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Herzkreislauf-Erkrankungen/ Arteriosklerose

A1
E-LDL Induce egr-1 mRNA and Activate EGR-1 Transcription Factor through Mitogen-Activated Protein Kinases

E. Wieland an d U. Bonitz

Abteilung Klinische Chemie, Georg-August-Universität Göttingen, Robert-Koch-Strasse 40, 37075 Göttingen

EGR-1, a zinc finger transcription factor is elevated in human atherosclerotic lesions. Enzymatically transformed low density lipoproteins (E-LDL) are potent inducers of egr-1 mRNA. The aim of the present investigation was to elucidate whether EGR-1 is activated by E-LDL and whether mitogen-activated protein kinase (MAPK) signalling is involved in both egr-1 gene expression and EGR-1 protein activation. For this purpose Mono-Mac-6 cells (1 x 10^7/ml) were incubated (60 min) with E-LDL (25 µg cholesterol/ml) in the presence (20 µM) and absence of the following MAPK inhibitors: PD98 059 (MEK 1), SP600 125 (SAPK/JNK), and SB203 580 (p38 MAPK). Gene expression was followed by quantitative real time PCR on the LightCycler using transcription elongation factor-2 (ef-2) as reference gene, and EGR-1 activation by electrophoretic mobility shift assay (EMSA) and densitometry.

Inhibition of the MEK1 pathway lead to both a decreased egr-1 mRNA expression and EGR-1 activation, whereas the p38 MAPK inhibitor did not prevent gene and protein activation. Inhibition of the SAPK/JNK pathway had no significant effect on EGR-1 activation but decreased egr-1 mRNA expression.

	Cells Ø E-LDL (n=9)	+ E-LDL (n=11)	+ PD98 059 (n=4)	+ SP600 125 (n=4)	+ SB203 580 (n=3)
egr-1 mRNA (copies egr-1/ef-2)	0.101* (0.055–0.133)	0.141 (0.095–0.503)	0.017* (0.009–0.079)	0.031* (0.023–0.034)	0.551 (0.421–1.631)
Area EMSA (arbitrary units)	40 552* (27 335–49 815)	53 161 (43 162–76 347)	36 207* (22 898–46 217)	52 984 (47 020–60 211)	81 669 (68 183–87 378)

Data are median (16.-84. percentile)* = $p < 0.05$ vs +E-LDL

These results suggest that EGR-1 is activated in response to E-LDL and that MEK1 signalling is involved in both egr-1 gene expression and EGR-1 activation, whereas JNK activation is only required for egr-1 mRNA expression. These findings are potentially relevant to atherogenesis and may provide clues for therapeutic interventions.

Schlüsselwörter: Arteriosklerose – Signaltransduktion

A2
Modified Lipoproteins and Acute Phase Reactants Induce Ligand Specific Clustering of Ban Innate Immunity Receptor Complex in Monocyte Rafts

G. Rothe, A. Pfeiffer, M. Torzewski, A. Böttcher, E. Orsó, W. Drobnik, G. Schmitz

Institut für Klinische Chemie und Laboratoriumsmedizin, Klinikum der Universität Regensburg

Cellular recognition of modified lipoproteins has been linked to the role of scavenger receptors in innate immunity while the cooperation of these receptors remains unclear. We recently showed by fluorescence resonance energy transfer (FRET), that the LPS receptor CD14 is clustered with the integrin associated protein CD47 and the Fc-gamma-receptors CD32 and CD64. Activation by LPS leads to co-association of CD14 with complement receptor 3 (CD11b) and the scavenger receptor CD36. Other ligands to CD14 also induce conformational activation of the cluster while they differ in the pattern of receptors which are recruited. Thus LPS, but not ceramide, induces co-clustering with Toll-like receptor 4 and Fc-gamma-RIIIa (CD16). The effects of both agonists are modulated by the membrane cholesterol content suggesting that rafts are an important determinant.

Interestingly, the same receptor complex which recognizes LPS or ceramide represents a recognition structure for modified lipoproteins and acute phase proteins. Thus, CD36 represents the major binding site for enzymatically modified LDL (E-LDL) and the two Fc-gamma-receptors bind CRP with high affinity. The same FRET approach as well as competition experiments revealed that in acute phase plasma cellular uptake of E-LDL comprises both direct recognition of E-LDL by CD36 and recognition of E-LDL/CRP-complexes by CD32 and CD64. Moreover, CRP accelerates E-LDL induced macrophage foam cell formation.

In conclusion, the cooperation of pattern recognition receptors within rafts represents a mechanism for the differential recognition of exogenous and endogenous ligands. Different patterns of receptor clustering most likely correlate to a ligand specific signal transduction.

Keywords: Monocytes, LDL, CRP

A3
Modifizierte Lipoproteine reduzieren die Hepatocyte Growth Factor Synthese in humanen koronaren glatten Muskelzellen

C. Haug, U. Zorn, A. Grünert, M.G. Bachem

Institut für Klinische Chemie, Universitätsklinikum Ulm

In zahlreichen Untersuchungen wurde gezeigt, dass Low Density Lipoproteine (LDL) bei der Entstehung und Progression arteriosklerotischer Läsionen eine wesentliche Rolle spielen. Hepatocyte growth factor (HGF) ist ein potenter Regenerationsfaktor für Epithel- und Endothelzellen und scheint darüber hinaus auch die Aktivität von Matrixmetalloproteinasen zu stimulieren. Wir haben den Einfluss modifizierter LDL und Very Low Density Lipoproteine (VLDL) auf die HGF-Synthese in kultivierten humanen koronaren glatten Muskelzellen untersucht. Nach Isolation mittels sequentieller Ultrazentrifugation wurde LDL durch Inkubation mit Kupfersulfat oxidiert. LDL und VLDL wurden mit Hilfe von Cholesterolesterase, Trypsin und Neuraminidase enzymatisch degradiert (eLDL,

eVLDL). Sowohl oxidiertes LDL (oxLDL) als auch enzymatisch degradiertes LDL und VLDL induzierten einen signifikanten dosisabhängigen Abfall der HGF-Freisetzung aus kultivierten koronaren glatten Muskelzellen (48 h Inkubation): oxLDL 0.1, 1 und 10 µg/ml 79.7 ± 6.1 %**, 71.5 ± 5.0 %***, 44.1 ± 5.2 %***, MW ± SEM, n = 13; eLDL 0.1, 1 und 2 µg/ml: 77.0 ± 10.9 %, 71.8 ± 12.4 %, 58.3 ± 2.4 %**, n = 3; eVLDL 1 und 2 µg/ml: 88.1 ±;6.1 %, 60.9 ± 2.6 %***, n = 11, während nach Inkubation mit höheren Konzentrationen von nativem LDL (1, 10 und 100 µg/ml) ein deutlich geringerer Abfall der HGF-Freisetzung beobachtet wurde (94.3 ± 8.8 %, 92.2 ± 4.4 %, 81.9 ± 6.2 %*, n = 13); *:$p < 0.05$, **:$p < 0.01$, ***:$p < 0.001$ versus Kontrolle; Kontrolle: 100 ± 3.5 %, n = 28. Bislang ist unklar, ob HGF bei der Atherogenese eine Rolle spielt. Auf Grund der hier dargestellten Ergebnisse wäre denkbar, dass die durch modifizierte Lipoproteine induzierte Reduktion der HGF-Freisetzung zu einer verminderten Endothelzellregeneration und somit zur Verstärkung der endothelialen Dysfunktion beitragen könnte.

Schlüsselwörter: LDL, Hepatocyte Growth Factor, glatte Muskelzellen

A4
The Cholesterol-Collagen-Ratio of Atherosclerotic Plaques Determined by NIR Spectroscopy as a Possible Indicator of Plaque Vulnerability

M. Scheibe, V. Neumeister, P. Lattke, W. Jaross

Institut für Klinische Chemie und Laboratoriumsmedizin, Universitätsklinikum C.G.Carus an der Technischen Universität Dresden

The particular danger associated with an arteriosclerotic plaque consists in the possible rupture of its cap. The aim of the following investigations was to use near infrared spectroscopy (NIRS) in analysing the amounts of total cholesterol and collagen in the plaques of human arteries. The question should be answered, whether these chemical parameters together with their ratio correlate with the histochemical parameters such as fatty deposits, cap thickness over the lipid core and the ratio of both as a possible indicator of plaque stability.

NIR spectra were recorded at 1000–2500 nm using a fiberglass probe from 118 sections of 36 human aortas. The chemical reference analysis of cholesterol and collagen was done with HPLC. The chemometric evaluation was performed by the partial-least-squares-method. Acceptable results were achieved for calibrations and evaluations. 38 further aortal sections were NIR-spectroscopically analysed and ordered in relation to histological findings of fatty deposits, cap thickness over the lipid core and the ratio of fatty deposits to cap thickness. The cholesterol concentrations spectroscopically determined correlate with the histologically estimated fatty deposits ($r = 0.887$, $p = 0.01$), the spectroscopically determined collagen concentrations with the cap thickness over the lipid core ($r = 0.441$, $p = 0.01$), and the ratio cholesterol to collagen with the ratio fatty deposits to cap thickness ($r = 0.575$, $p = 0.01$).

Our investigations show that the NIRS is an accteptable tool for quantitativ determination of the main compounds of the arterial wall. The cholesterol to collagen ratio might be useful for risk assessment of possible plaque rupturing.

Schlüsselwörter: Arteriosklerose, Nahinfrarotspektroskopie, Plaque

A5
Androgens Induce Proliferation and Enhance Steroid Hormone Metabolism via Specific Steroid Hormone Receptors on Vascular Smooth Muscle Cells

[1]M. Klouche, [2]L. Castagnetta, [2]G. Carruba, [3]H. Eckstein, [4]F.-X. Schmid, [5]C. Knabbe and [1]G. Schmitz

[1] Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg,
[2] Institute of Experimental Oncology, University of Palermo,
[3] Department of Vascular Surgery, Ludwigsburg,
[4] Department of Vascular and Thoracic Surgery,
[5] Robert-Bosch-Hospital, Stuttgart, Germany

Steroid hormones play an important role in the development and the progression of atherosclerosis. Moreover, steroids regulate macrophage foam cell formation, expression of proinflammatory cytokines, endothelial function and lipoprotein homeostasis. We show that testosterone (10^{-10} – 10^{-9} M) induces dose-dependent proliferation of primary vascular smooth muscle cells (VSMC). By contrast, oestrogen produced no mitogenic effect and even mediated toxic effects (≥ 10 nM). Testosterone was extensively metabolised by VSMC, as demonstrated by the generation of biologically active metabolites dehydrotestosterone and 3α17ß-androstandiol. Since the synthetic andogen agonist R1881, which is not metabolised, induced a comparable proliferation of VSMC, the metabolic products appear not to be essential for mitogenesis. The mitogenic effect of testosterone was abrogated in the presence of the antiandrogen 4OH-Flutamide. Notably, we demonstrated for the first time that human primary VSMC expressed the androgen receptor (AR), and the novel subtype oestrogen receptor-β (ERβ), while the classical ERα and the progesterone receptor (PR) were absent. Expression of the AR occurred in 52 % of the cells, while ERβ positive nuclei were noted in 27 %. ERβ was functional, since oestradiol induced IL-6 expression by VSMC. Taken together, testosterone mediated direct proatherogenic effects via induction of VSMC mitogenesis via the androgen receptor, while oestradiol may promote or retard lesion progression.

Keywords: atherosclerosis, pentraxins, IL-6, steroids

A6
Prognostische Wertigkeit kardialer Marker bei chronischer Niereninsuffizienz

D. Peetz, S. Schütt, G. Hafner G, K.J. Lackner

Institut für Klinische Chemie und Laboratoriumsmedizin, Universitätsklinik Mainz

Einleitung: Erhöhte Troponin-T-Konzentrationen (TnT) weisen in aktuellen Studien auf eine schlechte Prognose bei chronischer Niereninsuffizienz (CN) hin. Entsprechende Untersuchungen für Troponin I (TnI) waren in der Vergangenheit durch erhebliche analytische Mängel der Teste der ersten Generation (z. B. TnI-Stratus) limitiert. In der vorliegenden Studie wurde untersucht, ob myokardiale Schädigungen bei CN mit modernen TnI-Testen, die auch bei niedrigen Konzentrationen präzise messen, nachgewiesen werden können.

Methodik: 105 Patienten (48 w, 57 m, 60,8 ± 15,5 a) mit CN. Probennahme vor Dialyse. Teste: TnI-Stratus (Dade Behring), TnI-ACS und CKMBmass (Bayer), TnT (Roche). Klassifikation entsprechen der oberen Referenzbereichsgrenzen in normal oder erhöht: TnI-Stratus: 0,6 µg/l, TnI-ACS: 0,15 µg/l, CKMBmass: 0,5 µg/l, TnT:

0,037 µg/l. Beobachtungszeitraum von 6 Monaten für nicht-kardiovaskuläre und kardiovaskuläre letale sowie kardiovaskuläre nicht-letale Ereignisse.
Ergebnisse Erhöhte Werte: TnI-Stratus (2,9 %), TnI-ACS (32,4 %), CKMBmass (71,9 %), TnT (65,7 %). Cutoff-Werte zur Vorhersage der verschiedenen 6-Monatsereignisse: TnI-ACS (0,11 µg/l), TNT (0,056–0,1 µg/l), CKMBmass (0,63–0,74 µg/l). Ereigniswahrscheinlichkeiten für Marker-negative (< cutoff) und Marker-positive (≥ cutoff) Patienten:

	Ereignisse (%)					
	nicht kardiovaskulär letal		kardiovaskulär letal und nicht letal		kardiovaskulär letal	
	< cutoff	≥ cutoff	< cutoff	≥ cutoff	< cutoff	≥ cutoff
TnI-ACS	5,9	11,1	17,6	50,0	5,9	14,8
TnT	8,2	11,5	14,6	49,1	3,9	16,7
CKMBmass	4,7	10,7	19,4	43,1	2,4	17,0

Schlussfolgerung: TnT- und moderne TnI-/CKMBmass-Teste weisen bei einem erheblichen Anteil der CN-Patienten auf prognostisch relevante myokardiale Ischämien hin, die mit TnI-Testen der ersten Generation nicht erkannt wurden.

A7
Risk Factors, Fibrinogen v. Cholesterol: A New View

N. Weinstock[1], M. Ntefidou[1], R. McIntosh[2]., S. Lord[3]

[1] Institut fuer Labordiagnostik, St. Vincentius-Kliniken, Karlsruhe, Germany
[2] Scottish National Blood Transfusion Center, Edinburgh, UK
[3] Dept. of Pathology, University of North Carolina, Chapel Hill, USA

Objective: Most of the studies on myocardial infarction focus on the effect of lipoproteins and lipid therapy. The ongoing study was started to elucidate the role of fibrinogen as an independent risk factor of arterial and venous thromboembolic events and to focus on new aspects of the interdependency of lipoproteins and fibrinogen as risk factors.
Methods: 1) In an interlaboratory study 73 patients with venous thrombosis confirmed by phlebographic diagnosis were checked at the time of admission, at the second day, on the day of and about 6 months after dischargement. The classic plasma acute phase and thrombophilia parameters were measured and evaluated. 2) In myocardial infarction studies including our own, the ECAT, the Framingham and 7 more, the interest was focused on the specific role of fibrinogen as a risk marker versus the known lipid parameters. To get better comparison in fibrinogen measurements the new internationally established high fibrinogen standard was introduced.
Results: 1) The DVT study has put evidence that fibrinogen is one of the most important risk factors for DVT. It is independent from CRP, and the other coanalysed factors. 2) The evaluation of the myocardial infarction studies suggest for the first time that the risk connected with lipids – e.g. Cholesterol, LDL, HDL and possibly VLDL – may depend on fibrinogen concentration and that the role of fibrinogen might independent and dominating. These unexpected results are supported by the newly recognised pathophysiological roles of fibrinogen in coronary artery diseases.
Conclusion: Our results show that fibrinogen is a risk factor for venous and arterial thromboembolism. There is strong evidence that fibrinogen is more important as independent risk factor than the known lipid parameters. We strongly suggest to implement fibrinogen as a risk factor in all new studies on thromboembolic events. The necessary comparability and accuracy to demonstrate these effects can only be achieved using an international accepted high fibrinogen standard like the one recently approved by the working party of the GTH (Gesellschaft für Thrombose- und Hämostaseforschung) and supported by the ISTH/SSC (International Society on Thrombosis and Haemostasis/Scientific and Standardisation Committee).

A8
Lipoprotein-vermitteltes Signaling in Nervenzellen

M. Orth, F. Opitz, M. Leonhardt, M. Müllrick, T. Witzlack, T. Wenzeck, J. Thiery

Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik, Universitätsklinikum Leipzig (AöR), Liebigstraße 27, 04103 Leipzig

Die Lipoproteine des zentralen Nervensystems und ihre Rezeptoren in Nervenzellen besitzen aufgrund der Bluthirnschranke vom Plasmakompartiment unterschiedliche, spezifische Funktionen. Mit dieser Arbeit sollte die Frage geklärt werden, ob in einem Kokulturmodell der Lipoprotein-vermittelte Lipidtransfer zwischen Nervenzellen und Mikroglia die Funktion dieser Zellen beeinflusst und die Applikation von neurotropen Hormonen diese Effekte moduliert.

Mittels quantitativer Taq Man RT-PCR zeigte sich eine hohe Expression von ABCA1 in monozytären Zellen und in beiden Zellarten eine ähnliche, hohe, Expression von HMG-CoA-Reduktase und LDL-Rezeptor. Durch Kokultur kommt es zu einer verminderten ABCA1-Expression in monozytären Zellen und zu einer verminderten LDL-Rezeptor-Expression in Nervenzellen. Diese Interaktionen werden durch die Zugabe von Hormonen (T3, Testosteron, Serotonin) nicht wesentlich beeinflusst.

Nach Induktion von ABCA1 durch c-AMP kommt es zu einer ausgeprägten Sekretion kleiner Lipoproteine aus den monozytären Zellen. Erste Ergebnisse zeigen, dass die beobachteten Effekte über den MAPK-Pathway vermittelt werden.

Unsere Befunde weisen darauf hin, dass der Lipoprotein-vermittelte Lipidtransfer in Nervenzellen und Mikroglia das Zell-Signaling und den zellulären Lipidstoffwechsel beeinflusst. Diese Effekte können durch die untersuchten neurotropen Hormone allerdings nicht weiter moduliert werden.

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A9
Nukleosomen im Serum bei Patienten mit cerebralem ischämischem Infarkt

[1]S. Geiger, [1]S. Holdenrieder, [2]G. Hamann, [1]P. Stieber, [2]A. Danek, [2]M. Mayer, [1]A. Mitlewski, [1]D. Nagel, [1]D. Seidel

[1] Institut für Klinische Chemie,
[2] Neurologische Klinik, Klinikum der Universität München-Großhadern

Hintergrund: Als Zelltodparameter können zirkulierende Nukleosomen im Serum von Patienten mit Erkrankungen, die mit massiver Zellschädigung einhergehen, nachgewiesen werden. Wir untersuchten, ob und in welchem Umfang Nukleosomen nach cerebralem ischämischen Infarkt im Serum auftreten und ob die Kinetik während der ersten Woche mit der klinischen Symptomatik korreliert.
Methoden: Zur Bestimmung der Nukleosomenkinetik verwendeten wir den Cell Death Detection ELISA^{plus} (Roche Diagnostics). Insgesamt wurden Nukleosomen im Serum bei 24 Patienten mit neu aufge-

tretenem cerebralem ischämischen Infarkt bei Aufnahme in die Klinik und zweimal täglich während der ersten Woche bestimmt. Die funktionelle Beeinträchtigung der Patienten wurde durch den Barthels-Score (Beurteilung der selbständigen Erledigung alltäglicher Aufgaben) bei Aufnahme objektiviert.
Ergebnisse: Bei den meisten der untersuchten Patienten stieg die Nukleosomenkonzentration innerhalb der ersten Tage steil an und fiel im Verlauf der ersten Woche langsam ab. Für die Korrelation mit der klinischen Symptomatik wurden Patienten mit starker funktioneller Beeinträchtigung entsprechend einem Barthels-Index ≤ 30 (BI ≤ 30), von Patienten mit geringer funktioneller Beeinträchtigung und einem Barthels-Index > 30 (BI > 30) unterschieden. Patienten mit einem BI ≤ 30 hatten signifikant ($p < 0{,}05$) höhere Nukleosomen-Werte als Patienten mit einem BI > 30 am ersten Tag nach dem ischämischen Ereignis (Median 743 AU vs. 210 AU), am zweiten Tag (874 AU vs. 292 AU) und am dritten Tag (1333 AU vs. 428 AU), sowie für den Minimalwert (281 AU vs. 139 AU), den Maximalwert (1853 AU vs. 150 AU), die normierte Fläche unter der Kurve der Tage 1–3 (1323 AU/d vs. 375 AU/d), und der Tage 1–7 (1031 AU/d vs. 411 AU/d).
Schlussfolgerung: Die Nukleosomenkonzentration im Serum spiegelt die funktionelle Beeinträchtigung der Patienten nach cerebralem ischämischem Infarkt wider und kann als laborchemisches Korrelat für das Ausmaß der klinischen Symptomatik angesehen werden.
Schlüsselwörter: Nukleosomen, cerebraler ischämischer Infarkt

A10
A Novel Mutation in the Cardiac Troponin I Gene Causing a Benign Form of Hypertrophic Cardiomyopathy in a Large Kyrgyz Family

N. Bit-Avragim[2,3], H. Schmidt-Traub[1], B. Hoffmann[1], A. Perrot[2], R. Rudenko[3], D. Usupbaeva[3], Zh. Kabaeva[2,3], B. Imanov[3], B. Isalieva[3], A. Aldashev[3], K.J. Osterziel[2], M.M. Mirrakhimov[3], R. Gessner[1]

[1] Institut für Laboratoriumsmedizin und Pathobiochemie, Charité-CVK, Augustenburger Platz 1, D-13353 Berlin;
[2] Franz-Volhard-Klink, Charité-CBB, D-13122 Berlin;
[3] National Centre of Cardiology and Internal Medicine, Bishkek, Kyrgyzstan

Nine disease genes, all encoding for sarcomeric proteins, have been identified for familial hypertrophic cardiomyopathy (HCM). Mutations of the cardiac troponin I gene (cTnI) are responsible for about 1 % ofall cases of HCM. Clinical features and prognosis vary widely between different families.

We studied 147 unrelated patients with HCM of different ethnic origins (Caucasian and Central Asian) and 36 healthy Kyrgyz controls. Screening of all 8 coding exons of the cTnI gene was done by exon-specific PCR amplification, single strand conformation polymorphism (SSCP) analysis and direct fluorescence DNA sequencing.

We identified 11 individuals in one family of Kyrgyz origin with a novel missense mutation in exon 7 of the cTnI gene causing an Arg > Gln substitution at codon 162 (R162Q). All clinically affected individuals from this familiy are characterized by mild asymmetrical septal hypertrophy (max. wall septal thickness 15 mm) and pathological Q-waves in ECG. Systolic anterior motion of the mitral valve on echocardiography was registered only in one patient. Symptoms developed at a mean age of 27 ± 3 years. Myocardial hypertrophy was present in only 40 % of heterozygous carriers over 20 years. A pathological ECG was present in 80 %.

We identified a novel missense mutation (R162Q) in a large Kyrgyz family with HCM. The new mutation is characterized by mild asymmetrical septal hypertrophy and a benign course of the disease with low penetrance.

A11
Plasma N-terminal Pro-Brain Natriuretic Peptide in the Assessment of Left-Ventricular Dysfunction in Patients with Hypertrophic Cardiomyopathy

M. Rost, M. Speth, N. Katz.

Institut für Klinische Chemie und Pathobiochemie am Klinikum der Justus-Liebig-Universität Gießen

Objective: Pro-brain natriuretic peptide (pro-BNP) is secreted mainly by high wall tension and stress of the left ventricle. It is expected, that plasma levels of pro-BNP as well as its N-terminal fragment (NT-pro BNP) closely reflect impaired left ventricular function in patients with heart failure. In the present study we assessed the determination and the clinical significance of NT-pro BNP levels in patients with suspected hypertrophic cardiomyopathy.
Methods: The Roche/Elecsys ELICA was used to determine NT-pro BNP levels in 50 patients (median age 59 years) referred for echocardiography. The values obtained were correlated to NYHA classification, plasma creatinine levels, left ventricular ejection fraction (EF), left atrial wall diameter (LAD), left ventricular enddiastolic wall diameter (LVEDD) and left ventricular systolic wall diameter (LVSD) using regression analysis.
Results: There was a good correlation between the mean NT-pro BNP plasma levels and the functional NYHA classification ($r = 0.89$, $p < 0.01$) and the decrease of left ventricular EF ($r = 0.31$, $p < 0.01$). Moreover, regression analysis revealed a close association between NT-pro-BNP levels and higher plasmatic creatinine ($r = 0.32$, $p < 0.01$) and Iarger LAD ($r = 0.38$, $p < 0{,}01$). Despite the relationships obtained, we did not found a satisfactory correlation between NT-pro BNP plasma levels and the LVEDD ($r = 0.10$) and LVSD ($r = 0.25$) of the patients.
Conclusion: 1. NT-pro BNP increases proportionately to the left ventricular dysfunction and severity of the hypertrophic cardiomyopathy,

2. NT-pro BNP , however, has a poor predictive value for left ventricular systolic and diastolic dysfunction.
Keywords : Heart failure – N-terminal pro-brain natriuretic peptide – echocardiography

Hämatologie/Hämostaseologie/ Immunhämatologie

H1
Diagnostik des funktionellen Eisenmangels unter Anwendung verschiedener Hämatologiesysteme

S. Franck, M. Messinger, L. Thomas

Krankenhaus Nordwest, Zentrallabor, Frankfurt am Main

Beim funktionellen Eisenmangel (FEM) besteht ein Ungleichgewicht zwischen Versorgung und Bedarf der Erythropoese mit Eisen. Im Vergleich zu den biochemischen Markern und dem Blutbild, spiegelt der Hämoglobingehalt der Retikulozyten den FEM zeitgerecht wider.

Wir untersuchten 539 Patienten mit Anämie (Frauen < 12 g/dl, Männer < 13 g/dl) auf FEM durch Messung des Hämoglobingehaltes der Retikulozyten.

Gemessen wurde das Retikulozyten-Hb am Advia 120 (CHr) durch eine direkte Hämoglobinbestimmung vergleichend zu derjenigen am Sysmex XE 2100 (RET-Y). Der RET-Y ist der Mittelwert der Vorwärtsstreulichtintensität nach Fluoreszenzmarkierung der Retikulozyten. Im Vergleich zu den biochemischen Markern des Eisenstoffwechsels (Ferritin, löslicher Transferrinrezeptor) liegt ein FEM vor, wenn der Hämoglobingehalt der Retikulozyten am Advia < 28 pg und am Sysmex XE 2100 der Ret-Y < 165 ist.

Zur Erkennung des FEM betragen die Übereinstimmungen beider Meßsysteme bei Eisenmangelanämie, Anämie chronischer Erkrankungen, Tumorpatienten und Schwangeren jeweils 93, 93, 92 und 94 %.

Unsere Untersuchungen zeigen, daß der RET-Y eine vergleichbare Zuverlässigkeit zum CHr zur Erkennung des FEM besitzt.

Schlüsselwörter: Funktioneller Eisenmangel, Retikulozytenhämoglobin

H2
Fibrinogen Aachen I: eine neue Familie mit kongenitaler alpha 16 Arg → Cys Dysfibrinogenämie

D. Kunz, M. Meyer*, A.M. Gressner.

Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum, RWTH, Pauwelsstr. 30, 52074 Aachen, *Fachhochschule Jena, Fachbereich Medizin

Abnormales Fibrinogen wurde in einer bisher asymptomatischen Familie in drei Generationen gefunden. Zur Untersuchung kam ein 13-jähriger Junge mit im Rahmen einer Operationsvorbereitung auffälligem Spontan-Quick von 24 % (INR 2,7), einer grenzwertig verlängerten PTT, einer deutlich verlängerten Thrombinzeit (40 s) und eines berechneten Fibrinogens von 1,36 g/l. Bei der 39-jährigen Mutter des Jungen wurde 2 Jahre zuvor bei einem stationären Aufenthalt die Diagnose einer „Gerinnungsstörung bei Hypofibrinogenämie" gestellt.

Quick, TZ und Reptilasezeit waren bei Mutter und Sohn pathologisch. Eine detaillierte Analyse der Gerinnungsfaktoren ergab normwertige Aktivitäten für die Einzelfaktoren II–XIII. Bei beiden Patienten wurde ein Fibrinogen nach Clauss von < 0,5 g/l gemessen. Die Antigenbestimmung ergab 1,9 g/l (Mutter) und 1,7 g/l (Sohn). Diese Befundkonstellation führte zu dem dringenden Verdacht auf eine kongenitale Dysfibrinogenämie. Die molekulargenetische Untersuchung ergab den Nachweis der heterozygoten Mutation FGA-C1202 T. Diese führt zum Austausch der Aminosäure 16 Arg → Cys in der Alpha-Kette, was eine gestörte Freisetzung der Fibrinopeptide A zur Folge hat.

