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Autorenverzeichnis

Genetic markers for atherosclerosis, myocard infarction and stroke: Results from the Austrian MEDPED Project and Austrian Stroke Prevention Study

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Atherosclerosis is a multifactorial disease which in exceptional cases may be also caused by single gene abnormalities like in FH or FDB.

We are the central laboratory in Austria for the DNA analysis in the so called MEDPED project. This is an international multicenter study aimed at offering diagnosis and therapy for patients with monogenetic forms of hypercholesterolemia like FH and FDB. For FDB, three different mutations in apolipoprotein B gene are responsible, while in FH more than 500 distinct mutations in LDL-receptor(-R) gene are described so far. Screening for mutation in LDL-R gene represents a real practical challenge leading to the development of more time- and cost-effective screening methods. We identified in the Austrian population at least 40 different mutations in LDL-R gene. Twenty-three of these candidate mutations have been confirmed by DNA sequencing: 9 of them are present in other European populations while 14 represent new mutations. Because of the wide variety of mutations described in FH patients interpretation of genetic findings in terms of prognosis or response to lipid lowering therapy is not yet possible and necessitates international cooperation.

In the Austrian Stroke Prevention Study, a cooperation with the Dept. of Neurology at the Univ. Graz we investigate genetic factors predisposing to cerebrovascular abnormalities. Participants of this study were randomly selected from the official register of Graz; inclusion criteria were no neurological abnormality and age 44-75 years. The goal of ASPS is the assessment of association between risk factors and brain abnormalities including carotid atherosclerosis, MRI changes and cognitive status. Based on our results the C-T-148 polymorphism at the beta-fibrinogen gene and the paraoxonase Met-Leu-54 polymorphism are significant and independent predictors of carotid atherosclerosis. The frequency of the apolipoprotein ε2 allele was significantly higher in persons with silent ischemic brain damage.

Bedeutung der APC-Resistenz in der Thrombophiliediagnostik

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Die einzelnen Komponenten des Protein C Systems haben bei der laborchemischen Abklärung einer Thrombophilie eine große Bedeutung. Neben der häufigen Faktor V Mutation (Typ Leiden) verursachen angeborene Defekte von Protein C und S meist eine APC-Resistenz. Letztere sind im Gegensatz zur Faktor V Mutation selten, außerdem findet sich eine ausgeprägte genotypische und phänotypische Heterogenität. Erhöhte Fibrinogen- und Faktor VIII Spiegel führen ebenfalls zu einer Resistenz bei Zugabe von aktiviertem Protein C zum Patientenplasma. Eine Erhöhung dieser Faktoren findet sich unspezifisch bei akuten und -cbronischen Krankheitszuständen. Inwieweit hier das Ausmaß der APC-Resistenz eine klinische Bedeutung für den einzelnen Patienten hat, muß prospektiv geklärt werden. Eine persistierende Erhöhung der Faktor VIII Plasmaspiegel findet sich bei 20 bis 30% der Patienten nach stattgehabter venöser Thromboembolie ohne andere Hinweise auf eine chronische Entzündung.

Zusammengefaßt findet sich die Mehrzahl der bis jetzt bekannten laborchemisch faßbaren Thromboserisikofaktoren im Protein C System. Hereditäre und erworbene Veränderungen sind zu unterscheiden. Außerdem müssen die für den betroffenen Patienten unterschiedlichen Risiken der jeweiligen Befunde berücksichtigt werden. Im Gegensatz zu Defekten bei Protein C oder S wird die weit überwiegende Zahl der Faktor V Leiden Träger keine Thrombose erleiden.

Klinische Studien aus der Sicht des klinischen Studienadministrators

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Der klinische Studienadministrator ist ein neues Berufsbild in Europa, vergleichbar dem einer amerikanischen study nurse. Er soll den klinischen Prüfarzt bei seiner Arbeit unterstützen und ein Bindeglied zwischen Monitor, Prüfer, Patienten, Krankenhauspersonal und Auditor sein. Doch auch im Vorfeld und bei der Erledigung von Einreichungen, die zur Zulassung einer Studie notwendig sind, kann der Studienadministrator eingesetzt werden.

Durch die neuen strikten Richtlinien, die für klinische oder auch präklinische Studien von Seiten des Gesetzgebers und von Seiten der Pharmazeutischen Industrie vorgeschrieben sind, wird die Notwendigkeit für die Funktion und das Einsetzen eines Studienadministrators immer mehr erkannt.

- Ich möchte mit diesem Vortrag auf dieses neue Berufsbild aufmerksam machen und gleichzeitig einen kurzen Überblick über den Ablauf einer klinischen Studie bringen.

Seit 1997 können 20 diplomierte medizinisch-technische AnalytikerInnen eine Zusatzausbildung zum klinischen Studienadministrator aufweisen. Die Ausbildung, die auch 1999 stattfindet, umfaßt einen 6 tägigen theoretischen Kurs und 40 Stunden Praktika an ausgewählten Praktikumsplätzen. Diese Ausbildung ist die erste dieser Art in Europa und wird vom Bundesverband der diplomierten medizinisch technischen AnalytikerInnen Österreichs in Zusammenarbeit mit der Arbeiterkammer Wien durchgeführt. Die Ausbildung vermittelt relevante Gesetzesinhalte aus dem österreichischen Arzneimittelgesetz und dem Medizinproduktegesetz (bezogen auch auf EU Raum), definiert die Aufgaben von Sponsor, Monitor, Prüfer und Studienadministrator und vermittelt weitere Inhalte, die für das Verständnis einer klinischen Studie notwendig sind.

- An Hand des Ablaufs einer klinischen Studie werden die möglichen Aufgaben des Studienadministrators erklärt und es werden unter anderem folgende Begriffe definiert: Sponsor, Monitor, Prüfer, Ethikkommission, Standard Operating Procedures (SOP's), Investigator's Brochure, Serious Adverse Events (SAE), Case Report Form (CRF).

Poster-Abstracts

PV-1

The clinical value of automated urinary sediment analysis in the differential diagnosis of renal diseases

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Urinary sediment microscopy is a standard laboratory test, with thousands performed every day. However, this diagnostic procedure severely limits in the inter-observer variability.

The aim of this prospective case-control study was to perform a double-blinded comparison of stained cytology findings and concurrent automated dipstick testing with the evaluation of scattergrams of the fully automated cell analyzer Sysmex UF-100. Second-morning voided urine samples were freshly collected from 432 patients and stored for up to 6 hours at 4° C. For microscopic urinalysis a rapid 3-minutes eosin B/methyl blue stain was performed according to the protocol of the manufacturer. Without any knowledge of the UF-100 data and dipstick analyses qualitative and semiquantitative evaluation was done microscopically per 10 random selected high-power fields for the different cytological variants: glomerular, tubular, interstitial, reactive, inflammatory and neoplastic. In comparison with urine cytology the highest sensitivity and specificity of UF-100 were observed in the diagnosis of glomerular hematuria (91.1%/79.5%). Only a moderate agreement was seen between these two methods and dipstick testing (39.6% false-positive strips). For symptomatic urinary tract infections, a statistically significant agreement was found between WBC count, the frequency of high-count bacteriuria and the inflammatory epithelial response. The accuracy with which UF-100 urinalysis should predict renal tubular or interstitial changes was not sufficient to give a good discrimination.

In conclusion, the UF-100 cell analyzer is a valuable screening method for glomerular and inflammatory urinary disorders.

PV-2

Increased sensitivity for detection of low copy number human and chimpanzee associated GBV-C/HGV by direct RNA capture onto paramagnetic particles

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Since the discovery of the GB Virus-C/Hepatitis G Virus (GBV-C/HGV) as parenteral, perinatal and vertical transmissible agent associated with variety of diseases such as chronic or acute hepatitis, aplastic anemia, thrombocytopenia, cryoglobulinemia and hematological malignancy, numerous studies have rapidly increased insight in the biology of this virus. Diagnosis of GBV-C/HGV infection and viremia which is crucial for understanding of the virus pathogenesis can only be determined by specific detection of genomic RNA by RT-PCR. The success of application of RT-PCR relies on isolation/purification of the target RNA. Standard methods for preparation of viral nucleic acids for PCR involve multiple manipulations, need large quantities of test material and showed low sensitivity in the case of minimal virus concentration. To reduce these problems, more sensitive procedures have to be developed. We described a new assay for detection of minimal amount GBV-C/HGV in body fluids and cells. GBV-C/HGV RNA is captured onto streptavidin-coated magnetic beads by liquid hybridization with biotinilated complementary oligonucleotides. Serum and mononuclear cells were obtained from patients with different hematological diseases involved in GBV-C/HGV study and from a group of chimpanzees infected with GBV-$C_{troglodytes}$. The viral genomic sequence was determined parallel by the standard RNA isolation/RT-PCR and the new capture assay using oligonucleotides within the 5'UT-region of conservation for both human and chimpanzee associated GBV-C/HGV. The specificity of the assay is confirmed using different capture oligonucleotides. Sensitivity was determined by testing serial dilutions of a GBV-C/HGV positive samples The new RNA capture system allows virus RNA detection even in samples with titer below the detection limit of the standard RNA isolation and RT-PCR methods.

PV-3

Measurement of absolute concentration and viability of CD34$^+$ cells in cord blood and cord blood products

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The high number of nucleated red blood cells or nonviable cells in cord blood and its products may affect conventional flow cytometric methods for CD34$^+$ cell counting. We developed a simple flow cytometric no-wash procedure that avoids these shortcomings as it provides absolute CD34$^+$ cell counts and assesses cell viability. Samples were incubated with PE-labeled anti-CD34 [Becton Dickinson Immunocytometry Systems (BD)], and PerCP-labeled anti-CD45 (BD) in bead-containing TrueCOUNT tubes (BD). After red cell lysis with a fixative-free reagent, the impermeant nucleic acid dye YO-PRO-1 (Molecular Probes) was added and samples were analyzed on a single-laser FACSCalibur (BD). A comparison with the ProCOUNT progenitor cell assay (BD) in 57 samples revealed excellent correlation of results ($r = 0.98$, intercept -0.2 cells/µl, slope 1.01). Precision studies conveyed coefficients of variation of 6.4 and 8.9% at concentrations of 35 and 16 CD34$^+$ cells/µl, respectively. In untreated and leukocyte-enriched cord blood $4.5 \pm 3.8\%$ of CD34$^+$ cells were stained by YO-PRO-1, representing apoptotic or necrotic cells. In post-thawing cryopreserved samples this number increased to $10.4 \pm 5.5\%$. Isotype controls showed very low blank values of viable cells (0.1 ± 0.4 cells/µl, maximum 2.4) and seemed unnecessary. Replacing YO-PRO-1 with TO-PRO-3 facilitated four-color analysis of subpopulations of viable CD34$^+$ cells on a FACSCalibur equipped with a second laser. We found the proposed method to be a rapid, efficient and flexible procedure that improved validity of CD34$^+$ cell counts.

