete koagulacijskih analiza i programi vanjske procjene kvalitete

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Kontrola kvalitete je sveobuhvatni pojam koji opisuje sve poduzete mjere koje osiguravaju pouzdanost laboratorijskih pretraga i nalaza. Unutarnja kontrola kvalitete (engl. *internal quality control*, IQC) i vanjska procjena kvalitete rada (engl. *external quality assessment*, EQA) dvije su različite, no vrlo komplementarne sastavnice programa uspostavljanja kontrole kvalitete rada laboratorija. IQC se primjenjuje kako bi se ustanovilo provode li se brojne tehnike i procesi konzistentno tijekom određenog vremenskog razdoblja i na taj se način garantira dosljednost laboratorija iz dana u dan. EQA se provodi kako bi se otkrio stupanj podudarnosti između laboratorija i ostalih centara. U većim programima EQA kao što je npr. britanski NEQAS (UK NEQAS) za laboratorijsku koagulaciju s više od 1.000 registriranih centara za laboratorijske programe i više od 2.000 centara za program njege uz bolesnika retrospektivnom analizom rezultata dobivenih od uključenih laboratorija mogu se otkriti ne samo laboratoriji s niskom kvalitetom rada, već i reagensi te metode koji daju rezultate koji su nepouzdani te navode na pogrešan zaključak.
Kako bi se odredila kvaliteta rada laboratorija, za svaki se testni uzorak moraju odrediti ciljne vrijednosti. Postoji nekoliko referentnih metoda u hemostazi i teško je odrediti ekspertne laboratorije za velik broj metoda i tehnika koje se primjenjuju za svaku pretragu. Prema tome, UK NEQAS za laboratorijsku koagulaciju kao ciljnu vrijednost koristi medijan vrijednosti svih rezultata pristiglih od sudionika. Za testove probira, u kojima se koriste reagensi različite osjetljivosti, određuje se medijan specifičan za svaki pojedini reagens.
Programi EQA za laboratorijsku koagulaciju organizirani su u velikom broju država i kod tih se programa uzorci distribuiraju periodično ili po ciklusima centrima koji sudjeluju. Individualni programi variraju prema broju ponuđenih

S08-4

Quality assessment of haemostatic assays and EQA schemes

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Quality assurance may be used as an overall term to describe all measures that are taken to ensure the reliability of laboratory testing and reporting. Internal quality control (IQC) and external quality assessment (EQA) are two distinct, yet complementary components of a laboratory quality assurance programme. IQC is used to establish whether a series of techniques and procedures are performing consistently over a period of time, therefore ensuring day to day laboratory consistency. EQA is used to identify the degree of agreement between one laboratory's results and those obtained by other centres. In larger EQA schemes, such as UK NEQAS for Blood Coagulation with over 1000 registered centres for the laboratory based programme and over 2000 centres for the Point of Care Testing (POCT) programme, retrospective analysis of results obtained by participating laboratories permits the identification, not only of poor individual laboratory performance, but also those reagents and methods that produce unreliable or misleading results.
In order to determine laboratory performance, target values must be established for each test sample. There are few established reference methods in haemostasis, and it is difficult to identify expert laboratories for the wide range of methods and techniques used for each test. Consequently, UK NEQAS for Blood Coagulation uses the median value of all results returned by participants as the target value. For screening tests, where different reagent sensitivities are apparent, reagent specific median values are determined.
EQA programmes for Blood Coagulation have been established in a number of countries and in all of these programmes, samples are distributed to participating centres on a periodic or cyclical basis. Individual programmes vary considerably in the numbers of tests or

pretraga i analita, registriranih sudionika, izvoru uzoraka i učestalosti provedbe modula. Opće je prihvaćeno da što je baza podataka sudionika veća, to je bolja usporedba između grupa reagensa, odnosno između analiza rezultata pristiglih od sudionika. Opseg tih usporedbi koje provodi UK NEQAS za laboratorijsku koagulaciju je vjerojatno veći od opsega ostalih programa EQA za laboratorijsku koagulaciju, zbog obuhvaćanja barem 30 različitih pretraga.
Primarna funkcija EQA je ispitivanje znanja i vještina pojedinih laboratorija. To bi trebalo uključivati sve aspekte koagulacije i ponuđeni analiti bi trebali predstvljati najnovije spoznaje struke. Sukladno njihovoj primarnoj funkciji, dobro organizirani programi EQA mogu pružiti infomaciju o relativnim značajkama analitičkih procesa uključujući princip metode, reagense i instrumente. Programi mogu također otkriti i varijabilnost rezultata koja ovisi o metodi, temu o kojoj ćemo nešto više reći u ovom predavanju.
Tajnost podataka važna je značajka programa UK NEQA i informacije o radu pojedinih laboratorija ne smiju se otkrivati nikome osim ovlaštenog voditelja odjela ili njegovog zamjenika.
Poboljšan rad laboratorija nedvojbeno je vezan uz njegovo sudjelovanje u programima EQA. To se ne primjećuje samo u cjelokupnom radu svih laboratorija uključenih u program, čemu svjedoči smanjenje varijabilnosti rezultata između laboratorija, već i kod pojedinačnih laboratorija.