Einer Familienuntersuchung waren nur die Großeltern zugänglich, wobei bei der bisher asymptomatischen 68-jährigen Großmutter die Dysfibrinogenämie nachgewiesen wurde. Allen Betroffenen wurde ein Nothilfepass ausgestellt, um bei etwaigen Operationen oder stationären Aufenthalten Fehlinterpretationen der auffälligen globalen Gerinnungstests zu vermeiden.

Bei den derzeit weltweit ca. 300 betroffenen Familien wurde überwiegend dieser Defekt als Ursache der erblichen Dysfibrinogenämie gefunden. Er wurde in der Regel bei klinisch asymptomatischen Trägern oder aber bei Patienten mit einer milden Blutungssymptomatik beobachtet.

H3
Pyropoikilozytose und gestörte automatisierte Thrombozytenzählung nach Verbrennungstrauma

D. Kunz und A. M. Gressner.

Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum, RWTH, Pauwelsstr. 30, 52074 Aachen

Das EDTA-Blut einer 79-jährigen Patientin mit einem schweren Verbrennungstrauma wurde im Verlauf von 14 h nach dem akuten Ereignis zur Blutbildkontrolle bis zum Ableben in das Zentrallabor eingeschickt. Das EDTA-Blut wurde am CellDyn 4000 (Abbott GmbH) analysiert.

Zum Zeitpunkt der stationären Aufnahme wurde eine erhebliche Diskrepanz zwischen der Thrompozytenzählung mittels Impedanz (1070 G/l) im Vergleich zur optischen Streulichtmessung (1680 G/l) beobachtet. Außerdem lagen alle Erythrozytenindizes außerhalb der Referenzbereiche. Eine Überprüfung der Thrombozytenzahl mit der immunologischen CD61 Zählmethode am CD4000 ergab ein Ergebnis von 158 G/l. Eine mikroskopische Abschätzung mit der von Sutor eingeführten Leukozyten/Thrombozyten-Ratio (1) bestätigte das CD61-Ergebnis und zeigte zusätzlich eine massive Anisozytose und Poikilozytose der Erythrozyten mit dem Nachweis von zahlreicher Mikrozyten, die im CD4000 zur Überschätzung der Thrombozytenzahl führten.

Innerhalb der folgenden 3 h überschätzten die konventionellen Zählmethoden mit Ergebnissen von 300 bzw. 364 G/l im Vergleich zum CD61-Wert von 51 G/l weiterhin die Thrombozytenzahl.

Neben den bereits bekannten störenden Einflussgrößen auf die Thrombozytenzählung nach dem Impedanzprinzip im Vergleich zur optischen Streulichtmessung, wie Kryoglobulinen, leukämischen Zellfragmenten, Mikrozyten, Lipoproteinen, Riesenthrombozyten (2–4) etc., muss die Pyropoikilozytose, die unmittelbar nach einem akuten Verbrennungstrauma oder massiver Überwärmung der Patienten auftritt, ebenfalls beachtet werden. Korrekte Zählergebnisse liefern in diesen Fällen nur immunologische Zählmethoden bzw. eine mikroskopische Untersuchung des Blutausstriches.

1. Sutor AH. Pädiatrische Praxis 1995/96;50:43
2. Kunz D. Sem Thromb Hemost 2001;27:229
3. Kunz D et al. Br J Haematol 2001;112:584
4. von Ahsen N et al. Clin Chem 2001; 47,1858

H4
Determination of Platelet Function in Patients under Therapy with Acetylsalicylic Acid or Clopidogrel

J. Lotz, S. Kotulla, K. Lackner

Institut für Klinische Chemie und Laboratoriumsmedizin, Johannes-Gutenberg-Universität, Mainz

Introduction: In patients with ischaemic heart disease monitoring of platelet function under therapy with acetylsalicylic acid (ASS) or clopidogrel (CP) is not well established. Therefore, platelet function was determined in healthy volunteers and in patients with ASS or CP therapy by aggregometry and by the invitro-bleeding time on the PFA-100-function-analyzer.

Patients and methods: 77 healthy volunteers, 92 patients with a daily dose of 100 mg ASS and 17 patients with 75 mg CP were analyzed. Platelet aggregation was measured after induction with arachidonic acid (AA), ADP or collagen using a Packs-4 (Helena Diag-

nostika, Hartheim). The in vitro bleeding time was determined in a PFA-100 (Dade-Behring, Marburg) using collagen/epinephrine or collagen/ADP coated capillaries.
Results: In healthy volunteers cut-off values were determined for AA and ADP induced aggregation and in-vitro-bleeding time after exposure to coll/epi or coll/ADP coated capillaries. These were applied to the analysis of antiplatelet effects of the medication. In 86/92 patients with ASS therapy the aggregation induced by AA were below the cut-off, while only 60/92 patients had prolonged closure times in the coll/epi capillary of the PFA-100. ADP induced aggregation was reduced in 15/17 patients taking CP. However, prolonged closure times in the coll/ADP capillary were observed in only 4/17 patients.
Conclusion: According to our results, aggregometry after stimulation with AA is the method of choice to detect ASS effects, while aggregometry after stimulation with ADP is best suited for CP. The in vitro bleeding time is less sensitive for either substance no matter which platelet activator is used. Our data suggest that a relevant number of patients on ASS and CP have insufficient levels of platelet inhibition. Independent of the underlying cause, this may be clinically relevant.

H5
Referenzbereiche für die Thrombozytenaggregation in Abhängigkeit von der Thrombozytenzahl des plättchenreichen Plasmas

G. Töpfer, G. Friedel, K. Sauer, F. Hornig, U. Funke

Zentrallabor, Städt. Klinikum Görlitz GmbH, Zentrallabor, Kreiskrankenhaus Zittau

Einleitung: Die Fragestellung: „Liegt eine Thrombozytenfunktionsstörung vor“, wird nicht nur für Patienten mit Thrombozytenzahlen im Referenzbereich (150–450 G/L) gestellt, sondern auch bei verminderten Thrombozytenkonzentrationen. Außerdem hat zunehmend die Überfunktion der Thrombozyten (Sticky platelet Syndrome) Interesse gefunden, um u. U. thrombozytäre Gründe für eine Thromboseneigung (Thrombophilie) zu finden. Infolgedessen war unsere Aufgabe, für die Plättchenaggregation Referenzbereiche für unterschiedliche Thrombozytenzahlen (Konzentrationen) zu erstellen. Dabei wurden die Induktorkonzentrationen (ADP, Kollagen, Ristocetin, Epinephrin) so gewählt, dass eine Unterfunktion der Thrombozyten bei den Patientenaggregationen sichtbar wird, aber auch so gewählt (ADP, Epinephrin), dass eine Überfunktion erkennbar wird.
Durchführung: Von 40–60 gesunden Blutspendern ohne Gerinnungsstörung und mit Blutbild-Ergebnissen im Referenzbereich wurden innerhalb von 1–3 h nach der Blutentnahme die plättchenreichen Plasmen (PRP) mittels Thrombozytenaggregation untersucht. Die Thrombozytenzahl wurde so mit plättchenarmen Plasma (PAP) eingestellt, dass 300-, 200-, 150-, 100-, 75- und 50 G/L enthalten waren (außerdem die native Zahl).

Nach 5 Minuten Rühren bei 1000 U/min und 37 °C im PACKS-4-Aggregometer wurden als Aggregationsinduktor

ADP	7,5 µM/L	Progen/Immuno
Kollagen	5 µg/mL	Hormonchemie München
Ristocetin	1,36 mg/mL	Mascia Brunelli
Epinephrin	300 µM/L	Mascia Brunelli

hinzugegeben und die Abnahme der Trübung durch Aggregation 15 Minuten per Schreiber registriert. Für die Fragestellung „Sticky platelet Syndrome“ wurden die Plättchen nativ, mit 300-, 200- und 100 G/L mit den Auslösern ADP 3,75-, 1,5- und 0,5 µM/L (Progen/Immuno) und Epinephrin 10-, 1-, 0,5 µM/L (Mascia Brunelli) untersucht.

Ausgewertet wurden die Anfangsaggregation (slope) in %/min und die Maximalaggregation sowie die Desaggregation.
Ergebnisse: Der 10–90 %-Percentile-Bereich wurde als Referenzbereich definiert. Für ADP und Kollagen als Induktor besteht eine nahezu lineare Abhängigkeit, besonders des klinisch relevanten unteren Referenzbereichs-Grenzwertes von der Thrombozyten-Konzentration (50–300 G/L) für Anfangs- und Maximalaggregation. Für Ristocetin ist diese Abhängigkeit genauso wie für Epinephrin als Aggregationsauslöser geringer ausgeprägt und nur in einzelnen Bereichen deutlich (am stärksten zwischen 200 und 75 G/L). Bei der Fragestellung Sticky platelet Syndrome zeigt von 300 bis zu 100 G/L die höchste zulässige Referenz-Maximal- und Anfangs-Aggregation ebenfalls einen starken Abfall. Bei Epinephrin trifft dies nur für die Maximalaggregation ohne Ausnahme zu.
Schlussfolgerung: Die Referenzbereiche der Thrombozytenaggregation sind besonders bei ADP und Kollagen, aber auch bei Epinephrin und Ristocetin als Induktor von der Plättchenzahl abhängig. Bei ADP und Kollagen ist diese Abhängigkeit am PACKS-4 sogar nahezu linear.

H6
In-vitro-Simulation of Therapeutic Plasmatic Thrombolysis

T.W. Stief , R. Bünder, H. Renz.

Abt. für Klin. Chemie, Uni-Klinikum Marburg, 35033 MR

Introduction: A therapy form of thromboembolias is plasmatic thrombolysis. Several plasminogen (plgen) activators (PA) are clinically available, including urokinase (u-PA), t-PA, streptokinase (SK), SK-plgen-complex (PSAC), or mutants of t-PA such as reteplase (RP). There is fear of hemorrhagias in these PA. We simulated plasmatic thrombolysis, using the PA at relevant plasma conc. and determined plasmin (Pli) and PA.
Methods: Normal citrated plasma was spiked with 31–1000 IU/ml u-PA, 0.31 -10 µg/ml t-PA,125–4000 IU/ml SK, 12.5–400 IU/ml PSAC, 125–4000 U/ml (1 mg = 0.58 MU) RP. After 0–80 min (37 °C) 50 µl samples were withdrawn and added to 100 µl 1.5 M arginine, pH 8.7 and oxidized with 50 µl of the singlet oxygen ($^1O_2$) donor chloramine T® (CT; 20 mM). For determination of Pli, 10 µl thereof was incubated with 150 µl 1.5 M arg. and 100 µl of 20 mM CT-preoxidized pooled normal EDTA-plasma for 30 min. For determination of [PA + Pli]-activity, arg. was added after this incubation. 25 µl of 6 mM Val-Leu-Lys-pNA were added and ΔA was monitored, using a microtiterplate reader. [PA + Pli] – Pli = PA.
Results: The PA-conc. required to induce 25 % [ED_{25}] of the maximally inducible Pli-activity in plasma (max. 2 CTA-U/ml) after 10 min (37 °C) were: 500 IU/ml u-PA, 10 µg/ml t-PA, 100 U/ml PSAC, 3000 U/ml SK, 1000 U/ml RP. The activity half lives were: 30 min u-PA, 30 min t-PA, > 80 min SK, > 80 min PSAC, 50 min RP.
Discussion: The present study shows – for the first time – an in vitro simulation of the activity of plasmatic PA. At clinically used conc., RP induces the highest plasmatic Pli-activity. In contrast, at the clinically employed plasmatic conc. of u-PA (max. 50 IU/ml) – the physiol. PA of blood – tenfold less Pli-activity is generated. Due to unselective generation of plasmin in plasma, all PA are of some danger in inducing severe hemorrhagias. Clinical thrombolysis should include more physiological activators of thrombolysis, such as activators of neutrophils.

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H7
Aminoterminal Fragment of Recombinant Cellular Prion Protein Stimulates t-PA Activity Stronger than the Alzheimer Beta-Amyloid Peptide (1–42)

M. Praus, G. Epple, G. Kettelgerdes, E. Köttgen, R. Gessner

Institut für Laboratoriumsmedizin und Pathobiochemie, Charité Campus Virchow-Klinikum, Humboldt-Universität zu Berlin, Augustenburger Platz 1, D-13353 Berlin

The cellular prion protein (PrP^C), tissue-type plasminogen activator (t-PA) and plasminogen are expressed in synaptic membranes *in vivo*. There is evidence that in the central nervous system the fibrinolytic system is associated with excitotoxin-mediated neurotoxicity and Alzheimer's disease. Recently binding of the disease associated isoform of the prion protein PrP^{SC} to plasminogen and stimulation of t-PA activity have been reported. We showed before that Plasmin is able to cleave recombinant PrP^C at lysine residue 110 generating an NH_2-terminal truncated molecule that has been described to be a major product of normal PrP^C metabolism. Our results show that the NH_2-terminal part of PrP spanning amino acids 23–110 (PrP23–110) together with proteoglycans stimulates t-PA mediated plasminogen activation in vitro. The apparent rate constant was increased 57 fold in the presence of 800 nM PrP23–110. Furthermore, we compared the stimulation of t-PA activity by PrP23–110 and the Alzheimer beta-amyloid peptide (1–42). While the stimulatory activity of the Alzheimer peptide was independent of proteoglycans, PrP23–110 was approximately 4 and 37 fold more active in the absence or presence of proteoglycans, respectively. In summary, we have identified plasmin as one of the proteases that could be involved in normal PrPc metabolism. In addition, we showed that the NH_2-terminal cleavage product accelerates plasminogen activation in vitro. The cleavage of PrP^C by plasmin and the proteoglycan dependent stimulation of plasminogen activation mediated by t-PA could be part of an autoregulatory mechanism of pericellular proteolysis. This would be the first report of a measurable biological function of the PrPc.

H8
Quantification of Tissue Factor Pathway Inhibitor (TFPI) in Human Seminal Plasma and in Human Ovarian Follicular Fluid

E. Thyzel, C. Götting, T. Brinkmann, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany

Tissue factor pathway inhibitor (TFPI) is a multivalent serine protease inhibitor which plays a central role in the extrinsic pathway of blood coagulation. Because of the known hypocoagulable state of human ovarian follicular fluid, we have investigated TFPI levels in liquor folliculi and in human seminal plasma.

Total TFPI was measured in seminal plasma of 28 healthy ejaculate donors and 23 infertile patients with oligo-, astheno- or teratozoospermia and unfulfilled desire for children. Mean TFPI concentrations were determined as 3.75 ± 2.42 ng/mL and 2.70 ± 2.03 ng/mL respectively. In seminal fluid of infertile patients TFPI levels were significantly lower ($p < 0.07$) than in the control group of healthy ejaculate donors. TFPI levels were also determined in ovarian follicular fluid gained from the punctured follicles of superovulated women ($n = 70$) undergoing *in vitro fertilization* (IVF) and *intra cytoplasmatic sperm injection* (ICSI). The mean value was calculated as $330{,}21 \pm 158.44$ ng/mL. The TFPI levels measured in liquor folliculi were about 3.7-fold higher than the TFPI levels found in human blood.

In summary the high TFPI levels measured in human ovarian follicular fluid underline the physiological importance of the hypocoaguable state of human ovarian follicular fluid. The pathobiochemical reason for the observed low TFPI levels in human seminal plasma of infertile patients has to be further elucidated in future experiments.

Keywords: tissue factor pathway inhibitor (TFPI), seminal plasma, follicular fluid

H9
Expression of Wild Type TFPI and the [P151L] TFPI Mutant in Insect and Mammalian Cells

E. Thyzel, S. Siegling, C. Götting, T. Brinkmann, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany

Tissue factor pathway inhibitor (TFPI) is an important regulator of the extrinsic blood coagulation pathway. We recently discovered the first natural TFPI polymorphism (536C–>T) which leads to a proline to leucine exchange at position 151 of the mature protein and significantly correlates with a higher risk for venous thrombosis. To study the biochemical and pathophysiological role of this mutation we have developed a new model system for the transient expression of TFPI and its variants in insect and mammalian cells.

Wild type and [P151L]TFPI cDNA constructs were cloned into suitable expression vectors for protein expression in *High Five* insect cells and in *COS-1* mammalian cells. The cells were then transiently transfected and TFPI expression was monitored in the cell culture supernatant. In mammalian cells we measured expression rates ranging from 68.29 ng/mL for the wild type TFPI to 83.23 ng/mL for [P151L]TFPI. We were also able to express TFPI in insect cells with expression rates of up to 3.95 ng/mL for wild type TFPI and 5.24 ng/mL for [P151L]TFPI respectively. The specific inhibitory activity for recombinant wild type TFPI expressed in insect cells was determined as 0.167 U/ng and as 0.350 U/ng for the [P151L]TFPI. In mammalian cells the specific inhibitory activities for the recombinant proteins were 0.028 U/ng and 0.026 U/ng respectively.

In summary, we have succesfully expressed recombinant wild type TFPI and the [P151L]TFPI mutant in mammalian cells and for the first time in insect cells. Furthermore we have developed an efficient model system for the recombinant expression of wild type TFPI and other potential TFPI variants in *COS-1* and *High Five* cells to make it accessible for further biochemical and pathophysiological characterizations.

Keywords: tissue factor pathway inhibitor (TFPI), [P151L]TFPI, protein expression

H10
Erythropoietin and Cytokines in Patients with Nucleated Red Blood Cells in Blood

[1]A. Stachon, [1]K. Eisenblätter, [2]T. Holland-Letz, [1]M. Krieg

[1] Institut für Klinische Chemie, Transfusions- und Laboratoriumsmedizin; BG Kliniken Bergmannsheil; Universitätsklinik
[2] Abteilung für Biometrie; Ruhr-Universität Bochum

In adults the detection of nucleated red blood cells (NRBC) in the peripheral blood is generally associated with a poor prognosis. The underlying pathophysiologic mechanisms are widely unknown. In particular, in those patients the associated cytokine and growth factor profiles in blood have not been investigated. We report on the analysis of erythropoietin, IL-6, IL-12p70, IL-13, GM-CSF, and stem cell factor (all by ELISA) in 32 patients with NRBC in the peripheral blood (NRBC+). 75 patients without NRBC served as control (NRBC-). Controls and patients were matched for age, gender and treatment (internal medicine, general and accident surgery, cardiothoracic surgery, or neurology). By a multiple logistic regression the odds ratios for the detection of NRBC in blood of the following parameters were assessed: age, gender, body mass index, erythropoietin, IL-6, IL-12p70, IL-13, SCF, and GM-CSF.

The average NRBC concentration (mean ± SEM) in NRBC+ was 737 ± 160/µl. Average concentrations in NRBC+/NRBC- were as follows (pg/ml): erythropoietin: 169 ± 40/35(4, IL-6: 637 ± 188/ 106 ± 35, IL-12p70: 40 ± 11 ± 21(7, IL-13: 202 ± 141/75 ± 59, GM-CSF: 107 ±638/73 ± 32, stem cell factor: 496 ± 164/ 167(± 15. The multiple logistic regression analysis revealed a significant association of the appearance of NRBC in blood with increasing erythropoietin and IL-6 concentrations. Age, gender, body mass index, IL-12p70, IL-13, GM-CSF, and stem cell factor had no significant effect on the appearance of NRBC.

In summary, it is attractive to speculate that the appearance of NRBC in the peripheral blood is caused by elevated concentrations of erythropoietin and IL-6. However, further studies are needed to clarify this hypothesis.

Onkologie

O1
Immunchemische und funktionelle Charakterisierung von Pgp, MRP1 und LRP

A. Dorn-Beineke[1], D. Schadendorf[2] und M. Neumaier[1]

[1] Institut für Klinische Chemie und
[2] Dermato-Onkologische Kooperationseinheit des DKFZ, Fakultät für Klinische Medizin Mannheim, Universität Heidelberg, D-68135

Einleitung: Es ist trotz internationaler Konsensusbestrebungen noch nicht gelungen, die MDR (Multidrug-Resistenz)-Analytik im Rahmen der Pgp (P-Glykoprotein)-MDR und Non-Pgp-MDR zu vereinheitlichen und einen internationalen Vergleich zu ermöglichen.
Methodik: Wir charakterisierten acht Zelllinien und 56 gesunde Probanden immunchemisch und funktionell mittels Durchflußzytometrie mit dem Ziel, für die Diagnostik von Pgp, MRP1 (Multidrug-resistance associated protein 1) und LRP (Lung resistance protein) die optimalen Testdesigns auszuarbeiten sowie die Schwankungen der Protein-Expressionen- und Funktionen zu ermitteln. Wir weisen Gründe auf, weshalb sich in der gegenwärtigen Situation in der MDR-Diagnostik eine unmittelbare Vergleichbarkeit der Ergebnisse aus verschiedenen Laboratorien sehr schwierig gestaltet.
Ergebnisse: Wir zeigen, dass Pgp-Antikörper eine hohe Bindungsdiversität, besonders auf myeloischen Zellen, aufweisen. Die Zellaufbereitung spielt eine entscheidende Rolle. Der immunchemische Nachweis von MRP1 und LRP gestaltet sich weniger problematisch. Die Erfassung der Protein-Funktionen ist stark abhängig von der Sensitivität und Spezifität der eingesetzten Substrate und Converter. Hier können wir eine eindeutige Wertigkeit vornehmen. Die Protein-Expressionen von Pgp und MRP1 korrelieren nicht mit den Protein-Funktionen, welche die sensitivere Nachweismethode darstellen. Wir konnten Unterschiede in den Schwankungen von Protein-Expressionen- und Funktionen zwischen intrinsisch resistenten Zelllinien bzw. gesunden Probanden und sekundär resistenten Zelllinien herausarbeiten. Der Einsatz von sekundär resistenten Zelllinien zur Standardisierung von Testssystemen ist aufgrund der hohen Variationskoeffizienten von Tag zu Tag nicht anzuraten.
Keywords: P-Glykoprotein, MRP1, LRP

O2
Bedeutung der Multidrug-Resistenz und Apoptose bei CML-Patienten unter Therapie mit STI571 (Glivec®)

A. Dorn-Beineke[1], C. Herrle[1], C. Keup[1], T. Lahaye[2], A. Hochhaus[2], T. Lenz[3] und M. Neumaier[1]

[1] Institut für Klinische Chemie,
[2] Medizinische Klinik III, [3]Institut für Medizinische Statistik, Fakultät für Klinische Medizin Mannheim, Universität Heidelberg

Einleitung: Das 2-Phenylaminopyrimidinderivat STI571 wurde gezielt als selektiver Inhibitor von Tyrosinkinasen entwickelt. STI571 hemmt kompetitiv die Tyrosinkinasen BCR-ABL, ABL, TEL-ABL, PDGF-R, TEL-PDGF-R, TEL-ARG und c-kit und stellt ein neues therapeutisches Prinzip in der Behandlung von Neoplasien dar. Neben vielversprechenden Ergebnissen in Phase-I-und-II-Studien wurden jedoch auch Resistenzentwicklungen in vitro als auch in vivo beobachtet. Mehrere Hypothesen, darunter die Induktion eines Multidrug-Resistenz (MDR)-Phänotyps, wurden beschrieben.
Patientenkollektiv: Es wurden 36 CML-Patienten (30 Blastenkrisen, fünf Akzelerationsphasen, eine chronische Phase) untersucht. 30 Patienten wurden vor STI-Therapie, 32 Patienten unter STI-Therapie, und sechs Patienten wurden ein zweites Mal unter STI-Therapie untersucht.
Methoden: Die immunologische Bestimmung des erweiterten MDR-Phänotyps (Pgp, MRP1 und LRP) wurde mittels eines geeigneten Antikörpercocktails durchgeführt. Zur Testung der funktionellen Aktivitäten von Pgp und MRP1 wurden der Rhodamin123-Efflux- und der CalceinAM-Influx-Assay eingesetzt. Zur Apoptoseinduktion wurde ein in-vitro-Modell angewandt. Die Apoptose wurde mittels Annexin V und 7-AAD detektiert.
Ergebnisse: Die Studiendaten zeigen, daß ein genereller Einfluß von STI571 auf die Hochregulierung der MDR-Pumpen Pgp, MRP1 und LRP auf CD34+ Blasten von CML-Patienten nicht angenommen werden kann. STI fällt somit nicht unter die Gruppe der MDR-related drugs. Die Apoptoseinduktion bei CD34+ Blasten im in-vitro-Modell sagt die Apoptoseinduktion in vivo nicht voraus und eignet sich nicht als „Screening-Test".
Keywords: Multidrug-resistance, CML, STI571

O3
Radiofrequency-EMF (1800 MHz) and DNA Strand Breaks in Human Promyelocytic Leukaemia Cells HL-60

K. Schlatterer, R. Gminski , R. Tauber, R. Fitzner

Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin

Objective: DNA damaging effects of radiofrequency electromagnetic fields (RF-EMF) have been discussed controversely so far. Goal of this study was to examine, if structural alterations on the genetic level, related to cancerogenesis, are induced in the human promyelocytic leukaemia cell line HL-60. Analysis was focussed on the detection of DNA strand breaks by means of single cell gel electrophoresis (SCGE, Comet assay).
Methods: Cells were cultured in RPMI 1640 (10 % FCS, 37 °C, 5 % CO_2). RF-exposure [continuous wave, 1800 MHz, SAR 1.0 W/kg (main vector; n = 7 independent experiments), SAR 1.3 W/kg (n = 16), SAR 2.0 (n = 5)] and sham-exposure were performed double-blinded (35 mm Petri dishes; 7.5×10^5 cells/3 ml; T- and pH-control; 37 °C; 5 % CO_2) over 24 h. Subsequently, DNA damage was monitored by the Alkaline Comet assay. To assess DNA migration, 1000 ethidium bromide stained cells were classified into 5 comet categories, and a tail factor was calculated. For significance calculation the Student t-test was used at the level $p < 0.05$. Cytotoxic effects were examined by measuring cell viability with the MTT assay. Induction of apoptosis was monitored by annexin V and TUNEL assays.
Results: A small, but significant increase in DNA migration was detected in RF-exposed HL-60 cells (1800 MHz, 24 h, continuous wave) for 1.3 W/kg and 2.0 W/kg, respectively. On the other hand RF-EMF exposure under these conditions did not induce cytotoxicity or apoptosis. Hence, the DNA strand breaks reflect neither cytotoxic nor apoptotic effects of RF-EMF.
Conclusion: The results show that RF-EMF exposure may cause DNA strand breaks in human promyelocytic cells. Further experiments are under way to elucidate, whether effects of RF-EMF on DNA are direct or indirect, and if the effects observed are correlated with an altered DNA damage repair capacity.

O4
Nukleosomen im Serum zeigen frühzeitig die Effizienz einer Chemotherapie an

S. Holdenrieder[1], P. Stieber[1], J. von Pawel[2], H. Raith[3], D. Nagel[1], A. Mitlewski[1], B. Mahnert[1], K. Feldmann[3], D. Seidel[1]

[1] Institut für Klinsiche Chemie, Klinikum der Universität München-Großhadern
[2] Onkologische Abteilung,
[3] Institut für Klinische Chemie, Asklepios-Kliniken Gauting

Hintergrund: Die Zelltodendprodukte „Nukleosomen“ werden bei Patienten mit malignen Tumoren während Chemotherapie in erhöhten Konzentrationen im Serum vorgefunden. Aufgrund ihrer kurzen Verweildauer untersuchten wir, ob ihre Kinetik bereits zu einem frühen Zeitpunkt die therapeutische Effizienz anzeigen kann.
Methode: Mittels des Cell Death Detection ELISA^{plus} (Roche Diagnostics) wurden die Nukleosomenverläufe bei 279 Patienten mit fortgeschrittenem Bronchialkarzinom (212 NSCLC, 67 SCLC) während Chemotherapie gemessen. Dabei erfolgten die Bestimmungen an den Tagen 1, 3, 5 and 8 des ersten Zyklus' und vor jedem neuen Therapiezyklus. Das Ansprechen auf die Therapie (Response = Remission; Non Response = Progression und stable disease) wurde durch bildgebende Verfahren vor Beginn des dritten Zyklus' beurteilt. Zusätzlich wurden Tumormarker vor jedem Zyklus bestimmt (NSCLC: CEA und CYFRA 21–1; SCLC: CEA, NSE und ProGRP).
Ergebnisse: In der univariaten Analyse bei Patienten mit NSCLC hatten Responder signifikant ($p < 0{,}05$) niedrigere Nukleosomenwerte als Non Responder für die Fläche unter der Kurve der Tage 1–8 (AUC 1–8), die Basiswerte vor dem zweiten (BV2) und dritten (BV3) Zyklus sowie stärkere Abfälle der Basiswerte von Zyklus 1 zu 2 (BV1–2) und von Zyklus 1 zu 3 (BV1–3). Außerdem unterschieden CYFRA 21–1 (BV1, BV2, BV3, BV1–2, BV1–3) und CEA (BV1–2) signifikant zwischen beiden Gruppen.