PV-4

90K/Mac-2BP is an independent clinical parameter during HCV infection

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The serum protein designated 90K/Mac-2BP has been found at elevated concentrations in the sera of patients with various types of cancer and viral infections. The importance of the 90K serum concentrations to predict the response towards interferon-α treatment for HCV infection was demonstrated recently (Artini et al. J Hepatol 1996;25:212-217). Their data prompted us to utilize a new ELISA for soluble human 90K/Mac-2BP to monitor the serum concentrations of this protein in our HCV positive patients. Seventy HCV-PCR and anti-HCV antibody positive patients were analyzed for their serum levels of AST, ALT, GGT, ChE, HCV-viral load, viral subtypes, and 90K. On correlation of age and 90K levels, we found a superficial correlation that was resolved to rather be a strong dependence of 90K on disease severity/duration which increases with age. Multiple correlation analysis demonstrated the independent nature of 90K concentrations, underscoring the potential high utility of this new marker. Our data strengthen the initial report about the scavenger receptor protein 90K/Mac-2BP as an independent predictor of disease severity during hepatitis C infection.

PV-5

Cytomegaloviral load in the urine of HCMV congenitally infected infants and the effects of antiviral therapy

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Sequential urine samples from 20 congenitally infected infants were analyzed for cytomegaloviral load using quantitative PCR. Modulations in viral load were compared between 11 infants treated with antiviral therapy and 9 non-treated infants. 6/9 (66%) of the treated infants became PCR negative during therapy, 3 of whom remained PCR negative for 2 months following cessation of therapy. In contrast, all nine of the non-treated infants remained PCR positive throughout surveillance and at no time did the viral load fall below $10^{4.0}$ genomes/ml of urine despite comparable follow-up times. Therapy with ganciclovir resulted in a significant reduction in the viral load per day during therapy with a median of $10^{3.74}$ genomes/ml urine/day compared to a median of $10^{6.91}$ genomes/ml urine/day in untreated infants ($p>0.01$, Mann-Whitney U test) when considering all samples analyzed. The mean clearance rate of HCMV in urine following ganciclovir therapy was 1.65 days. The sustained effect of a short course of antiviral therapy on cumulative virus load has implications for the future use of antiviral interventions in congenital HCMV infection.

P-1

Evaluation of soluble CD44 splice variant v5 in breast cancer patients according to the criteria of a tumor-associated marker

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Aberrant expression of CD44 splice variants has been detected on a variety of human tumor cells. In breast cancer a correlation between the overexpression of specific CD44 isoforms and poor prognosis has been reported. The aim of the study was to evaluate the performance characteristics of soluble CD44 splice variant v5 (sCD44v5) in breast cancer patients at diagnosis and in the patients' follow-up. Serum levels of sCD44v5 were determined in 147 healthy volunteers, in 53 patients with nonmalignant breast disease, in 85 patients with breast cancer at presentation, in 13 patients with recurrence and in 73 patients with active metastatic disease. A commercially available sandwich-type ELISA (Bender MedSystems Vienna) was used for the quantitative measurements of sCD44v5.

Statistically, the levels of sCD44v5 at presentation in stages I-IV, in benign disease, and in a female control group were not different significantly. The use of stored samples in patients who have remained tumor free has given evidence of a relatively stable intra-patient value, however, considerable between-patient variation of sCD44v5 was observed. In patients with active metastatic disease, elevated levels of sCD44v5 (>58 $ng.ml^{-1}$) were detected in 50% of the cases with

a marked elevation in only 26% (> 115 ng.ml^{-1}). In these cases, sCD44v5 correlated with tumor burden and serum levels fell during clinical response to cytoreductive therapy. Comparison with CA15-3 in the patients' follow-up revealed sCD44v5 to be less sensitive concerning lead time, discovery of recurrent disease, percentage of patients with raised levels in metastatic disease and the extent of amplification of serum levels during progressive disease. The clinical interpretation of sCD44v5 determinations was further limited because of elevated serum levels in smokers and chronic inflammatory disease. In conclusion, sCD44v5 measurements cannot be recommended as a tumormarker in breast cancer patients in routine diagnosis due to the limited diagnostic sensitivity and specifity of the test.

P-2

Einflüsse einer Östrogentherapie bei menopausalen Patientinnen mit reduzierter Knochendichte auf Marker des Knochenstoffwechsels

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Zur Erfassung eines erhöhten Knochen-Umsatztes stehen heute neben alkalischer Phosphatase (AP) und Harn-Hydroxyprolin mehrere neue biochemische Marker zur Verfügung: Die Knochenresorption kann u.a. durch Harn-Metaboliten der Quervernetzungsregionen der Typ-1 Kollagenketten (Pyridinoline [Pyr], Deoxypyridinoline [D-Pyr], C- bzw. N- Telopeptide [CTX, NTX]) und die Knochenbildung z.B. mit Osteocalcin (OCAL) erfaßt werden. Wir haben untersucht, ob diese verschiedenen Marker eine Beurteilung der Effekte einer antiresorptiven Therapie ermöglichen.

109 Patientinnen (>6a in der Menopause) mit und ohne regelmäßige Östrogenmedikation (ERT) wurden durch Knochendichte-Messungen (Hologic 2000) der Lendenwirbelsäule (L1-L4, a,p) und des linken Oberschenkelhalses mittels T-Score, entsprechend den WHO Kriterien, in 3 Diagnose-Gruppen eingeteilt: normal (N), Osteopenie (OPEN), Osteoporose (OPOR). Daher erhielten wir folgende Patientengruppen [Anzahl]: N[6], OPEN[40], OPOR[42], OPEN+ERT[13] und OPOR+ERT[8]. Pyr, D-Pyr (Pyrilinks, Pyrilinks-D, Metra), CTX (Crosslaps, CIS) und NTX (Osteomark, Ostex) wurden auf Harn-Kreatinin normiert, aus Serum wurde OCAL (CIS) und AP (Boehringer Mannheim) bestimmt. Auf signifikante Unterschiede ($p<0.05$) zwischen den Gruppen wurde mit dem Mann-Whitney-U-Test geprüft:

	Pyr	D-Pyr	CTX	NTX	OCAL	AP
OPEN/OPOR	n.s.	n.s.	n.s.	s.	n.s.	n.s.
OPEN/OPEN+ERT	s.	s.	s.	s.	s.	n.s.
OPOR/OPOR+ERT	n.s.	n.s.	s.	s.	s.	n.s.

ERT führte zu einer Verringerung des Knochen-Umsatztes (Resorptions- und Bildungsmarker ↓). CTX, NTX und OCAL zeigten eine gute Trennschärfe zwischen den Gruppen. Nur CTX und NTX korrelierten signifikant mit der Knochendichte der Lendenwirbelsäule von Patientinnen ohne ERT und sind daher den anderen Resorptionsmarkern überlegen.

P-3

Evaluation of four different automated methods for determination of whole blood cyclosporin A concentrations

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Cyclosporin A (CsA) is a widely used and potent immunosuppressant drug with a narrow therapeutic index. Therefore CsA concentrations have to be closely monitored. There are various immunological automated methods, such as an enzyme multiplied immunoassay (EMIT) or a fluorescence polarization immunoassay (FPIA for TDx) for CsA whole blood determinations, available. Recently, two new automated methods, a FPIA for the AxSYM by Abbott and a CEDIA enzyme immunoassay by Boehringer Mannheim, were introduced. In addition, Dade Behring improved their EMIT assay by changing the pretreatment reagents. The present study should evaluate all three new methods in comparison with high performance liquid chromatography (HPLC) and the FPIA for the TDx analyzer. We measured whole blood CsA concentration of 179 patients after kidney (49), bone marrow (40), heart/lung (50) and liver (40) transplantation. Within- and between-run precisions were determined for all methods. CsA concentrations, determined with the four methods mentioned, were correlated with HPLC results. In addition, we determined the crossreactivity patterns of all methods tested. We also compared the results of all methods in different patient groups. This study compares and summarizes the advantages and disadvantages of 4 commercially available immunological methods for CsA whole blood measurement.

P-4

NSAID ibuprofen reduces significantly leukocyte transendothelial migration

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Background: During inflammation leukocytes playing a tremendous role in the defense against bacterial infections. The non steroid anti-inflammatory drug ibuprofen is able to reduce inflammatory processes and is a widely used drug. The influence of ibuprofen on TNF-α-induced expression of adhesion molecules on endothelial cell is well investigated and published previously by our group.

Aim of the study: Investigation of the functional consequence of our morphological findings. Therefore we studied human white blood cell (WBC) migration through human endothelial cell monolayers (ECM) under ibuprofen.

Methods: Whole blood was taken from healthy female and male volunteers. Human umbilical vein endothelial cells (HUVEC) were cultured on fibronectin coated microporous membranes until an ECM was achieved. A migration assay was performed. The influence of ibuprofen (0.01 mM, 0.1 mM, 1 mM, 2 mM) was investigated (n=5). Untreated leukocytes through untreated endothelial cell monolayers were used as control. The Student's *t*-test and variance analyzes were done and a $p<0.05$ was regarded as significant.

Results: Ibuprofen (2 mM) showed a reduction of leukocyte through endothelial cells to 35.6 ± 5.5% ($p<0.05$) when both cell types, WBC and ECM were treated, similar to the situation after an i.v. resorption. The treatment of either leukocytes (87.1 ± 17.6%, $p>0.05$) or endothelial cell monolayers (62.1 ± 9.4%, $p<0.05$) showed that endothelial cells were mainly mediated. Using lower concentrations of ibuprofen demonstrated a dose dependent effect by showing a lower reduction.

