

ity in nondiseased) was calculated as: $DOR = \text{Likelihood ratio (+) / Likelihood ratio (-)} = [\text{Sens}/(1-\text{Spec})] / [(1-\text{Sens})/\text{Spec}]$ from the Sensitivities and Specificities of 59 reports. Statistical analysis was performed with the program Meta-Disc.

Results and conclusion: Tests potentially useful tend to have DOR well above 20. LH (+) greater than 10 or LH (-) less than 0.1 have the potential to alter clinical decisions. LR (+) between 5 and 10, and LH (-) between 0.1 and 0.2 often provide useful additional information. Tests with LH ranging from 0.33 to 3 rarely alter clinical decisions. The results of DOR in our study were between 6.77 (ED, adults) and 14.8 (ICU, neonates) showing that the diagnostic performance of the PCT in different settings is moderate (low to intermediate, median DOR under 20) thus not lending support to its widespread use.

P04-15

Serum copper, ceruloplasmine and zinc concentrations in a hospital working population

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Background: Copper and zinc are essential micronutrients for humans, even though they are not included in routine analyses. The objective of this study is to measure copper and zinc in a hospital working population and to evaluate the need of measure copper and ceruloplasmine together.

Material and methods: We recruited 395 employees (64 men and 331 women). Serum copper ($\mu\text{g/dL}$) and zinc ($\mu\text{g/dL}$) concentrations were measured using flame atomic absorption spectrometry. In addition we measured ceruloplasmine (mg/dL) by immunonephelometry in the 100 non menopausal women (14 under oral contraceptive treatment).

Results: The mean of serum copper was $118.2 \mu\text{g/dL}$ (SD: 26.4). Copper percentiles (5, 25, 50, 75, 95) were: 84, 102, 114, 127, 178 $\mu\text{g/dL}$. Among the non menopausal women (100), the mean of ceruloplasmine was 36.8mg/dL (SD: 14.4). Women under oral contraceptive treatment had serum ceruloplasmine (66.3, SD: 13.6) and copper (204.9, SD: 19.2) higher ($P < 0.01$) than those who did not take contraceptives (32.1, SD: 8.4; 108.9, SD: 20.5). However, no significant differences were found in the calculated free copper concentrations between both groups of women. The serum zinc mean was $88.3 \mu\text{g/dL}$ (SD: 10.6). Zinc percentiles (5, 25, 50, 75, 95) were: 71, 82, 88, 95, 105 $\mu\text{g/dL}$.

Conclusion: We found an appropriate copper and zinc status in this population. Since ceruloplasmine concentrations influence copper concentrations, it is necessary to measure both of them in order to correctly interpret serum copper in clinical practice. It would be desirable to establish reference values for zinc, copper and ceruloplasmine in Spanish population.

P05 – Diabetes mellitus

P05-01

Comparison of HbA1c results by two immunoturbidimetric methods

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Background: The measurement of HbA1c as a biomarker for long-term monitoring of glycemic control in diabetic patients demands high analytical precision. The aim was to evaluate analytical performance of immunoturbidimetric method for measurement of HbA1c (Tina-quant Hemoglobin A1c Gen. 2) on Cobas Integra 400 plus analyzer (Roche) before introduction into routine use.

Methods: Within-run and day-to-day imprecision was determined using two commercial control samples (PeciNorm and PeciPath, Roche) and one patient sample with value near the target point for good glycemic control (7%; 54 mmol/L). Method comparison was obtained with parallel measurement of patients samples (N = 75) on Dimension Xpand (Siemens) analyzer (immunoturbidimetric method traceable to DCCT) used routinely in our laboratory. Statistical analysis was performed using MedCalc 12.2.1. software.

Results: Coefficients of variation for within-run imprecision ranged (N = 25): PeciNorm 0.95%, PeciPath 0.82%, and patient sample 1.16%. Coefficients of variation for day-to-day imprecision ranged (N = 20): PeciNorm 0.95%, PeciPath 0.54%, patient sample 0.72%. Cumulative intralaboratory imprecision for low and high commercial controls and a patient sample was 1.4%, 1.0% and 1.3% respectively. The correlation coefficient (r) between two analyzers was $r = 0.98$. The parameters of Passing-Bablok regression showed systematic differences among analytical systems (95% CI, $a = -1.343$ to -0.400 ; $b = 1.000$ to 1.143). Systematic differences evaluated by Bland-Altman method were Cobas – Dimension (95% CI = -0.93 to 0.28).