Bei Patienten mit SCLC hatten Responder signifikant niedrigere Werte als Non Responder für die AUC 1–8 und die Basiswerte vor dem ersten (BV1) und zweiten (BV2) Zyklus. Ferner diskriminierte ProGRP (BV1–2) zwischen beiden Gruppen.

In der multivariaten Analyse, in die alle univariat signifikanten klinischen Parameter eingingen, wurden bei Patienten mit NSCLC die Nukleosomen (AUC 1–8), CYFRA 21–1 (BV1), das Stadium und Alter, bei Patienten mit SCLC die Nukleosomen (BV1 und BV2) als unabhänige Prädiktoren der therapeutischen Effizienz ermittelt.
Schlussfolgerung: Somit eignen sich beim nicht kleinzelligen Bronchialkarzinom die Nukleosomen im Serum zusammen mit CYFRA 21–1- und klinischen Parametern für die frühzeitige Einschätzung der Effizienz einer Chemotherapie, beim kleinzelligen Bronchialkarzinom isoliert die Nukleosomen vor Beginn der Therapie sowie vor Applikation des 2. Zyklus'. Dadurch kann eine stärkere Individualisierung der Therapie erreicht werden.
Schlüsselwörter: Nukleosomen, Chemotherapie, Bronchialkarzinom

O5
Nukleosomen im Serum korrelieren mit der Therapieeffizienz bei Radiotherapie

S. Holdenrieder[1], Andreas K.[1], P. Stieber[1], R. Wilkowski[2], D. Nagel[1], D. Seidel[1]

[1] Institut für Klinische Chemie,
[2] Institut für Strahlentherapie und Radioonkologie, Klinikum der Universität München-Großhadern

Hintergrund: Nukleosomen treten in erhöhten Konzentrationen spontan im Serum von Patienten mit malignen Erkrankungen sowie während Chemo- und Radiotherapie auf. Wir untersuchten, ob ihre Kinetik mit dem klinischen Ansprechen auf Radiotherapie korreliert und eine Beurteilung der Therapieeffizienz erlaubt.
Methode und Patienten: Zur Bestimmung der Nukleosomen kam der Cell Death Detection ELISA^{plus} (Roche Diagnostics) zur Anwendung. Es wurden 16 Patienten mit malignen Erkrankungen (6 Brochialkarzinom, 4 HNO-Karzinom, 4 Lymphom, 2 kolorektales Karzinom), die eine mehrwöchige Radio- (N = 8) oder Radiochemotherapie (N = 8) erhielten, untersucht. Blutabnahmen wurden vor der Therapie, 3, 6, 24 Stunden, 3 und 7 Tage nach Therapiebeginn sowie wöchentlich im weiteren Therapieverlauf vorgenommen. Der Therapieerfolg wurde durch bildgebende Verfahren am Therapieende erfaßt. 10 der Patienten erreichten eine Remission, ein Patient stable disease und 5 Patienten waren progredient.
Ergebnisse: Die Konzentration der Nukleosomen stieg bei den meisten Patienten kurz nach Therapiebeginn steil an und fiel nach unterschiedlich langer Latenz wieder langsam ab. Patienten mit einer Remission zeigten einen deutlich früheren Abfall der Werte als Patienten mit einer Progression (im Median 1 Tag vs 4 Tage). Zudem hatten sie

signifikant ($p < 0{,}05$) niedrigere Minimalwerte während der Therapie (27 AU vs 158 AU), Endwerte nach Therapie (23 AU vs 336 AU) und eine kleinere Fläche unter der Kurve der ersten 8 Tage (AUC 1–8/d: 242 AU/d vs 1096 AU/d).
Schlussfolgerung: Die Kinetik von Nukleosomen im Serum korreliert mit dem Ansprechen auf Radiotherapie und kann zur frühzeitigen Beurteilung der Therapieeffizienz herangezogen werden.
Schlüsselwörter: Nukleosomen, Effizienz, Radiotherapie

O6
The TATA-Less Promoter of Murine LI-Cadherin Interacts with the Caudal-Related Homedomain Protein Cdx2 and Hepatocyte Nuclear Factor 1 (HNF1)

M. Danevad, A. Weimann, R. Jung, M. Praus, E. Gimpel, M. Wendeler, R. Geßner

Institut für Laboratoriumsmedizin und Pathobiochemie, Charité Campus Virchow-Klinikum, Humboldt-Universität zu Berlin, Augustenburger Platz 1, 13353 Berlin

The LI-cadherin is a structurally divergent member of the cadherin superfamily with seven instead of five extracellular cadherin repeats. It is exclusively expressed in the small and large intestine but not the upper gastric tract. Although lacking from the healthy gastric epithelia, LI-cadherin becomes strongly expressed during intestinal metasplasia and in gastric adenocarcinomas. It serves thus as an early and highly sensitive marker for those disorders. Whether LI-cadherin is-directly involved in the phenotypic transition of the epithelial cells is not yet resolved.

In order to analyze the regulation of LI-cadherin expression we have cloned, sequenced and functionally characterized the murine LI-cadherin promoter.Two transcriptional start sites 18 bp apart could be identified by 5'-RACE and primer extension analysis. Sequencing revealed that the LI-cadherin promoter lacks a TATA-box. Promoter activity was tested with luciferase reporter gene constructs in different cell lines. The main promoter activity could be assigend to the first 200 bp upstream of the start site. The 5'-flanking region of the murine LI-cadherin gene contains binding sites for the intestine specific Cdx homeodomain protein Cdx2 and hepatocyte nuclear factor HNF1. Both proteins bound to the murine LI-cadherin promoter and supershifted in EMSA assays indicating a putative organ-specific function. A comparison of the human and murine promoter region showed conserved binding sites for Cdx2 and HNF1. Mutational analysis demonstrated specific SP1 binding to the only GC-box found. Potential recognition sites for AP-1, HFH3, GATA-1,2,3, OCT1, RFX1 and c-myb are present in the murine LI-cadherin promoter.

O7
TFF3 Induces Down-Regulation of E-Cadherin

D. Meyer zum Büschenfelde, R. Tauber and O. Huber

Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin

Objective: TFF (trefoil factor family)-peptides represent a small family of three secreted polypeptides with a highly conserved trefoil-like structure defined by three characterisitc disulfide bridges. They exhibit motogenic and protective function in mucinous epithelia. In this context we here investigated whether the E-cadherin/β-catenin adhesion system is affect in TFF3-transfected HT29B6 cells.
Methods: HT29B6 cells were transfected with an expression vector for FLAG-tagged TFF3. After selection, stable clones were picked and migratory behaviour of cells in response to TFF3 was analyzed in motility assays. Expression of E-cadherin and β-catenin was examined by Western blotting.
Results: Stable clones expressed TFF3 as examined by immunoprecipitation of FLAG-TFF3 from cell culture supernatants with anti-FLAG-antibodies. TFF3-transfected cells showed enhanced migratory activity in wound-filling assays. Cells migrating into the wound revealed a more spindle shaped morphology. In Western Blots, TFF3-transfected cells exhibited reduced E-cadherin expression. β-catenin expression was less affected.
Conclusion: Up-regulated expression of TFF-peptides contributes to enhanced motogenic activities of cells during epithelial restitution. This is at least in part mediated by a down-regulation of the E-cadherin cell-cell adhesion system. The molecular mechanisms involved in TFF3-mediated down-regulation of E-cadherin are under investigation.

O8
Migration-Modulating Effects of Thrombin via Protease-Activated Receptor-1 (PAR-1) in Human Prostate-Derived Cells

J. Liu, M. Steiner, P. Schuff-Werner

Universität Rostock, Institut für Klinische Chemie & Pathobiochemie, D-18057 Rostock, Germany

Several lines of evidence suggest that thrombin contributes to cancer progression via its main cellular receptor, protease-activated receptor 1 (PAR-1).

In the present study, we analysed the expression profile of thrombin receptors in the human prostate cancer cell line DU 145 and SV40-immortalized human prostate epithelial cells (PNT1A) by RT-PCR and flow cytometry. In addition, migration-modulating effects of exogeneously added thrombin on the cells were assessed using an *in vitro* wound healing assay.

RT-PCR experiments demonstrated the expression of PAR-1 mRNA in DU 145 and PNT1A cells. In contrast, mRNA specific for the additional thrombin receptors PAR-3 and PAR-4 was not detectable. PAR-1 protein localized on the cell surface was demonstrated by flow cytometry using a monoclonal antibody (WEDE15). *In vitro* wound healing assays revealed dose-dependent effects of thrombin on wound area closure patterns. In growth-arrested DU 145 and PNT1A cells, thrombin at low concentrations (0.1–0.5 U/ml) gave rise to closure of the wound area after 24 h whereas thrombin at high concentration (1.0 U/ml) was without significant effect on cellular migration.

Our results suggest that thrombin-mediated PAR-1 activation modulates migration of prostate cancer cells which might contribute to the molecular pathogenesis of prostate cancer.

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Keywords: prostate cancer, thrombin

O9
cPSA, der bessere Marker in der Diagnostik der Prostataerkrankungen

G. M. Oremek, J. Binder, H. Sauer-Eppel, F. Eden

Zentrallabor, Zentrum der Inneren Medizin Theodor-Stern-Kai 7, D-60590 Frankfurt/Main

Es stellt sich die Frage, inwieweit in der Früherkennung des Prostatakarzinoms das cPSA aussagefähiger ist, als die bisherige Kombination von fPSA und tPSA totales PSA. 250 Patienten der Urologischen Klinik wurden in die Studie eingeschlossen. Für das cPSA wurde ein cut-off von 2,3 ng/ml ermittelt. Bei einem tPSA zwischen 2,0–4,0 ng/ml kann cPSA mit einem cut-off von 2,3 ng/ml eine 2 % Tumorsensitivität aufweisen, die histologisch belegbar ist. Für diesen Konzentrationsbereich ist das neue cPSA der Marker der Wahl. Die herkömmlichen Methoden erreichen diese Sensitivität nicht. Im Vergleich zu der Aussagekraft von fPSA könnten 30 % an Biopsien eingespart werden. Bei einem tPSA-Konzentrationsbereich von 4,1–10 ng/ml erwies sich das cPSA in 71 % der Fälle spezifischer als die bisherige PSA-Quotenbildung. Das cPSA erwies sich als robuster Marker bei Manipulationen der Prostata, es kommt zu keiner Verfälschung der Meßwerte durch eine digitale rectale Untersuchung. Ein weiterer Vorteil ist die analytische Stabilität bei Transport und Lagerung. Das cPSA ist stabil im Falle der Lagerung bei –20 °C. Das fPSA sinkt um 10 % ab. Zusammengefaßt kann mitgeteilt werden, daß das neue cPSA ein Marker mit hoher Spezifität ist, gerade in Konzentrationsbereichen von 2,0–4,0 ng/ml und im Graubereich von 4,0–10 ng/ml.

O10
Pro-Gastrin-Releasing Peptide (ProGRP) ein Tumormarker bei kleinzelligen Bronchialkarzinom

G. M. Oremek, H. Sauer-Eppel

Zentrallabor, Zentrum der Inneren Medizin Theodor-Stern-Kai 7, D-60590 Frankfurt

Gastrin-Releasing Peptide (GRP) kann beim Menschen sowohl im gesamten Gastrointestinaltrakt, im Nervensystem und insbesondere im Bronchialtrakt nachgewiesen werden, wo es bereits zur immunhistochemischen Klassifizierung der Bronchialkarzinome eingesetzt wird. Yamaguchi et al entwickelten eine enzymimmunologische Methode zur Bestimmung des stabilen rekombinanten ProGRP einen Vorläufer von GRP im Serum. Untersucht wurden Seren von 200 gesunden Probanden im Alter von 19–70 Jahren, sowie 300 Seren von Patienten mit benignen und malignen Lungenerkrankungen. Bei den Probanden lag die Konzentration von ProGRP im Bereich von 1,7–43,9 ug/ml. Im Vergleich zur Kontrollgruppe zeigt sich bei Patienten mit chronischer Bronchitis, Sarkoidose, Fibrose, Bronchialem Plattenepithelkarzinom und großzelligem Bronchialkarzinom keine relevante Erhöhung der ProGRP-Konzentration im Serum. Deutlich erhöht waren die Konzentrationen bei Patienten mit einem kleinzelligen Bronchialkarzinom. Die Konzentrationen für diese Gruppe lagen im Bereich von 85 pg/ml bis 2950 pg/ml. Die hohe Sensitivität (75 %) und Spezifität (95 %) der ProGRP-Bestimmung bestätigt die Ergebnisse bei kleinzelligen Bronchialkarzinomen. Der neue Tumormarker liefert für die klinische Routine wertvolle Hilfe in der Diagnostik und Verlaufskontrolle von kleinzelligen Bronchialkarzinomen.

O11
Proliferation Rate, Structural Features and Clinical Significance of Cathepsin B and Cathepsin L in Operated Lung Cancer Patients

K. Kayser[1], N. Richter[1], P. Hufnagl[1], G. Kayser[2], J. Kos[3], and B. Werle[4]

[1] Universität Heidelberg
[2] Institut für Pathologie, Universität Freiburg
[3] Jozef-Stefan-Institute, Dept. of Biochemistry and Molecularbiology, Ljubljana, Slovenia
[4] Zentrallabor des Klinikums der Universität Heidelberg

Introduction: Cathepsins such as cathepsin B and cathepsin L are thought to be useful as prognostic indicators for patients suffering from lung cancer. The aim of this study was to immunohistochemically investigate the expression of cathepsin B and cathepsin L in different histologies and various cells types of lung cancer. The data were correlated with clinical pathological parameters, the proliferation rate Ki-67 (MIB), quantitative structural features of tumor cells and finally, the prognosis of the patients.
Material and methods: Histological slides obtained from formalin fixed, paraffin-embedded tissue of 120 potentially curative resected lung cancer specimens were quantitatively analysed with an automated image analysing system.
Results: The expression of cathepsin B was most frequently present in large cell anaplastic carcinomas, followed by adeno and squamous cell carcinomas. In small cell carcinomas no cathepsin B could be recognized. That of cathepsin L was less frequently seen, and mainly expressed in macrophages and adenocarcinoma tumour cells. No convincing association between the expression of cathepsin B and cathespin L and the proliferation rate could be noted. The structural entropy remarkably differs between the histological cell types and for both cathepsin B and cathepsin L expression. The expression of cathepsin B is of prognostic significance in non-small cell lung cancer, and ranges directly after the pN and pT stages in multivariate statistical analysis. In contrast to cathepsin B, cathepsin L inversely correlate with the prognosis of the patient.
Conclusion: In concordance with the literature, the expression of cathepsin B should be considered as a significant prognostic feature in contrast to that of cathepsin L. Its contribution to the patients outcome is still questionable.
Keywords: lung cancer, prognostic factors, cathepsin B

O12
CYFRA 21-1 and CEA in the Differential Diagnosis of Pleural Effusions

H. Weisser[1], A. Fisseler-Eckhoff[3], K. Rasche[4], G. Schultze-Werninghaus[4], K.-M. Müller[2], M. Krieg[2]

[1] Institute of Medical Microbiology, University Clinic Jena;
[2] Institute of Clinical Chemistry, Transfusion and Laboratory Medicine,
[3] Institute of Pathology,
[4] Division of Pneumology, University Clinic Bergmannsheil, Bochum, Germany

The diagnostic value of CYFRA 21-1 and CEA was evaluated in malignant (mesothelioma, n = 41, nonmesothelioma, n = 42), paramalignant (n = 31) and benign pleural effusion (n = 40). Serum (S) and pleural fluid (PF) levels of CYFRA 21-1 and CEA were determined by means of two commercial enzyme immunoassays. Repunctures were included. Differences in the median values between the

groups were checked using Kruskall-Wallis test followed by Dunn's multiple comparison test.

Significant differences were found among the four groups with regard to levels of CYFRA 21–1 and CEA in S and PF:

Tumor marker [ng/ml]	1. Benign	2. Malignant mesothelioma	3. Malignant nonmesothelioma	4. Paramalignant
S				
CYFRA 21-1	1.8 (1.2–2.6)	3.6 (2.0–6.6)	6.1 (3.1–25.3)	2.8 (1.3–5.5)
CEA	1.8 (1.1–2.6)	1.2 (0.9–2.2)	5.7 (2.5–19.7)	2.4 (1.3–6.5)
PF				
CYFRA 21-1	11.3 (3.0–24.7)	117.0 (42.2–629.2)	87.0 (33.5–425.0)	10.1 (3.8–27.7)
CEA	0.8 (0.4–1.5)	0.6 (0.4–1.2)	38.0 (6.8–204.5)	1.2 (0.6–13.3)

Data are expressed as the median, 25 % – 75 % percentiles
S-CYFRA 21-1: $p < 0.0001$ between groups 1–4; $p < 0.05$ 1 vs 2, 1 vs 3, 3 vs 4
S-CEA: $p < 0.0001$ between groups 1–4; $p < 0.05$ 1 vs 3, 2 vs 3, 2 vs 4
PF-CYFRA 21-1: $p < 0.0001$ between groups 1–4; $p < 0.001$ 1 vs 2, 1 vs 3, 2 vs 4, 3 vs 4
PF-CEA: $p < 0.0001$ between groups 1–4; $p < 0.05$ 1 vs 3, 2 vs 3, 2 vs 4, 3 vs 4

Best discrimination between benign and malignant effusions was obtained by CYFRA 21-1 in PF (cut-off 65 ng/ml: 62 % sensitivity, 95 % specificity). Considering malignant effusions alone, best discrimination between mesothelioma and nonmesothelioma was obtained by CEA in PF (cut-off 2.3 ng/ml: 83 % sensitivity, 95 % specificity). Moreover, in mesothelioma the CYFRA 21-1 levels in PF seems to be associated with the histological type with higher values in epitheloid tumors as compared to sarcomatoid and biphasic tumors.

Thus, CYFRA 21–1 and CEA in the PF can be a useful addition in the differential diagnosis of pleural effusions.

O13
In vitro Histoculture as a Model System for Therapeutic Approaches in Colon Carcinoma

M. Volkmann, T. Lehnert[1], J. Cornelis[2], W. Drope, M. Borho, M. Hofmann, H. Weckauf[3], W. Fiehn and W.J. Hofmann[3]

Zentrallabor,
[1] Chirurgische Universitätsklinik,
[3] Pathologisches Institut, Universität Heidelberg;
[2] Deutsches Krebsforschungszentrum, Heidelberg.

In vitro histoculture was performed in primary colorectal cancer to evaluate a possible use in preclinical testing of new therapeutic approaches. In 20/23 cases, histocultures could be established from histologically confirmed colorectal carcinoma. After 3–6 days, a 7–14 day period of experimental treatment with 5-Fluorouracil or Doxorubicine was performed. In addition to MTT endpoint, glucose levels and CEA secretion in the cell culture supernatant were measured and correlated with tumor grading as well as histological examinations comprising semiquantitative measurement of viable tumor cells, proliferation index and p53 status. As a pilot experiment, productive infection with autonomous Parvovirus H1 was established in one of the tumours as demonstrated by RT-PCR.

Histological examination and measurements in the culture supernatant showed viable tumour tissue during culture periods of up to 42 days with, on average, constant glucose consumption and sustained CEA secretion into the culture superatant at an average 50 % of the pre-treatment value. MTT-endpoint as well as glucose consumption showed a dose dependant range of chemosensitivities in the examined tumors for 5-Fluorouracil, but no sensitivity to Doxorubicine.

We conclude that in vitro histoculture is a potential model system for the pre-clinical testing of new therapeutic approaches for colorectal carcinoma. While "non-invasive" measurements can be used to evaluate cell viability and differentiation, divergent results compared to the MTT endpoint in some samples indicate a possible complementary role of both assays in the quantitation of the effect of experimental treatment.

Keywords: Colon carcinoma, histoculture, parvovirus;

O14
Preoperative Serum Levels of CEA, CA 19-9 and CA 242 and their Prognostic Significance in Colorectal Cancer

D. Hofmann, P. Stieber, U. Lau-Werner[1], D. Nagel, H. Hornung[1], D. Seidel

[1] Institut für Klinische Chemie, Chirurgische Klinik, Klinikum der Universität München-Großhadern

Objective: The carcinoembryonic antigen CEA is the tumour marker with the highest sensitivity for the early detection of recurrent disease in colorectal cancer. CA 19-9 as well as CA 242 are clearly inferior to CEA concerning sensitivity but in contrary to CEA proved to be independent prognostic factors in colorectal cancer at time of primary diagnosis. The aim of this study was now to evaluate the prognostic value of CEA, CA 19-9 and CA 242 based on the same patient samples.

Patients and Methods: Preoperative serum levels of CEA (MEIA, AxSYM, Abbott), CA 19-9 (Elecsys, Roche Diagnostics) and CA 242 (EIA, CanAg). from 142 patients with colorectal carcinoma were investigated.

We choose the corresponding medians (CEA: 2.3 ng/mL, CA 19-9: 15.9 U/mL, CA 242: 9.5 ng/mL) of all samples investigated as well as the 75th percentile (CEA: 8.7 ng/mL, CA 19-9: 36.4 U/mL, CA 242: 22.3 ng/mL) as cut off values for the prognostic evaluation.

Results: Patients with CEA < 2.3 ng/mL showed significantly ($p < 0.0001$, log-rank-test) better survival rates than those with CEA > 2.3. The same power of discrimination was found for CEA-values of 8.7 ng/mL. Patients with CA 19-9 < 15.9 U/mL (< 36.4 U/mL) showed significantly ($p = 0.0003$ resp. $p < 0.0001$) better survival than those with CA 19–9 > 15.9 U/mL (> 36.4 U/mL).

For CA 242 only the 75th percentile (22.3 ng/mL) discriminated the two patient groups significantly ($p < 0.0001$).

Conclusion: All three markers provided significant prognostic information in this univariate analysis. A multivariate analysis comparing these findings with other prognostic factors like staging, lymph node involvement and distant metastases is planned.

Keywords: CEA, CA 19-9, CA 242, Colorectal Cancer, Prognosis, Tumour marker

O15
Measurement of Fecal Tumor M2-PK Concentrations in Patients with Gastrointestinal cancer

P.D. Hardt, M. Toepler, B. Ngoumou, J. Rupp, H.U. Kloer

Justus-Liebig-Universität Gießen, Med. Klinik III und Poliklinik, Gießen

Pyruvate kinase (PK) plays an important role in the glycolytic pathway. The conventional form of PK is a tetrameric molecule, which

shows a high affinity for the substrate phosphoenolpyruvate. In tumor cells, however, the tetrameric PK M2 isoenzym is predominantly present in a dimeric form, which is called Tumor M2-PK. Tumor M2-PK is over-expressed by a wide range of different tumors. This appears to be linked to the different metabolic requirements shown by tumor cells. As a result of their rapid proliferation, these cells are using a metabolic "shortcut" to "save energy" for cell multiplication. It is this change that is utilised by the Tumor M2-PK test to detect and monitor tumors, since it uses monoclonal antibodies specific to the dimeric isoform Tumor M2-PK to quantify its level in body fluid samples.

Tumor M2-PK has been measured with a commercially available ELISA (ScheBo®· Biotech AG, Gießen, Germany).

Groups	n	Mean [U/ml]	Median [U/ml]	Range [U/ml]
GI Cancer total	18	79,5	43,6	0,0–353,9
Colon Cancer	8	118,7	42,9	1,9–353,9
Rectum Cancer	6	63,7	54,5	5,0–170,4
Gastric Cancer	4	24,6	24,9	4,7–43,93
Adenoma	8	6,5	3,4	0,0–21,3
Controls	48	0,3	0,0	0,0–2,5

It is possible to detect Tumor M2-PK in stool samples of patients with GI cancer (specificity: 100 %, sensitivity: 94,7 %). Stool samples of patients with colorectal cancer showed remarkably higher values of Tumor M2-PK than samples of patients with gastric cancer, adenoma or healthy controls. Further studies regarding the determination of Tumor M2-PK in stool are in progress.

Keywords: pyruvate kinase, tumor M2-PK, gastrointestinal cancer

O16
Is CA 15-3 Indeed More "Specific" for Breast Cancer than CEA?

B. Mahnert, D. Lässig, P. Stieber, M. Untch[1], H. Fürst[2], S Holdenrieder, K. Hofmann, D. Nagel, A Schalhorn[3]

[1] Institut für Klinische Chemie, Frauenklinik,
[2] Chirurgische Klinik,
[3] Medizinische Klinik III, Klinikum der Universität München-Großhadern

CA 15-3 has the reputation to be – in contrary to CEA – mainly released in breast cancer patients. Our aim was to investigate whether this general opinion is based on real scientific data.

Patients and methods: CEA was determined using the AXsym system (Abbott), CA15-3 using the Elecsys system (Roche Diagnostics). Our calculations are based upon the CEA and/or CA 15-3 values of 11 443 persons where either one or both of the markers were determined routinely and the clinical background is known.

Results: The medians of CEA- and CA 15-3-values in healthy individuals were 1.0 ng/mL and 14.4 U/mL, the 95th percent. 2.9 ng/mL and 23.3 U/mL. By dividing the values by the 95th percent. of healthy individuals we achieved an objective comparison for the extent of release of the two markers (multiples of 95th percent.). Especially in gastrointest. (up to 18-fold), gyn. (up to 15-fold) and urol. (up to 7 fold) diseases the CEA-release could be significantly increased. The CA15-3-release was mainly elevated for lung and gyn. diseases (up to 6-fold). The corresponding 95th percent. were for CEA resp. CA 15-3 6.3 ng/mL and 40 U/mL in gastroint., 4.5 ng/mL and 39 U/mL in gyn., 3.9 U/mL and 28 U/mL in breast, 5.5 ng/mL and 120 U/mL in lung and 6.5 ng/mL and 24 U/mL in urol. diseases. The 100 % *tumor specificity* was reached for CEA at 53 ng/mL and for CA 15-3 at 120 U/mL.

Second we calculated the *sensitivities* on the basis of 95th percent. in healthy individuals and obtained for CEA resp. CA 15-3 47 % and 32 % in gastroint., 27 % and 68 % in gyn., 26 % and 32 % in breast, 60 % and 40 % in lung and 27 and 35 % in urol. cancers. There was a significant correlation to UICC staging in breast cancer for CEA and CA 15-3 (UICC I: 18 each; UICC II: 21 % and 25 %; UICC III: 32 % and 43 %; UICC IV: 61 % and 76 %). To find unknown primaries CEA did not allow a differentiation concerning tumor localization as the highest values were reached by various cancers.

Conclusion: CEA as well as CA15-3 are released by various benign and malignant diseases. In breast cancer CEA and CA15-3 have comparable sensitivities. There is no correlation between the two markers ($r = 0.234$) in breast cancer leading to a significant increase in sensitivities for the combination.

O17
Antiestrogenic Regulation of the TGF-beta 2 Promoter

J. Beisner, M. Buck, K. Pfizenmaier, C. Knabbe

Robert-Bosch-Krankenhaus, Stuttgart, Germany, Institut für Zellbiologie und Immunologie, Universität Stuttgart, Germany

Antiestrogens are able to inhibit the growth of breast cancer cells and are therefore effectively used for the endocrine treatment of hormone dependent breast cancer. Breast cancers that initially respond to antiestrogens often acquire resistance during therapy. The mechanisms that confer antiestrogen resistance still remain unknown.

We have previously shown that antiestrogens act through activation of transforming growth factor beta, an important regulator of cell growth and differentiation. TGFβ2 mRNA levels are induced under antiestrogen treatment. To obtain more information about the endocrine regulation of the transcriptional activation of TGFβ2 we performed promoter-reporter assays. A TGFβ2 promoter element (–778/+63) transiently transfected in MCF-7 cells showed a 12fold activation after treatment with the pure antiestrogen ICI 182 780. Treatment with 4-Hydroxytamoxifen increased the expression only 1,7 fold. The results of the reporter assays reflect the effects observed for mRNA expression.

Sequencing analysis of the TGFβ2 gene promoter region revealed several point mutations and a heterozygous insertion of 4 bases (CTTC) at position –245 in MCF-7 cells. A promoter construct that did not include the insertion showed a reduced induction (4 fold) by the antiestrogen ICI suggesting a possible transcription factor binding site that is mediating a hormonal regulation of the TGF-beta-2-gene. In T47D cells we could not observe a difference in ICI induced activation of the two promoter constructs. One possible reason for the heterogeneous effects of the insertion on antiestrogen induction could be cell-type specific differences in endogenous level of transcription factors.

Our data demonstrate that antiestrogenic regulation of the TGFβ2 gene is mediated through a transcription factor binding site in the promoter around position –245. Loss of a four base insertion in this region leads to reduced sensitivity to antiestrogens.