Conclusion: Ibuprofen was identified as a potent inhibitor of leukocyte migration through endothelial cell monolayers. Our previously published changes of adhesion molecules on endothelial cells could be confirmed with this functional migration test. The inhibition of the leukocyte migration may contribute to the anti-inflammatory actions of the drug. (This work is published in: Life Science 1998; 62:1775-1781)

P-5

Ketamine significantly affects leukocyte trans-endothelial migration

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Background: In long term ventilated patients at intensive care unit anesthetic drugs like ketamine are needed over a longer time period. Polymorphonuclear leukocytes play a major role in host defence. They migrate rapidly to the site of infection and destroy invading bacteria. During migration through endothelial cell monolayers, leukocytes undergo morphological changes from rounded, relatively smooth cells to elongated, ruffled cells with pseudopodia. The influence of anesthetic and analgesic drugs on leukocytes is well documented.

Aim of the study: We investigated the effect of ketamine on the leukocyte migration through a monolayer of endothelial cells in vitro.

Methods: Seven independent experiments from different donors were done. Investigation of the influence of ketamine (0.3 ng/ml, 3 ng/ml and 30 ng/ml) to the migration of human leukocytes through human endothelial cell monolayers. Human endothelial cell monolayers and/or human leukocytes were preincubated with clinically relevant (3 µg/ml), higher (30 µg/ml) and lower concentrations of ketamine (0.3 µg/ml) and the amount of leukocyte migration after 3 hours were measured in a fluorometer.

Results: The migration of leukocytes through monolayers of endothelial cells under the clinically relevant concentration of ketamine was reduced to 59 ± 9.8% ($p<0.05$) when leukocytes but not the endothelial cell monolayers were preincubated with ketamine. Leukocyte migration was reduced to 92 ± 7.3% ($p>0.05$) when only monolayers of endothelial cells and to 52 ± 8.8% ($p<0.05$) when both leukocytes and monolayers of endothelial cells were treated with ketamine. The higher and lower concentrations showed a dose dependent effect.

Conclusion: Ketamine is able to reduce significantly the migration of leukocytes through endothelial cell monolayers. The use of different dosages revealed a dose-dependent effect. The current model allowed treatment of one cell type, leukocyte or endothelial cell. Ketamine influences mainly leukocytes rather than endothelial cell monolayers.

(This work is published in Critical Care Medicine, Sept. 1998)

P-6

Prostaglandin E_1 stimulates leukocyte migration through human endothelial cell monolayers

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Background: Investigating the interactions between the cells of the vascular wall and blood components is essential to understand normal vascular biology and pathophysiological processes during inflammation. Leukocytes have to pass a monolayer of endothelial cells (ECM) to migrate into the extravascular tissue.

Aim of the study: The aim of the current study was to investigate whether prostaglandin E_1 (PGE_1) influences the process of leukocyte migration.

Methods: Human umbilical endothelial cells were cultured on microporous membrane filters until achievement of a monolayer for investigation of leukocyte migration. Polymorphonuclear leukocytes (PMNL) were isolated from healthy volunteers and PMNL migration was studied under the influence of PGE_1 using different concentrations (50, 500, 5000 ng/ml). The migration rate of untreated PMNL through untreated ECM was used as control and set as 100%.

Results: The effect of therapeutic plasma concentrations of PGE_1 (500 ng/mL) on PMNL migration through monolayers of endothelial cells showed an increase up to 205 ± 7.8% ($p<0.05$ compared to control), when both cell types ECM and PMNL were treated simulating the situation after an i.v. injection. When treating solely PMNL, the migration rate was not significantly increased (120 ± 9.2%). The treatment of solely endothelial cell monolayers increased the amount of leukocyte migration up to 145 ± 10.2% ($p<0.05$ compared to control). The influence of different dosages of PGE_1 (50 ng/mL, 500 ng/mL, and 5000 ng/mL) showed a dose dependet effect.

Conclusion: Prostaglandins seem to affect the migration of leukocytes through endothelial cell monolayers. This finding supports the theory that prostaglandins may play a major role during inflammation. Clinical studies are warranted to confirm this hypothesis.

P-7

Reduced transendothelial leukocyte migration under the plasma expander hydroxyethyl starch

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Background: Leukocytes play a tremendous role during inflammatory processes. Leukocytes have to pass a monolayer of endothelial cells (ECM) to migrate from the vascular space into the extravascular tissue to avoid microorganisms. Hydroxyethyl starch (HES) is frequently used in cryopreservation, leukapheresis and in infusion as volume expander during trauma.

Aim of the study: The aim of the current study was to investigate HES influences on leukocyte migration.

Methods: Whole blood was taken from healthy volunteers. The neutrophils were isolated using a ficoll-percoll method. Human umbilical vein endothelial cells were isolated and cultured on microporous membranes to achieve a endothelial cell monolayer (ECM). ECM and neutrophils were pre-incubated HES (200/0.5) using different concentrations (1%, 5%, 10%). The amount of leukocyte migration was measured after 3 hours using a migration assay as previously described by our group. In separate experiments only one type of cell, neutrophils or ECM were pre-treated with HES. All experiments were performed n=5 and a p-value < 0.05 were considered as significant. Statistical analysis were done using the student's *t*-test and variance analysis.

Results: The migration rate of untreated PMNL through untreated ECM was used as control and set as 100%. In clinically relevant concentrations, HES was able to significantly decrease leukocyte migration through endothelial cell monolayers (HES 10%: 62 ± 12% SD, $p<0.05$). The use of different concentrations of HES showed a dose-dependent effect. In this assay, both cell types (PMNL and ECM) were treated simultaneously, simulating the clinical situation after an i.v. injection. The treatment of one cell type, only PMNL (HES 10%: 89 ± 9% SD, $p<0.05$) or only ECM (HES 10%: 76 ± 2% SD, $p<0.05$), suggests that the influence is rather on endothelial cells than on leukocytes.

Conclusion: We investigated the cellular interaction between both cell systems, leukocytes and endothelial cells simultaneously in the presence of HES. HES is able to reduce significantly the migration of leukocytes through endothelial cell monolayers when both cell systems are pre-treated simultaneously.

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P-8

C5a receptor mediated basophil and skin mast cell chemotaxis

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Background: The complement product C5a is a potent chemotaxin involved in the directed migration of leukocytes. Mast cell and basophils are proinflammatory leukocytes which have the capacity to migrate against chemotaxins, and to accumulate in areas of ongoing inflammation.

Aim of the study: In the present study, the chemotactic effect of recombinant human (rh) C5a on human tissue mast cells (MC), primary blood basophils, the human mast cell line (HMC-1), and the basophilic cell line (KU-812) have been investigated.

Materials: Chemotaxis of tissue mast cells (foreskin MC, n=4; lung n=3), primary blood basophils (healthy volunteers, n=17), HMC-1 and KU-812 cells against rh-C5a was measured using a chemotaxis assay previously described by our group.

Results: Chemotaxis of HMC-1 cells, and KU-812 cells, but not lung mast cells were induced through rh-C5a. The effects of rh-C5a on basophils and mast cells were dose-dependent (effective concentration range: 10^{-7} M to 10^{-11} M) and could be inhibited by a blocking antibody directed against the C5a receptor (CD88). In contrast to the chemotactic responses, histamine release was only elicited in the high concentration range of rh-C5a. As assessed by double staining technique, flow cytometry, and antibodies against CD88, foreskin mast cells, blood basophils, HMC-1, and KU-812 cells were found to express C5a receptor/CD-88.

Conclusion: Our data show that C5a induces chemotaxis in human basophils and foreskin mast cells are heterogenous cells in terms of migratory responses against C5a. Whether the interactions between C5a and CD88 on mast cells or basophils play a role in inflammatory or allergic reactions associated with accumulation of such cells, remains to be elucidated.

P-9

Anti-Filaggrin Antikörper: Sinnvolle Ergänzung der routinemäßigen Rheumaserologie

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Die chronische Polyarthritis (cP; rheumatoide Arthritis) ist teilweise schwer zu diagnostizieren, da in der Frühphase der Erkrankung die typischen röntgenologischen Veränderungen fehlen und sich die routineförmige Labordiagnostik auf unspezifische Entzündungsmarker und häufig krankheitsassoziierte Autoantikörper beschränkt. Die höchste Spezifität zur cP zeigen die sogenannten anti-Keratin-Antikörper (Berthelot JM et al., Rev Rhum Engl Ed 1995), die als Antikörper definiert sind, die mit den schuppenförmigen Zellen des Stratum corneum des Ratten-Ösophagus reagieren. Neuere Arbeiten zeigen, daß die anti-Keratin-Antikörper mit den cP-assoziierten Anti-Filaggrin-Antikörper ident sind (Simon M et al., Clin Exp Immunol 1995).

Wir haben in einer retrospektiven Studie Sera von Patienten unserer Rheumaambulanz und Sera von Patienten, die sich zu einer Badekur im Heilstollen in Badgastein-Böckstein aufhielten, auf anti-Fiaggrin-Antikörper untersucht, die diagnostische Spezifität überprüft und die Bestimmung in unser Routineprogramm übernommen.

Anti-Filaggrin-Antikörper wurden durch indirekte Immunfluoreszenz auf Ratten-Ösophagusschnitten (zuerst selbst angefertigt, dann kommerziell über die Firma Immco Diagnostics Inc., Buffalo, USA, bezogen) nachgewiesen (Sera 1:4 mit PBS verdünnt aufgetragen, Antikörper mit FITC-markiertem anti-human IgG Konjugat der Firma Inova, San Diego, USA, nachgewiesen).

Insgesamt wurde 139 Sera untersucht. In den Proben von 94 Patienten mit cP (108 Frauen, 31 Männer) waren 34 Proben positiv (36%). Von 45 Patienten mit anderen rheumatischen Erkrankungen wurde 1 Probe (Diagnose: Verdacht auf Spondylitis ankylosans) als positiv beurteilt. In den Sera der cP Patienten war die Häufigkeit der Filaggrin Antikörper unabhängig vom Vorhandensein eines Rheumafaktors (Chi–Quadrat Vierfeldertest, $p = 0.058$).

Durch die hohe Spezifität, die einfache Handhabung und die verhältnismäßig gut als positiv erkennbaren Muster ist der Test auf anti-Filaggrin-Antikörper für das Routinelabor zu empfehlen.

P-10

Viral load increase in HIV due to poor compliance - a case report

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The quality and duration of survival for patients with HIV infection has improved since AIDS was first recognized in the early 1980s. Although much of this improvement is due to more effective management of the opportunistic infections the substantial contribution is made by antiretroviral therapy.