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analytes offered, registered participants, the source of samples and frequency of distribution. It is generally accepted that the larger the participant database, the more robust may be the comparisons between reagent groups, and therefore the analysis of results for individual participants. The scope of these comparisons by UK NEQAS for Blood Coagulation is possibly wider than many other Blood Coagulation EQA schemes, due to the inclusion of at least 30 different tests in the laboratory based programme alone.
The primary function of EQA is proficiency testing of individual laboratories. This should include all aspects of coagulation and analytes offered should represent the current "state of the art". In conjunction with their primary function, well established EQA programmes can also provide information concerning the relative performance of analytical procedures, including the method principle, reagents and instruments. EQA schemes may also identify method-dependent variability of results, a point that will be covered in this presentation.
Confidentiality is an important feature of UK NEQA Schemes, and information regarding individual laboratory performance is not divulged to anyone other than the nominated head of the department or their deputy.
Improved performance has been clearly linked to ongoing participation by a laboratory in EQA programmes. This has been seen not only in overall performance, evidenced by a reduction of the variability of results between laboratories, but also in respect of individual laboratories.

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S08-5

Poremećaji hemostaze u pretilosti

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S08-5

Disorders of hemostasis in obesity

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Pretilost je poznati metabolički čimbenik rizika za nastanak ateroskleroze, kardiovaskularne bolesti (CVD) i posljedične arterijske tromboze. Patofiziologija arterijske tromboze u CVD je složena i multičimbenična, a u njenom nastanku i progresiji hemostatski sustav ima važnu ulogu.
Rezultati dosadašnjih ispitivanja pružaju sve više podataka o prokoagulantnom (protrombotičkom) stanju koje je posljedica poremećaja hemostatskog sustava u pretilih osoba. Poremećaji hemostatskog sustava sinergističkim međudjelovanjem s drugim metaboličkim čimbenicima rizika (inzulinska rezistencija, hiperinzulinemija, dislipide-

Obesity is a known metabolic risk factor for atherosclerosis, cardiovascular disease (CVD) and consequent arterial thrombosis. Pathophysiology of arterial thrombosis in CVD is complex and multifactorial, in whose development and progression the hemostatic system has an important role.
The results of investigations to date provide more and more data on procoagulant (prothrombotic) state that is a consequence of disorders in hemostatic system in obese subjects. Disorders of hemostatic system by synergistic interactions with other metabolic risk factors (insulin

mija, hipertenzija), često prisutnim u pretilih osoba, dodatno doprinose povećanju sveukupnog rizika za CVD. Povezanost metaboličkih (aterosklerotskih) i protrombotičkih čimbenika u nastanku i progresiji kardiovaskularne bolesti uvelike objašnjava složene patofiziološke procese koji dovode do stvaranja aterosklerotskog plaka, rupture plaka i posljedične arterijske tromboze.

Poremećaji sustava zgrušavanja i fibrinolize u pretilih osoba uključuju sve sastavnice hemostatskog sustava: disfunkciju vaskularnih endotelnih stanica, promjene funkcije trombocita, promjene plazmatske faze zgrušavanja i poremećaj fibrinolitičkog sustava. Pomak fiziološke hemostaze prema protrombotičkom stanju rezultat je pojačane agregabilnosti trombocita, hiperkoagulabilnosti i smanjene fibrinolitičke aktivnosti.