O18
Cystatin C as GFR marker in Patients with Monoclonal Gammopathy

W. Hofmann[1], W.G. Guder[2], M. Garbrecht[3]

[1] Institut für Klinische Chemie und Immunologie, Städtisches Krankenhaus München-Neuperlach
[2] Institut für Klinische Chemie, Städtisches Krankenhaus München-Bogenhausen
[3] 4. Med. Abteilung, Städtisches Krankenhaus München-Neuperlach

Introduction: Different strategies to detect renal involvement in patients with Bence-Jones proteinuria were used in routine. Usually renal dysfunction is monitored by creatinine in serum and total protein in urine.With Cystatin C in serum and urine protein differentiation (IgG, albumin, α_1-microglobulin) more sensitive markers are available to detect renal dysfunction.
Material and Methods: 44 patient materials (serum, urine) were sent for the question: Monoclonal Gammopathy (MG) ? in our laboratory. For 28 patients a MG was diagnosed by immunofixation. From these 28 patients 17 showed light chain Kappa or Lambda in urine.

Free Kappa, Lambda light chain in serum and urine, Cystatin C in serum and total protein, IgG, albumin, α_1-microglobulin in urine were analysed on a BN-Prospec protein analyser. Creatinine in serum and urine was measured enzymatically on an advia 1650.
Results: In patients with MG and MG with Bence-Jones proteinuria significantly higher Cystatin C concentrations (> 1,2 mg/L) were found than in patients without MG. In contrast creatinine (> 1,2 mg/dL) was not significantly different in patients with MG from patients without MG.

α_1-Microglobulin as tubular marker and total protein in urine were increased in 10 of 11 patients with Bence-Jones Proteinuria and elevated serum Cystatin C concentration.
Conclusion: Cystatin C is more often and higher increased than creatinine in patients with MG, specially with Bence-Jones proteinuria. Therefore Cystatin C appears to be a more sensitive marker to screen for reduction of GFR in patients with MG. Further studies are necessary to validate this first observation.
Keywords: Monoclonal gammopathy, Cystatin C, GFR

O19
Kappa and Lambda Light Chain in Serum and Urine in Patients with Monoclonal Gammopathy

Hofmann, W.[1], Guder W.G. [2], Bradwell A.R[3], Garbrecht M.[4]

[1] Institut für Klinische Chemie und Immunologie, Städtisches Krankenhaus München-Neuperlach
[2] Institut für Klinische Chemie, Städtisches Krankenhaus München-Bogenhausen
[3] Department of Immunology, Medical School, University of Birmingham, B152TJ, UK
[4] 4. Med. Abteilung, Städtisches Krankenhaus München-Neuperlach

Introduction: Different strategies to detect Bence-Jones proteinuria were used in routine. Usually Bence-Jones proteinuria (BJP) was detected by immunofixation, the "gold standard" in medical laboratories. Other authors recommend to measure total kappa and lambda light chains or screen by forming an urinary albumin/total protein (alb/TP) ratio. In this study we measured free kappa, lambda light chains (flc) quantitatively in serum and urine.
Material and Methods: 44 patients, 28 with a monoclonal gammopathy were examined. From these 28 patients, 17 showed a light chain excretion in urine (BJP). Free and total kappa, lambda light chains in serum and urine, total protein and albumin in urine were analysed on a BN-Prospec protein analyser .
Results: All 17 patients with a BJP were discovered by the in- or decreased ratio of serum free kappa/lambda light chains (k/l < 0,36 or > 1,0). On the other hand 3 of 17 patients with BJP showed an unsuspicious urinary free k/l ratio (0,46–4,0) and 4 of 16 an unsuspicious urinary total kappa/lambda ratio (criterias: total k/l: < 1,0 or > 5,2, total kappa + total lambda > 10 mg/l). The used strategy to measure only albumin and total protein in urine (criterias: TP > 300 mg/L, alb /TP < 0,3) was positive in only 5 of 17 cases.
Conclusion: Measuring flc in serum seems to be a new and more sensitive method for detecting monoclonal light chain production. All patients with BJP were detected correctly. Measuring free or total kappa/lambda light chains in urine may overlook cases of BJP. The strategy for detection BJP by albumin and total protein seems too insensitive.
Keywords: Bence-Jones proteinuria, light chain

Stoffwechsel-, Knochen- und Bindegewebs-Erkrankungen

S1
Regulation of the MMP/TIMP System during Experimental Liver Regeneration

R. Lichtinghagen, M. Wehmeier, C. Sallmann, S. Schalk, M.P. Manns, M. J. Bahr

Depts. Clinical Chemistry and Gastroenterology, Hepatology and Endocrinology, Medizinische Hochschule Hannover, Hannover, Germany

Background: During the process of liver regeneration the hepatic extracellular matrix reconstitutes and sinusoids are newly formed. The aim of our study was to gain insight into the regulation of matrix metalloproteinases (MMPs) and their specific inhibitors (TIMPs) in matrix remodeling and neoangiogenesis during experimental liver regeneration.
Materials and methods: A rat model of 2/3 hepatectomy (HE) was performed. Rat livers were excised at definite time points (0, 1, 2, 3, 5, 7, 10, 14 d). The expression of MMP-2, -3, -9, -13, -14, TIMP-1, smooth-muscle-alpha-actin (SMA, a marker of hepatic stellate cell activation), and GAPDH was determined using real-time-RT-PCR (LightCycler) from hepatic mRNA preparations (50 ng/assay). Results from 2/3 hepatectomy were compared to sham-operated (sham) objects.
Results: MMP-2, -3 und -13 were weakly expressed without significant differences between 2/3HE und sham. MMP-9 was below the detection limit. A clear regulation could be shown for MMP-14, TIMP-1 und SMA. Hepatic TIMP-1 was upregulated after 1 d with a decline to initial values starting at day 3. MMP-14 follows the TIMP-1 regulation with a delay of one day. The upregulation of SMA started on day 2 and was sustained until day 5.
Conclusions: The matrix metalloproteinase/TIMP system appears to be regulated differentially during liver regeneration. Interestingly, the early upregulation of MMP-14 and TIMP-1 was not be accompanied by a parallel activation of hepatic stellate cells, as demonstrated for the process of liver fibrosis.

S2
Erstdiagnostik des Morbus Gaucher mittels Saurer Phosphatase, ACE und Ferritin

G. Kappert[1], N. Brill[2], S. vom Dahl[2], D. Häussinger[2], H. Reinauer[1]

[1] Institut für Klinische Chemie und Laboratoriumsdiagnostik,
[2] Klinik für Gastroenterologie, Hepatologie und Infektiologie, Heinrich-Heine-Universität Düsseldorf

Beim M. Gaucher (Zerebrosidlipidose) liegt ein autosomal-rezessiv vererbter Mangel an Betaglukosidase vor, der infolge einer Abbaustörung zur Speicherung von Glukozerebrosiden v. a. in Retikulumzellen (Gaucher-Zellen) mit entspr. Organvergrößerung führt. Neben der Analytik von Zerebrosiden in Knochenmarkzellen und Enzymaktivitätsbestimmungen in Leukozyten od. Fibroblasten spielt die Bestimmung von Saurer Phosphatase (SP), Angiotensin Converting Enzyme (ACE) und Ferritin zur Erstdiagnostik ein wichtige Rolle. Wir untersuchten daher, ob mit alleiniger Bestimmung von SP, ACE und Ferritin eine sichere Erstdiagnose des M. Gaucher möglich ist:

Dazu wurden SP, ACE und Ferritin bei 51 gesunden (w:m 30:21; 20–68a) und bei 70 unbehandelten Erkrankten (w:m 32:38; 3–74a) gemessen.

Die Referenzbereiche definiert als 5.–95. Perzentil des Normalkollektives waren: SP 3,0–6,1 U/l; ACE 16,6–38 U/l; Ferritin 8,5–282 µg/l. Die AUC der ROC-Kurve fiel für Ferritin kleiner (95 %-KI: 0,906–0,981)) als für SP (0,978 (95 %-KI: 0,947–1) und ACE (0,988 (95 %-KI: 0,970–1) aus. Bildet man aus allen Parametern dividiert durch den jeweiligen Referenzbereich die Summe, so erhöht sich die AUC auf 0,999 (95 %-KI. = 0,995–1). Hierdurch war eine Sensitivität von 100 %, eine Spezifität von 98 % und ein Youden Index Y von 0,980 zu erreichen.

Somit ist eine sichere Erstdiagnostik des M. Gaucher allein an Hand einer Berechnung gewährleistet, in welche drei einfach zu bestimmende Parameter eingehen.

S3
Heparin is a Predominantly Non-Competitive Inhibitor of Xylosyltransferase

J. Kuhn, S. Müller, M. Schöttler, T. Brinkmann, C. Götting, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen

Human UDP-D-xylose:proteoglycan core protein β-D-xylosyltransferase-I (XT-I) is the key enzyme in the biosynthesis of heparin, heparan sulfate and chondroitin sulfate. The enzyme initiates the formation of these glycosaminoglycans by transfer of xylose from UDP-xylose to consensus serine residues of the proteoglycan core protein. Recently, we have isolated the human XT-I, cloned the corresponding cDNA and expressed the recombinant XT-I in CHO-K1 cells.

A direct interaction of XT-I with heparin was demonstrated by heparin affinity chromatography on POROS 20 HE using the native enzyme from JAR choriocarcinoma cells and the recombinant XT-I. The XT activity was completely retained on the heparin matrix and at least 0.5 M NaCl in the mobile phase was required to elute the enzyme. A synthetic peptide containing the consensus XT-I acceptor sequence was used for the investigation of the kinetic mechanism of XT-I mediated xylosylation. The K_m-values of the native XT-I and of the recombinant XT-I for the synthetic peptide was determined as 20.8 µM and 22.3 µM, respectively. XT activity was strongly inhibited by heparin and the kinetic study revealed that this inhibition was predominantly of the non-competitive type. The K_i-value was determined to be 0.1 µM. Further investigations will have to focus on the in vivo effects of the observed strong interaction of circulating heparin and the posttranslational xylosylation in humans.

Keywords: Xylosyltransferase-I, heparin, proteoglycan

S4
Expression of Xylosyltransferase I Variants Identifies Essential Cysteine Residues Critical for the Enzymatic Activity

S. Müller, M. Schöttler, J. Kuhn, T. Brinkmann, C. Götting, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum NRW, Universitätklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany

Proteoglycans are abundant molecules present in the extracellular matrix and on the cell surface and are necessary for maintaining the structural integrity of connective tissue. Xylosyltransferase I (XT-I) initiates the biosynthesis of glycosaminoglycan chains in proteoglycans. We have shown previously that XT-I is a biochemical marker of the elevated proteoglycan biosynthesis in connective tissue diseases, although the structure and the catalytic mechanism of this enzyme remains to be elucidated.

The human XT-I gene encodes a protein with 14 cysteine residues. In order to clarify, which of these cysteine residues are critical for the enzymatic activity, we now have developed a system for the expression of an enzymatically active XT-I-V5 epitope fusion protein in High Five insect cells. Vectors for the expression of XT-I variants were generated by substitution of cysteine against alanin codons using site-directed mutagenesis. We have successfully cloned 14 vectors with one of each cysteine residue mutated. High Five insect cells were then transiently transfected with the cloned vectors and the expression of XT-I was monitored in the cell culture supernatant for 96 hours. The recombinant enzyme was detected by measuring the XT-I activity and by western blot analysis using anti-V5-HRP antibodies.The enzyme activity of the XT-I variants [Cys118Ala]XT-I and [Cys313Ala]XT-I was reduced to less than 1 % of the wild type XT-I activity, although the proteins were expressed at comparable levels.

In summary, we have identified two cysteine residues of XT-I which are essential for the enzymatic activity. This is an important prerequisite for the identification of potential disulfide bonds and structure-function relationships affecting the enzymatic activity.

Keywords: Proteoglycan; Xylosyltransferase; connective tissue

S5
The Mammalian Xylosyltransferase Genes Descend from a Common Ancient Gene

M. Schöttler, S. Müller, J. Kuhn, T. Brinkmann, C. Götting, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany

Xylosyltransferase I (XT-I) is the initial enzyme involved in the biosynthesis of the glycosaminoglycan chains in proteoglycans. In pre-

vious studies we have shown that XT-I is a biochemical marker of the elevated proteoglycan biosynthesis in connective tissue diseases.

We now could locate the human XT-I gene on chromosome 16p13.1 using radiation hybrid mapping. It spans over 300 kb and consists of 11 exons. The human XT-II gene was assigned to chromosome 17q21.3-22 and also consists of 11 exons but the gene size is only 15 kb. The exon size and the exon/intron junctions are highly conserved in both genes. In addition, we analyzed the murine xylosyltransferase genes by scanning the mouse genome databases and by the analysis of BAC clones isolated from a 129 mouse genome library probed with a cDNA fragment homologous to exon 2. We could localize the mouse XT-I and XT-II genes on chromosome 7 band F3 and chromosome 11 band D respectively. The exon-intron structure of the mouse XT-I gene revealed that the gene spans 289 kb and consists of 11 exons. The mouse xylosyltransferase genes also show highly conserved exon/intron junctions. We now were able for the first time to identify the premier exon of the murine XT-I gene, which codes for the cytoplasmatic tail and the transmembrane domain of this type II transmembrane protein.

Our results let us conclude that the XT-I and XT-II genes evolutionary descend from a common ancient xylosyltransferase gene. The investigation of the complete xylosyltransferase genes is an important prerequisite for the generation of the xylosyltransferase knock-out mouse as a model system to study the pathobiochemistry of connective tissue and the biological role of the proteoglycans.

Keywords: Xylosyltransferase, gene structure, proteoglycan

S6
Der BONN-Risk-Index: eine sichere, schnelle und kostengünstige Methode zur Bestimmung des Calciumoxalat-Kristallisationsrisikos im Harn

N. Laube, I. Briese, M.E. Schmidt, A. Hesse

Experimentelle Urologie, Universitätsklinikum Bonn

Durch geeignete Metaphylaxestrategien kann die Rezidivrate für Harnsteinpatienten in vielen Fällen deutlich gesenkt werden. In Zeiten knapper werdender Finanzmittel treten zunehmend die volkswirtschaftlichen Aspekte des Steinleidens und der Metaphylaxe in den Vordergrund der Diskussion. Aus diesem Grund ist es wichtig, mit einem möglichst geringen Aufwand das Kristallisationsrisiko im Harn eines Steinpatienten hinreichend genau zu erfassen.

Der BONN-Risk-Index[1,2], BRI, ist eine erfolgreich eingesetzte Methode zur Bestimmung des CaOx-Kristallisationsrisikos. Der BRI wird mit Hilfe eines In-vitro-Kristallisationsexperiments an *unbehandelten* Nativurinen bestimmt; daher wird das individuelle Konzentrationsverhältnis der vorhandenen inhibitorisch und promotorisch wirkenden Substanzen bei der Risikobestimmung *vollständig* berücksichtigt. Der Einfluß der Metaphylaxe auf das Kristallisationsrisiko wird von der BRI-Methode sicher, schnell und kostengünstig erfaßt.

110 Proben von Steinpatienten und 96 Proben von Gesunden wurden umfassend analytisch untersucht. Neben dem BRI wurden zusätzlich die relative CaOx-Übersättigung[3], RS, und der AP-Index[4] bestimmt. Für jeden Index wurde als Leistungskriterium eine Diskriminanzanalyse durchgeführt; in einem ersten Ansatz wurde als Maß der einfache Overlap zwischen beiden Gruppen ermittelt. Für die Indizes wurden jeweils die individuellen Kosten, angelehnt an die relativen Aufwände nach Bewertung der *Deutschen Krankenhausgesellschaft*, berechnet. Die Ergebnisse belegen die unterschiedlichen Leistungen und Kosten der untersuchten Indizes: der Overlap nimmt in der Reihenfolge BRI → AP → RS zu, die Kosten betragen: BRI = 24 €; AP = 25 €; RS = 56 €.

[1] Urol Res 2000 (28):274
[2] J Chem Inf Comput Sci 2002 (42):633
[3] J Urol 1985 (134): 1242
[4] World J Urol 1997 (15):176

DFG-Projekt: He-1132/11-4
Schlüsselwörter: Stoffwechselerkrankungen

S7
Modellrechnungen zum Einfluß in-vivo befindlicher CaOx-Steine auf das Analysenergebnis lithogener Substanzen im Harn

N. Laube[1], S. Hergarten[2], M. Pullmann[2], M.E. Schmidt[1], A. Hesse[1]

[1] Experimentelle Urologie, Universitätsklinikum Bonn
[2] Geodynamik und Physik der Lithosphäre, Universität Bonn

Es wird angenommen, daß Harnsteine, welche sich im Harntrakt befinden, kontinuierlich wachsen. Daher ist das Ergebnis einer Urinanalyse, welche bei einem harnsteintragenden Patienten durchgeführt worden ist, an den lithogenen Substanzen der wachsenden Harnsteinart systematisch verarmt. Die analytisch korrekt bestimmte Harnzusammensetzung führt bei einer Nichtberücksichtigung des Abreicherungseffekts zu einer „falsch niedrigen" Interpretation der vorliegenden Ausscheidungswerte der lithogenen Harnbestandteile. Der tatsächliche Krankheitszustand des Patienten wird dadurch zu optimistisch bewertet und notwendige therapeutische Maßnahmen zu niedrig angesetzt, oder, in extremen Fällen, sogar unterlassen, da die pathologisch relevante Harnzusammensetzung nicht bekannt ist.

Zwei sich ergänzende mathematische Modelle werden motiviert und hergeleitet. Das zu erwartende Ausmaß des Abreicherungseffekts wird anhand verschiedener Eingangsparameter berechnet.

Bei einem innerhalb eines ½ Jahres auf 0,5 cm^3 gewachsenen Stein sind Abweichungen der gemessenen Oxalsäurekonzentration eines typischen grenzwertig „normooxalurischen" Harn von der tatsächlichen in Abhängigkeit des verwendeten Modells von −7 % bis −60 % zu erwarten. Die relativen CaOx-Übersättigungen der korrigierten Urinanalysen steigen gegenüber dem Wert der unkorrigierten Analyse um 46 % bzw. 78 %.

Die vorgestellten Ergebnisse sind von hoher klinischer Relevanz. Stoffwechseluntersuchungen bei Harnsteinpatienten können nur dann ein korrektes Ergebnis liefern, wenn diese „steinfrei" sind. Andernfalls sollte bei der Interpretation der Urinanalyse eine Korrektur erfolgen.

DFG-Projekt: He-1132/11-4
Schlüsselwörter: Stoffwechselerkrankungen, Methodenvergleich/ Qualitätskontrolle

S8
New Parameters in the Diagnosis of Renal Osteodystrophy in Haemodialysis Patients

H.G. Wahl[1], St. Graf[2], M. Bandorski[3], D. Bandorski[3], and W. Fassbinder[2]

[1] Institut für Laboratoriumsmedizin,
[2] Medizinische Klinik III, [3]Medizinische Klinik II, Klinikum Fulda, Germany

Inadequate control of serum phosphorous (P) and calcium (Ca) levels leads to various complications in haemodialysis (HD) patients, including renal osteodystrophy, cardiovascular and soft tissue calcifi-

cation. Tartrate-resistant acid phophatase isoform 5b (TRAP) has been shown to be associated with osteoclastic activity in non-uraemic patients. We therefore investigated whether a novel serum immunoassay specific for TRAP 5b (BoneTrap Assay, Medac, Germany) could be used as a specific marker for activated bone resorption in HD patients. In addition, C-terminal telopeptide of type I collagen (β-CrossLaps), Osteocalcin and intact parathyroid hormone were measured in EDTA plasma (Elecsys β-CrossLaps, PTH and N-MID Osteocalcin, Roche Diagnostics, Germany). Pre-dialysis plasma and serum samples were drawn from 163 HD patients twice within a period of 6 months. Statistical analysis was done for 126 patients with a complete set of data (67 men and 59 women, age 66.3 ± 13.0 years, HD treatment 5.0 ± 4.5 years, mean ± SD). Mean levels (± SEM) of TRAP 5b (5.79 ± 0.30 U/l), β-CrossLaps (1.56 ± 0.11 ng/ml) and osteocalcin (294.4 ± 32.9 ng/ml) were clearly elevated. Intact PTH ranged from normal to very high levels (mean value 227 ± 24 ng/l) with normal to slightly elevated levels of Ca, P and Ca x P. TRAP 5b and β-CrossLaps showed a strong positive correlation. There were only small changes in Ca, P, Ca x P, PTH and TRAP 5b levels after six months, but we identified two subgroups with significant changes in TRAP 5b. One group (n = 20) showed a significant increase of 2.9 ± 0.4 U/l and the other (n = 14) a decrease of 4.8 ± 0.7 U/l. Both groups showed only a small decrease in PTH levels. As direct parameters of bone resorption TRAP 5b and β-CrossLaps might show relevant changes at earlier stages than the conventionally used indirect parameters.

Keywords: renal osteodystrophy, haemodialysis, bone resorption markers

Immunologie/Allergologie

I1
A Novel Approach for Automatized Classification of HEp-2 Immunofluorescence Patterns

U. Sack, S. Knöchner, [1]Petra Perner, [2]U. Pigla, Manja Kamprad

Institute of Clinical Immunology and Transfusion Medicine, University of Leipzig,
[1] Institute of Computer Vision and Applied Computer Sciences,
[2] Biotectid GmbH, Leipzig

In diagnosis of autoimmune diseases, indirect immunofluorescence with HEp-2-cells presents the major screening method. This procedure allows to scan a broad range of autoantibodies and to classify them by fluorescence patterns. The identification of the patterns is currently done manually with the help of a microscope. The major disadvantage of this method is the lack of automatized solutions. Furthermore, need for standardization and documentation is hampered by the common microscopic technique.

We have established a novel system for automatized classification of HEp-2 fluorescent patterns. Designed as an assisting system, representive patterns are acquired by an operator with a digital microscope camera and transferred to a personal computer. A software package was developed based on a novel image analysis and feature extraction algorithm. The generated data set was then subject to machine learning techniques to find out relevant features for a correct classification of autoantibodies.

Our results show, that identification of positive fluorescence and discrimination between most important autoantibody entities can be performed by this system. The decision tree as generated in the learning phase can be integrated in the laboratory system. To enable the usage of such a system in routine diagnostics, accuracy of this system must be further improved. At the moment, more then 80 percent of samples can be classified in a correct way. Furthermore, overall speed of the system must be enhanced.

We conclude that the established system can be used as an automatic inspection module. The integrated software for machine learning allows to permanently improve reliability of classification as well as number of recognized autoantibodies.

I2
EUROBLOT.ax – A Membrane-Based Rapid Assay for the Detection of Autoantibodies Against Extractable Nuclear Antigens (ENA)

W. Meyer, K. Sperling, M. Siegemund, W. Schlumberger, W. Stöcker

EUROIMMUN GmbH, Lübeck, Germany

Flow through assays with antigen coated membranes allow quick determination of serum antibodies. Diluted patient serum, labeled second antibody and chromogenic substrate subsequently are allowed to flow by capillary force through the membrane. Initial trials to detect autoantibodies against ENA by flow through assays showed a low sensitivity due to the high flow rate of sample and reagents. This problem was solved by introduction of a small pore membrane which reduces the flow rate and results in a sensitivity comparable to classic immunoassays.

For the new EUROBLOT.ax, a membrane with a high binding capacity was coated with separate spots of the purified antigens RNP/Sm, Sm, SSA, SSB, Scl-70 and Jo-1. The arrangement of this membrane together with the second small pore non-binding membrane in a plastic housing with an open cavity oriented towards the top allowed the addition of the reagents onto the antigen-coated side of the membrane and their flow through this membrane with a flow rate of 0.07 ml/(min × cm^2). 140 sera from patients with different rheumatic diseases and 50 sera from healthy blood donors were incubated and results were compared to the EUROASSAY, a well-established line immunoblot.

The arrangement of membranes with different properties in the EUROBLOT.ax results in a reduced flow rate of the reagents thereby increasing sensitivity and signal strength. The EUROBLOT.ax exhibited 100 % sensitivity with respect to the EUROASSAY for all anti-ENA antibodies except anti-Scl-70 (97 %); specificity was over 97 % for all six anti-ENA antibodies.

The EUROBLOT.ax allows detection of anti-ENA antibodies with a high sensitivity within 15 minutes. With the arrangement of membranes with different properties the flow rate can be optimized for other antigen-antibody systems allowing the investigation of a broad spectrum of antibodies under optimal conditions.

I3
The Diagnostic Value of Anti-Nucleosome Antibody Testing in Systemic Lupus Erythematosus: Comparison of a 1st and 2nd Generation ELISA

Schlüter B[1], Kaucikaite L[1], Schotte H[2], Willeke P[2], Bonsmann G[3], Gaubitz M[2], Assmann G[1]

[1] Inst. für Klin. Chemie u. Labormedizin,
[2] Med. Klinik B, [3]Dermatol. Klinik, Univ. Münster

Anti-nucleosome antibodies (ANuA) are sensitive serologic markers of systemic lupus erythematosus (SLE). They are also found in systemic sclerosis (PSS) and mixed connective tissue disease (MCTD)

limiting their diagnostic specificity for SLE. However, this may critically depend on the purity of antigens used in ANuA assays. We evaluated 2 ELISA tests with differently prepared nucleosomes (Fa. Euroimmun, Germany, Test A (1st gen.): from nuclease S7 treated chromatin; Test B (2nd gen.): from additionally purified chromatin devoid of histone H1, Scl-70 and non-histone proteins). A large cohort of patients (113 SLE, 122 non-SLE collagenoses, 21 syst. vasculitis, 94 syst. arthritis, 42 organ-specific autoimmunopathies, 40 viral hepatitis or HIV infection) and 127 healthy blood donors were studied. The overall diagnostic sensitivity of both assays was comparable (A: 58,4 %; B: 54,0 %) and showed an about twofold increase in active versus inactive SLE disease states (according to SLEDAI score, $p < 0.005$). Sensitivity was not influenced by the presence of renal involvement. SLE sera which had been tested negative for anti-dsDNA antibodies by Farr-RIA were ANuA positive in 18,8 % (A) or 15,6 % (B), respectively. The diagnostic specificity of both assays ranged from 97–100 % compared to healthy controls or non-collagenose diseases. When non-SLE collagenoses served as controls, specificity of test A declined to 83,9–87,5 % while it remained at 95,1–99,0 % for test B. ROC-analysis revealed a significant better differentiation between SLE and PSS/CREST by test B ($p = 0.015$). We conclude that tests for ANuA are valuable tools in the diagnosis of SLE. High diagnostic specificity can be obtained even in clinically relevant patient collectives through optimally purified antigen preparations.

I4
TREC Analysis Faciliates the Determination of T-cell Migration and Proliferation in Mice

W. Wetzel[1], G.K. Przybylski[2], P. Gronek[2], C.A. Schmidt[2], R. Geßner[1]

[1] Institut für Laboratoriumsmedizin und Pathobiochemie,
[2] Medizinische Klinik – Hämatologie und Onkologie, Charité Campus Virchow-Klinikum, Humboldt-Universität zu Berlin, Augustenburger Platz 1, 13353 Berlin

T cell differentiation is characterized by sequential rearrangements of the T cell receptor (TCR) genes resulting in the generation of Tcell receptor excision circles (TRECs). TRECs do not replicate during mitosis and are diluted out with each cell division. Recently, quantitative analysis of the TCRD-TRECs, generated during recombination of the TCRα chain, has been used to determine the proliferative history of T cell populations in humans.

To study T cell proliferation and migration in vivo we measured TRECs in 129P2/OlaHsd and C57BL/6 mice after immunzation with Ovalbumin. We compared the TREC counts in T cells from primary and secondary lymphatic organs and analyzed the effect of immunization on the TREC counts. We found differences of TREC numbers in thymus in both mouse strains indicating differences in thymic maturation of T cells (573 TRECs per 1000 CD 3 positive cells in 129 mice versus 133/1000 in C57BL/6 mice). In the secondary lymhatic organs we detected 20–70 TRECs per 1000 CD 3 positive cells and thus conclude that the cells had divided approximately 4–5 times since TCR recombination. After immunization the fraction of TREC-positive T cells as well as the total number changes in efferent and non-efferent lymph nodes. We also found changes of TREC numbers in the thymus indicating a systemic immune response.

We conclude that it is possible to utilize TREC determination to monitor T cell proliferation and migration in mice. It does not intervene with the immune response as the injection of BrdU or labeled T cells may do. TREC determination gives independent information in addition to T cell counts in lymphatic organs and in blood. On the basis of the data obtained it is possible to summarize T cell proliferation and migration.

Infektion/Entzündung/Sepsis

E1
CD14 Promotorpolymorphismen und zelluläre Immunkompetenz bei Patienten mit chronischer Herzinsuffizienz

D. Kunz, S. Krüger*, U. Janssens* und A.M. Gressner

Institut für Klinische Chemie und Pathobiochemie,
* Medizinische Klinik I, Universitätsklinikum, RWTH, Pauwelsstr. 30, 52074 Aachen

Bei ödematösen Patienten mit chronischer Herzinsuffizienz (CHI) wurden erhöhte Lipopolysaccharidkonzentrationen (LPS) gefunden. Ferner wurden bisher kontroverse Daten zum Einfluss von LPS auf die Leukozytenaktivierung von CHI-Patienten publiziert. Neben der Entwicklung einer LPS-Toleranz wurde auch eine gesteigerte zelluläre Suszeptibilität gegenüber LPS postuliert, was aus höheren Serumspiegeln von proinflammatorischen Zytokinen bei CHI abgeleitet wurde.