It was originally thought that very little virus replication occurred during clinical latency. However, studies of lymphoid tissue using PCR analysis for HIV RNA and in situ hybridization for individual virus expressing cells clearly demonstrated that HIV replication occurs throughout the cause of HIV infection, even during clinical latency. Measuring the levels of HIV-1 in plasma is important in determining the effectiveness of a potential therapeutic regimen, and may be critical for the therapeutic management of infected patients. Most patients find it difficult to comply with highly complex schedules of assessment and medication. Therefore a number of factors may contribute to the therapeutic failure of antiretroviral therapy, the development of viral drug resistance is of concern.

In this case we describe therapy monitoring in a HIV-patient treated with the following substances: AZT, Lamivudin, Norfinavir. Viral load was determined since 1997. When therapy was started - a continuous decline in viral load was found, until in June 1998 viral load was raising to a level of $3,2 \times 10^5$. Because of this unexpected increase viral load was determined one month later - with the result of a reduction by 1.6 log/ml. This effect was proven one month later, then the value was lowered again by 1.4 log/ml.

However the reason for all these discrepancies was that the patient had stopped therapy for about two months.

This follow up shows, that determination of viral load in HIV patients is a very suitable parameter not only for the effectiveness of therapy but also for checking the compliance of patients. Because of the problems of side effects due to antiviral agents, a therapy drop-out has to be taken into account in cases of such unexpected increases in viral load. All data will be presented in detail.

P-11

Lyme Disease - Comparison of two Elisa Systems (IgG, IgM) to Western Blot and Clinical Data

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Lyme disease is a global tick borne disease caused by infection with Borrelia burgdorferi sensu lato. The disorder develops in stages and with different manifestations involving mainly the skin, the nervous system and the joints. The diagnosis is based on the recognition of typical clinical signs and is assisted by laboratory tests.

A lot of different assays are available detecting IgG and IgM antibodies. However great differences are described between the distinct assay systems, partially due to the different genotyps of Borrelia b. (B sensu stricto = genotyp 1, B garinii = genotyp 2, B afzelii = genotyp 3) and their distinct distribution over the world. In this study we evaluated the Enzygnost IgG and IgM Elisa (Behringwerke, Marburg) and compared it to the Lyme disease Elisa IgG and IgM (MRL Diagnostics, Cypress, CA). Elisa results were confirmed by Western blot analysis (IgG, IgM, Gull Laboratories, Fresenius, Bad Hamburg GE). IgM Western blot analysis was considered to be positive if there was either a band in the 23 kDa and/or in the 39 kDa region.

A group of 100 selected patients was investigated with respect to clinical as well as laboratory data. Like anticipated, great differences were found with respect to Elisa results mainly within the IgM assays. In only 60% of our patient group consistent results were optained. The Behring Enzygnost Elisa showed about 20% false positive results, when compared to western blotting analysis, whereas the Lyme disease Elisa from MRL demonstrated opposite results with a better specificy but about 20% false negative results versus 3% false negative with the Behring assay. Within all other IgM negative results with the Enzygnost test system only one patient showed clinical relevant signs of Lyme disease. This patient was also negative for IgG Elisa as well as WB.

Therefore our conclusion out of this study is, that we would suggest a more sensitive test system for screening, followed by Immunoblot for positive results. However if there ist a strong clinical evidence for a Borrelia infection also negative Elisa results should be proofed by Immunoblot. All results will be presented and discussed in detail.

P-12

B-cells show immunophenotypical signs of activation in patients with alcoholic liver cirrhosis

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To evaluate the hypothesis of B-cell activation in alcoholic liver cirrhosis (ALC), we measured the expression of the antigens CD5, CD23, CD24, CD40, and HLA-DR on peripheral blood B-cells of 20 patients with ALC and 20 healthy volunteers. In ALC, B-cells showed significantly increased expression of CD23 ($P < 0.0001$), CD40 ($P = 0.038$), and HLA-DR ($P = 0.012$), as well as a decreased expression of CD24 ($P < 0.0001$). Furthermore, the percentage of $CD5^+$ B-cells was reduced in ALC (13 ± 13 vs. 20 ± 9; $P = 0.006$). We could not find a significant correlation between the activation-induced immunophenotypic changes and serum IgG, IgA, IgM levels, or the Child-Pugh stage. The results of this study clearly indicate that B-cells in patients with ALC are in a state of activation thus supporting the hypothesis of *in vivo* stimulation being the reason for B-cell hyperactivity in ALC. The mechanism of this stimulation cannot be ascertained by our study but the pattern of B-cell antigen expression was consistent with T-cell-mediated activation.

P-13

Neopterin - a prognostic parameter in females with breast cancer

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Neopterin, which is produced in large quantities by human monocytes/macrophages upon stimulation by interferon-α, has turned out to be a sensitive marker for monitoring Th1-cell immune response in humans. Increased neopterin concentrations were described in patients with virus infections, autoimmune disorders and during allograft rejection. In patients with malignant diseases serum and urine neopterin concentrations are of prognostic value. In this study, the predictive power of urinary neopterin concentrations in females with breast cancer was examined.

Patients and methods: 129 females with breast cancer at the moment of diagnosis were investigated. Tumor histology and routine laboratory parameters were concomitantly examined. Urinary neopterin and creatinine concentrations were determined by an automated high-performance liquid chromatography technique. The thereby resulting urinary neopterin/creatinine ratios were referred to the individual sex and age dependent reference values generated earlier.

Patients were followed up for up to 13 years, and the ability of all variables to predict fatal outcome was assessed by calculating product limit estimates and multivariate Cox's proportional hazards model.

Results: Urinary neopterin values were elevated in 18% of the patients and did not correlate with tumor size or lymph node status. Neopterin levels were influenced by the presence of distant metastases ($p=0.04$) and by tumor differentiation ($p=0.01$). In univariate analysis, the presence of distant metastases ($p<0.001$), neopterin ($p<0.001$), tumor size ($p=0.001$) and lymph node status ($p<0.01$) were significant predictors of survival. 17/23 patients with elevated (mean survival time: 43 months), but only 39/106 with normal (mean survival time: 107 months) urinary neopterin levels died from malignancy during the observation period. By multivariate analysis, a combination of the variables presence of distant metastases ($p<0.001$), neopterin ($p=0.03$) and lymph node status ($p=0.01$) was found to jointly predict survival.

Discussion: Urinary neopterin concentrations provide valuable prognostic information in females with breast cancer. Neopterin is not a tumor marker in the usual sense of the word. The predictive power of elevated neopterin in patients with malignancies might be explained by a chronic immune stimulation, which is unable to eliminate the stimulating agent, namely the tumor. On the other hand, a capacity of neopterin derivatives to induce proto-oncogene expression, such as c-fos gene, could be discussed.

P-14

The prognostic impact of urinary neopterin in patients with malignancies of the oral cavity

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Upon stimulation by interferon-g, human monocytes/macrophages produce neopterin which has turned out to be a sensitive marker for monitoring Th1-cell immune response in humans. Besides, high neopterin concentrations in serum and urine of patients with malignant diseases have been described to be associated with poor survival. We therefore studied patients with malignancies of the oral cavity to compare urinary neopterin levels with their outcome.

Patients and methods: 23 patients with squamous cell carcinoma of the oral cavity at the moment of diagnosis and 12 treated patients with the same disease at the moment of the recurrence of the tumor were investigated. Tumor histology and routine laboratory parameters were concomitantly examined. Urinary neopterin and creatinine concentrations were determined by an automated high-performance liquid chromatography technique. The thereby resulting urinary neopterin/creatinine ratios were referred to the individual sex and age dependent reference values generated earlier.

Patients were followed up for up to 4 years, and the ability of all variables to predict fatal outcome was assessed by calculating product limit estimates and multivariate Cox`s proportional hazards model.

Results: Urinary neopterin values showed no statistically significant correlation with tumor differentiation, tumor size or patients` age, but they were significantly higher in patients with a recurrent tumor ($p=0.025$). In univariate analysis, only neopterin ($p=0.01$) and the variable recurrent versus first-diagnosed tumor ($p=0.005$) were significant predictors of survival. 14/19 patients with elevated, but only 4/16 with normal urinary neopterin levels died from malignancy during the observation period. By multivariate analysis, a combination of neopterin ($p<0.01$) and the variable recurrent versus first-diagnosed tumor ($p=0.06$) was found to jointly predict survival.

Discussion: Urinary neopterin concentrations provide valuable prognostic information in patients with squamous cell carcinoma of the oral cavity. Neopterin is not a tumor marker in the usual sense of the word. The predictive power of elevated neopterin in patients with malignancies might be explained by a chronic immune stimulation, which is unable to eliminate the stimulating agent, namely the tumor. On the other hand, a capacity of neopterin derivatives to induce proto-oncogene expression, such as c-fos gene, could be discussed.

P-15

Neopterin - a prognostic parameter in surgery for lung cancer

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Neopterin is released in large amounts by human monocytes/macrophages upon stimulation with interferon-γ. The biosynthetic pathway is similar to tetrahydrobiopterin which is used in metabolism as cofactor for amino acid mono-oxygenases. Neopterin is not further converted in the organism and is excreted via the kidneys. Elevated levels of neopterin in serum and urine are detected in viral infections including HIV, autoimmune disorders and allograft rejection episodes. Therefore, determination of neopterin has been established as a sensitive monitor for cell-mediated immune activation in, e.g., virus infection and autoimmune disorders. Moreover, in some malignant tumors, increased neopterin levels were found to be associated with worse prognosis of survival. Here we tested the attitude of neopterin for the prognostic value of survival in patients with lung cancer.

Patients and Methods: From 110 lung cancer patients (103 non small cell lung cancer, 7 small cell lung cancer) preoperative urine concentrations of neopterin per creatinine were measured at the time of diagnosis, and these values were correlated to postoperative survival. The median age at diagnosis was 64 years. Median follow-up of patients was 17.4 months. Infectious diseases were excluded by clinical observation and routine laboratory parameters. Clinical staging and health status allowed in all patients surgical intervention with lung resection.