Važno obilježje pretilosti jest kronično subkliničko upalno stanje uzrokovano djelovanjem proupalnih citokina (TNF-alfa, IL-6) iz adipoznog tkiva. Upala i hemostatski sustav tijesno su povezani patofiziološki procesi koji sinergistički djeluju i moduliraju međusobnu aktivnost. Stanje kronične upale u pretilih osoba pokazalo se značajnim čimbenikom poremećaja u hemostatskom sustavu. Proupalni citokini iz adipoznog tkiva izravno uzrokuju disfunkciju endotela narušavanjem ravnoteže u sintezi i djelovanju vazoaktivnih supstanci s proupalnim i prokoagulantnim te protuupalnim i protukoagulantnim svojstvima. U disunkciji endotela čimbenici koji potiču vazokonstrikciju, proupalno, prokoagulantno i protufibrinolitičko stanje (tromboksan A2 (TXA2), von Willebrandov čimbenik (vWF), čimbenik aktivacije trombocita (PAF), inhibitor aktivatora plazminogena-1 (PAI-1)), prevladavaju utjecaj čimbenika sa suprotnim djelovanjem. Disfunkciju endotela potiču i drugi popratni metabolički poremećaji (hiperinzulinemija i dislipidemija) često prisutni u pretilih osoba.

Poremećaj funkcije trombocita u pretilosti očituje se pojačanom aktivacijom i posljedičnom agregacijom trombocita, što dodatno doprinosi protrombotičkom stanju. Pokazano je da u pretilih osoba izostaje fiziološki inhibitorni učinak inzulina na agregaciju trombocita, a u pretilih osoba s inzulinskom rezistencijom povećana je osjetljivost trombocita na aktivaciju fiziološkim agonistima agregacije (ADP, trombin, kolagen). Pojačanoj aktivaciji trombocita izravno doprinosi i disfunkcija endotelnih stanica povećanom sintezom čimbenika aktivacije i agregacije trombocita (vWF, PAF, TXA2) uz istodobno smanjenu sintezu inhibitora agregacije (prostaciklin, dušikov oksid).

Promjene plazmatske faze zgrušavanja u pretilosti obilježava povećana aktivnost određenih čimbenika zgrušavanja: fibrinogena, tkivnog čimbenika (TF), čimbenika VII (FVII) i VIII (FVIII). Porast fibrinogena primarno je rezultat kroničnog upalnog stanja potaknutog djelovanjem proupalnih citokina iz adipoznog tkiva na povećanu sintezu u jetri. Disfunkcija endotela i aktivacija upalnih stanica dovode do pojačanog izražaja TF na membrani stanica i izla-

resistance, hyperinsulinemia, dyslipidemia, hypertension), often present in obese subjects, additionally contribute to the overall risk for CVD. The connection of metabolic (atherosclerotic) and prothrombotic risk factors in development and progression of CVD largely explaines complex pathophysiological processes that cause atherosclerotic plaque, plaque rupture and consequent arterial thrombosis.

Disorders of coagulation and fibrinolytic systems in obese subjects include all constituents of hemostatic system: dysfunction of vascular endotehelial cells, alterations of platelet function, alterations of plasma coagulation phase and abnormality of fibrinolytic system. The shift of physiological hemostasis toward prothrombotic state is the result of enhanced platelet aggregability, hypercoagulability and decreased fibrinolytic activity.

Important feature of obesity is a chronic subclinic inflammatory state caused by action of proinflammatory cytokines (TNF-alfa, IL-6) from adipose tissue. Inflammation and hemostatic system are close related pathophysiological processes that sinergistically act and modulate the activity of each other. Chronic inflammatory state in obese subjects turned out to be a significant factor of disorders in hemostatic system. Proinflammatory cytokines from adipose tissue directly cause endothelial dysfunction by disturbing a balance in the production and action of vasoactive substances with proinflammatory and procoagulant and antiinflammatory and anticoagulant properties. In endothelial dysfunction factors that promote vasoconstriction, proinflammatory, procoagulant and antifibrinolytic state (thromboxan A2 (TXA2), von Willebrand factor (vWF), platelet activating factor (PAF), plasminogen activator inhibitor-1 (PAI-1)) prevail the impact of factors with opposite actions. Endothelial dysfunction is also stimulated by other accompaning metabolic disorders (hyperinsulinemia and dyslipidemia) often present in obese subjects.