Conclusion: Method demonstrated high reproducibility meaning that is suitable for long-term follow-up of diabetic patients. If method is changed, constant bias should be corrected for improved results agreement.

P05-02

Renal tubular markers for predicting early stage diabetic nephropathy in patients with type I diabetes

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Background: Early detection of renal dysfunction is important since this is the first step in the pro-

gressive loss of renal functions. The aim of this study was carried out to evaluate the correlation between the duration of diabetes, metabolic control (glycemic control, HbA1c) and the urinary excretion of tubular marker: β 2-microglobulin, α 1 microglobulin as LMWP and lysosomal enzyme N-acetyl B-D-glucosaminidase for early detection of subclinical nephropathy.

Materials and methods: This study consisted of 46 randomly selected patients with type 1 diabetes mellitus that diagnosed in Department of Endocrinology and with a normal serum creatinine (< 80 mmol/L) and no signs of clinical nephropathy. 30 healthy controls were randomly select. Biochemical parameters glucose, HbA1c, serum and urinary creatinine, creatinine clearance and urinary excretion of low molecular weight proteins and urinary N- β -D glucosaminidase were measured with standarised methods in all cases and controls.

Results: The study showed a significant increase in β -2Microglobulin ($P < 0.001$), α -1Microglobulin ($P < 0.01$), NAG ($P < 0.01$), HbA1C ($P < 0.001$) in diabetic patients compared to control. There was a positive correlation between the duration of diabetes and β 2-microglobulin ($P < 0.001$), NAG ($P < 0.05$), HbA1c ($P < 0.001$). There was a significant positive correlation between glycemic control (HbA1C) and β -2microglobulin, α -1microglobulin and N-acetyl- β -D-glucosaminidase ($P < 0.001$, $P < 0.01$, $P < 0.05$) respectively.

Conclusion: Tubular markers appear to be useful in early detection of diabetic nephropathy with positive correlation with the duration of type 1 diabetes and glycemic control (HbA1c).

P05-03

IL-12 concentrations in the aqueous humor and serum of diabetic retinopathy patients

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Introduction: Previous studies suggest that inflammation plays an important role in pathogenesis of diabetes. Cytokines may play positive or negative role in immunological reactions. Among many cytokines, interleukin 12 (IL-12) is known to be a strong pro-inflammatory cytokine.

Materials and methods: A total of 76 participants were enrolled in this study and classified in 4 groups: 23 diabetic patients with non-treated retinopathy, 17 diabetic patients with treated retinopathy, 12 diabetic patients without retinopathy, and 24 healthy control patients. Serum and aqueous humor samples were taken for the analysis of IL-12 concentration.

Results: There was a significant difference between the groups in IL-12 concentrations in the aqueous humor (χ^2 (3,N=76) = 27.137; $P = 5.5 \times 10^{-6}$) with highest values measured in the non-treated diabetic retinopathy group (12.40 pg/mL). No significant differences in IL-12 serum concentrations between the groups were found ($F = 0.405$, $P = 0.750$). Correlation analysis of IL-12 concentrations in the serum and aqueous humor showed association between the two variables only in non-diabetic patients ($P = 0.003$).

Conclusion: To the best of our knowledge, this is the first study to show a significantly higher concentration of pro-inflammatory cytokine IL-12 in the aqueous humor of non-treated diabetic retinopathy patients in comparison with diabetic patients treated for retinopathy, diabetic patients

without retinopathy or with healthy individuals. Because the serum levels of IL-12 did not differ considerably between the studied groups, it is possible that this is due to its local production and secretion.