Results: 27 patients (24.5%) died of tumor progression. 16 of these patients (57%) had preoperatively elevated neopterin levels in urine. After surgery 66 patients had pathological stage I of lung cancer. Univariate analysis (Mantle Cox) showed a significantly worse prognosis of survival ($p<0.0001$) for patients whose neopterin level were > 212 µmol/mol creatinine, representing the 4th quartile of the distribution of neopterin, compared to those with lower levels. Of eleven patients with stage I lung cancer and neopterin > 212, nine died of tumor progression (82%) in the observed period ($p<0.0001$).

Conclusion: Preoperative urine neopterin is a sound prognostic parameter for survival in patients with lung cancer, and this is true independently of tumor stage at diagnosis. Even in patients with stage I lung cancer, the predictive value of neopterin was significant. Neopterin is hypotesized to be more elevated in aggressive tumors due to a high degree of immunological challenge. In patients with high risk for lung surgery or reduced respiratory function, detection of preoperative urinary neopterin may represent a merit diagnostic possibility influencing the decision of surgery.

P-16

Diagnosis of familial hypercholesterolemia (FH) by means of DNA analysis of LDL-receptor gene mutations

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Diagnosis of heterozygous familial hypercholesterolemia, an autosomal dominant genetic disorder with an estimated prevalence of 1/500 is difficult, because plasma lipid levels overlap with those of the general population. Using DNA tests to confirm the diagnosis of FH have suggested that between 15-20% of adult subjects may be misdiagnosed by cholesterol testing alone (Ward, 1996). A proportion of children who are at risk for FH (carriers of a mutation causing FH) may present initially with lipid levels within a normal range and elevated levels may develop at a later stage (Kessling et al, 1990).

Within the MED-PED project we were able to detects 25 children with proven LDL-receptor gene mutation: 21 heterozygous, 1 homozygous and 4 ApoB variants. At least 5 children would have been missed using the common criteria for first degree relatives of patients with FH (> 220 mg/dl Cholesterol). Using the criteria for the general population (> 270 mg/dl) 10 adolescents would have been missed.

Thus, diagnosis of FH by means of DNA analysis of LDL-receptor mutations seems to be an appropriate diagnostic tool for early detection of children and adolescents who are at later risk for cardiovascular diseases. This procedure enables prevention strategies even in adolescents.

P-17

Ist Lipoprotein(a) wirklich ein Akutphasen-Protein?

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Lipoprotein(a) [Lp(a)] ist ein atherogenes Lipoprotein, das strukturell dem low-density Lipoprotein ähnlich ist. Hohe Plasmakonzentrationen an Lp(a) gehen mit einem erhöhten Risiko für koronare Herzkrankheit (KHK) einher und die Bestimmung des Plasmaspiegels wird zur Risikoabschätzung verwendet. Transiente Konzentrationsanstiege von Lp(a) wurden bei Tumorpatienten und bei postoperativen Patienten berichtet. Kürzlich wurde bei Patienten mit beschleunigter BSG (>50 mm/h) eine 7-fach höhere Konzentration als bei Gesunden gefunden, und es wurde empfohlen Lp(a) Bestimmungen zur KHK Risiko Abschätzung nur nach Ausschluß einer Akutenphase Reaktion (APR) durchzuführen (W-K Min et al. Clin Chem 43, 1891, 1997).

Bedingt durch einen Fehler in den Laboranforderungen wurde bei stationären Patienen (KHK n=12, Tumor n=5, Pulmonalembolie, Asthma bronchiale, Niereninsuffizienz je 1) über mehrere Wochen neben dem gewünschten Chemogramm und C-reaktiven Protein (CRP) als APR-Parameter auch Lp(a) bestimmt. Bei 20 Patienten mit APR (CRP >10 mg/L) wurden 7 bis 19 Paare von CRP und Lp(a) gemessen, 10 Patienten waren während der Beobachtung auch ohne APR (CRP < 5). CRP und Lp(a) wurden für jeden Patienten nach Passing-Bablock korreliert, wobei 4 Patienten eine signifikante Korrelation zeigten, 2 mit pos. Regressions-Koeffizienten (r=+0.7, 0.6) und 2 mit neg. Koeffizienten (r=-0.9, -0.8). Die Summe aller 20 Koeffizienten ergab eine mittleres r von -0.1. Die Korrelation von Lp(a) mit anderen Parametern der klinischen Chemie zeigte bei 5 Patienten lediglich eine signifikante Korrelationen zur Cholinesterase (r= +0.61 bis +0.96, MW +0.81), für alle Patienten war der gemittelte r-Wert +0.44.

Diese Ergebnisse weisen darauf hin, daß Lp(a) - wenn überhaupt - nur gering während APR verändert ist, bei eingeschränkter Leberfunktion hingegen vermindert ist.

P-18

Statistical Comparison of 5 Commercially Available Enzyme Immunoassays for Detection of HCMV-specific IgM Antibodies

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In the last years the number of commercially available ELISA kits for the detection of HCMV- specific IgM antibodies has increased rapidly. The aim of the present study was to evaluate five commercial ELISA kits for the serological diagnosis of HCMV-infection. We compared these methods –namely the ABBOTT, the NOVUM, the GULL, the MEDAC and the BIOTEST immunoassay- for their imprecision, diagnostic effectiveness and interference with substances eventually producing cross-reactions with HCMV-IgM (EBV-IgM, rheumatoid factor). In addition, repeated measurements taken from samples of renal and heart transplant recipients with active HCMV infection were analysed to compare the temporal development of the CMV extinctions measured with the five assay systems. Sensitivity and specificity were assessed based on concordance of the five diagnostic systems, antigenemia results and clinical plausibility. A quantitative comparison of the extinctions with commonly used statistical methods (Passing-Bablok regression, Altman-Bland plot) and a ROC analysis were performed too. The results of the study demonstrated that there are great differences in diagnostic power as well as crossing reactions with EBV-IgM and rheumatoid factor between the tested commercially available ELISAs. The outcome of the quantitative comparison showed a very poor agreement between the five ELISAs. The results of the performed ROC analysis showed ROC curves with partially statistically different areas. The shapes of the curves indicated that the placement of the assay specific cut-off points were not always optimally chosen.

P-19

Cystatin C - Ein sensitiver Parameter in der Frühdiagnostik von Nierenfunktionsstörungen

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Cystatin-C, ein Mitglied der Cystein-Proteinaseinhibitoren, ist ein kleinmolekulares Protein (13kD), welches von allen kernhaltigen Zellen mit konstanter Rate gebildet wird (house-keeping gene). Wegen des niedrigen Molekulargewichtes wird Cystatin-C glomerulär filtriert und proximal tubulär vollständig reabsorbiert. Der periphere Serum Cystatin-C Wert korreliert somit direkt mit der glomerulären Filtration und ist, im Gegensatz zu Kreatinin, unabhängig von Muskelmasse, Lebensalter, Geschlecht und Körperlänge. Zur Messung von Cystatin-C sind schnelle nephelometrische und turbidimetrische Testsysteme verfügbar. In der vorliegenden Studie wurde Serum und Plasma Cystatin-C bei einem Kollektiv von 210 pädiatrischen und adulten Patienten mit unterschiedlichen nephrologischen Erkrankungen mittels eines Behring Nephelometers (BNA) als Parameter zur Früherfassung einer Nierenfunktionsstörung evaluiert. Cystatin-C wurde mit dem Plasma-Kreatinin und mit der glomerulären Filtrationsrate (GFR, nach Schwartz'scher Formel) statistisch verglichen. Sowohl Serum als auch Plasma Cystatin-C zeigten eine signifikant bessere Korrelation mit der GFR als Kreatinin. Weiters konnten bei Patienten mit normalen Kreatinin und GFR-Werten, welche konsekutiv eine glomeruläre Nierenerkrankung entwickelten, initial erhöhte Cystatin-C Werte nachgewiesen werden.

Zusammenfassend ergibt sich aus den vorliegenden Daten, daß die nephelometrische Bestimmung von Cystatin-C im Vergleich zum bisherigen „Gold-Standard" Kreatinin, eine gleich schnelle, aber sensitivere Methode zur Früherfassung von Nierenfunktionsstörungen darstellt.

P-20

Ausscheidungsprofile von Benzodiazepinen und Opiaten im Harn bei Patienten im Drogenentzug

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Studienziele: Erstellung standardisierter Ausscheidungsprofile von Benzodiazepinen (BE) und Opiaten (OP) im Harn von Patienten im Rahmen eines stationären Drogenentzuges. Zur Identifizierung eines objektivierbaren Maßes für den Verdünnungszustand des Harns wurden in den Proben Kreatininkonzentration und Osmolalität bestimmt. Darüber hinaus wurden auch Serumproben zur Validierung des homogenen Enzymimmunoassays (EMIT II®, Behring-Syva) für dieses Material auf Drogen untersucht.

Patienten/Methodik: Insgesamt konnten 48 Patienten erfaßt werden, von denen im Verlauf der 1. Woche nach Aufnahme in das Drogenentzugsprogramm täglich Harnproben gesammelt wurden und in der 2. Woche alle 2 Tage. Blutabnahmen für die Serumanalytik erfolgten am 1. und 4. Tag.

Ergebnisse: Insgesamt konnten 360 Harn- und 90 Serumproben standardisiert gesammelt und ausgewertet werden. In bezug auf den Drogenkonsum handelte es sich um 19 reine OP-, 3 reine BE- und 26 Mischkonsumenten. OP-positive Harnproben sanken nach durchschnittlich 3,6 Tagen (Bereich 1 bis 8) unter den Cut-off (300 ng/ml) des Testsystems. Beim BE-Testsystem (Cut-off 200 ng/ml) wurden die Proben innerhalb von durchschnittlich 4,2 Tagen (Bereich 1 bis 10) negativ.

Fazit: Unsere Daten unterstützen das Therapiekonzept, daß nach Beendigung des Drogenkonsums BE- und OP-Konzentrationen in den abgegebenen Harnproben innerhalb von spätestens 2 Wochen unter den jeweiligen Testsystem-Cut-off zur Unterscheidung zwischen „positiven" und „negativen" Proben absinken. Als Maß für die Harnverdünnung sind sowohl Kreatininkonzentration als auch Osmolalität geeignet. Bezüglich eines Cut-offs des Harnkreatinins erscheinen 30 mg/dl als praxistauglich. Zur Analyse von Serumproben war das unmodifizierte Testsystem geeignet, wobei die Ergebnisse bei der BE-Testung bessere Übereinstimmungen mit den Harnwerten zeigten.