Disorder of platelet function in obesity manifests as enhanced platelet activation and consequent aggregation, that additionally contribute to the prothrombotic state. It has been shown that a physiological inhibitory effect of insulin on platelet aggregation is absent in obese subjects and in those with insulin resistance there is an increased platelet susceptibility with physiological aggregation agonists (ADP, thrombin, collagen). Dysfunction of endothelial cells contribute also to strengthened activation of platelets by increased synthesis of factors that promote platelet activation and aggregation (vWF, PAF, TXA2) with simultaneous reduced synthesis of inhibitors of aggregation (prostacyclin, nitric oxide).

Alterations of plasma coagulation cascade in obesity are characterized by increased values of certain coagulation factors: fibrinogen, tissue factor (TF), factor VII (FVII) and factor VIII (FVIII). Elevated fibrinogen level is primarily the

ganja velike količine TF krvi, što rezultira pojačanom aktivacijom sustava zgrušavanja putem aktivacije FVII.
Dobro utvrđen poremećaj u sustavu hemostaze u pretilih osoba je smanjena fibrinolitička aktivnost obilježena sistemnom hipofibrinolizom, primarno zbog povećane sinteze PAI-1. Porast PAI-1 u plazmi pretilih osoba najvećim je dijelom rezultat prekomjerne sinteze u adipocitima posredovane proupalnim citokinima, a dijelom i disfunkcije endotela koja je uzrok povećane sinteze PAI-1 u endotelnim stanicama.
U predavanju će biti prikazani rezultati dosadašnjih ispitivanja o poremećajima hemostatskog sustava u pretilosti.

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result of chronic inflammatory state stimulated by action of proinflammatory cytokines from adipose tissue on enhanced fibrinogen synthesis in liver.
Dysfuntion of endothelial cells and activation of inflammatory cells cause increased expression of TF at the cell membranes, with exposing large amounts of TF into blood and increasing activation of coagulation through FVII activation.
Well established disorder of the hemostatic system in obese subjects is diminished fibrinolytic activity characterized by systemic hypofibrinolysis, primarily due to increased synthesis of plasminogen activator inhibitor-1 (PAI-1). Increased PAI-1 plasma level in obese subjects is mostly the result of excess production in adipocytes mediated by proinflammatory cytokines, and partly of endothelial dysfunction that cause increased synthesis of PAI-1 in endothelial cells.
The results of investigations to date about disorders of hemostatic system in obesity will be presented in the lecture.

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S09 – Ateroskleroza

S09-1

Patofiziologija akutnog koronarnog sindroma: od slabog plaka do ranjivog bolesnika

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Nekoliko je čimbenika uključeno u tranziciju između stabilne i asimptomatične koronarne arterijske bolesti (CAD) i akutnog koronarnog sindroma (ACS). Među brojnim i kompleksim mehanizmima, puknuće aterosklerotskog plaka smatra se glavnim okidačem koji izaziva taj proces. Međutim, jednako je učestala i pojava napuklina ili krvnih ugrušaka uz pojavu jednostavne erozije fibroznog plaka. Taj se fenomen može smatrati rezultatom endotelne aktivacije erozije: aktivirani endotel se može mijenjati od antitrombotskog i antiadhezivnog ka protrombotskom.
Stvaranje plaka je dugoročan proces koji započinje infiltracijom lipida i leukocita te vodi ka remodeliranju arterija. Nakon toga, plak počinje urastati u krvnu žilu te dolazi do sužavanja lumena krvne žile i smanjenja protoka krvi. Aterosklerotski plak je podložan disrupciji, sastoji se od jezgre bogate lipidima u središnjem dijelu ekcentrično zadebljane intime, na kraju s tankom fibroznom kapom. U jezgri se također nalaze i pjenaste stanice markofaga s pu-

S09 – Atherosclerosis

S09-1

Pathophysiology of acute coronary syndromes: from vulnerable plaque to vulnerable patient

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Several factors are implicated in the transition between stable or asymptomatic coronary artery disease (CAD) and ACS. Among the multiple and complex mechanisms, plaque rupture is considered the trigger event in this process. However, equally common is the finding of fissures or of thrombi superimposed on simple erosion of fibrous plaque. This phenomenon can be considered as a result of endothelial activation or erosion: activated endothelium, in fact, may change from antithrombotic and antiadhesive to prothrombotic.
Plaque formation is a long-standing process, initiating with lipid and leukocyte infiltration and leading to the artery remodelling. Subsequently the plaque starts to grow into the vessel and there is a reduction of the vessel lumen and of the blood flow. Atherosclerotic plaque prone to dysruption, consists of a lipid-rich core in the central portion of the eccentrically thickened intima, limited by a