P05-04

Evaluation of obesity influence on lipid profile in type II diabetes mellitus

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Background: There are an increasing number of obese people with type II diabetes mellitus (DM II) associated with dyslipidemias and cardiovascular problems. This study evaluated the obesity influence, by calculating body mass index (BMI) on lipid profile in subjects with DM II.

Materials and methods: 70 patients with DM II, aged ≥ 18 years, 35 obese (O) and 35 non obese (NO), selected from medical patient's records of Faro Hospital' Diabetology Service. Were analyzed total cholesterol (TC), LDL, HDL, triglycerides (TG), fasting plasma glucose and glycosylated hemoglobin (HbA1c). The statistical processing included the independent student t-test, linear coefficient correlation and Pearson chi-square.

Results and conclusions: The values obtained showed that mean of TC and TG are slightly higher in the group O related to NO (O: TC = 181.43 mg/dL and TG = 143.6 mg/dL; NO: TC = 180.60 mg/dL and TG = 126.3 mg/dL). LDL, HDL, fasting glucose and HbA1c, showed higher values in NO group (O: LDL = 100.9 mg/dL, HDL = 44.89 mg dL, glucose = 175.4 mg/dL, HbA1c = 7.9%; NO: LDL = 103.9 mg/dL, HDL = 46.63 mg/dL, glucose = 188.6 mg/dL, HbA1c = 8.2%). We concluded that there are significant differences between groups but obesity was not a crucial factor of influence on lipid profile, with the patients under study.

P05-05**Non-HDL cholesterol as a predictor of impaired glucose metabolism**

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Background: Non-HDL cholesterol (non-HDL-C), recently used as a risk factor for cardiovascular disease and surrogate for apolipoprotein B level, may be a useful indicator of early disturbances of glucose metabolism, reflected by glycated hemoglobin (HbA1c), vitamin 25(OH)D3 and HOMA-IR values, in healthy, non-obese individuals.

Materials and methods: Study consisted of 82 healthy, non-diabetic subjects, aged 25-40 years (49 women and 33 men). HbA1c in the whole blood, plasma fasting glucose and serum total cholesterol (TC), HDL-C, triglycerides (TG), insulin, C-reactive protein (CRP), total bilirubin, apolipoproteins B100 and AI (apoB, apoA-I), vitamin 25(OH)D3 and antropometric measurements (BMI, waist to hip ratio-WHR) were performed. LDL-C, non-HDL-C, HOMA-IR and atherogenic indexes (TC/HDL-C, LDL-C/HDL-C, apoB/apoAI) were calculated. Subjects were divided by tertiles of non-HDL-C.

Results: Significantly higher values of BMI, glucose, insulin, HbA1c, HOMA-IR, total bilirubin, TG, non-HDL-C (127.6 vs. 141 mg/dL; $P < 0.001$) were found in men compared to women. In the whole study group lipids, except HDL-C, apoB, HOMA-IR, HbA1c, atherogenic and anthropometric indexes were increased and 25(OH)D3 was decreased in subsequent non-HDL-C tertiles. Although non-HDL-C showed the strongest correlation with traditional lipid parameters, apoB and atherogenic indexes, it has been also significantly related with WHR ($r = 0.46$; $P = 0.012$), glucose ($r = 0.42$; $P = 0.016$), HbA1c ($r = 0.38$; $P = 0.04$), HOMA-IR ($r = 0.37$; $P = 0.04$) and 25(OH)D3 ($r = -0.39$; $P = 0.027$).

Conclusion: Relationship, observed in young healthy non-obese individuals, between non-HDL-C and glucose, HOMA-IR, 25(OH)D3 and HbA1c may reflect an early impairment of glucose metabolism and the risk of diabetes and metabolic syndrome in the future.

P05-06**HbA1c – Portuguese EQAS Schemes (2003-2011)**

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Objectives: Of the different objectives of the external evaluation tests, we present the performance evaluation of laboratories participating in PNAEQ, for hemoglobin A1c schemes, since 2003, with respect to the different methodologies used to determine this parameter.