In der vorliegenden Studie wurden die HLA-DR-Expression (BD Biosciences) der Monozyten und die ex vivo Induktion von TNF-α nach Vollblutstimulation mit LPS (Milenia Biotec) bei Patienten mit CHI ($n = 35$) vor und nach kontrollierter Belastung im Vergleich zu gesunden Kontrollen ($n = 20$) gemessen. Zusätzlich wurde der CD14 (–260) Promotorpolymorphismus ($n = 100$ je Gruppe) mit dem Lightcycler (Roche) analysiert.

Die HLA-DR-Expression der Monozyten unterschied sich nicht bei Patienten und Kontrollen. In beiden Gruppen wurde jedoch nach Belastung ein geringfügiger Anstieg beobachtet. Die Leukozyten der Patienten zeigten vor Belastung eine höhere Empfindlichkeit gegenüber der Stimulation mit LPS, erkennbar an einer signifikant höheren *ex vivo* Induktion von TNF-α (50 pg/ml LPS: 440 ± 291 versus 315 ± 138 pg/ml TNF-α; 500 pg/ml LPS: 1031 ± 625 versus 715 ± 348 pg/ml TNF-α). Bei den Patienten war der Genotyp TT (–260) CD14 signifikant häufiger vertreten als bei den gesunden Kontrollen. Eine Korrelation zwischen CD14 (–260) Genotyp und LPS-stimulierter TNF-Produktion wurde aber nicht gefunden.

Diese Ergebnisse zeigen erstmals in einem funktionellen Ansatz die gesteigerte Suszeptibilität der Leukozyten von CHI Patienten gegenüber LPS. Dies spricht bei diesen Patienten gegen die Entwicklung einer LPS-Toleranz.

E2
Induktion der Genexpression von Häm-Bindendem Protein 23 (HBP23)/Peroxiredoxin (Prx) I durch Tetradecanoylphorbol Acetat (TPA) in Makrophagen. Funktionelle Charakterisierung der 5'-flankierenden Promotor-Region

A. Hess[1], A.P. Neuschäfer-Rube[2], N. Katz[1], T. Kietzmann[2], S. Immenschuh[1]

[1] Inst. für Klinische Chemie und Pathobiochemie, Justus-Liebig-Universität Gießen, 35392 Gießen;
[2] Inst. für Biochemie I, Georg-August-Universität Göttingen, 37073 Göttingen

Antioxidativ wirksame Proteine spielen eine protektive Rolle gegen die Toxizität von oxidativem Stress bei Entzündungsreaktionen. In Makrophagen wird die Genexpression des antioxidativ wirksamen

Proteins HBP23, das auch als Prx I bezeichnet wird, durch zahlreiche Stress Stimuli induziert. HBP23/Prx I weist hohe Affinität für das Prooxidans Häm sowie Thioredoxin-abhängige Peroxidase Aktivität auf. Ziel dieser Studie war es, die molekularen Mechanismen der stress-abhängigen Induktion des HBP23/ Prx I Gens aufzuklären. Es konnte gezeigt werden, dass die HBP23/Prx I Genexpression in kultivierten Gewebemakrophagen der Leber und der Makrophagenzelllinie RAW264.7 durch den Phorbolester TPA transkriptional induziert wurde. Um *cis*-regulatorische DNA-Elemente der TPA-abhängigen Regulation zu identifizieren, wurde die proximale Promotor 5'-flankierende Region des Ratten HBP23/Prx I Gens kloniert. Die Luciferase Aktivität von Reportergen Konstrukten mit dem klonierten DNA-Fragment wurde in transfizierten RAW264.7 Zellen durch TPA induziert. Mittels Deletions- und Mutationsanalyse konnte gezeigt werden, dass die TPA-abhängige Heraufregulation der Promotoraktivität durch zwei proximale Aktivator Protein (AP)-1 Bindungsstellen vermittelt wurde. Überexpression von dominant-negativem c-Jun inhibierte und Wild-Typ c-Jun verstärkte die TPA-abhängige HBP23/Prx I Reportergenaktivität. Durch Hemmung der TPA-abhängigen HBP23/ Prx I Promotoraktivität durch Protein Kinase C (PKC) Inhibitoren und Überexpression von dominant negativem Ras konnte gezeigt werden, dass ein PKC/Ras-Signalweg an der Induktion des HBP23/ Prx I Gens durch TPA beteiligt ist.

E3
Molecular Diagnosis of Infectious Endocarditis

C. A. Jantos, J. Dreier, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany.

Infective endocarditis (IE) is a life-threatening disease which is difficult to diagnose. Blood cultures which allow the recovery of the etiologic agent are regarded as the most important laboratory test. However, in a significant proportion of patients blood cultures remain sterile due to the presence of slow-growing, fastidious bacteria or the administration of antibiotics. However, identification of the causative agent of IE is mandatory for appropriate antimicrobial therapy. Therefore, this study was undertaken to determine whether broad-range bacterial PCR with subsequent sequencing may prove useful to establish the etiology of IE.

Heart valves removed from patients with suspected IE were examined for the presence of bacteria by PCR and culture. Broad-range 16S rDNA PCR was used for the amplification of bacterial DNA from tissue specimens. Amplicons were sequenced and the sequence was compared with DNA sequence databases and the results of culture.

Bacterial DNA was detected in 20 heart valves removed from 25 patients with suspected IE. The most common pathogens identified were *Streptococcus bovis* (7), *Streptococcus anginosus* (3) and *Staphylococcus aureus* (2). Of 20 PCR-positive valves 11 yielded a positive result also by culture. With the exception of two cases the bacteriologic findings confirmed the molecular results. In 2 of 5 valves with negative PCR results, a possible pathogen was recovered by culture. Thus, in 9 patients the causative organism was only detectable by molecular methods.

In conclusion, results of this study demonstrate that broad-range PCR with subsequent sequencing is useful for the detection of non-culturable causative organisms in patients with IE. This approach may help to improve patient outcome through the administration of appropriate antimicrobial therapy.

Keywords: infective endocarditis, 16S rDNA PCR

E4
Expression and Purification of a Human L-Selectin IgG Fusion Protein

J. Dernedde, B. Volz and R. Tauber

Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin

Objective: The leukocyte adhesion molecule L-selectin (CD62L) is a type I membrane protein expressed on microvilli of leukocytes. L-selectin mediates the initial steps of leukocyte attachment to vascular endothelium, a prerequisite of subsequent extravasation and might hence be of value for the diagnosis of inflammatory diseases. For studies on the binding mechanism and for the development of diagnostic assays we rely on biologically active L-selectin protein. Here we demonstrate the expression of L-selectin as an IgG fusion and subsequent purification of functional protein.
Methods: The extracellular part of L-selectin was fused to the hinge region of human IgG and cloned into a mammalian expression vector. Expression of the fusion was achieved in transfected K562 cells. The fusion protein was then purified on protein A sepharose and biologically active material was further isolated by affinity chromatography on the synthetic ligand SO_4-Tyr-sLex.
Results: Recombinant L-selectin IgG chimera was stably expressed in K562 cells at levels of approximately 1 µg/ ml. Whereas only about 40 % of the protein showed binding to the synthetic ligand SO_4-Tyr-sLex, this highly pure protein fraction was capable to enrich the natural ligands PSGL-1 and CD34 from KG1a cells.
Conclusion: By example of an L-selectin IgG chimera expressed in K562 cells, it could be shown that during expression and purification procedure only part of the molecules retains their biological function. Applying ligand binding affinity chromatography as a final purification step pure biologically active protein was isolated, suited for L-selectin-ligand binding assays.

E5
Identifizierung neuartiger leukozytärer Liganden des Adhäsionsrezeptors L-Selektin

G. Harms[1], G. Grelle[2], J. Dernedde[1], R. Tauber[1]

[1] Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin
[2] Max-Delbrück-Zentrum für Molekulare Medizin, Robert-Rössle-Str. 10, 13125 Berlin

Die Diapedese von Leukozyten in entzündete Gewebe ist ein wichtiger Teilschritt der Immunantwort. Sie wird eingeleitet durch transiente Kontakte der Leukozyten mit den Endothelzellen der Gefäßwand bzw. mit bereits an der Gefäßwand arretierten Leukozyten. Diese heterotypische bzw. homotypische Zell-Zell-Interaktion wird durch das auf den Mikrovilli der Leukozyten exprimierte L-Selektin und seine Liganden vermittelt. Ziel der Arbeiten war die Identifizierung der noch weitgehend unbekannten an der homotypischen Leukozyten-Leukozyten-Adhäsion beteiligten L-Selektin-Liganden auf Leukozyten.

Aus U937-Zellen wurden Fraktionen integraler Membranproteine und membranassoziierter Proteine präpariert und affinitätschromatographisch auf L-Selektin-Agarose fraktioniert. Aus der Fraktion integraler Membranproteine wurden zwei L-Selektin-Liganden isoliert: Das Sialomuzin-PSGL-1, der bislang einzige bekannte leukozytäre L-Selektin-Ligand, und das Glycoprotein CD34. Aus der Fraktion membranassoziierter Proteine wurden Polypeptide isoliert,

die mittels Massenspektroskopie als Nukleolin [1] und Hsc70 identifiziert wurden. Die Identität beider Proteine wurde durch den Nachweis ihrer Reaktivität mit monoklonalen Antikörpern im Immunblot verifiziert. Durch kovalente Markierung mit Sulfo-NHS-SS-Biotin gelang der Nachweis, dass beide Proteine neben ihrer nukleolären bzw. zytoplasmatischen Lokalisation auch auf der Oberfläche intakter Zellen lokalisiert sind. Auf der Zelloberfläche exponiertes PSGL-1, Nukleolin und Hsc70 binden spezifisch L-Selektin aus der Extrazellular-Flüssigkeit bzw. auf der Oberfläche benachbarter Zellen. Untersuchungen zum Mechanismus der L-Selektin-Ligand-Bindung zeigten, dass Nukleolin wie Hsc70 mit sialylierten O-Glycanen postranslational modifiziert werden, die in Analogie zu PSGL-1 und anderen bekannten L-Selektin-Liganden direkt an der Bindung beteiligt sein könnten. Weiterhin konnte die Expression von Nukleolin und Hsc70 auf den myeloischen Zelllinien K562 und HL60 und der hämatopoetischen Progenitor-Zelllinie KG1a sowie auf frisch aus dem Blut isolierten Leukozyten nachgewiesen werden.

Die Ergebnisse zeigen, dass myeloische Zelllinien und Leukozyten neben dem Sialomuzin PSGL-1 mit Nukleolin und dem Chaperon Hsc70 neuartige Liganden des leukozytären Adhäsionsrezeptors L-Selektin exprimieren. Die Ergebnisse weisen darauf hin, dass Nukleolin und Hsc70 zusammen mit PSGL-1 an der homotypischen Leukozyten-Leukozyten-Adhäsion im Rahmen der Immunantwort beteiligt sind.

[1] Harms et al. (2001) Identification of nucleolin as a new L-selectin ligand. Biochem. J. **360**, 531–538.

E6
Plasma Levels of Bioactive Lipids as Prognostic Markers in Septic Patients

W. Drobnik, G. Liebisch, F.X. Audebert[1], D. Fröhlich[1]
T. Glück[2], P. Vogel[3], G. Rothe, G. Schmitz

Institut für Klinische Chemie und Laboratoriumsmedizin,
[1] Klinik für Anästhesiologie;
[2] Klinik für Innere Medizin I,
[3] Klnik für Chirurgie; Universität Regensburg

Objectives: Bioactive lipids including lysophosphatidylcholine (LPC) and ceramide are present in human plasma and are known to act as hormone-like agonists, which modulate cellular processes via activation of G-protein coupled receptors or the regulation of receptor clustering. In the current project we analyzed the diagnostic and prognostic value of bioactive lipids in plasma form patients with sepsis.
Methods and Results: 119 patients fulfilling at least 3 SIRS criteria were included in the study and disease course was reported up to 30 days. Clinical data concerning survival were available for 102 patients from whom 20 patients and 19 patients died within the first 11 days or between day 11 and day 30, respectively. Plasma samples were obtained at day 1, day 4 and day 11. Plasma levels of LPC and ceramide as well as their respective precursor molecules, e.g. phosphatidylcholine (PC) and sphingomyelin (SPM), were determined by quantitative tandem mass spectrometry. Ceramide and LPC concentrations were normalized for plasma SPM and PC levels, respectively. Increased ceramide levels and decreased LPC concentrations were found to be significant predictors of sepsis related death. The prognostic value of the ceramide to LPC ratio was comparable to the SAPSII score at day 1 and even better at day 11.
Conclusions: Plasma bioactive lipids are novel and innovative parameters in the risk assessment of septic patients.
Keywords: sepsis, bioactive lipids, diagnostic value

E7
Quantifizierung des endogenen Cannabinoids 2-Arachidonoyl-Glyzerin

G. Malchau, G. Kühn und K. Wielckens

Institut für Klinische Chemie der Universität zu Köln

Endogene Cannabinoide wie Anandamid oder 2-Arachidonoylglyzerin (2-AG) haben nicht nur Wirkungen im ZNS, sondern z. B. auch an Blutgefäßen und -zellen. So konnte gezeigt werden, dass Cannabinoide Thrombozyten aktivieren oder im Schock den Blutdruck senken. Im peripheren Blut spielt dabei im Gegensatz zum ZNS offenbar 2-AG die Hauptrolle. Allerdings werden die Untersuchungen dadurch behindert, dass 2-AG sich während der Extraktion zu 1-AG umlagert und eine Isomer-Verteilung von 10:1 zugunsten des 1-AG entsteht, unabhängig davon, ob primär 1- oder 2-AG vorhanden war. Somit war es bisher nicht möglich, die tatsächliche 2-AG-Konzentration in der Probe zu bestimmen. Folglich ist eine Quantifizierungsmethode erforderlich, bei der die Isomerisierung verhindert wird. Zu diesem Zweck wurde ein Verfahren entwickelt, bei dem das Probenmaterial zuerst azetyliert wird, um die OH-Gruppen – und damit die Umlagerung – zu blockieren. Danach folgt die Extraktion mit Chloroform/Methanol, eine Reverse Phase Dünnschichtchromatografie und die Quantifizierung mit Hilfe von GC-MS. Als interner Standard wird deuteriertes 2-AG verwendet, das der Primärprobe zugefügt wird. Bei der Evaluierung der Methodik zeigte sich, dass ohne die Azetylierung 1-AG und 2-AG – wie erwartet – im Verhältnis von 10:1 in der Probe vorlagen. Wurde Serum oder Plasma hingegen mit Azetanhydrid behandelt, finden sich deutlich höhere Anteile von 2-AG, was den Schluss zulässt, dass die Isomerisierung während der Aufarbeitung tatsächlich verhindert und 2-AG quantifiziert werden kann. Damit steht eine Technik zur Verfügung, die dazu beitragen könnte, die physiologische und pathophysiologische Rolle des endogenen Cannabinoids 2-AG näher zu charakterisieren.

E8
Pharmakokinetik von Meropenem bei neutropenischen Patienten mit Fieber

S. Hoppe[1], H. Schwörer[2], A. Beckmann[2], H. Grüning[1], P.D. Niedmann[1], C. Binder[3], X. Schiel[4], M. Oellerich[1], L. Binder[1]

[1] Abt. Klinische Chemie/
[2] Gastroenterologie-Endokrinologie/
[3] Hämatoonkologie, Georg-August-Universität Göttingen;
[4] Abt. Hämatoonkologie, Ludwig-Maximilians-Universität, München; Deutschland

Das Carbapenem Meropenem zeigt ein breites antibakterielles Spektrum gegen grampositive, gramnegative und anaerobe Erreger und wird in der empirischen Therapie neutropenischer Patienten mit Fieber eingesetzt. Ziel der Studie war die Untersuchung der pharmakokinetischen Variabilität von Meropenem bei febrilen neutropenischen Patienten (NP) im Hinblick auf seine therapeutische Wirksamkeit. 10 NP wurden in die Studie eingeschlossen. Die Patienten erhielten 8-stündliche Dosierungen von 1000 mg Meropenem. Bei jedem Patienten wurden während eines Dosierungsintervalls 5 bis 8 Plasmaproben entnommen. Maximumproben wurden innerhalb von 12 Minuten nach Infusionsende, Minimumproben unmittelbar vor der nächsten Dosis entnommen. Die Meropenemkonzentrationen wurden mittels Reversed-Phase-HPLC gemessen.

Die Maximumkonzentrationen von Meropenem lagen bei den NP bei 57.6/57.9 (37.9–72.7) mg/l [Mittelwert/Median (Range)], die Minimumkonzentrationen bei 1.9/0.8 (< 0.1–6.2) mg/l. Die gefunde-

nen Maxima unterschieden sich somit um einen Faktor 1.9, die Minima um einen Faktor größer als 62. Es bestand ein linearer Zusammenhang zwischen Meropenem- und Kreatininclearance (R = 0,82). NP mit einer Kreatininclearance > 100 ml/min unterschritten die MIC90 von Pseudomonas aeruginosa (4 mg/l) bereits nach 40/43 (22–53)%, die MIC90 von Enterobacteriaceae (< 2 mg/l) nach 57/59 (29–80)% des Dosierungsintervalls. NP mit einer Kreatininclearance < 100 ml/min lagen unter 4 mg/l bzw. 2 mg/l nach 61/52 (44–89)% bzw. 82/84) (56–100)% des Dosierungsintervalls.

Die überwiegend zeitabhängige Bakterizidie unter Meropenem erfordert eine Überschreitung der MIC über einen Zeitraum von mindestens 60–70 % des Dosierungsintervalls. Dies wird bei NP mit normaler Nierenfunktion häufig nicht erreicht. Die renal bedingte pharmakokinetische Variabilität von Meropenem kann daher die Ursache eines pharmakodynamischen Therapieversagens darstellen.

E9
In vitro Activities of Moxifloxacin, Levofloxacin, Quinupristin/Dalfopristin, Linezolid, and Vancomycin against Clinical Isolates of Staphylococci

H. Weisser, W. Pfister, S. Eick, E. Straube

Institute of Medical Microbiology, University Clinic of Jena, Germany

Despite advances in antibacterial therapy, the incidence of infections caused by multiresistant gram-positive bacteria has increased during the last years. Thus, there is a need for highly acitive antimicrobial agents, especially for therapy of infections caused by methicillin-resistant staphylococci. The aim of this study was to determine the in vitro activities of moxifloxacin (MOX), levofloxacin (LFX), quinupristin/dalfopristin (SYN), linezolid (LIZ), and vancomycin (VAN) against staphylococci. A total of 7245 isolates of staphylococci were recovered from clinical specimens. These isolates included 4072 methicillinsusceptible S. aureus (MSSA), 212 methicillin-resistant S. au-reus (MRSA), 868 methicillin-susceptible S. epidermidis (MSSE),1015 methicillin-resistant S. epidermidis (MRSE), 198 methicillin-susceptible S. haemolyticus (MSSH), 439 methicillin-resistant S. haemolyticus (MRSH), 304 methicillin-susceptible strains of other coagulase-negative staphylococci (MSCoNS), and 137 methicillin-resistant strains of other coagulase-negative staphylococci (MRCoNS). Minimal inhibitory concentrations (MIC) were determined by broth microdilution (MICRONAUT-S-GENARS, Merlin, Bornheim-Hersel). MICs values at which 50 % (MIC_{50}) and 90 % (MIC_{90}) of the isolates were inhibited were evaluated

	MIC (mg/L)									
	MOX		LFX		SYN		LIZ		VAN	
Bacteria	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
MSSA	0.063	1	0.125	2	0.5	1	1	2	1	1
MRSA	2	4	4	16	1	1	1	2	1	1
MSSE	0.125	2	0.25	8	0.5	0.5	0.5	1	1	1
MRSE	1	2	4	8	0.5	0.5	0.5	1	1	2
MSSH	0.125	2	0.125	8	0.5	1	0.5	1	0.5	1
MRSH	1	4	4	16	0.5	1	0.5	1	1	2
MS CoNS	0.125	0.5	0.25	1	0.5	1	0.5	1	0.5	1
MR CoNS	1	4	4	16	0.5	1	0.5	1	1	2

Apart from some elevated MIC values, especially of MOX and LFX, the antimicrobials tested exhibited good activities against methicillin-susceptible as well as methicillin-resistant staphylococci.

E10
Extracorporeal Plasma Perfusion for Selective Removal of Endotoxin (LPS) in Septic Patients

S. Bengsch[1], K.-S. Boos[1], D. Inthorn[2], D. Seidel[1]

[1] Institute of Clinical Chemistry,
[2] Department of Surgery, University Hospital Grosshadern, Munich, Germany

Severe sepsis is a life-threatening pathological condition and is still the most frequent cause of death in intensive care units in maximum-care hospitals. However, innovative blood-purification procedures using selective adsorbents to remove pyrogens appear to offer a promising therapeutic approach.

In this context we designed an open monocentric pilot study without a control group. Patients suffering from severe sepsis and with a lipopolysaccharide (LPS) concentration ≥ 0,3 EU/ml were elected for adsorption apheresis in addition to the standard sepsis therapy in the ICU. Plasma was obtained by hollow fiber filtration of whole blood and perfused through a cartridge (DEAE-modified cellulose sheets, B. Braun Melsungen AG). The plasma cleared of LPS of gram-neg. bacteria and of lipoteichoic acid (LTA) of gram-pos. bacteria was remixed with the cellular blood components and reinfused into the patient.

83 treatments administered to 15 patients revealed an effective and selective reduction of the LPS plasma concentration by 40 % as a result of adsorption apheresis. Comparison of initial levels and 48 hours after a serie of 5 apheresis treatments showed that CRP, PCT, LBP and APACHE II score dropped significantly. Overall, adsorption apheresis using the described adsorber has proved to be a promising therapeutic tool for patients with severe sepsis.

Drug Monitoring/Toxikologie/ Pharmakologie

D1
Biochemical Parameters for Alcohol Consumption in Patients from a Medical Ward

M. Zogbaum[1], T. Bulang[2], F. Kruschke[2], J. W. Harasymiw[3], H. Porst[2], T. Demant[1]

[1] Institut für Labormedizin und
[2] III. Medizin. Klinik, Krankenhaus Dresden-Friedrichstadt, D-01067 Dresden, Germany,
[3] Alcohol Detection Services Inc., Brookfield, WI 53045–8156, USA

We investigated whether in patients from a medical ward alcohol consumption can be estimated by either carbohydrate-deficient transferrin (CDT%) or by a score derived from a panel of 25 routine lab parameters (EDAC: Early Detection of Alcohol Consumption, *Alcohol Clin Exp Res 25:228–235, 2001*).
Methods: Consecutive patients were assigned to one of three groups: i) established alcohol abuse (n = 12), ii) diseases possibly related to alcohol consumption (n = 18), iii) alcohol-independent diseases (n = 11). The daily alcohol intake was derived from a detailed case history. Patients with low credibility in alcohol self appraisal were eliminated from the study. CDT% was determined by a TIA assay (Bio-Rad). The EDAC score was calculated by linear discriminant function (LDF) analysis.
Results: The reported alcohol intake per day was expectedly different in the three patient groups (medians: 350 vs 20 vs 6 g/d; $p < 0.001$), likewise the CDT% values were significantly different (3,6 vs 2,0 vs 1,7 %; $p = 0.008$) in contrast to the EDAC scores (0,35 vs 0,12 vs 0,05; n.s.). In all patients only the CDT% values but not the EDAC sores were correlated with reported alcohol intake (CDT%: r = 0,53, p = 0,001) ; EDAC: r = 0,18, n.s.). For alcohol intake > 40 g/d the CDT% assay (Cut-off: 2.6 %) had a specificity of 0.73 and a sensitivity of 0.67, positive and negative predictive values were 0.59 and 0.79. For the EDAC score (Cut-off: 0,33) the respective values were: specificity 0.65 and sensitivity 0.53, positive and negative predictive values were 0,47 and 0,71.
Conclusion: None of the two tests allows for a sufficiently reliable estimate of alcohol consumption in medically ill patients. Whereas CDT% may provide some supportive information the EDAC score in its present form is not suitable for this clinical set-up.
Keywords: alcohol abuse, carbohydrate-deficient transferrin (CDT%), Early Detection of Alcohol Consumption (EDAC) score

D2
Analytical Evaluation of the EMIT® 2000 Tacrolimus Assay on the VIVA Analyzer

D. Hermsen, H. Reinauer

Institut für Klinische Chemie und Laboratoriumsdiagnostik, Heinrich-Heine-Universität Düsseldorf

Objective: Tacrolimus is an immunosuppressive drug used to prevent organ transplant rejection. The purpose of this study was to evaluate the new EMIT® 2000 Tacrolimus assay on the VIVA analyzer (Dade Behring Inc.) and to perform a method comparison (MC) study with the established Tacrolimus microparticle immunoassay (MEIA, ABBOTT Diagnostics). Methods and Results: The EMIT assay employing a homogenous enzyme immunoassay technique and the MEIA method performed on the IMx® analyzer were used according to the manufacturer's instructions. Within-run and between-run CVs ranged between 5 % –10 % and 8 % –23 % respectively using commercial controls (2–19 ng/mL) and EDTA whole blood pools (5–10 ng/mL). The analytical and functional sensitivity were found < 1,6 ng/mL and < 3,5 ng/mL, respectively. The dilution linearity was observed between 3–27 ng/mL. Accuracy was assessed by comparing LC-MS/MS target values of commercial controls with mean values of a between-run obtained by the EMIT method. Recoveries ranged between 113 %–116 %. The MC study was performed using 160 EDTA whole blood specimens of kidney, liver and allogenic bone marrow transplant patients. EMIT results correlated well (r = 0,966) with the MEIA method. The Passing-Bablok regression analysis yielded the following equation: EMIT = 0,973 (range 0,926–1,016) × MEIA –1,112 (range –1,531 – –0,791 ng/mL). Regression data of the organ transplant subgroups were comparable with results of the complete collective. The negative bias was not unexpected due to differences in immunoassay standardization and probably due to the hematocrit interference of the MEIA method. Conclusion: The EMIT assay is a useful and reliable tool for therapeutic drug monitoring of Tacrolimus in transplant patients. Additionally, the VIVA analyzer allowed a quick analysis of few samples with a low turnaround time and also has an unrestricted run size.
Keywords: Drug monitoring, Tacrolimus, evaluation

D3
A Simple High-Performance Liquid Chromatographic Method for Determination of Thiopurine Methyltransferase (TPMT) Activity in Erythrocytes

D. Indjova-Hristova[1], M. Shipkova[2], P.D. Niedmann[2], D.A. Svinarov[1], M. Oellerich[2]

[1] Dept. of Clinical Laboratory, Medical University, Sofia, Bulgaria;
[2] Dept. of Clinical Chemistry, Georg-August-University, Göttingen, Germany

Background: Genetic polymorphism of the S-methylation pathway catalysed by TPMT is known to be responsible for variation in the metabolism, toxicity, and therapeutic efficacy of thiopurine drugs. We describe a new simple, nonradioactive HPLC method for determination of TPMT activity in isolated erythrocytes (Ery), based on the conversion of 6-mercaptopurine (pH 7.5, 37 °C) to 6-methylmercaptopurine (6-MMP) using S-adenosyl-L-methionine as methyl donor.

Methods: The incubation step was stopped by a mixture of trichloracetic acid/acetonitrile containing the internal standard 4-aminoacetophenone. 6-MMP was quantified by absorbance at 290 nm after chromatographic separation on a Zorbax SB-Phenyl column (5 µm, 4.6 × 250 mm) using mobile phases (flow rate 1.1 mL/min) consisting of acetonitrile, phosphate buffer pH 3.0, triethylamine and dithiothreitol.
Results: The assay was linear up to 50 nmol/(mL Ery × h) and the detection limit was 0.3 nmol/(mL Ery × h). The extraction efficiency of 6-MMP was 95–103 % (n = 3) and its analytical recovery ranged between 98.4 % and 101.1 % (n = 12). The within-day imprecision using pooled human erythrocytes (n = 12) was 4.4 % at a TPMT activity of 15.6 nmol/(mL Ery × h) and 4.9 % at 6.0 nmol/(mL Ery × h). The between-day imprecisions (n = 12) were 6.8 % and 7.5 % nmol/(mL Ery × h), respectively. A very good agreement was found between TPMT activity determined with this method (y) and an widely used radiochemical procedure (x), ($r = 0.94$; $n = 130$; $y = 0.502 + 0.946x$; $p < 0.05$).