P-21

Standardisierung des Nachweises erythrozytärer Antikörper

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Die obligate Durchführung eines Antikörper-Suchtests (AKST) bei jeder Blutgruppen-Untersuchung gilt mittlerweile als „europäischer Standard". Die Durchführung ist zumeist in nationalen Richtlinien verankert, jedoch hinsichtlich Ausmaß und Art der minimalen Untersuchungen unterschiedlich geregelt. Das Testzellpanel sollte - bezüglich klinisch relevanter Antikörper - homozygote Antigeneigenschaften aufweisen, um die Auffindung schwacher Antikörper zu gewährleisten. Daraus leitet sich die Verwendung von zumindest 3 Testzellen ab.

Seit Jänner 1997 findet in unserem Labor ein standardisiertes Schema Anwendung. Der AKST wird mit 2 Testzellen bei Raumtemperatur im Coombs- Milieu, sowie mit 3 weiteren Testzellen jeweils bei 37°C im 2-Stufen-Enzymtest (Papain) und im indirekten Coombstest durchgeführt. Dieser breite Suchtest erlaubt eine richtungsweisende Aussage zur weiteren Differenzierung über das optimale Reaktionsmedium und das Erkennen von Mehrfachantikörpern. Ausgehend von den Reaktionen im AKST ist das weitere diagnostische Vorgehen in 5 Flußdiagrammen festgelegt.

Im Zeitraum bis September 1998 wurden 8048 Patientenproben untersucht. 6,3% reagierten im AKST positiv. Bei 2,6% konnten ein/oder mehrere Antikörper spezifiziert werden. (Im Bereich der orthopädischen Abteilung stieg dieser Prozentsatz auf 4,3%; bei vortransfundierten Patienten dieser Abteilung gegen 8%.)

Der Anteil von Patienten, bei denen kein Antikörper spezifiziert werden konnte, die nur mit papainisierten Testzellen reagierten lag bei 71%. Dem steht gegenüber, daß 33% der spezifizierten Antikörper (vorwiegend Rhesus-AK) nur im Papain-Test nachweisbar waren.

Die angeführten Flußdiagramme sind beim Verfasser erhältlich.

P-22

Enzymimmunoassays für proBNP Fragmente (8-29) und (32-57)

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Brain natriuretisches Peptid (BNP) ist ein Mitglied der Familie der natriuretischen Peptide, die für die Homöostase des Salz- und Flüssigkeitshaushalts des Körpers mitverantwortlich sind. BNP wird bei der Freisetzung aus dem Ventikel in die biologisch aktive Form BNP 32 und ein 76 Aminosäuren langes N-terminales Fragment proBNP (1-76) gespalten.

Für die Immunisierung von Schafen wurden Teilsequenzen mit hoher Immunogenität ausgewählt: proBNP (8-29) und proBNP (32-57). Für die Entwicklung der Tests wurden die für das jeweilige Antigen spezifischen Antikörper aus dem Rohserum über Immunaffinitätschromatographie gereinigt. Mikrotiterplatten wurden mit einem fc-spezifischen anti-Schaf IgG beschichtet, verbleibende Bindungsstellen wurden abgeblockt.

Im ersten Schritt des Assays binden die gereinigten spezifischen Antikörper reproduzierbar mit dem fc-Teil an dem auf die Platte beschichteten Antikörper. Der nächste Inkubationsschritt ist eine kompetitive Reaktion des unmarkierten Peptids in Standard oder Probe und dem biotinylierten Peptid (Tracer) mit dem im Unterschuß vorhandenen spezifischen anti proBNP Antikörper. Streptavidin-HRPO und TMB werden zur Quantifizierung eingesetzt.

ProBNP Immunreaktivität wurde in Serum, Plasma und Urin gefunden. Wiederfindungen der synthetischen Peptide und Linearität der Assays wurden untersucht. Die Nachweisgrenzen (95% B/Bo) der beiden Assays liegen bei 4 fmol/ml für Nt-proBNP (8 - 29) und 1 fmol/ml für mid-proBNP (32 - 57). Die Referenzbereiche im Plasma für Gesunde betragen bis zu 400 fmol/ml (Nt-proBNP) und bis zu 100 fmol/ml (mid-proBNP)

Mit diesen Assays ist eine einfache, sichere und reproduzierbare Bestimmung von proBNP Immunreaktivität in Serum, Plasma und Harn möglich.

P-23

Enzym Immuno Assay für 25-Hydroxyvitamin

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25-Hydroxycalciferol (25-OH-D) stellt den Hauptmetaboliten von Vitamin D im Blutkreislauf dar. Die Hydroxylierung findet beim Menschen primär in der Leber statt. Die analytisch wichtigen Formen von 25-OH-D entstehen einerseits aus Vitamin D_3, welches endogen aus dem durch Einwirkung von UV-Licht auf die Haut gebildeteten Provitamin D3 gebildet wird, und anderseits aus dem synthetischen Vitamin D_2 welches in manchen Ländern Nahrungsmitteln zugesetzt wird. Da nur geringe Mengen 25-OH-D in der Niere zu Dihydroxy-Vitamin D Metaboliten umgesetzt werden gilt 25-OH-D als verläßlicher Indikator für den Vitamin D Status.

Wir haben einen neuartigen kompetitiven Bindeproteinassay im Mikrototiterplatten-format unter Verwendung humanem Gc-Globulins (Vitamin D Bindeprotein) und eines biotinylierten 25-OH-D Analogons als Tracer entwickelt. 50µl Serum oder Plasmaproben werden mit 200µl vorgekühltem Äthanol gefällt und 10 µl des Überstandes im Assay eingesetzt. Das 25-OH-D in der Probe konkurriert mit dem über Streptavidin an die Festphase gebundenen Tracer um die Bindung an das Gc-Globulin. Die Detektion des gebundenen Gc-Globulins erfolgt über einen Peroxidase markierten Antikörper und TMB. Das Detektionslimit (0-Standards + 2SD) liegt bei 5nmol/l (2ng/ml), der höchste Standard beträgt 312 nmol/l (125ng/ml). Damit ist es möglich Hypovitaminosen klar vom (saisonal schwankenden) Referenzbereich zu differenzieren. Auch sonnenbedingt stark erhöhte 25-OH-D Spiegel können direkt bestimmt werden. Der Test kann innerhalb von 5 h bei Raumtemperatur oder über Nacht bei 4°C durchgeführt werden. Die Wiederfindung von synthetischem 25-OH-D liegt zwischen 78 und 88%. Der Assay korreliert gut mit einem kommerziell erhältlichen Radioimmunoassay (I^{125} markiertem Tracer) bzw. mit einem Bindeproteinassay mit tritiiertem 25-OH-D).

P-24

Sandwich-ELISA zur Bestimmung von proatrialem natriuretischem Peptid [proANP (1-98)] in Serum, Plasma und Harn

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In der Literatur wird berichtet, daß die Konzentrationen verschiedener Fragmente von proANP bei Herzinsuffizienz in Relation zum Schweregrad der Herzmuskelschwäche stark ansteigen und daher als nicht invasive in der Zirkulation Marker von Interesse sind.

Bei der Freisetzung von ANP wird das Prohormon in einen N-terminalen Teil von 98 Aminosäuren pro-ANP (1-98) und das biologisch aktive α-ANP (99-126) zu gleichen Teilen gespalten. ProANP (1-98) hat eine deutlich längere Halbwertszeit im Blutkreislauf als das biologisch hochwirksame α-ANP, seine Konzentrationen sind höher und reagieren wenig sensitiv auf die pulsatile Fluktuation der Konzentration von α-ANP. Die Menge an proANP (1-98) spiegelt die Gesamtmenge des sezernierten ANP wieder.

Der hier vorgestellte Sandwich-Immunoassay mißt mit hoher Spezifität und Sensitivität proANP (1-98) in Serum, Plasma und Harn in einem weiten Meßbereich von 5 fmol/ml bis 1000 fmol/ml. Die eingesetzten Antikörper sind immunaffinitätschromatografisch gereinigt und wurden mittels Epitopmapping ausgewählt. Der Erstantikörper, spezifisch für die Teilsequenz pro-ANP (13 - 17) ist auf einer Mikrotiterplatten beschichtet. Der biotinylierte Zweitantikörper, spezifisch für die Teilsequenz proANP (88 - 93) bildet mit dem Erstantikörper und dem Analyten einen Sandwich. Nach Inkubation mit Streptavidin-Peroxidase erfolgt die Farbreaktion mit TMB.

Bei einem Probeneinsatz von 20 µl Serum oder Plasma wird eine Nachweisgrenze von 100 fmol/ml Probe erreicht. Wiederfindung von synthetischem Peptid und Verdünnungslinearität sind beschrieben. Die Konzentrationen von ProANP (1-98) in einem Kollektiv von Gesunden sind signifkant niedriger als in Patienten der NYHA-Klassen I bis IV. Diskriminierung ist auch innerhalb der NYHA-Klassifizierung möglich.

P-25

Identification of two novel mutations in the ß subunit of the human epithelial sodium channel (hENaC)

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Mutations in the carboxy termini of the ß subunit (hßENaC) and the γ subunit (hγENaC) of hENaC have been identified in patients with Liddle's syndrome, an autosomal dominant trait characterized by severe hypertension, hypokalemia, suppressed plasma renin activity and low aldosterone levels. However, besides these classical characteristics, patients may present atypically, without laboratory abnormalities. To test the hypothesis that mutations in the hßENaC or the h-γENaC genes could be present in patients with end-stage renal disease (ESRD) 256 patients undergoing chronic hemodialysis treatment at the University Hospital of Vienna were screened by polymerase chain reaction (PCR) followed by single-strand conformation polymorphism (SSCP) analysis for putative variants of these genes. Using SSCP and direct sequence analysis, we identified two novel heterozygous mutations at codon 567 (1700C>T) and codon 592 (1776G>A) of hßENaC in two patients from Eastern Europe resulting in a substitution of Ala567Val, whereas the Thr at codon 592 was not exchanged. By contrast, all other. hemodialysis patients did not show any mobility shift on SSCP gels compared to healthy controls. Mutations in the carboxy terminus of hßENaC and hγENaC are likely to result in increased sodium reabsorption in the kidney and consecutive elevation of blood pressure levels. Thus, the novel Ala567Val mutation of the present study may have contributed to progression to ESRD by alteration of blood pressure.