Results: The methods used by participants PNAEQ since 2003 are: immunoturbidimetry, LPLC, HPLC and colorimetry, with an average participation of 21.5%, 8.1%, 60.1% and 3.2% respectively. The method of ion exchange chromatography was mentioned by the laboratories from 2004 to the present date with an average attendance of 4.2%. In 2009 the immunoinhibition method was used by only 1.7%, which did not allow statistical evaluation (number of participants less than 3). It is found that the HPLC method is used by most laboratories with increased use over the years (43% to 75%), and also has a lower CV% (pathological level of 2.7 to 5.2% and normal level of 3.9 to 10.3%), with an increase in performance in the last two years (CV% around 3.5%). For immunoturbidimetry and chromatography methods, the CV% calculated did not vary significantly with different concentrations of the samples.

Conclusion: The lower coefficient of variation was observed in the pathological level, being HPLC the

method with lowest values. For methods of chromatography and immunoturbidimetry the calculated CV% have a large variation with the concentration of the sample. It is found that the HPLC method is what is closest to the value of CV% indicated on Standard 033/2011 of Portugal 's Health General Directorate (Prescription and determination of glycated hemoglobin A1c).

P05-07

Vitamin D levels and its relationship with fasting glucose in pregnancy

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Background: Vitamin D is implicated in several physiologic processes. Lower levels of Vitamin D 25-OH have been inversely associated with maternal glycemia but association with risk of gestational diabetes (GDM) are controversial. The aim of this work is to study the relationship between vitamin D levels and fasting glucose between 24-28 weeks of gestation, using a cutoff ≥ 92 mg/dL according to recommendations of the American Diabetes Association (ADA) and the International Association of Diabetes and Pregnancy Study Groups (IADGSP).

Materials and methods: 136 women were screened for gestational diabetes between 24-28 weeks during the summer months. Serum basal glucose and vitamin D were measured in an Olympus AU2700 autoanalyzer (Beckman) and Liaison (Dia Sorin), respectively. Patients were separated in groups depending on vitamin D levels (< 10 mg/dL severe insufficiency, 10-20 mg/dL insufficiency, 20-40 mg/dL deficiency and > 40 mg/dL sufficiency). Statistical analysis was performed using SPSS-15. T-test and Chi-squared test were used to compare groups.

Results: 70 pregnant women had insufficient levels of Vitamin D (< 20 ng/mL) and 24 had impaired fasting glucose values (≥ 92 mg/dL). No statistical differences were found between vitamin D levels of pregnant women with normal or impaired fasting glucose (20.6 mg/dL and 19.2 mg/dL respectively). No significant differences were found among vitamin D groups comparing diabetic and non diabetic patients.

Conclusions: In our population, no association was found between vitamin D deficiency and impaired fasting plasma glucose using cut-off 92 according to ADA criteria.

P05-08

Implications of variable preanalytical procedures for the diagnosis of diabetes mellitus in Croatia

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Background: Preanalytical sample handling is of utmost importance for the accurate glucose results in screening and diagnosis of diabetes mellitus. The effect of in vitro glycolysis should be minimized by immediate cooling of blood samples for glucose analysis and plasma separation from the cells within 30 min from sampling. An effective glycolysis inhibitor is recommended for any delayed processing. The aim of this study was to evaluate current practice regarding preanalytical steps of glucose measurement in Croatian laboratories.

Materials and methods: A structured, anonymous survey has been conducted among the participants of the Croatian Chambers of Medical Biochemists' continuous education course „The role of laboratory in diagnosis and management of diabetes melli-

tus" in September 2011. The survey considered: type of primary sample, use of glycolysis inhibitors, sampling location, sample storage conditions and average time of plasma separation after venipuncture.

Results: A total of 56 participants (32%, 30%, 11% and 11% from the primary, county- and teaching-hospitals, and private laboratories, respectively) were included in the survey. Venous serum was the most prevalent primary sample type (62%), and glycolysis inhibitors were used in 22% of the laboratories. Laboratory only, and combined laboratory and family physician's office were equally reported (43%) as the sampling location. Plasma/serum separation within 30 min is performed in 34% of the laboratories, whereas delayed processing (30-60 min, 1-2 h and >2 h) was reported by 37%, 20% and 9% of the laboratories, respectively.