Conclusion: The new HPLC method for determination of TPMT activity in Erys is a rapid and reliable procedure, which can be successfully used for both routine and scientific investigations.
Keywords: Drug monitoring, Thiopurine drugs, TPMT activity

D4
Thiopurine Methyl Transferase (TPMT) *3A, *3B and *3C Haplotyping by Combined Long Range PCR, Intramolecular Ligation and Real Time ASA-PCR

N. von Ahsen, E. Schütz[1], S. Hartung, R. Andag, M. Oellerich

Abt. Klinische Chemie, Georg-August-Universität, Robert-Koch-Str. 40, 37075 Göttingen und [1]ChronixBiomedical, Benicia, CA, USA

Objective: The TPMT enzyme deficiency causes a pharmacogenetic trait characterized by intolerance to thiopurine drugs (6-MP, 6-TG, azathioprine). Subjects at risk are identified by determination of TPMT activity. Genotyping for the most common mutations is necessary in certain cases (e. g. after blood transfusion) but difficult and equivocal, since mutations on different exons in either cis (*3A) or trans combined (*3B and *3C) can only be discriminated by phenotyping, or RNA based haplotyping with its inherent shortcomings.
Methods: We simplified and modified a method by McDonald et al., (Pharmacogenetics 12:93). A 9 kB amplicon comprising the mutations indicative for TPMT*3A, *3B or *3C was generated from genomic DNA by PCR with primers containing a terminal *Sal*I restriction site. PCR products were digested and ligated intramolecularly thereby approximating the mutation sites from 9 kB to 900 bp. A second PCR passing the ligation site was performed consecutively. The resulting PCR product was diluted and used in an ASA-PCR reaction on the LightCycler. Primers for the ASA-PCR contained one additional destabilizing mismatch 3 bp from the 3' end. The PCR product and genotype was detected with hybridization probes as described before (Schütz et al., Clin Chem 46:1728).
Results: Amplification in the first round PCR was possible in 50 of 50 samples. The allele specific amplification allowed for definitive genotype assignment based on the combined interpretation of amplification and hybridization probe melting point.
Conclusion: We report a robust method for TPMT haplotyping suitable for clinical molecular diagnostics. The advantage over conventional genotyping method is a definitive assignment of TPMT*3A, *3B or *3C alleles.
Schlüsselworte: TPMT, Genotyping, Thiopurine pharmacogenetics

D5
The C3435T and G2677 Polymorphisms of the *MDR1* Gene as well as the G69 866A Polymorphism of the *CYP3A5* Gene Do Not Influence Cyclosporine C2 Concentrations in Renal Transplant Recipients

N. von Ahsen[1], C. Grupp[2], M. Oellerich[1], V. W. Armstrong[1]

[1] Departments of Clinical Chemistry,
[2] Nephrologie and Rheumatologie, Georg-August University, Robert-Koch-Straße 40, D-37075 Göttingen

P-glycoprotein (P-gp), a product of the *MDR1* gene and the cytochrome P450 3A (CYP3A) subfamily enzymes are major determinants of cyclosporine (CsA) bioavailability. Single nucleotide polymorphisms (SNP) in exons 26 (C3435T) and 21 (G2677T) of the *MDR1* gene are linked to altered P-gp activity. An intronic SNP (G6986A) of the *CYP3A5* gene leads to alternative splicing of CYP3A5 transcripts and absence of the CYP3A5 protein.

The objective of this study was to determine whether these polymorphisms influence CsA absorption as reflected by 2-hour post-dose (C2) concentrations.

Forty two stable (>6 months posttransplant) renal transplant recipients (median age 50 yrs; range 23–75 yrs) were investigated. EDTA whole blood samples were drawn 2 hrs after the Neoral® (Novartis) dose. C2 concentrations were determined using the Emit assay (Dade Behring). Dose-adjusted concentrations were calculated by dividing the measured C2 level by the corresponding 12-h dose on a mg/kg basis. The *MDR1* C3435T, G2677T and the *CYP3A5* G6986A polymorphisms were genotyped using homogeneous hybridisation probe assays on the LightCycler™ (Roche).

The frequencies of the variant alleles containing the polymorphisms were as follows: *MDR1* C3455T 48.8 %; G2677T 34.5 %; *CYP3A5* G6986A 10.7 %. These frequencies are similar to those reported for Caucasian populations. The dose adjusted mean (SD) C2 levels for the polymorphisms were: *MDR1* 3435 CC (n = 12) 419 (143) µg/L, CT (n = 19) 398 (149) µg/L, TT (n = 11) 514 (163) µg/L; *MDR1* 2677 GG (n = 18) 404 (137) µg/L, GT (n = 19) 446 (152) µg/L, TT (n = 5) 496 (167) µg/L; *CYP3A5* 6986 GG (n = 34) 421 (138))µg/L, GA (n = 7) 500 (193) µg/L, AA (n = 1) 422 µg/L. One-way ANOVA revealed no significant differences (p gt;0.1) between the dose-adjusted C2 levels in the respective groups.

We conclude that these allelic variants in the MDR 1 and CYP3A5 genes are not major determinants of CsA bioavailability from Neoral®.
Keywords: Ciclosporin, MDR, cytochrome P450

D6
Die Bioverfügbarkeit von Cyclosporin A und Tacrolimus in Abhängigkeit vom Polymorphismus C3435T im MDR-Gen bei nierentransplantierten Patienten

A. Strate, K. Reuner, H. Felten, F. Litfin, S. Kreft, H. Patscheke

ZLMT, Städtisches Klinikum Karlsruhe

p-Glykoprotein (pGP) ist ein Membranprotein, das hydrophobe Pharmaka aus der Zelle transportieren kann und im Multi-Drug-Resistance-(MDR-)Gen kodiert ist (1). Die Bioverfügbarkeit (BV) dieser Pharmaka hängt von der Funktion des pGP und damit auch von Poly-

morphismen im MDR-Gen ab: Gesunde Träger des T-Allels an der Position 3435 im Exon 26 des MDR-Gens exprimieren intestinal weniger pGP und zeigen eine deutlich höhere BV von oral verabreichtem Digoxin als Träger des C-Allels (2). In der vorliegenden Studie wird untersucht, ob die BV von Cyclosporin A (Cysp A) und Tacrolimus bei nierentransplantierten Patienten mit dem Polymorphismus C3435T im MDR-Gen zusammenhängt. Dazu wurde bei 39 ambulant betreuten Patienten (29 Patienten mit Cysp A, 10 Patienten mit Tacrolimus) mit Hilfe der PCR der Genotyp bestimmt und die dosisbezogene Vollblutkonzentration von Cysp A bzw. Tacrolimus berechnet. Die Genomanalyse ergab eine Allelfrequenz des T-Allels von 57 % (43 % homozygot, 28 % heterozygot); ein signifikanter Unterschied bei der dosisbezogenen Vollblutkonzentration konnte weder für Cysp A noch für Tacrolimus gefunden werden, obwohl zyklische Peptide wie das Cysp A von pGP transportiert werden (1). Möglicherweise ist ein direkter Zusammenhang zwischen BV und Genotyp wegen multipler Interaktionen mit Pharmaka aus der Begleitmedikation nicht erkennbar. Die Bestimmung des Polymorphismus C3435T im MDR-Gen kann die Konzentrationsbestimmung bei Einstellung der immunsuppressiven Therapie bei nierentransplantierten Patienten nicht ersetzen.

1. Ambudkar et al. Annu Rev Pharmacol Toxicol. 1999:361–98
2. Hoffmeyer et al. Proc Natl Acad Sci. 2000; 97:3473–8

D7
Genotying of CYP2D6 and CYP2C19 Alleles in Psychiatric Patients and Its Clinical Relevance for Practical Psychopharmacotherapy

R. Lichtinghagen, S. Kropp, K. Winterstein, W. Bautsch, U. Schneider

Depts. Clinical Chemistry and Psychiatry, Medizinische Hochschule Hannover

Background: Cytochrome-P-450 CYP2C19 and CYP2D6 polymorphisms were evaluated in a study group of psychiatric in-patients. We assessed the impact of slow metabolizers on the duration of hospital stay.
Methods: 229 patients were genotyped for CYP2C19 *2, CYP2D6 *3, *4 and *5 on the LightCycler. Detailed clinical information was obtained from 55 patients (schizophrenia (n = 22), depression (n = 33)).
Results: For CYP2C19 we found 3.9 % poor metabolizers and an allele frequency of 15.9 % (*2). CYP2D6 revealed 4.8 % slow metabolizers (3.9 % homozygosity (*4), 0.9 % compound heterozygosity) and allele frequencies of 0.9 % (*3), 17.3 % (*4), and 6.3 % (*5), respectively. Patients carrying CYP2D6 or CYP2C19 slow metabolizer alleles stayed significantly longer in the hospital (median 58 vs. 38 days; $p < 0.005$), while there was no different clinical global impression (CGI) between the groups. Patients with CYP2C19*2 alleles received significantly more antidepressant drugs (median 2 vs. 1, $p < 0.05$)
Conclusion: Polymorphisms of cytochrome-P-450 mediated drug metabolism do have an impact on the therapeutic success in psychopharmacology. As it seems, patients with CYP2C19 and CYP2D6 polymorphisms have more complicated treatments and stay longer in the hospital. Genotyping might help to find the right drug for the individual patient, and might reduce psychological stress with the ongoing effort to stabilize the patient. The knowledge of CYP slow metabolizers in the individual case might reduce hospitalization due to a facilitated therapy.

Massenspektrometrie

M1
Analyse der Glycane des Serumtransferrins bei Tumorpatienten und Alkoholikern mittels HPAEC-PAD und MALDI/TOF-MS

M. Lipkowski[1], L. Dibbelt[1], M. Seyfarth[1], H.S. Conradt[2]

[1] Zentrallabor Klinische Chemie, Universität Lübeck
[2] Proteinglycosylierung, Gesellschaft für Biotechnologische Forschung, Braunschweig

Veränderungen der Glycosylierungsmuster von Plasmaproteinen wurden bei vielen Tumorerkrankungen beschrieben. Es ist noch ungeklärt, ob diese Veränderungen nur Folgen krankheitsbedingter Störungen oder auch eine der Ursachen von Krankheitssymptomen sind.

Um mögliche Glycanstruktur-Variationen zu untersuchen und ihre diagnostische Aussagekraft zu prüfen, wurden Transferrin-Glycane, die aus dem Serumtransferrin von 20 Tumorpatienten, 5 klinisch gesunden Personen und 8 Patienten mit chronisch erhöhtem Alkoholkonsum isoliert worden waren, mittels *high-pH anion exchange chromatography with pulsed amperometric detection* (HPAEC-PAD) und *matrix-assisted laser desorption/ionisation time of flight*-Massenspektrometrie (MALDI/TOF-MS) analysiert. Bei der Auftrennung der desialylierten Glycane mittels HPAEC-PAD traten bis zu 7 Peaks auf, die chromatographisch und massenspektrometrisch als Kohlenhydrat-Strukturen identifiziert werden konnten. Der Vergleich der prozentualen Anteile dieser Fraktionen zwischen klinisch gesunden Personen und Tumorpatienten zeigte nur geringe Unterschiede in der Zusammensetzung der Glycane. Patienten mit einem Primärtumor oder Metastasen in der Leber, aber auch Personen mit chronisch erhöhtem Alkoholkonsum zeigten eine erhöhte Fucosylierung der Glycanketten.

Für eine routinemässige diagnostische Anwendung der genannten Methoden erscheinen jedoch die Unterschiede zwischen Tumorpatienten, Alkoholikern und klinisch gesunden Personen nach unseren Befunden zu gering zu sein.

M2
Congenital Disorders of Protein N-Glycosylation: Characterization of Carbohydrate Side Chains by Mass Spectrometry

K. Gempel, S. Stadler, S. Hofmann, B.F. Pontz, K.-D. Gerbitz, M.F. Bauer.

Institut für Klinische Chemie, Molekulare Diagnostik und Mitochondriale Genetik, Akademisches Lehrkrankenhaus München-Schwabing, Kölner Platz 1, D-80804 München

The CDG syndromes (Congenital Disorders of Glycosylation) are inborn defects affecting the assembly and maturation of sugar side chains of glycoproteins. Protein glycosylation is a multi-step process involving a variety of sugar transferases which are located in the ER and Golgi apparatus and act on proteins *en route* the secretory pathway. Ten different enzymatic or transport defects of N-glycosylation have been described, resulting in structurally altered carbohydrate side chains. Here we describe a child with CDG syndrome type IIa, involving a defect of the UDP-GlcNAc:α6-D-mannoside β1→2-N-acetylglucosaminyl-transferase II located in the Golgi apparatus. The clinical phenotype includes psychomotor retardation, dysmorphic features and EEG abnormalities. The patient presented here is the

first with a compound heterozygous genotype (C578T mutation causing a premature stop; C1286 G exchange leading to Asp→His substitution). Ion exchange chromatography of serum transferrin revealed a characteristic pattern with disialotransferrin representing the predominant species while fully glycosylated tetrasialotransferrin was virtually absent. Electrospray ionization mass spectrometry of purified serum transferrin of the patient and of normal controls revealed a mass difference of 1431 Da. This difference corresponds to the proposed loss of the trisaccharide Sia-Gal-GlcNAc within each carbohydrate side chain of the transferrin. In conclusion, the analysis of serum transferrin by a combination of ion exchange chromatography and mass spectrometry has allowed to correctly assign the enzymatic defect and to elucidate the underlying gene mutations.

M3
Direct Injection and On-Line Solid-Phase Extraction of Biofluids for LC-MS/MS Analysis

C.T. Fleischer, M. Vogeser, K.-S. Boos, D. Seidel

Institute of Clinical Chemistry, University Hospital Großhadern, D-81366 Munich, Germany

In bioanalytical LC-MS the main drawback of many protocols either is a time-consuming and/or labour-intensive sample pretreatment step or it is an instable ionisation yield due to an inadequate clean-up procedure. The fact that no interfering sample components are visible in most MS/MS chromatograms does not mean that they are not present. Matrix components co-eluting with the analyte(s) of interest may disturb the ionisation process by suppressing or enhancing the efficacy of the ionisation of the analyte investigated. Thus, residual matrix components strongly diminish the accuracy of quantitation. In addition, the biological variability, i. e. the varying composition of the same biological matrix also effects the reproducibility of a LC-MS(MS) method. This holds especially for the abundant low MW components in a complex biofluid. Therefore, not only high MW components (e.g. proteins) should be removed prior to LC-MS/MS but also low MW compounds which potentially interfere with the ionisation process. Towards this we developed an on-line SPE platform based on automated column-switching and tailor-made SPE column packings. For size-selective fractionation of biofluids and simultaneous extraction of low MW compounds by reversed-phase (RP) partitioning (C-18, C-8, C-4) we investigated Restricted Access Materials (RAM) [1]. RAMs are characterized in that they have a defined size-selective exclusion barrier and a non-adsorptive outer particle surface towards macromolecular matrix components (e. g. proteins, nucleic acids, polysaccharides). Low-molecular compounds (e. g. drugs) have free access to the binding centers and thus can be extracted and enriched prior to their analytical separation and MS detection. A RAM-SPE column (e.g. LiChrospher®ADS, Merck KGaA, Germany) allows the direct, multiple injection and fractionation of crude, complex samples such as hemolysed blood, plasma, serum, milk, saliva, urine, fermentation broth and cell-culture or tissue homogenate supernatant.

Finally, we succeeded in speeding up the RAM fractionation step (e. g. extraction of 50 µl of human plasma in less than 60 seconds) by applying an optimised column size and flow-rate, respectively. Examples will be given for the fully automated analysis of immunosuppressiva and antibiotics.

In conclusion, tailor-made SPE-column packings and hardware allow a robust and rugged operation of on-line (high speed) SPE-(LC)-MS(MS).

[1] Boos, K.-S., Grimm, C.-H., TrAC 18 (1999) 175
[2] Boos, K.-S., Fleischer, C.T., Chimia 55 (2001) 42–45

Keywords: Sample Preparation, Solid-phase extraction (SPE), Mass spectrometry

M4
Analysis of Drugs in Urine by Stir Bar Sorptive Extraction (SBSE) and Thermal Desorption GC-MS

H.G. Wahl[1,2], C. Peterfi[2], A. Hoffmann[3] and H.M. Liebich[2]

[1] Institut für Laboratoriumsmedizin, Klinikum Fulda,
[2] Medizinische Universitätsklinik Abt. IV, Tübingen,
[3] Gerstel GmbH, Mülheim a. d. Ruhr, Germany

The techniques used for the analysis of drugs include solvent and solid phase extraction with or without derivatization prior to GC, GC-MS, HPLC or CE. Also, solid phase microextraction (SPME) can be used for direct GC-MS analysis of drugs in biological samples. Here we present a new extraction technique, the so called Stir Bar Sorptive Extraction (SBSE). A small stir bar coated with polydimethylsiloxane (Twister, 20 mm length, 1.3 mm i.d., Gerstel GmbH, Mülheim, Germany) is placed directly in the sample (5 ml urine or plasma) and stirred for 0.5 hours. During this time analytes are extracted into the PDMS phase, which acts as an immobilized liquid phase. The stir bar is then removed and placed into a thermal desorption unit. Heating of the stir bar releases the extracted compounds into a GC-MS system for subsequent analysis. The analytical system consists of a thermodesorption system, a temperature programmable vaporization inlet (TDS, CIS, Gerstel) and a GC-MS (GC 5890 series II, MSD 5971, Agilent Technologies, USA). So far good results were obtained for barbiturates (butalbital, amo-, pento- and secobarbital) and benzodiazepines (Nordazepam) in urine. Quantification of barbiturates was done in single ion monitoring mode (SIM, ions m/z 168 and m/z 156 for quantitation and ions m/z 124, 141 and m/z 167 as qualifier ions). Calibration curves (spiked urine samples) for butalbital, amo-, pento- and secobarbital in the concentration range from 1.0 to 120.0 ng/ml were linear with coefficients of correlation higher than 0.99. Limit of detection was 12ng/l with GC-MS in SIM mode. SBSE proved to be a powerful tool for qualitative and quantitative analysis of barbiturates in urine. With almost no sample preparation this is a very fast and yet highly sensitive (low ppt level) method.

Keywords: drugs, GC-MS, Stir Bar Sorptive Extraction (SBSE)

M5
Humanbiomonitoring: Bestimmung von Pentachlorphenol in Plasma und Urin mittels GC/MS

J. Hardt, W. Ehret

Institut für Laboratoriumsmedizin, Mikrobiologie und Umwelthygiene (ILMU), Klinikum Augsburg, Stenglinstr. 2, 86156 Augsburg.

Pentachlorphenol (PCP) ist cancerogen und wurde bis zum Verbot durch die Pentachlorphenol-Verbotsverordnung (1989) auch in Deutschland weitläufig als Holzschutzmittel eingesetzt. Die PCP-Belastung von Personen kann durch ein Humanbiomonitoring, also die Analyse des PCP im biologischen Material quantifiziert werden.

Wir verwenden für die Analytik des PCP ein von der Deutschen Forschungsgemeinschaft (DFG) geprüftes und publiziertes Verfahren mit $^{13}C_6$-PCP als internem Standard (DFG 1999). Plasmaproben werden nach Proteinfällung und Urinproben nach Säurehydrolyse mit Hexan extrahiert. PCP wird anschließend in Kaliumcarbonatlösung zurück extrahiert und mit Essigsäureanhydrid derivatisiert. Die Ester werden in Hexan ausgeschüttelt und mittels Kapillargaschromatographie/Massenspektrometrie quantifiziert (Bestimmungsgrenzen: 0,5 µg/l Urin, 1 µg/l Plasma).

Mit diesem Verfahren untersuchten wir (von Mai 2000 bis Mai 2002) Plasmaproben von 224 Patienten (Alter: 2 bis 81 Jahre) der Umweltambulanz am Klinikum Augsburg. Außerdem wurden Urinproben von 124 dieser Patienten analysiert. Das QM-System des ILMU ist nach DIN EN ISO 9001 zertifiziert.

Die Massenspektrometrie ermöglicht den Einsatz des isotopenmarkierten internen Standards und damit sehr gute Zuverlässigkeitsdaten des Verfahrens (z. B. Präzision von Tag zu Tag: unter 4 % bei 10 µg/l Plasma). Im Median wurden im Plasma der Patienten 1,4 µg/l PCP gemessen und im Urin 0,6 µg/g Kreatinin. Lediglich drei Patienten wiesen eine PCP-Konzentration oberhalb des Referenzwertes von 12 µg/l Plasma auf.

Literatur: DFG, Analyses of Hazardous Substances in Biological Materials, Volume 6, Wiley-VCH 1999.

Schlüsselwörter: Pentachlorphenol, Gaschromatographie/Massenspektrometrie

M6
Determination of Free Serum Cortisol by the Use of Liquid-Chromatography Tandem Mass-Spectrometry after Equilibrium Dialysis (ED-LC-TMS)

M. Vogeser

Institut für Klinische Chemie, Klinkum der Universität München
Direktor: Prof. Dr. med. Dr. h. c. Dietrich Seidel

Background: Protein binding of cortisol is highly variable, especially in severely ill patients with typically low concentratins of the corticosteroid binding globulin. Therefore, direct measurement of free serum cortisol is physiologically more appropriate than measurement of total cortisol. We aimed to develope a highly specific method for the determination of bioactive cortisol.
Methods: Equilibrium dialysis of serum samples was performed by use of self assembled cells based on 2 mL reaction vials; 1 mL of serum was separated from 200 µL of dialysis buffer in the lid of the vial by a dialysis membrane. After overnight incubation at 37 °C the dialysate was recovered and precipitated with methanol/zinc sulfate containing tri-deuterated cortisol as the internal standard. The supernatant was then submitted to automated solid phase extration and analytical chromatography by use of column-switching with on-line transfer to a triple stage MS-system applying electrospray ionization in the positive mode. The following transitions were monitored: cortisol, 363 > 309; d3-cortisol, 366 > 312. Sera that were sampled in the perioperative period of 12 patients undergoing cardiac surgery were investigated with this method.
Results: The method proved convenient, rugged and precise with an total coefficient of variation of 8.1 %. In cardiosurgical patients preoperatively 5.7 % of total cortisol was found as free cortisol, in contrast to 21.2 % on the first postoperative day.
Conclusions: Our newly developed ED-LC-TMS method for the quantification of free serum cortisol is efficient and may serve as a reference method for the development of immunoassays for routine measurement of free serum cortisol. Application of the method to samples of intensive care patients demonstrated the highly variable protein binding of cortisol.

M7
Simultaneous Quantification of Psychotherapeutic Drugs in Human Plasma by Tandem Mass Spectrometry.

M. Wood and M. Morris

Clinical Applications Group, Micromass UK Ltd, Manchester M23 9LZ, UK.

Recent figures indicate that approximately a quarter of the world's population will suffer from a diagnosable mental disorder at some point in their lives. Depression, schizophrenia, anxiety disorders and epilepsy are amongst the most common conditions in developed countries.

Treatment usually comprises psychotherapy and psychotherapeutic medication. Therapeutic drug monitoring of the latter is necessary to evaluate compliance, potential for toxicity, to verify therapeutic concentrations and to establish individual target concentrations in patients who are responding well to therapy. We have developed a simple and rapid LC-MS/MS method that allows the simultaneous quantification of several psychotherapeutic agents in plasma.

Calibrators were prepared by adding various psychotherapeutic drugs including; tricyclic antidepessants (TCA's), selective serotonin reuptake inhibitors (SSRI's) and antipsychotics to blank plasma. For all of the analytes, responses were linear ($R^2 > 0.99$) over the range investigated (0.1–500 µg/L). Limits of detection of 1 µg/L (or better) were obtained, which is in accordance with the required sensitivity for the monitoring of these particular compounds.

The developed method was successfully used to analyse plasma samples that had been collected from patients currently receiving various psychotherapeutic drugs. These results are presented in addition to the accuracy and precision of the developed method. The technique has also been applied to the analysis of whole blood samples.

M8
Simultaneous Measurement of Cyclosporin A and Creatinine Concentrations in Low-Volume Capillary Blood (Finger-Prick) Samples Using Liquid Chromatography Electrospray-Ionisation Tandem Mass Spectrometry

D. Cooper[1], B. Keevil[2], D. Tierney[2], A. Machall[3], N. Yonan[3] and M. Morris[1]

[1] Clinical Applications Group, Micromass UK Limited, Manchester, M23 9LZ, UK.
[2] Department of Clinical Biochemistry and
[3] Transplant Unit, Wythenshawe Hospital, Manchester, UK.

Routine monitoring for the immunosuppressive drug cyclosporin A (CsA) is required to maintain an effective, sub-toxic drug concentration. Current practice is to measure trough concentrations of CsA but several studies have demonstrated that the CsA concentration 2 hours post-dose (C2) correlates well with full AUC measurements and provides a better indicator of drug exposure. The time of sampling (2hr ± 10 min) is critical and this may present difficulties in a busy clinic. C2 values for CsA will be well above the range of most currently available CsA assays thus presenting difficulties in the laboratory.

The use of capillary blood (finger-prick) samples for CsA monitoring will simplify sample collection and will allow unskilled staff or the patients themselves to obtain accurately timed samples with

minimum resources. However, it is usual to measure serum creatinine at the same time to monitor the potential nephrotoxic effects of CsA. To resolve these difficulties we have developed and validated a simple and rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of CsA and creatinine using capillary blood.

M9
Rapid Identification of Bacteria by Intact Cell Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry

D. Dare, H. Sutton, C. Keys[1], H. Shah[1], T. McKenna[2] L. Pollack[2]

Manchester Metropolitan University, Manchester, UK.
[1] Public Health Laboratory, London, UK.
[2] Micromass UK Ltd, Manchester, UK.

Preliminary work has demonstrated that MALDI-TOF-MS spectra from intact cells of a range of different bacteria produced by different operators at three different laboratories are highly reproducible, provided appropriate protocols are followed. Since these spectra are representative of surface components present on the bacterial cell, they can be used to generate a unique pattern of peaks for each bacterium. This has enabled a database to be generated, which can be used for the rapid identification of these bacteria. The database consists of 703 entries, containing a variety of species of 76 different genera. The bacteria within the database are all strains of known provenance provided by the UK National Collection of Type Cultures.

In order to validate the database, the Medical Identification Service Unit of the Central Public Health Laboratory Service, together with the Manchester Metropolitan University, produced parallel sets of data. One set was used to produce the database and the other set to challenge the database entries. The results of these with respect to the genera *Aeromonas, Bacillus, Burkholderia, Cedecea, Corynebacterium, Moraxella, Salmonella, Staphylococcus, Yersinia* are presented, a total of 65 strains. Accurate identification was obtained for genus (n = 1); species (n = 10); subspecies (n = 12); and strain (n = 36). These results demonstrate the enormous potential of this technique for the rapid identification of bacteria.

Molekulare Diagnostik

MD1
Isolation of Bacterial DNA from Clinical Samples

C.H. Stülten, M. Orendi, M. Susa, C. Knabb

Robert-Bosch-Krankenhaus, Auerbachstr. 110, 70376 Stuttgart

Rapid diagnosis and specific treatment is essential for critically ill patients suffering from bacterial infections. The main goal of our work is to expedite diagnosis by applying multiparametric molecular assays. As a first step we developed a method to isolate bacterial DNA from body fluids as well as swabs. Pure cultures of Staphylococcus aureus and Streptococcus pneumoniae were isolated from clinical specimen. A dilution series was set up by defined amounts of bacteria and sterile body fluids. Bacteria were then lysed with lysozyme and proteinase K. DNA was isolated using the Qiagen Blood Mini Kit. Eluats were tested for bacterial DNA by real-time PCR using a light cycler instrument (Staph. aureus, femA se: 56'-AAG CGC ATA ACA AGC GAG AT 3'; femA as: 5'-TGC ATA ACT TCC GGC AAA AT-3'; 199-bp fragment) and 16SrRNA (Strep. pneumoniae, Strep F: 5'-TCA CCA Agg CgA CgA TAC AT-3'; Strep A 5'-ACA CAC gTT CTT CTC TTA CAA Cag A-3'; 191-bp fragment). Detection limits were 10 fg (i.e. 1 CFU) and 100 fg DNA (i.e. ~ 6 CFU) for Staph. aureus and Strep. pneumoniae, respectively. Bacterial DNA was isolated and identified within 3h from body fluids as well as swabs. As few as 10 CFU of Staph. aureus/ml in serum, plasma, blood, urine, swabs and 100 CFU/ml in blood cultures and pleural effusions were detected. For Strep. pneumoniae 100 CFU/ml were detectable in serum, plasma, blood, urine and pleural effusions.

We developed a sensitive method for isolation of bacterial DNA from various body fluids and swabs. Thus, our method is suitable for isolation and detection of bacteria from various specimen, particularly from blood cultures, and may be applied to enhance diagnosis of infectious diseases.

Keywords: bacteria, molecular diagnostic

MD2
Molecular Diagnostic of Pseudoxanthoma Elasticum (PXE): A Real-Time PCR Method for the Detection of PXE-Causing Mutations

V. Schulz, A. Grundt, C. Szliska*, T. Brinkmann, C. Götting, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, and *Dermatologische Klinik, Krankenhaus Bethesda, Freudenberg, Germany

Pseudoxanthoma elasticum (PXE) is a heritable disorder of the connective tissue characterized by the calcification of elastic fibers of the skin, eyes and cardiovascular system. Recently, PXE-causing *ABCC6* gene polymorphisms were found. The most prevalent one in european PXE patients is the 3421C→T (R1141X) mutation resulting in a truncated protein.