P-26

Clinical heterogeneity of hereditary homocystinuria due to cystathionine ß synthase (CBS) deficiency

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CBS is an enzyme involved in the metabolism of the toxic amino acid homocysteine. CBS deficiency is an autosomal recessive disorder characterized by premature vascular occiusive disease, ectopia lentis, mental retardation, osteoporosis,. and homocystinuria. We report on a 25-year old male patient presenting with stenoses of both iliac arteries, thrombotic occlusion of the tibia-fibular trunk, slight mental retardation, subluxatio lentis, excessively high urinary homocystine concentration, and a reduced activity of CBS in skin fibroblast cultures. To characterize the causative genetic defect, total RNA was isolated from peripheral blood, reverse transcribed (RT) into cDNA, followed by direct sequencing of RT-PCR products covering the entire CBS coding region. We identified a 833T>C (Ile278Thr) and a 1058C>T (Thr353Met) gene mutation. Compound heterozygosity for these mutations has been most recently reported in an Australian male patient, who, by contrast, did not show symptoms of vascular occlusive disease. We therefore have screened our patient for additional genetic vascular disease risk factors. However, our patient neither showed the 1691G>A mutation in the coagulation factor V, nor the 20210G>A mutation in the prothrombin gene, or the 677C>T mutation in the *MTHFR* gene. We conclude that compound heterozygosity for the 833T>C and the 1058C>T transition in the CBS gene causes homocystinuria. Among patients with identical mutations, however, the clinical presentation may be heterogeneous.

P-27

Semiquantitative PCR analysis of expression of endothelial cell adhesion molecules responsive to inflammatory stimuli and age-modified fibronectin

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Patients with diabetes and end-stage renal disease frequently experience atherosclerotic and infectious complications. Advanced glycation endproducts (AGE) are supposed to con-tribute to atherosclerosis in these patients. We hypothesized that the interaction of AGE and inflammatory mediators may result in up-regulation of cell adhesion molecules (CAM) involved in atherogenesis. Because Northern blot analysis is laborious, we have used a semiquantitative PCR protocol to investigate the effect of AGE fibronectin and IL-1α on mRNA levels of E-selectin, ICAM-1, VCAM-1, and PECAM-1 in umbilical cord vein endothelial cells cultured in the presence or absence of AGE fibronection, soluble AGE, and IL-1α for 2.5 h or 10 h. AGE fibronectin significantly up-regulated the IL-1α induced relative mRNA level of E-selectin (at 2.5 h and 10 h), ICAM-1 and VCAM-1 (both at 2.5 h), whereas PECAM-1 showed a significant decrease of mRNA levels at both time points. This significant IL-1α induced reduction of PECAM mRNA was independent of AGE-fibronectin. Furthermore, we observed an increase in relative transcript levels of E-selectin, ICAM-1, VCAM-1 and PECAM-1 after incubation with AGE fibronectin and soluble AGE. In summary, we demonstrate that matrix glycation and inflammation up-regulates gene expression of endothelial CAM which may contribute to the increased atherosclerotic disease morbidity and mortality in patients with diabetes or chronic renal failure.

P-28

Erythropoietin inducible genes in human vascular endothelial cells

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The incidence of hypertension is increased in patients receiving recombinant human erythropoietin (rHuEPO) therapy. The mediators responsible for this adverse effect are largely unknown. To gain more information about potential side-effects of rHuEPO we used differential mRNA display in cultured human umbilical vein endothelial cells (HUVEC) that were exposed to rHuEPO (500 units / ml) for 2 hours. Among 107 mRNA / cDNA fragments which were differentially displayed we identified 10 genes that were up-regulated by rHuEPO as was confirmed by semiquantitative reverse transcriptase PCR in two independent cell-culture experiments. The genes coded for proteins 1.) interacting with the vascular system (thrombospondin 1; a 20 kDa myosin regulatory light chain; endothelin-1; relative increase of mRNA levels: 53.1% 42.1%, 12.1%), 2.) involved in gene transcription and translation (c-myc transcription factor PuF; S19 ribosomal protein; tryptophanyl-tRNA synthetase; increase of mRNA levels: 33.3%, 38.5%, 50.8%), 3.) related to energy transfer (NADH dehydrogenase subunit 6; cytochrome C oxidase subunit 1; increase of mRNA concentrations: 45.4%, 51.1%), and 4.) regulating signal transduction (protein tyrosine phosphatase G1; mono-ADP-ribosyltransferase; increase of transcript levels : 63.8%, 22.2%). Expression of eight of these genes in HUVEC is reported here for the first time. In summary, we identified 10 rHuEPO responsive genes which might be involved, at least in part, in side-effects of rHuEPO therapy.

P-29

A homogeneous time resolved fluoroimmunoassay for chorionic gonadotropin using TRACE® technology: KRYPTOR-hCG+β.

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We developed a homogeneous time resolved fluoroimmunoassay for total hCG (chorionic gonadotropin) based on TRACE® technology for use on KRYPTOR, a fully automated multiparametric random-access analyser. TRACE® is an innovative methodology based on non-radiative energy transfer between an antibody linked to the Europium cryptate (the donor) and a second antibody linked to the XL 665 (the acceptor) induced by the formation of the Ag/Ab complex.

Results are obtained using 26 µl of serum incubated at 37°C with two labelled conjugates after only 14 minutes. A master curve is stored on board and a calibration is made every 2 weeks with 1 calibrator. The direct reading range is 0-2000 IU/l. Higher values up to 3.10^6 IU/l may be detected in a few seconds of incubation by means of kinetic analysis and are automatically diluted. The detection limit based on imprecision profile has been assessed as being 2 IU/l with a probability of 95%.

Within-run CVs are 4.3% at 20.6 IU/l, 1.5% at 224 IU/l and 0.7% at 891 IU/l. Between-runs, between-lots, between-calibrations CVs are 2.3% at 21.1 IU/l, 3.3% at 919 IU/l, 3.1% at 1498 IU/l. No cross reaction was detected with human FSH, TSH, LH, GH, PL. The calibration is made against the 3rd IS WHO 75/537.
A comparative study (n = 104) was carried out with the AxSYM analyser : KRYPTOR-hCG+β = 1.11 x AxSYM - 4.91 (r^2 = 0.91). Normal values have been determined on a panel of 4043 sera of pregnant women from 4th to 18th week after LMP.

P-30

A homogeneous time resolved fluoroimmunoassay for luteinizing hormone using TRACE® Technology: KRYPTOR-LH

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A homogeneous time resolved fluoroimmunoassay for Luteinizing Hormone based on TRACE® was developed for KRYPTOR, a fully automated multiparametric random-access analyser. TRACE® is an innovative methodology based on non-radiative energy transfer between an antibody linked to the Europium cryptate (the donor) and a second antibody linked to the XL 665 (the acceptor) induced by the formation of the Ag/Ab complex.

Results are obtained after 19 minutes incubation at 37°C of 50µl serum or plasma without citrate, with the two conjugates. One master curve is stored on board and is recalibrated every 2 weeks with 1 calibrator. The direct reading range is 0-250 mIU/ml. Higher values up to 2500 mIU/ml may be detected in a few seconds of incubation based on kinetic analysis and automatically diluted. The detection limit calculated using imprecision profile has been assessed as being 0.3 mIU/ml with a probability of 95%. Within-run CVs are 7% for 3.3 mIU/ml, 1.4% for 28.7 mIU/ml and 1.3% for 122 mIU/ml. Between-runs, between calibrations CVs are 8.4% for 3.5 mIU/ml, 4% for 28 mIU/ml and 2.2% for 165 mIU/ml. No cross reaction is detected with hCG, FSH, hPL,and hGH. The calibration is made against 2nd IS WHO 80/552. A comparative study (n= 200) was carried out with the VIDAS and AxSYM analysers:
KRYPTOR-LH = 0.93 VIDAS-LH + 0.18 r^2 = 0.98
KRYPTOR-LH = 0.59 AxSYM-LH + 0.37 r^2 = 0.95
(The AxSYM-LH calibration is made against 1st IRP 68/40).

P-31

A homogeneous time resolved fluoroimmunoassay for follicle stimulating hormone using TRACE® technology: KRYPTOR-FSH

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A homogeneous time resolved fluoroimmunoassay for Follicle Stimulating Hormone based on TRACE® was developed for KRYPTOR, a fully automated multiparametric random-access analyser. TRACE® is an innovative methodology based on non-radiative energy transfer between an antibody linked to the Europium cryptate (the donor) and a second antibody linked to the XL 665 (the acceptor) induced by the formation of the Ag/Ab complex.

Results are obtained after 19 minutes incubation at 37°C of 50µl serum or plasma without citrate, with the two conjugates. One master curve is stored on board and is recalibrated every 2 weeks with 1 calibrator. The direct reading range is 0-250 mIU/ml. Higher values up to 2500 mIU/ml may be detected in a few seconds of incubation based on kinetic analysis and automatically diluted.

The detection limit calculated using imprecision profile has been assessed as being 0.4 mIU/ml with a probability of 95%. Within-run CVs are 5.8% for 4.6 mIU/ml, 2.5% for 95 mIU/ml and 0.6% for 171 mIU/ml. Between-runs,between-calibrations CVs are 11% for 4.5 mIU/ml, 3.1% for 19.2 mIU/ml and 2.5% for 173 mIU/ml. No cross reaction is detected with hCG, LH, and hGH. The calibration is made against 2nd IRP WHO 78/549. A comparative study (n = 200) was carried out with the VIDAS and AxSYM analysers:
KRYPTOR -FSH = 0.92 VIDAS-FSH + 0.08 r^2 =0.97
KRYPTOR -FSH = 0.97 AxSYM-FSH + 0.30 r^2 = 0.97.

P-32

A homogeneous time resolved fluoroimmunoassay for prolactin using TRACE® technology: KRYPTOR-PROLACTIN

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A homogeneous sandwich fluoroimmunoassay of prolactin has been developed using TRACE® technology. This assay has been designed for KRYPTOR, a fully automated multiparametric random-access analyser. TRACE® is an innovative methodology based on non-radiative energy transfer between an antibody linked to the Europium cryptate (the donor) and a second antibody linked to the XL 665 (the acceptor) induced by the formation of the Ag/Ab complex.