Conclusions: Preanalytical procedure for glucose measurement in Croatia urgently needs improvement and harmonization.

P05-09

Use of urinary protein:creatinine ratio in advanced stages of chronic kidney disease in diabetes

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Background: Albumin:creatinine ratio (ACR) is the recommended method for the detection of incipient kidney disease in diabetic patients. However, protein daily excretion rate is used for the quantification and monitoring of proteinuria in more-advanced stages of chronic kidney disease. We wanted to determine whether measurement of protein:creatinine ratio (PCR) can be used as an alternative to protein excretion rate in detecting clinically significant proteinuria in diabetic patients.

Materials and methods: 48 diabetic patients were included in the study. We measured ACR and PCR in random urine samples and protein contents in 24-h urine samples. All measurements were made on Olympus AU600. Urinary albumin was measured by immunoturbidimetric method, while urinary creatinine and protein were measured by the spectrophotometric Jaffe and pyrogallol red method, respectively. Clinically significant proteinuria was considered to be present with the ACR \geq 30 mg/mmol which is approximately equivalent to PCR 50 mg/mmol. All results were analyzed with MedCalc 9.4.2.0. statistical software (MedCalc Software bvba, Mariakerke, Belgium).

Results: ACR was \geq 30 mg/mmol in 28/48 diabetic patients. Sensitivity was 100% and specificity was 95.0% for PCR as an indicator of clinically significant proteinuria at a cut-off value of 50 mg/mmol. Protein daily excretion rate as an indicator of clinically significant proteinuria gave lower values for sensitivity and specificity at the cut-off value of $>$ 0.5g/24h; they were 78,6% and 85,0% respectively.

Conclusions: The protein:creatinine ratio performed better as an indicator of clinically significant proteinuria than the 24-urine collection method in diabetic patients.

P05-10

Relationship between PAI and CRP in diabetic patients

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Background: CRP is a risk marker of cardiovascular disease and his role in promoting atherosclerosis has been recently investigated. Previous studies show that CRP and hyperglycemia induce PAI-1 expression and suggest PAI-1 to cause a hypofibrinolytic state in atherosclerotic plaques. Therefore, we investigated CRP, PAI and other inflammatory parameters in diabetics.

Materials and methods: Forty-one diabetic (aged 65 ± 8.95) patients with HbA1c (average 9.98%; range 5.9-15.6) were enrolled. We analyzed routine inflammatory parameters (CRP, WBC, ESR, fibrinogen) and compared them with PAI activity. We excluded diabetics with CRP $> 20 \mu\text{g/L}$. Statistical analysis was performed by SPSS 19 (IBM, Armonk, New York, SAD).

Results: PAI showed significant relationship with CRP in diabetic subjects ($r = 0.303$; $P = 0.05$). No correlation was found between PAI and other inflammatory parameters. PAI did not correlate with HbA1c. Previous research showed that hyperglycemia increased PAI expression in cell cultures but that has not been confirmed in our study.

Conclusion: Previous studies have demonstrated that CRP induces PAI expression in human and bovine aortic endothelial cells. Our results show correlation between CRP and PAI in our patients. If further research confirm these findings, it is possible that PAI will be recognized as a novel risk marker of cardiovascular disease in diabetes mellitus.

P05-11

Tissue transglutaminase antibodies and HLA haplotypes in type 1 diabetic Lithuanian children

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease. Celiac disease (CD) is the second most prevalent autoimmune condition accompanying T1D. It may be partially explained by shared

genetic susceptibility to HLA haplotypes. The aim of our work was to determine prevalence of tissue transglutaminase antibodies (TTG Ab) in T1D and healthy control children and to assess whether positivity of TTG Ab is related to CD associated HLA haplotypes.

Materials and methods: 271 children with T1D and 289 healthy control children were serologically screened for the presence of TTG Ab IgG class. Genetic testing was performed for 30 children with T1D and positive TTG Ab, 137 children with T1D and negative TTG Ab, and 4 TTG Ab positive control children.