For the detection of this mutation we now have developed a real-time PCR method. Primers and allele-specific hybridization probes were designed for the amplification of exon 24 of the *ABCC6* gene. The genotype discrimination was performed by hybridization melting curve analysis and FRET detection. All results were verified by DNA sequencing and RFLP analysis. Using this method we have analyzed DNA samples from 54 German PXE patients for the occurrence of the 3421C→T mutation. 4 of these patients (7.4 %) were found to be homozygous and 21 (38.9 %) to be heterozygous carriers of the trait. 24 unaffected or not yet affected relatives were also analysed. The genotype frequencies were 62.5, 37.5 and 0 for wildtype, heterozygote and homozygote. Blood donors (n = 50) used as control group were all negative for the 3421C → T mutation. DNA samples from patients with thrombotic (n = 60) and cardiological diseases (n = 56) were also investigated and were found to be wildtype genotype.

In conclusion, we have developed a rapid and reliable method for the detection of the 3421C → T mutation in the *ABCC6* gene of PXE patients. As myocardial infarcts occur in PXE patients at early age this method can be easily employed for screening of young patients with myocardial infarcts of unknown origin.

Keywords: Pseudoxanthoma elasticum, *ABCC6* gene, real-time PCR

MD3
Identification of New Pseudoxanthoma Elasticum (PXE) Associated Mutations in the Human *ABCC6* Gene Using DHPLC Analysis

V. Schulz, A. Grundt, C. Szliska*, T. Brinkmann, C. Götting, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, and *Dermatologische Klinik, Krankenhaus Bethesda, Freudenberg, Germany

Pseudoxanthoma elasticum (PXE) is a heritable disorder of the connective tissue affecting the extracellular matrix of the skin, eyes and cardiovascular system. Recently, the PXE candidate gene *ABCC6* was identified and PXE-associated polymorphisms in the *ABCC6* gene were detected.

In order to identify new mutations in the *ABCC6* gene we have analysed DNA samples from 54 German PXE patients and 24 unaffected or not yet affected family members, which is the largest collection of PXE patients and relatives available in Germany. The exons 15, 24 and 26–29 of the *ABCC6* gene were amplified by PCR and the DNA fragments were subjected to DHPLC analysis and DNA sequencing. Finally the results were verified by RFLP analysis. Using this approach we have identified 5 new PXE-associated mutations. 2 novel polymorphisms G3341A, Arg1114His and C3389T, Thr1130Met were detected in exon 24 of 2 patients suffering from PXE. A T3715C mutation (Tyr1239His) was identified in exon 26 of a PXE patient and a G3932A mutation (Gly1311Glu) was found in exon 28 of another affected person. In exon 29 a novel deletion (4182delG) resulting in a truncated protein was detected. This mutation was observed in 3 PXE patients in homozygous and heterozygous state. Blood donors investigated by RFLP analysis and used as control group were all negative for the mentioned mutations.

Our results demonstrate that DHPLC analysis is a well-suited method for the detection of new PXE-associated mutations in the human *ABCC6* gene and that different *ABCC6* gene polymorphisms are PXE-causing.

Keywords: Pseudoxanthoma elasticum, *ABCC6* gene polymorphism, DHPLC analysis

MD4
Detection of Low Copy Viremia of Hepatitis B Virus by Individual NAT in an anti-HBC and anti-HBS Positive Blood Donor

J. Dreier, M. Kröger, J. Diekmann, K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen

The HBV testing policy of blood donors in some European countries, e.g. Germany, is focussed on HBsAg, however anti-HBc for routine screening is not prescribed. HBV infected individuals are authorised for blood donation five years after complete healing, if they are tested HBsAg negative, anti-HBs positive (titer > 100 IU per liter), HBV-DNA negative in a sensitive NAT, and with normal alanine aminotransferase (ALT) level.

Here we describe the details of a low copy HBV viremia that we investigated retrospectively. A clinically inconspicuous 35-year-old female blood donor with anti-HBc antibodies seroconverted to anti-HBs positive (titer > 10.000 IU per liter) after vaccination. Blood donations were repeatedly tested HBV-DNA negative by minipool NAT. We have now re-evaluated all donor samples stored for follow up examinations with a more sensitive individual-donation NAT. HBV viremia was detected in several samples with a concentration ranging from < 10 to 260 IU per ml. Actually, the viremia was confirmed with 1.346 IU per ml. Sequencing of PCR amplification products from the S region of the HBV genome preclude false positive results from contaminating PCR products.

The sequence analysis of the HBsAg "a" determinant characterized the HBV isolate as genotype A, subtype "ayw" without vaccine escape mutations.

In the look-back procedure no post-transfusion hepatitis B was detected in patients received whole units of blood or plasma from these PCR-positive, HBsAg-negative, and anti-HBc-positive donations.

In conclusion, low copy HBV viremia in anti-HBc positive blood donations is not detectable by pool HBV NAT and was only identified by enhanced-sensitivity individual PCR assays. Our results show that due to periodic HBV aviremia or low copy virus replication, additional routine testing for anti-HBc will eliminate the risk of HBV transmission by HBsAg negative blood.

Keywords: isolated anti-HBc, blood screening, HBV PCR

MD5
High Sensitivity Detection of Tumor Cells in Peripheral Blood of Colorectal Cancer Patients by a Reverse Transcription PCR Method

A. Krehan, M. Stefan, O. Böcher, P. Steffens and S. Waschütza

AdnaGen AG, Ostpassage 7, D-30853 Hannover-Langenhagen, Germany

Despite advances in diagnostic approaches, 30–40 % of patients with colorectal cancer develop local or distant tumor relapse during follow-up. Therefore, early detection of tumor cells circulating in peripheral blood is necessary for identification of micrometastases.

Most techniques based on detection of expression markers by RT-PCR are very sensitive but do not allow sufficient standardization[1]. One problem of these techniques is the insufficient specificity due to pseudogenes[2] and illegitimate transcription[3] of markers in non malignant blood cells.

We established an RT-PCR assay with increased specificity for detecting tumor cells in peripheral blood of colorectal patients. To achieve this we combined the selection of tumor cells with an antibody mix with RT-PCR techniques for the detection of mRNAs encoding for tumor specific markers.

Moreover, we were able to overcome the critical aspect of degradation of RNA during storage and transport of samples. Due to the instability of cellular RNA *in vitro* preserving the RNA expression profile is essential for reliable analysis of gene expression.

We developed a reagent, which stabilized the expression profile of tumor markers for up 2 days at temperatures ranging from 5 °C to 45 °C.

Preliminary results of a pilot study showed occurence of tumor cells in blood of carcinoma patients despite resection of primary tumors.

This innovative method is an option for clinicians as a predictive tool with respect to metastases and may result in an appropriate selection of patients for adjuvant therapy.

[1] Jung, R., Soondrum, K., Krüger, W. Neumeier, M. (2001) Recent Results Cancer Res 158:32–39
[2] Ruud, Pl., Fostad, O., Hoving, E., (1999) Int J Cancer 80(1): 119–25
[3] Chelly, J., Concordet, J.P., Kaplan, J.P., Kahn, A. (1989) Proc Natl Acad Sci USA 86(8):2617–21

Keywords: RT-PCR, disseminated tumor cell detection, colon cancer

MD6
A Simple Protocol for Obtaining LOH Information for Routine Tumour Diagnosis

[1,2]C. Beetz, [1,2]S. Horn, [2]A. Hartmann, [1]T. Deufel, [2]S. Patt, [1]M. Kiehntopf

[1] Institut für Klinische Chemie und Laboratoriumsdiagnostik,
[2] Institut für Pathologie, Universitätsklinikum Jena, Bachstraße 18, 07740 Jena

The diagnosis of tumour specimens is still based largely on aberrant histology and immuno-histochemistry. Further aberrations include loss and gain of genomic material. Some of these imbalances are characteristic for different tumour entities. A combined occurrence has in some cases been correlated with prognosis and/or response to therapy, by this providing a basis for further sub-typing. Genome wide screening methods used for obtaining the original results (e. g. CGH) are costly and time intensive. PCR-based microsatellite analysis, on the other hand, is a simple procedure to detect loss of predefined regions.

Using gliomas, we developed a protocol by which loss of heterozygosity (LOH) can be rapidly assigned to a clinical sample. It combines Chelex-based extraction of DNA from blood and from microdissected quick sections with fluorescence-based detection of PCR products. This protocol is of possible relevance to routine molecular diagnosis of tumours in several aspects: a) material for molecular analysis does not need to be collected and fixed separately, b) rapid DNA extraction is feasible and prevents degradation, c) tumour substrate is the same as in the first quick section analysis, and d) results may be obtained already intra-operatively. In addition, once extracted, high quality DNA sufficient for more than 200 PCR reactions can be stored for later use in more detailed analyses. We also present a basic set of microsatellite markers that were chosen to (a) be unambiguously interpretable, (b) show optimum informativity, (c) be apt for multiplexing, and (d) represent many genomic regions in order to detect at least some cases of LOH (control for purity of tumour DNA). This basic set can be extended to include specifically the regions of interest for the tumour entity to be investigated.

keywords: LOH, tumour, routine diagnosis

MD7
Expression der Telomerase in Plasmazellen: Gibt es einen Unterschied zwischen MGUS und Plasmozytom?

M. Schenkel, M. Scholz, D. Zech und K. Wielckens

Institut für Klinische Chemie der Universität zu Köln

Monoklonale Gammopathien werden bei bis zu 2 % der Patienten nachgewiesen. In den meisten Fällen handelt es sich dabei die sog. monoklonale Gammopathie unbestimmter Signifikanz (MGUS), die meistens „benigne“ verläuft, aber auch in ein *Plasmozytom* übergehen kann. Die Abgrenzung zwischen MGUS und Plasmozytom ist mitunter problematisch, weil bisher eindeutige Laborparameter fehlen. Patienten mit *MGUS* müssen ein Leben lang überwacht werden, um einen möglichen Übergang zu einem Plasmozytom frühzeitig zu erkennen. Auch für die Überwachung der MGUS-Patienten wäre eine Methode sehr hilfreich, die frühzeitig die den Übergang zum Plasmozytom anzeigt. Ein in malignen Zellen häufig exprimiertes Enzym ist die *Telomerase*. Die bisherigen Befunde bzgl. der Telomerase-Aktivität maligner Plasmazellen sind allerdings uneinheitlich. Doch gibt es Hinweise, dass maligne Plasmazellen eine erhöhte Telomerase-Aktivität aufweisen. Um zu testen, ob die Telomerase-Konzentration von Plasmazellen als Marker für Malignität und zur Abgrenzung von MGUS und Plasmozytom einsetzbar ist, wurde eine Methode entwickelt, bei der im ersten Schritt Plasmazellen durch Bindung an *CD-138*-Antikörper – gekoppelt an magnetische Mikrobeads – angereichert werden. Nach Extraktion der Gesamt-RNA wurde die Telomerase mRNA am Lightcycler (Roche) nachgewiesen. Nach der Optimierung gelang es mit diesem Verfahren auch extrem geringe Anzahl von malignen Plasmazellen zu isolieren und die Telomerase-mRNA in diesen Zellen nachzuweisen. Die Validierung der Methodik an normalen Probanden ergab, dass in keinem Fall Telomerase-mRNA nachweisbar war. Demgegenüber zeigten Patienten mit Plasmozytom in der Regel einen positiven Befund. Erste Untersuchungen bei Patienten mit MGUS lassen hoffen, dass das hier beschriebene Verfahren die Unterscheidung von MGUS und Plasmozytom erleichtern könnte.

MD8
Molecular Diagnosis in Acute Hepatic Porphyrias

U. Groß[1], H. Puy[2], R. Gerlach[1], J. Lamoril[2], J.C. Deybach[2] and M.O. Doss[3]

[1] Klinik und Poliklinik für Neurochirurgie, Universitätsklinikum Frankfurt,
[2] Centre Français des Porphyries, Hôpital Louis Mourier, Colombes,
[3] Abteilung für Klinische Biochemie, Marburg.

Introduction: The mutated genes of porphyria patients can be detected with the help of molecular investigations.
Methods: Enzymeactivities were measured in blood cells. Molecular diagnosis was established by DGGE and direct sequencing. Results: The diagnosis of acute hepatic porphyrias was established by the enhanced excretion of heme precursors. Five different mutations in five patients (3 males, 2 females; age: 23–44 y) with acute intermittent porphyria (AIP) and lowered porphobilinogen deaminase activity [48 ± 3 % (n = 5); normal: 57 ± 11 pkat·g^{-1} (n = 274); $\bar{x} \pm$ SD] were found. Four of these were new mutations. Three are single base substitutions [(R16C), exon 3; (V202L), exon 10; (T → A, IVS13 + 2), intron 13]. Two are frameshifts (del^{215}GA, exon 6; ins^{886}A, exon 14) which produce a stop codon. Two new mutations in the coproporphyrinogen oxidase (CoproOx) gene of two patients (females; age: 27 and 30 y) with hereditary coproporphyria (HCP) were found:

$C^{854} \rightarrow T$, exon 4; $ins^{857}A$ in the same exon. Both women had a decreased CoproOx activity [65 and 49 %; normal: 138 ± 21 pkat·g^{-1} ($n = 50$); $\bar{x} \pm SD$]. In each patient as well as in 6 subclinical family members DGGE analysis showed a mutated heterocygotic pattern. Conclusion: Our results confirm the allelic heterogeneity of mutations in patients with AIP and HCP. A molecular diagnosis may be a helpful tool, if the excretory parameters are in a limiting value in subclinical relatives. [This study was supported by the German Research Association (grant GR 1363/2–2)]
Keywords: porphyria, mutations, diagnosis.

MD9
Intestinal HCO_3^- Secretion: Interaction of the CFTR Binding Adapter Protein CAP70 with the Cl^-/HCO_3^- Exchanger DRA and Possible Consequences of this Interaction for Cystic Fibrosis

H. Rossmann[1], G. Lamprecht[2], K. Yahya[1], P. Jacob[2], C. Neff[2], S. Baisch[2], J. Biber[3], U. Seidler[2], and W. Fiehn[1]

[1] Zentrallabor, Universität Heidelberg;
[2] Abt. Innere Med. I, Universität Tübingen;
[3] Physiologisches Institut, Universität Zürich-Irchel

CAP70 has been shown to be an adapter protein coupling characteristic C-terminal sequences of transport and channel proteins, e.g. CFTR (cystic fibrosis transmembrane regulator). CFTR and the Cl^-/HCO_3^- exchanger DRA (down regulated in adenonma) are thought to play a major role in small intestinal HCO_3^- secretion. We hypothesized that CAP70 mediates the formation of macromolecular complexes in the brush border membrane (BBM) of enterocytes, containing one or more CFTR and DRA molecule(s).

DRA was localized to the BBM of dueodenum, ileum, and colon by immunoblot using membrane preparations from rabbit mucosae. Preincubation of the anti-DRA antibody with the recombinant DRA protein, blocked the DRA signal. Simultaneously a 70 kD band appeared in duodenum and ileum, which was restricted to the BBM and was missing in the colon. Deletion of the C-terminal 4 amino acids of the recombinant DRA protein and repetition of the described overlay experiment resulted in the disappearance of the 70 kD band suggesting a specific protein-protein interaction between DRA and a DRA binding protein. Incubation of a parallel blot with an anti-CAP70 antibody revealed a band, which displayed the size and the expression pattern expected for the DRA binding protein. In contrast, neither CAP70 mRNA and protein expression nor the described overlay band was observed in the colon. This data suggests that CAP70 represents the observed DRA binding protein. In vitro protein-protein interaction studies (far Western, interaction ELISA) using recombinant protein of CAP70 and each of its four PDZ domains revealed specific coupling of the DRA C-terminus to the 2. PDZ domain, while CFTR is known to interact with PDZ 1, 3, and 4.

CAP70 and CFTR both interact with CAP70 in the BBM of small intestinal mucosa. While CFTR binds to PDZ 1, 3, and 4 of CAP70, DRA interacts with the 2. PDZ domain. Thus CAP70 is able to mediate the assembly of macromolecular complexes, which may result in coordinated activity or facilitate regulatory processes. This data may contribute to elucidate the pathomechanism of the GI manifestations of cystic fibrosis.

Keywords: Cystic fibrosis, molecular pathogenesis, HCO_3^- secretion

MD10
FcγRIIa (CD32) His-Arg-Polymorphism Does Not Affect CRP Concentration

R. Conradi, K.J. Lackner

Institut für Klinische Chemie und Laboratoriumsmedizin, Universitätsklinikum Mainz

FcγRIIa (CD32) has been described as a high affinity receptor for CRP on human monocytes and neutrophils. A common G/A polymorphism which results in arginine (R) to histidine (H) exchange at amino acid position 131 in the extracellular domain of FcγRIIa, affects binding of CRP to CD32. The R131-allele has higher affinity to CRP than H131.

In this study we analyzed the effect of the FcγRIIa-131-polymorphism on the CRP-blood concentration.

Methods: CRP concentrations and FcγRIIa genotypes were determined in 233 patients and 142 presumably healthy volunteers. Genotyping was performed using the FRET technology on the LightCycler™ (Roche).

Results: The mean CRP blood concentration was 7,37 (0,21–129) mg/l in patients and 2,84 (0,13–53,06) mg/l in the control group. In the patient group the genotype was in 32 % HH (control group: 25 %), in 25 % (20 %) RR and in 43 % (55 %) HR, respectively.

Comparing the genotypes of patients with blood concentration of CRP, the HH genotypes had the highest CRP concentrations: The means (and medias) were HH: 10,59 (3,62), HR: 5,16 (2,93), RR: 6,96 (2,79) mg/l. The differences were statistically not significant. If the groups were divided into quartiles according to CRP levels, again no consistent differences in the frequency of genotypes were observed.

Conclusion: We found no significant difference of genotypes for CD32 H131R-polymorphism between patients and controls. Within the groups there was no consistent association of the H131R-polymorphism with CRP serum concentration. Even though the polymorphism affects CRP binding to CD32, it has no effect on CRP serum levels.

MD11
The Intron 6 G/T Polymorphism of c-myb and the Risk for In-Stent Restenosis

M. Praus[1], M. Gross[2], J. Waigand[2], J. Krämer[2], E. Köttgen[1], R. Geßner[1]

[1] Institut für Laboratoriumsmedizin und Pathobiochemie, Charité Campus Virchow-Klinikum, Humboldt-Universität zu Berlin, Augustenburger Platz 1, D-13353 Berlin and
[2] Franz-Volhard-Klink, Charité Campus Berlin-Buch, Humboldt-Universität zu Berlin, Wiltbergstraße 50, D-13122 Berlin

The principle pathogenic mechanisms leading to the development of atherosclerosis are long-term accumulation of lipids and cell proliferation.Restenosis after stent deployment is driven by short-term smooth muscle cell (SMC) proliferation. The proto-oncogene c-myb encodes a transcription factor involved in regulation of the cell cycle and lipid accumulation in vivo.

485 patients undergoing coronary stenting and angiographic follow-up after 6 months were analyzed with quantitative coronary angiography and genotyped for a G/T-single nucleotide polymorphism in intron 6 of the c-myb gene. To study the functional role of c-myb *in vitro* and *in vivo* we used a recombinant adenovirus to overexpress a dominant negative mutant of c-myb in cultured human aortic smooth muscle cells and in rat carotid arteries.

Competitive inhibition of c-myb by adenoviral mediated gene transfer of a mutant-myb protein reduced smooth muscle cell proliferation in vitro by 26 % and neointima formation in rat carotid arteries by 74 % compared to control virus. In our patient cohort restenosis > 50 % occurred in 27.6 % of those with at least one G-allele and in 20.8 % of those without (p = 0.10). After adjustment for the independent risk factors diabetes mellitus, reference lumen diameter, smoking, dyslipidemia and number of diseased vessels, the results showed a trend towards statistical significance (p = 0.08). Our results indicate that c-myb plays a role in smooth muscle cell proliferation and that the G/T-nucleotide transversion polymorphism in intron 6 of the c-myb oncogene might be an independent risk factor for human in-stent restenosis.

MD12
Mutations in the TAFI Gene Are Not Associated with Higher Risk of Venous Thrombosis

[1]Kostka H, [2]Schellong S, [3]Kuhlisch E, [1]Siegert G

[1] Institut für Klinische Chemie und Laboratoriumsmedizin und
[2] Medizinische Klinik und Poliklinik III des Universitätsklinikums an der TU Dresden
[3] Institut für Medizinische Informatik und Biometrie der Medizinischen Fakultät der TU Dresden

Objectives: The TAFI (thrombin-activatable fibrinolysis inhibitor) level is genetically determined. The LETS study shows that elevated TAFI levels form a mild risk factor of venous thrombosis. The aim of this study was to assess the risk of deep venous thrombosis (DVT) of polymorphisms in the TAFI gene.
Methods: This study includes 130 patients with DVT and 130 age- and sex-matched healthy controls. The TAFI level was measured in controls by a ELISA from Affinity Biologicals Inc. (Ontario, Canada). The TAFI mutations 505G > A (Ala147Thr) and + 1542C > G were determined by RFLP.
Statistics: Fisher's exact test was used to compare frequencies for genotype combination TAFI 505/+1542 between patients with DVT and controls. Odds ratios for association of DVT with TAFI genotypes were estimated by logistic regression models.
Results: The mean TAFI levels between men and women were not different (48.6 % vs. 52.9 %, n. s.). In the controls the TAFI levels between the various TAFI genotypes were signifikant different (p < 0.0005). The highest TAFI levels were found in 505AA/ + 1542CC carriers (73.5 %) and the lowest in 505GG/+ 1542GG carriers (10.8 %). In relation to the genotype 505GG/+ 1542GG all other determined TAFI genotypes were not associated with a higher thrombotic risk.
Conclusion: The mutations 505G > A and + 1542C > G in the TAFI gene had no influence on the thrombotic risk in this study.
Keywords: Thrombosis, TAFI genotypes

MD13
Real Time PCR Analysis of the VDR *Bsm* I Polymorphism

K. Schlatterer [1], O. Landt [2], F.H. Perschel [1], R. Fitzner [1], R. Tauber [1]

[1] Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin
[2] TIB MOLBIOL, Tempelhofer Weg 11–12, 10829 Berlin

Introduction: The vitamin D receptor (VDR) is essentially involved in cell growth and differentiation of different target tissues mediated by 1,25-$(OH)_2$ D3. Approaches to delelop genetic markers for the assessment of fracture risk and to identify target molecules for the prevention of bone disease, are promising. Several VDR alleles with single nucleotide polymorphisms (SNPs) are known, and are controversially discussed regarding their relevance as for the heritable components of bone density. *Bsm*I, *Apa*I and *Taq*I polymorphisms between exons 8 and 9 of the VDR gene 3' region are associated with circulating levels of the osteoblast-specific protein osteocalcin. For the VDR *Bsm*I polymorphism (45 082 GAATGC → T) the BB genotype is described to be associated with low bone mineral density (BMD). Whereas for genotypization classical RFLP-analysis is time-consuming, real-time PCR provides a rapid test alternative.
Methods: Genomic DNA was isolated from leukocyte nuclei by the QIAamp Blood Kit (Qiagen). LightCycler® real-time PCR was performed in capillaries.

Primers (0.3 µM): sense VDR_b F TAGGGGGGATTCTGAGGAACTA, antisense VDR bsm A AGTTTTGTACCCTGCCCGC.

Probes (0.6 µM): sensor b AGTATTGGGAATGCGCAGGCC-F, anchor LC Red640-TCTGTGGCCCCAGGAACCCTG-P, 1 µl buffer (10 x), 1.25 µl $MgCl_2$ (25 mM), 10 mM dNTP, 1.9 µl H_2O, 0.2 µl BSA (240 ng/l), 1.25 U Taq, 1µl DNA.

PCR: initial denaturation at 97 °C, 2min; 40 cycles denaturation (97 °C, 5 s, 20 °C/s), annealing (62 °C for 5 s, 20 °C/s), extension (72 °C, 10 s, 20 °C/s). Melting curve analysis: 1 cycle of 95 °C for 2s (20 °C/s) and 52 °C for 1s (20 °C/s), followed by ramping to 80 °C at 0.2 °C/s, continuously monitoring the fluorescence signal.
Results and conclusion: By use of this assay the VDR *Bsm*I polymorphism can be detected by a rapid one step analysis with a high separation of the bb (m. p. 61.2 °C), the BB (m. p. 68.1 °C) and the heterozygous genotype. This method offers interesting time- and cost-saving advantages for a diagnostic strategy in bone disease patients.

MD14
Identification of Two Patients with Unusual LightCycler Melting Curves in Multiplex Genotyping of the Hemochromatosis H63D/ C282Y Gene Mutations

R. Weiskirchen, C.G. Tag, and A.M. Gressner

Institut für Klinische Chemie und Pathobiochemie – Zentrallaboratorium, RWTH-Universitätsklinikum, Pauwelsstr. 30, D-52074 Aachen

The molecular diagnosis of *HFE* mutations can be performed by seveal techniques. Most of these tests are based on restriction fragment length polymorphism (RFLP) analysis, allele-specific amplification strategies, heteroduplex technologies, single strand confirmation polymorphisms (SSCP) analysis, radial capillary array electrophoresis or most recently on mircrovolume fluorimeters with rapid temperature control. The LightCycler™ (Roche Diagnostics) is a combined

microliter volume thermal cycler with an integrated fluorimeter, which offers a high-throughput, semi-automatic method allowing fast genotyping of the known *HFE* mutation sites. In this method, a short fragment harboring the particular polymorphic site is amplified by a polymerase chain reaction (PCR) and the genotypes are determined by the fluorescence resonance energy transfer (FRET) principle. Following amplification, a 3'-fluorescein-labeled probe and a 5'-LightCycler™ Red 640-labeled probe are hybridized to the same strand internal to the unlabeled PCR-primers and the emitted FRET-signals are monitored as the temperature increases. The obtained melting point (T_m) of the duplex depends on the presence or absence of mismatches. Accordingly, when mismatches are present, the observed T_m of the hybrid is lower. A quantitative LightCycler™ analyzing software subsequently transfers the FRET signals to specific melting curve graphs. Because of its simplicity the method has become an important tool in routine diagnosis of the *HFE* mutations, which are associated with hereditary hemochromatosis. We used the LightCycler™ technology for simultaneous detection of the H63D and C282Y mutations of the *HFE* gene in patients with a higher prevalence for hemochromatosis. In our cohort we identified two siblings with a variant pattern of the *HFE*-LightCycler™ melting profiles preventing allelic discrimination. We demonstrate that in these patients DNA sequencing or RFLP analysis is necessary to unequivocally assign the correct *HFE* genotype.

Keywords: HFE, Liver

Methodenevaluation/ Qualitätskontrolle

Q1
Möglichkeiten und Grenzen der Qualitätskontrolle bei der Bestimmung des 17 α-OH-Progesterons.

U. Meier, C. Schnabel C, A.M. Gressner und D. Kunz

Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum, RWTH, Pauwelsstr. 30, 52074 Aachen

Das 17α-OH-Progesteron (17OHPR) ist ein labormedizinischer Leitparameter bei der Diagnostik und Verlaufskontrolle des Androgenitalen Syndroms (AGS). Bei der Interpretation der Werte ist zu beachten, dass neben der Alters- und Geschlechtsabhängigkeit dieser Parameter einer zirkadianen Rhythmik unterliegt und erhebliche Schwankungen während des Menstruationszyklus auftreten. Neben den Basalwerten wird zur Diagnostik des Late-Onset AGS und zum Nachweis eines heterozygoten 21-Hydroxylase-Mangels der ACTH-Kurztest durchgeführt. Pathologische Werte mit einem Anstieg des 17OHPR um mehr als 2,6 µg/l ziehen oftmals weitere molekulargenetische Untersuchungen nach sich. Daher ist eine adäquate Spezifität der Immunoassays zum Nachweis des 17OHPR zu fordern. Die Herstellerinformationen sollten auf alle bekannten Störfaktoren und Einflussgrößen hinweisen, die zu Limitationen des Testes führen. Externe und interne Qualitätskontrolle sollten so angelegt sein, dass schnellstmöglich Fehler der Methode erkannt werden.

Bei der vorliegenden Untersuchung wurden >500 17OHPR-Werte der ersten sechs Monate der Jahre 2000–2002 im delta-check gegenübergestellt. Die Bestimmung erfolgte mit einem RIA der Firma DSL ohne Extraktion. Die interne Qualitätskontrolle war unauffällig, es wurden alle Ringversuche im genannten Zeitraum bestanden.

Der Median der Analysenergebnisse betrug 2000 0,82 µg/l (MW: 2,20 µg/l, n = 67), 2001 1,00 µg/l (MW 2,54 µg/l, n = 228) und 2002 1,47 µg/l (MW 3,26 µg/l, n = 267). Die Firma DSL kann keine Erklärung für dieses Phänomen liefern, interne und externe Qualitätskontrolle haben diesen kontinuierlichen Drift zu höheren Analysenergebnissen bei gleichem Patientengut maskiert. Extraktionen des Analyten führten zu extrem veränderten (erniedrigten) 17OHPR-Konzentrationen und damit zu deutlich veränderter Interpretation des ACTH-Kurztests. Die hier vorgestellten analytischen Erfahrungen belegen, dass weder die interne noch die externe Qualitätskontrolle in der Lage sind, die aufgezeigten analytischen Probleme offenzulegen. Nur die enge Zusammenarbeit mit dem Kliniker sowie retrospektive Δ-Checks können analytische Unzulänglichkeiten der geschilderten Art demaskieren.