One of the more interesting KRYPTOR features is the automatic dilution and immediate analysis of high value serum samples, allowing the user to have a result for all levels of concentration.

Results are obtained after 19 minutes'incubation at 37°C of 26 µl of sample with the two conjugates.One master curve is stored on board and is recalibrated every two weeks with one calibrator.The reagent can be stored 29 days on board after opening.

The direct reading range is 0 to 4,600 µIU/ml (220 ng/ml). The automatic dilution system allows immediate results up to 1,000,000 µIU/ml (48,000 ng/ml).

The detection limit, calculated using the imprecision profile, has been assessed as being 5 µIU/ml (0.24 ng/ml) with a probability of 95%.

Within-run precisions are 3% for 120 µIU/ml (5.7 ng/ml), 0.9% for 567 µIU/ml (27 ng/ml) and 0.6% for 3,100 µIU/ml (148 ng/ml). Between-runs, between-calibrations assays show CV at 4.7% for 110 µIU/ml (5.2 ng/ml), 2.6% for 575 µIU/ml (27.4 ng/ml), 2.9% for 3,065 µIU/ml and 3.5% for 3,785 µIU/ml (180 ng/ml).

Dilution tests show recovery percentages between 91% and 104%.

The calibration is made against the 3rd international standard WHO 84/500.

Kryptor-Prolactin allows the precise and automatic measurement of a sample up to 1,000,000 µIU/ml (48,000 ng/ml) within 19 minutes.

P-33

A homogeneous time resolved fluoroimmunoassay for ferritin using TRACE® technology: KRYPTOR-FERRITIN

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We developped a homogeneous sandwich fluoroimmunoassay for Ferritin using TRACE® technology. This assay has been designed for KRYPTOR, a fully automated multiparametric random-access analyser. TRACE® is an innovative methodology based on non-radiative energy transfer between an antibody linked to the Europium cryptate (the donor) and a second antibody linked to the XL 665 (the acceptor) induced by the formation of the Ag/Ab complex.

The results are obtained after 19 minutes of incubation at 37°C of 26µl of sample with the two conjugates and without any washing step. A factory master curve is recalibrated every two weeks with one calibrator. The assay working range is 0-1,200 ng/ml. Higher values up to 500,000 ng/ml are detected in the first few seconds of incubation and are automatically diluted without any delay. The antibody specificity was studied through isotype recovery test and shows a complete recognition of the isoferritins containing L subunit. The detection limit calculated using the imprecision profile has been assesed as being 0.6 ng/ml with a probability of 95%. Within-run CVs were 1.6% at 26.6 ng/ml, 0.6% at 449 ng/ml and 0.5% at 1040 ng/ml, between-runs CVs were 4.6% at 23.6 ng/ml, 2.5% at 352 ng/ml and 3.6% at 1094 ng/ml. Dilution tests clearly demonstrated straight linearity up to 1200 ng/ml. Moreover, interference study confirms the total ability of the test to be performed on hemolysed sera without any crossreaction.

In conclusion, this rapid, sensitive and accurate ferritin assay allows a reliable clinical investigation of iron status in anemia.

P-34

Oxidativer Stress und Antikörper gegen oxidiertes LDL (oLAb) in der ersten Periode des Lebens

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Die oxidative Modifikation von LDL durch freie Radikale soll einen der wichtigsten Faktoren für die Pathogenese der Atherosklerose sein. Grundsätzlich unterscheidet man zwei Arten von protektiven Mechanismen gegen den „Risikofaktor oxidiertes LDL":

a) Wirkung von Antioxidantien wie Tocopherol

b) Bildung von spezifischen Antikörpern (oLAb), die die Elimination von oxidiertem LDL begünstigen.

Während der Geburt stehen Mutter und Kind unter einem starken oxidativen Stress. Dies konnte durch die Untersuchung von 20 Neugeboerenen und ihren Müttern demonstriert werden: Es besteht hier eine signifikante Erhöhung von Malondialdehyd (Kotrollen 3.2+/-0.5 umol/l, Müttern 5.11+/-1.92 umol/l; $p<0.001$; Neugeborene 6.46+/-1.16umol/l, $p<0.0001$). Hingegen unterscheiden sich die oLAb Konzentrationen bei Kindern unmittelbar nach der Geburt (427+/-284 U/l), von jenen der Müttern (391+/-279 U/l) und Kontrollen (421+/-172 U/l) nicht. Möglicherweise ist hier auch der diaplazentare Transport zu berücksichtigen. Am Ende des 3.Monats konnte bei den Kindern eine beinahe Verdoppelung der oLAb Konzentration beobachtet werden (815+/-469 U/l, ($p<0.001$).

Dieses Phänomen sollte bei Diskussion der frühzeitigen Faktoren bei Entstehung der Atherosklerose berücksichtigt werden.

P-35

Die Messung des komplexierten PSA - eine Alternative zur PSA-Ratio?

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Einleitung: Für das Bayer Immuno I System wurde eine Testmethode entwickelt, die die direkte, quantitative Messung des an alpha-1-antichymotrypsin (ACT) gebundenen PSA, dem komplexierten PSA (cPSA), ermöglicht.

Es besteht die Hoffnung, sich mit der direkten Messung des cPSA, die Doppelanalyse: gesamtes PSA - freies PSA, ersparen zu können.

Studie: Von 985 Männern (ohne Anamnese) wurde das gesamten PSA mit einem Abbott - Assay (Axsym) bestimmt.

239 Seren hatten einen gesamt PSA Wert zwischen 2,0 und 10,0 ng/ml.

Diese wurden einer Messung des freien PSA mit Ratio-Berechnung und weiters einer Vergleichsmessung mit Bayer Immuno I gesamt PSA und komplexierten PSA unterzogen.

Wegen der verschiedenen Grenzwertsetzung der PSA Ratio, bei PSA < 4,0 gilt 0,1 und zwischen 4,0 und 10,0 gilt 0,25, wurde diese Einteilung berücksichtigt.

Ergebnis der Vergleichsmessungen:

	Abbott-Ratio < 0,1	Abbott-Ratio < 0,25	Bayer cPSA > 3,75
PSA 2,0 - 4,0 (N=132)	6		19
PSA 4,0 - 10,0 (N=107)		78	92
CPSA < 3,75 (N=129)	7		

Diskussion: Der rein labor-analytische Methodenvergleich bestätigt die theoretische Überlegung, wonach mit der quantitativen Erfassung eines Analyts mit fixem Grenzwert (cPSA) nur eine ähnliche, jedoch nicht wirklich mit einer Ratio (zusätzlich: variabler Grenzwert) vergleichbare Aussage getroffen werden kann.

Über Sensitivität und Spezifität des neuen cPSA Assays von Bayer im Vergleich zur PSA-Ratio von Abbott sollen weitere Studien (mit klinischen Daten) Auskunft geben.

Zwei NRW Unternehmen verbinden Schlüsseltechnologien zum Produkt

MERLIN und microParts - entwickeln ein vollautomatisiertes Mikrobiologie System auf Chip Basis

Förderung durch das Land NRW

Vorbildliche Betreuung durch die BioGenTec

Die NRW-Unternehmen **MERLIN Gesellschaft für mikrobiologische Diagnostika GmbH** und **microParts Gesellschaft für Mikrostrukturtechnik mbH** entwickeln gemeinsam Produkte für die Medizintechnik.

Es werden die Schlüsseltechnologien Mikrostruktutechnik und Biotechnik kombiniert, um neue Möglichkeiten im Bereich der medizintechnischen Diagnostik und pharmazeutischen Wirkstoffscreening zu schaffen.

Die MERLIN Diagnostika GmbH - das einzige deutsche Unternehmen, das automatisierte mikrobiologische Systeme im Halbmikromaßstab entwickelt, produziert und vertreibt - stellt sich der neuen Technologie-Herausforderung.

Umfangreiche Studien zeigen, daß der Trend im medizinisch-mikrobiologischen Bereich und in der Diagnostik immer mehr zur Vollautomatisation geht. Neben der allgegenwärtigen Kosteneinsparung ist die ständig steigende Zahl multiresistenter pathogener Mikroorganismen sowie die wachsende Zahl von Infektionen durch Krankheitserreger mit hoher natürlicher Antibiotikaresistenz als Ursache für diese Entwicklung zu sehen. Als Folge muß die Resistenzentwicklung laufend überwacht werden.

MicroParts ist ein führendes Unternehmen der Mikrostrukturtechnik. Ein Arbeitsschwerpunkt von microParts ist die Entwicklung von mikrofluidischen Komponenten für die Medizintechnik. Dabei werden Kapillarstrukturen, Kanäle, strukturierte Reaktionsbereiche, Detektionsbereiche und andere fluidische Elemente mit Abmessungen im Mikrometerbereich auf einem „Chip" integriert. Diese fluidischen Chips werden in großen Stückzahlen kostengünstig durch Kunststoffabformungen gefertigt. Somit können komplexe biochemische Prozesse auf diesem Chip („Labor auf einem Chip") kostengünstig zum einmaligen Gebrauch realisiert werden.

In Zusammenarbeit wird die fortschrittliche neue Generation miniaturisierter Identifizierung- und Resistenzbestimmungssysteme entwickelt. In bisher einmaliger Weise wird das umfangreiche know how in der Mikrobiologie und Biotechnologie mit völlig neuen Methoden und Produkten der Mikrosystemtechnik und Mikroelektronik zu einem vollautomatisierten, miniaturisierten Analysensystem zusammengeführt.

Kernstück der Entwicklung ist eine zum Patent angemeldete Kunststoff-Mikrotitrationsplatte im Chipformat.

Für das Auswertegerät dieses Chips werden die Mikrospektrometer von microParts eingesetzt, um die Reaktion auf dem Chip zu analysieren.

Schon jetzt zeichnet sich ab, daß MERLIN und microParts durch dieses neue Konzept der Mikrosystemtechnik völlig neue Anwendungsgebiete in vielen Bereichen der Medizin- und Gentechnik offenstehen.

So sieht es auch das Land NRW und fördert dieses Vorhaben mit 40% der Projektkosten.

Als vorbildlich beurteilt MERLIN in diesem Zusammenhang die umfassende unbürokratische Betreuung und Beratung durch die landeseigene BioGenTec sowie durch die BEO.

Die Förderung durch das Land belegt den hohen Stellenwert neuer Technologien in der Wirtschaftspolitik von Nordrhein-Westfalen.

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