Results: 11.1% of the diabetic children and 1.4% healthy controls were found to be positive for TTG Ab ($P < 0.001$). HLA DR3-DQA1*0501-DQB1*0201 or/and DR4-DQA1*0301-DQB1*0302 were found in 93.3% of TTG Ab positive and in 77.4% TTG Ab negative diabetic children ($P = 0.047$). There were no significant differences in the frequency of heterozygotic DR3-DQA1*0501-DQB1*0201 and DR4-DQA1*0301-DQB1*0302 haplotype between TTG Ab positive and TTG Ab negative diabetic children. DR4-DQA1*0301-DQB1*0302 homozygotic haplotype was found in 6.7% of TTG Ab positive but wasn't found in TTG Ab negative diabetic children ($P = 0.002$). All ($N = 4$) TTG Ab positive control children were heterozygotic for DR3-DQA1*0501-DQB1*0201 haplotype ($P < 0.05$).

Conclusions: This study showed higher prevalence of TTG Ab in children with T1D than in their healthy counterparts. TTG Ab positive diabetic children significantly more frequently had homozygotic DR4-DQA1*0301-DQB1*0302 haplotype and control children – heterozygotic DR3-DQA1*0501-DQB1*0201 haplotype.

P05-12**Bariatric surgery treatment for type 2 diabetes**

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Background: Bariatric surgery treatment should be considered in cases of extreme obese patients with type 2 diabetes, who could not reduce body mass through physical activity, diet and pharmacotherapy.

Materials and methods: There are two female patients shown with type 2 diabetes regulated by oral hypoglycemic agents, who were subjected to gastric sleeve resection.

Results: A 55 year old patient had BMI of 43.5 kg/m², blood glucose 10 mmol/L, HbA1c 7.4 %, score for beta-cell function assessment (HOMA-B) 34%, preoperative insulin sensitivity (HOMA-S) 56.4%, ghrelin level 807.7 pg/mL, and leptin level 35 ng/mL. A year after surgery BMI was reduced to 32.7 kg/m², whereas blood glucose (5.9 mol/l) and HbA1c (6%) were normalized without hypoglycemic agents. HOMA-B increased to 95% and HOMA-S to 60.1%. Ghrelin increased to 864 pg/mL and leptin decreased to 8.1 ng/mL.

A 44 year old patient had BMI 44.9 kg/m², blood glucose 8.2 mol/L, HbA1c 6%, HOMA-B 51%, HOMA-S 55.5%, ghrelin 603 pg/mL, and leptin 29.3 ng/mL. A year after surgery BMI was reduced to 35.3 kg/m², whereas blood glucose (4.3) and HbA1c (4%) were normalized without hypoglycemic agents. HOMA-B increased to 130.1% and HOMA-S to 102.6%. Ghrelin increased to 1008 pg/mL and leptin decreased to 12.3 ng/mL.

Conclusions: Reduction of body mass leads to improvement of pancreatic beta cell function and therefore better diabetes control. Bariatric surgery is a promising method for treating diabetes in obese patients.

P06 – Education**P06-01****Do we need complex education courses in special fields of laboratory medicine?**

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Objective: The fast progress in knowledge about coagulation disorders and new pharmacological agents for treatment and haemorrhagic and thrombotic events are the reason for complex education for clinicians and laboratory staff.

Materials and methods: From Sept 2006 up to May 2012 we organized 10 CME courses "Clinical and laboratory aspects of coagulation disorders" 72 h duration (Lectures – 26 h; Seminars + exam – 24 h; Practical classes in laboratory and clinical departments – 22 h).

Results: 58 persons were educated – 38 laboratory professionals and 20 clinicians (cardiologists, gynaecologists, internists, clinical pharmacologist) from 8 cities and 34 medical centres. The better experience was to educate both – laboratory staff and clinician from the same medical centre in the same time. One year after finishing the course the questionnaire was sent to participants: the usefulness were marked by 94%, "important for every day work" – 86%, "wish to repeat in a 5 years" – 93% of responders. The more important themes: genetics of thrombophilia, laboratory testing in acquired and inherited bleeding and thrombotic disorders (risk factors, reasons, predisposition), DIC, laboratory control of antithrombotic and anti-