Q2
Determination of Diagnostic Decision Limits

R. Haeckel and W. Wosniok

Zentralkrankenhaus Sankt-Jürgen-Straße, Bremen

Diagnostic decision limits (DL) are more important for disease management than limits of reference intervals (RI). RI are usually determined with a healthy population, and therefore, can only serve as a rough orientation if decision limits are not available.

DL are usually determined by the ROC procedure (optimizing specificity and sensitivity) or the efficiency rate (ER, maximizing the number the true positives and true negatives). Another concept is the determination of the discordance rate (DR), which was recently developed for method comparison studies. The new concept (Clin Chem Lab Med 2001;39:A60) is based on the estimation of the well known efficiency in relation to the distribution of the analyte quantity in the population for which the test is applied. All 3 concepts were compared with each other using the conversion of decision limits from one sample system to another one, as e.g. the conversion of decision limits for diabetes mellitus determined with venous plasma to those to be used for venous blood or capillary blood.

Comparison of decision limits (mmol/l, mg/dl in parenthesis) determined with the 3 techniques for venous and capillary blood in comparison with venous plasma after a 2 hours glucose load (number of data pairs = 155). The decision limit for VP was fixed at 11.1 mmol/l according to the WHO recommendation:

Conversion procedure	venous blood mmol/l (mg/dl)	capillary blood mmol/l (mg/dl)
ER	9.60 (173)	10.10 (182)
ROC curve	9.71 (175)*	10.65 (192)*
DR	9.54 (172)	10.32 (186)
* estimated or not possible due to tied values		
WHO	9.99 (180)	11.10 (200)
Derived of concentration ratios	9.67 (174)	10.00–11.45 (180–206)

Conclusion: The decision limits for glucose in capillary and venous blood recommended by WHO should be reduced. The advantage of the discordance rate in comparison with the other techniques are fewer tied values, that less experimental data are required and that epidemiological, analytical and disease state informations are considered.

Q3
Reference Intervals and Cut-Off Values for Hyperthyroidism as Determined by the TSH-Ultra-II Assay and the new TSH-3rdGeneration Assay on the AxSYM Analyser

W. Hubl[1], C. Baker[1], J. Schmieder[2], E. Gladrow [3] and Th. Demant[1]

[1] Institut für Klinische Chemie und Labormedizin, Krankenhaus Dresden-Friedrichstadt, D-01067 Dresden (hubl-wa@khdf.de).
[2] Praxis für Labormedizin, D-01239 Dresden-Prohlis.
[3] Abbott GmbH & Co KG, D-65205 Wiesbaden

We established reference intervals for a new TSH 3rd-generation assay in comparison to the TSH-Ultra-II assay on the AxSYM analyser (Abbott GmbH & Co KG) and determined TSH cut-off values for patients with hyperthyreoidism.
Subjects: Serum samples were collected from: 1.) healthy adults (n = 188), no history of thyroid disorders. 2.) euthyroidic patients from a medical practice (n = 230). 3.) hospital patients (n = 270) without thyroid disorders or non-thyroidal illness (NTI). 4.) patients with hyperthyreoidism (n = 50).
Results: Reference intervals for healthy adults were determined as 0.43–4.58 mIU/l by the TSH-3rd gen assay and 0.34–3.85 mIU/l by the TSH-Ultra-II assay ($p < 0.001$, $r = 0.968$, $y = 1.2967 \times + 0.1343$). In euthyroidic out-patients TSH ranged from 0.45–4.53 mIU/l and 0.39–3.63 mIU/l, respectively ($p < 0.001$, $r = 0.989$, $y = 1.2517 \times + 0.0754$), hospital patients had ranges of 0.39–4.50 mIU/l and 0.30–3.64 mIU/l ($p < 0,001$, $r = 0.978$, $y = 1.2686 \times + 0.0864$). Hospital patients had significantly lower values than healthy controls and out-patients. Results were the same in control groups aged 10–50 years or 51–95 years. A comparison of 97.5 %-percentiles in patients with hyperthyroidism showed a significantly higher value for the TSH-3rd gen assay than for the TSH-Ultra-II assay (0.086 vs. 0.042 mIU/l). Results from this study were distinctly different from information provided by the assay manufactures (Reference values TSH 3rd-gen: 0.47–4.64 mIU/l, TSH-Ultra-II: 0.49–4.67 mIU/l).
Conclusion: These results confirm the need for locally determined reference values for TSH and demonstrates that reference values and cut-off points have to be redefined with the introduction of a new generation TSH assay.
Keywords: TSH-AxSYM, reference intervals.

Q4
Qualitätskontrolle von Harnsteinanalysen: Bilanz der Ringversuche 1980 bis 2001

A. Hesse[1], R. Kruse[2], W.-J. Geilenkeuser[2], H.-J. Steffes[1], M. Schmidt[1]

[1] Experimentelle Urologie, Urologische Klinik, Universitätsklinikum Bonn
[2] Referenzinstitut für Bioanalytik, Bonn, Deutsche Gesellschaft für Klinische Chemie e. V.

Die Prävalenz des Harnsteinleidens hat in den letzten 20 Jahren von 4 % auf 5 % zugenommen. Nur auf der Basis einer genauen Harnsteinanalyse kann eine erfolgreiche Rezidivprophylaxe eingeleitet werden. Daher wurde frühzeitig erkannt, dass durch die Qualitätskontrolle mittels Ringversuchen die Laboratorien ihre Methoden überprüfen sollten. Seit 1980 wurden jährlich zwei Ringversuche angeboten.

Methode: Als Testsubstanzen wurden jeweils synthetische Substanzen verwendet. Pro Ringversuch wurden 4 Proben verschickt, wobei Reinsubstanzen und Zweistoffgemische eingesetzt wurden. Als Ergebnis wurde die richtige qualitative Angabe und eine halbquantitative Bestimmung bei Mischungen in 10 % Abstufungen verlangt.

Von den Teilnehmern wurden folgende Analysenmethoden angewendet: Chemische Analyse, Infrarotspektroskopie, Röntgendiffraktion und evt. Polarisationsmikroskopie in Kombination.
Ergebnisse: In allen Ringversuchen hatten die chemischen Methoden die größten Fehlerquoten. Für Reinsubstanzen lag die Fehlerquote der chemischen Analyse zwischen 6,5 % (Cystin) und 71 % (Xanthin), bei der IR-Spektroskopie waren es 0,9–3,7 % und bei der Röntgendiffraktion 0–6,8 %. Die Infrarotspektroskopie wurde verstärkt eingesetzt (Tabelle 1).

Tabelle 1: Veränderung des Methodenspektrumes zur Harnsteinanalyse

	1980	1984	1989	1994	1999	2001
	n = 103	n = 117	n = 113	n = 100	n = 84	n = 79
Chemische Analyse	87,4 %	73,5 %	59,3 %	51 %	23,8 %	12,7 %
Infrarot-spektroskopie	7,8 %	19,7 %	31,0 %	31,0 %	69,0 %	78,5 %
Röntgen-diffraktion	4,9 %	6,8 %	9,7 %	7,0 %	7,2 %	8,8 %

Zusammenfassung: Die Ringversuche für Harnsteinanalysen haben wesentlich zur Qualitätsverbesserung und Methodenbereinigung bei der Harnsteinanalyse beigetragen.
Schlüsselwörter: Qualitätskontrolle, Harnsteinanalysen

Q5
Der [$^{13}C_2$]Oxalat-Absorptionstest – Bestimmung von Referenzwerten der intestinalen Oxalat-Absorption

S. Voss[1], G.E. von Unruh[2], T. Sauerbruch[2], A. Hesse[1]

[1] Experimentelle Urologie, Klinik und Poliklinik für Urologie,
[2] Innere Medizin, Universitätsklinikum Bonn

Einleitung: Die renale Oxalat-Ausscheidung ist beim Calciumoxalat(CaOx)-Steinleiden einer der wichtigsten Promotoren. Unter Anwendung des [$^{13}C_2$]Oxalat-Absorptionstestes sind Referenzwerte für die intestinale Oxalat-Absorption bei 120 gesunden Probanden ermittelt und 100 CaOx-Rezidiv-Steinpatienten getestet worden.
Methodik: Ein Test dauert zwei Tage. Der erste Tag dient zur Standardisierung. Am Morgen des zweiten Tages wird die Kapsel mit [$^{13}C_2$]Oxalat eingenommen. Die angesäuerten Urinproben werden mit Ethylacetat extrahiert und die organischen Säuren mit MBDSTFA derivatisiert. Die Messung des [$^{13}C_2$]Oxalates (Maß für die Absorption) erfolgt mit Hilfe der GC/MS.
Ergebnisse: Die mittlere Oxalat-Absorption lag bei den Probanden bei $8,0 \pm 4,4$ % und bei den CaOx-Steinpatienten bei $10,2 \pm 5,3$ % ($p < 0,001$). Der Referenzbereich der 120 gesunden Probanden lag zwischen 2,2 und 18,5 %. Zwischen der Oxalat-Absorption von männlichen und weiblichen Probanden bzw. männlichen und weiblichen Patienten ist kein signifikanter Unterschied ermittelt worden.
Diskussion: Mit dem [$^{13}C_2$]Oxalat-Absorptionstest wurde an Gesunden ein Referenzwert für die Oxalat-Absorption ermittelt, der signifikant niedriger als die Absorption bei CaOx-Steinpatienten ist. Dieser Test kann ohne Einschränkung in der Forschung, Diagnostik und Therapiekontrolle angewandt werden.

DFG-Förderung: (Un 91/1-3)
Schlüsselwörter: GC/MS, [$^{13}C_2$]Oxalat-Absorption, Referenzwerte

Q6
SNP-Analysen zur Bestimmung genetisch bedingter Unterschiede in der medikamentösen Therapie

M. Lustig[1], L. Kruska[2], K. Buttler[1], M. Heisinger[1], S. Waschütza[1], E. Schnakenberg[1]

[1] AdnaGen AG, Ostpassage 7, 30853 Langenhagen,
[2] Labor Stein, Wallstr. 10, 41061 Mönchengladbach

Individuelle Unterschiede bei der Therapie mit Medikamenten ('non-responder', Resistenzen, Toxizitäten) werden seit vielen Jahren beobachtet und können genetische Ursachen haben. In den vergangenen mehr als zehn Jahren konnten Korrelationen zwischen enzymatischen Eigenschaften und der genetischen Variabilität einzelner Gene (z. B. Cytochroms P450, N-Acetyltransferasen, Thiopurin S-Methyltransferase u. v. m.) aufgezeigt werden. Das genetische Monitoring bietet die Möglichkeit z. B. vor Beginn einer Tumortherapie mit dem Chemotherapeutikum 5-Fluorouracil das geschwindigkeitsbestimmende Gen Dihydropyrimidin Dehydrogenase hinsichtlich des Vorliegens einer 165 Bp großen Deletion zu untersuchen. Träger dieser Mutation können Unverträglichkeitserscheinungen in Form von schweren Blutbildveränderungen beispielsweise bei der Therapie colorektaler Tumoren aufweisen. Weiterhin liegen Untersuchungen vor, die berichten, daß bereits heterozygote Träger einer Mutation im Gen der Thiopurin S-Methyltransferase therapiebedingte Nebenwirkungen nach Gabe von Thiopurinen zeigten. Nach Reduzierung der Thiopurindosis wurden bei heterozygoten als auch bei homozygoten Mutationsträgern keine hämatotoxischen Auffälligkeiten beobachtet. AdnaGen hat für zahlreiche Enzympolymorphismen real-time PCR-basierende Testsysteme (NAT2, GSTM1, GSTT1, GSTP1, TPMT, DPD, CYP2C9, CYP2C19, CYP2D6, mEH, PON1 u. a.) entwickelt, die zur Vermeidung genetisch bedingter unerwünschter Arzneimittelnebenwirkungen eingesetzt werden können. Darüber hinaus führt AdnaGen eigene pharmakogenetische Untersuchungen durch. Im Rahmen einer Pilotstudie an 75 Patienten mit einer Carbamazepin-Therapie wurde der Polymorphismus der Gene GSTM1 und GSTT1 untersucht. Erste Ergebnisse zum Einfluss dieser Gene im Stoffwechsel von Carbamazepin werden präsentiert.

Q7
Evaluation of the TOSOH AIA-PACK Troponin I 2 G Assay: A Second Generation Test for the Detection of cTnI

T. Brinkmann and K. Kleesiek

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen

We have evaluated the cardiac troponin I (cTnI) enzyme immunoassay AIA-PACK cTnI 2 G (Eurogenetics Tosoh). Evaluation of interassay precision at different cTnI levels (0.30–6.70 ng/ml, n = 29) showed coefficients of variation (CV) between 5.00 % and 4.71 % for cTnI on the AIA-600 II instrument. The functional and analytical sensitivity were determined with 0.02 ng/ml and 0.04 ng/ml, respectively.

Comparative cTnI measurements of serum samples were performed on the AIA-600 II analyser (Eurogenetics Tosoh) and the Dimension RxL analyser (Dade Behring). The cTnI concentrations of the patient sera (n = 226) ranged from 0 ng/ml to 114 ng/ml. Regression analysis showed a close correlation between both assays (RxL = 0.96 AIA + 0.38; r = 0.960).

To induce proteolytic *in vitro* degradation of the cTnI molecule, 20 different serum samples were incubated at 37 °C for 24h and cTnI was analysed with both test systems. Comparison of the measured cTnI concentrations obtained before and after incubation showed identical values on the AIA-600 II. However, decreased cTnI values were obtained on the Dimension RxL analyser after the incubation period ranging from 10 to 50 % of the initial values. The AIA-PACK cTnI 2 G assay uses two monoclonal antibodies against epitopes in the proteolytically stable region of the cTnI molecule (epitopes around position 40 and 90 of the cTnI amino acid sequence) explaining the stability of this assay.

Keywords: Troponin I, immunoassay

Q8
Evaluation of Troponin I, CK-MB and Myoglobin Assays on the Innotrac Aio! Immunoanalyzer

H.G. Wahl, E. Eckert, I. Nikolay, N. Trabandt, and J. Bohner

Institut für Laboratoriumsmedizin, Klinikum Fulda

The Innotrac Aio! cardiac marker assays are based on an a concept in which all the reagents are provided in a dry stable form within a single cup. Biotinylated monoclonal antibodies (mAb) are preimmobilised to the streptavidin surface of the cup. A separating layer prevents direct contacts of the europium labelled tracer mAb with the capture mAb. To perform the assay, the sample and the buffer are added into the cup and incubated for 15 minutes at 36 °C under continous shaking. After washing and drying steps the signal of the fluorescent Europium Lanthanide chelate is read (time-resolved fluorescence). Total analysis time is 18 minutes for all tests with a throughput of 70 samples per hour. Samples can be whole blood, plasma or serum with sample volumes of 5 µl (Myoglobin), 10 µl (CK-MB) and 20 µl (Troponin I). Within-run imprecision (n = 20, patient samples) was determined for 3 different concentrations (cut-off, elevated and high) for all three analytes. The coefficients of variation (CVs) were in the range from 5.3 to 6.5 % (Myoglobin), 5.8 to 9.3 % (CK-MB) and 3.8 to 8.9 % (Troponin I). Between-run imprecision (n = 10, controls) showed CVs in the range from 8.7 to 10.1 % (Myoglobin), 5.5 to 14.4 % (CK-MB) and 10.7 to 12.1 % (Troponin I). Linearity was tested for troponin up to 49.9 ng/ml (r = 0.997) myoglobin up to 2165 ng/ml (r = 0.9988) and CK-MB up to 178 ng/ml (r = 0.9948). For method comparison patient samples were also measured at the Stratus CS (Dade Behring) and Axsym (Abbott Diagnostics). Data analysis (Passing/Bablok and Bland/Altman) showed good correlation for all analytes with significant different slopes for all three Troponin I methods in the Bablock/Passing regressions. The good analytical performance shown here and the high practicability of the Innotrac Aio! Immunoanalyzer are particularly of interest for point-of-care testing.

Keywords: cardiac markers, point of care testing

Q9
Evaluation zweier neu überarbeiteter Troponin I Immunoassays

C. Haug, M.G. Bachem, A. Grünert

Institut für Klinische Chemie, Universitätsklinikum Ulm

Im Hinblick auf die zunehmende invasive Diagnostik und Therapie des akuten Koronarsyndroms ist die Optimierung von Troponin Immunoassays im unteren Messbereich von großer Bedeutung. Die kürzlich veröffentlichten Konsensusdokumente der European Society of Cardiology und des American College of Cardiology empfehlen die Entscheidungsgrenze für erhöhte Troponin-Konzentrationen bei der 99 % Perzentile einer Referenzpopulation anzusetzen und fordern, dass die Unpräzision in diesem Bereich ≤10 % betragen sollte.

Unter diesem Aspekt haben wir zwei neu überarbeitete Immunoassays für kardiales Troponin I (cTnI) evaluiert: Dimension® cTnI (Dade Behring, Deutschland) und AIA-PACK cTnI 2nd-Gen (Eurogenetics Tosoh, Belgien). Alle in Heparin-Plasma und Serum von gesunden Probanden gemessenen cTnI-Konzentrationen lagen jeweils innerhalb der von den Herstellern angegebenen Referenzbereiche (97.5 % Perzentile, Dimension® cTnI: µg/l, AIA-PACK cTnI 2nd-Gen: ≤ 0.04 µg/l). Die Intra-Assay-Variationskoeffizienten von cTnI-Werten oberhalb der von den Herstellern ermittelten 99 % Perzentilen (0.07 und 0.06 µg/l) lagen unter 17 % und nahmen mit zunehmender cTnI-Konzentration ab. In den meisten Proben ergab der AIA-PACK cTnI Assay höhere Werte, jedoch zeigte sich zwischen den mit beiden Tests gemessenen Konzentrationen eine signifikante Korrelation (Dimension® 2.61 ±;8.31 µg/l (MW±SD), AIA-PACK: 3.02 ± 8.67 µg/l, n = 208; r = 0.969, p < 0.001). Zusammenfassend wiesen beide Tests eine akzeptable Gesamtperformance auf, jedoch wurde insbesondere mit dem Dimension® cTnI Assay die in den Konsensusdokumenten geforderte Präzision im Bereich der 99 % Perzentile nicht erreicht. Diese Ergebnisse zeigen, dass die analytische Variabilität niedriger Troponin-Konzentrationen sorgfältig überwacht und bei diagnostischen und therapeutischen Entscheidungen berücksichtigt werden muss.

Schlüsselwörter: Troponin I, Enzymimmunoassay, akutes Koronarsyndrom

Q10
Clinical Evaluation of the LIAISON Troponin I Assay

W. Hubl[1], A. Graf[2], J. Eberhard[2], E. Rauhut[3] and Th. Demant[1]

[1] Institut für Klin. Chemie und Labormedizin und
[2] II. Medizin. Klinik, Krankenhaus Dresden-Friedrichstadt, D-01067 Dresden, (hubl-wa@khdf.de).
[3] Byk & DiaSorin GmbH, D -61231 Dietzenbach

The goal of the study was the clinical evaluation of a new Troponin I (TnI) assay on a LIAISON immunoassay analyzer (Byk & DiaSorin), and a methodological comparison with the TnI assay on an AxSYM immunoassay analyzer (Abbott GmbH & KG).

Patients: Patients with acute myocardial infarction (AMI) (n=69), post coronary angioplasty (PTCA) (n = 26), normal coronary angiograms (n = 33), unstable angina (n = 6), pulmonary embolism (n = 5) and healthy controls (n=59).

Results: TnI values with the LIAISON assay were 0.005–0.034 µg/l for healthy controls (median 0.006 µg/l, 99th-percentile 0.034 µg/l). The 99th-percentile fell between the functional sensitivity level at 0,03 µg/l and the level for 10 % imprecision (0,06 µg/l). TnI measurements for patients with normal angiograms produced a 99th-percentile cutoff at 0,019 µg/l. Patients with AMI had TnI values of 0,051–34,4 µg/l (2,5–97,5 %-percentile). Patients treated with PTCA showed the typical increase of TnI levels (0,0117 µg/l (before) vs. 0,0200 µg/l (after), p = 0,025). Both TnI assays were highly correlated, however the values measured on the LIAISON were less than 10 % of the values measured on the AxSYM (r = 0,978, y = 0.077 ×–1,66). Two of the six patients with unstable angina pectoris and one of the five patients with pulmonary embolism produced elevated TnI levels.

Conclusions: The LIAISON TnI assay produced a high analytical sensitivity with low values for functional sensitivity in the 99th-percentile reference range. Absolute values were found to be considerably lower compared to the AxSYM assay. Both tests were able to discriminate well between patients with acute myocardial infarction and healthy controls and both were able to detect slight TnI elevations in patients suffering from unstable angina pectoris or pulmonary embolism.

Keywords: Troponin I, LIAISON analyzer, clinical evaluation.

Q11
Stability of BNP Measured by the Triage Assay

H. Stiegler, Y. Fischer, A. Germing*, M. Krieg

Institut für Klinische Chemie, Laboratoriums- und Transfusionsmedizin,
* Klinik für Kardiologie, BG-Kliniken Bergmannsheil, Universitätsklinik, Bochum

Background: Plasma concentrations of BNP are elevated in patients with congestive heart failure (CHF) and have been shown to accurately predict clinical severity and left ventricular ejection fraction in those patients. A new fluorescence immunoassay for quantitative determination of BNP in EDTA anticoagulated whole blood was introduced recently (Triage BNP, Biosite Diagnostics). This assay allows immediate measurement of BNP at the point of care (POC). Nevertheless widespread applicability of its determination outside the POC, i. e. in a centralized clinical laboratory mainly depends on the stability of the analyte. Therefore we aimed to investigate the stability of BNP measured by the Triage assay.

Methods: 4 patients with symptomatic CHF and 2 healthy subjects were included. Blood samples were collected into anticoagulated plastic tubes with addition of EDTA. Sample portions of each patient were either immediately measured or stored as plasma or whole blood at different temperatures (+4° C and room temperature) up to 72 hours. For within-run imprecision EDTA anticoagulated whole blood was measured 11 times on the same day using the same assay lot and unchanged calibration. Between day imprecision was obtained by measurement of EDTA plasma on 11 consecutive days using the same assay lot and unchanged calibration.

Results: Coefficients of variation (CV) for within-run imprecision were 9.4 % (between day imprecision: 11 %, concentration: 40 pg/ml), 13 % (13 %, 450 pg/ml) and 15 % (16 %, 800 pg/ml), respectively. A pronounced decline of BNP concentrations was noted in plasma and whole blood after 4 hours independent of storage temperature.

Conclusion: BNP is sufficiently stable for up to 4 hours at room temperature. This allows measurement of BNP outside the POC, i. e. in a centralized clinical laboratory.

Q12
Development of an ELISA Specific for the Soluble Form of the Transferrin Receptor

K. Dassler, M. Kaup, R. Tauber and H. Fuchs

Institut für Klinische Chemie und Pathobiochemie, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany

Objective: The soluble form of the transferrin receptor (sTfR) is generated by cleavage by an unknown protease. The major form of sTfR found in human serum is cleaved between Arg-100 and Leu-101 within the stalk region, which keeps the extracellular domain of the TfR at a distance of 2.9 nm from the plasma membrane [Fuchs et al. (1998) Structure 6:1235–43]. Although the serum concentration of sTfR is altered in several diseases and used for diagnostic purposes the regulation and function of the shedding process is unknown.

Methods: In this study we developed an ELISA for the quantitation of sTfR-release from leukocytic cell lines into the supernatant. For capturing the sTfR a 96-well plate was coated with a monoclonal antibody specific for the extracellular domain of the TfR (OKT9). Bound TfR was quantified using a polyclonal anti-TfR antibody (pAB063) and a peroxidase-labeled swine anti-rabbit IgG.
Results: The sTfR-ELISA is sensitive in the low picomolar range as we could show using purified human placental TfR as standard. The basal sTfR-levels from several leukocytic cell lines measured correspond well to the sTfR detected by immunoprecipitation as reported earlier by our group [Kaup et al. (2002) Biol. Chem. 383:1011–20]. Furthermore, we show that the constitutive sTfR-release from HL60 cells can be inhibited by metalloprotease inhibitors and a specific furin inhibitor indicating that the TfR-shedding protease is activated by a furin-like proprotein convertase. Whereas stimulation of the cells by the phorbol ester PMA did not alter sTfR-release significantly in most cell lines, the phosphatase inhibitor pervanadate led to a marked increase in TfR-shedding.
Conclusion: Our results reveal that the sTfR-ELISA is a sensitive and reliable tool to study the TfR-shedding process in leukocytic cell lines.

Q13
Determination of Carbohydrate Deficient Transferrin (CDT) in Infancy and Youth

V. Neumeister[1], T. Hirsch[2], E. Kuhlisch[3], S. Bergmann[1], G. Siegert[1]

[1] Institut für Klinische Chemie und Laboratoriumsmedizin,
[2] Klinik für Kinder- und Jugendheilkunde,
[3] Institut für Medizinische Informatik und Biometrie Universitätsklinikum C.G.Carus an der TU Dresden

Since several years the determination of CDT isoforms indicating and estimating an alcohol abusus has been established. It is known problem that alcohol abuse of children begins frequently at early ages and, until now, no normal CDT values for children have been determined. For adults the normal reference value published by Bio Rad (Munich, Germany) is a serum concentration < 2.6 %CDT. The aim of the present study was to etablish the distribution of CDT values in infancy and youth.

The sera of 215 children with ages between 0.3 to 18 years, who exhibited no signs of liver and metabolic disease, and of course, who drank no alcohol were analysed. The determination of CDT was performed with the assay % CDT TIA (turbidimetric assay, Co. Bio Rad, Germany). Statistical calculation were done using the software SPSS.

The mean value % CDT was 2.24 (min. 1.3, max. 3.7). No general significant difference (p = 0.599) was observed between the % CDT values of male and female. In the age group of 6–10 years, significantly higher % CDT values were measured compared to the group of 0–5 years (p = < 0.0005), as well as to the group 11–14 years (p = 0.049), and to the group 15–18 years (p = 0.019). However, females of the age group 15–18 years showed significantly higher %CDT values than the males of the same group (p = 0.030).

Our investigations show that the published reference value of < 2.6 %CDT should not be applied to children, because in our investigations the corresponding 97.5 percentiles were found to be substantially higher in all investigeted groups. The 97.5 percentiles of all healty samples is 3.20 % CDT. However, HELANDER published 2001 a study of adults % CDT with a recommendation of normal % CDT < 3.0 (Helander et al. Alcohol & Alcoholism.2001,36,406–412).
Schlüsselwörter: CDT, Alkohol, Referenzwerte

Q14
Determination of Lactate in Neonatal Blood Samples by a New Portable Device (Accutrend Lactate)

H. Schlebusch[1]; C. Henkel[1]; M. Deperschmidt[1]; I. Paffenholz[2]; B. Gambke[3]

[1] Universitäts-Frauenklinik Tübingen,
[2] Universitäts-Frauenklinik Bonn,
[3] Roche Diagnostics GmbH, Mannheim

A simple, rapid and reliable method is desirable, if lactate has to be determined routinely in a labour ward or a neonatal intensive care unit. However, most of the current methods need relatively large samples volumes and laboratory facilities to obtain results. Moreover, incomplete inhibition of glycolysis is a frequent problem. We therefore evaluated a new handheld device, which enables lactate determinations in 20 µl whole blood using a test strip that employs a lactate oxidase – mediator reaction. With the test principle used in the system the plasma lactate concentration is measured. However, the result can also be displayed with respect to whole blood, using an implemented conversion function. Lactate was measured in 116 neonatal blood samples (cord blood, venous blood, capillary blood) using Accutrend Lactate (AL) and, after addition of NaF and centrifugation, by Vitros 250 for comparison. Lactate concentration ranged from 1,5 to 20.5 mmol/l, hematocrit from 25 % to 60 %. Passing-Bablok regression analysis resulted in AL = –0.319 +1.008 × Vitros, r = 0.988, i. e. AL values are on average 3 % lower than Vitros values, which is clinically not meaningful. The hematocrit of the samples had no influence on the results. Moreover, measurements were not affected by bilirubin (< 270 µmol/l), moderate hemolysis (< 400 µmol/l) and pO2 (range tested 42–286 mm Hg, using tonometered cord blood samples). Within series as well as day-to-day precision was very satisfactory showing CV's between 1,3 % and 9,5 %. Accutrend Lactate proved to be a reliable instrument which is easy to perform and very suitable for the determination of lactate in neonatal samples.
Keywords: Lactate, Neonates, POCT

Q15
The Influence of Storage and Temperature on the S-100B Concentration in Human Blood

O. Kopetsch, A. Raabe, R. Gerlach, H. Vatter, P. Gebhart, U. Groß and V. Seifert

Klinik und Poliklinik für Neurochirurgie, Klinikum der Johann-Wolfgang-Goethe-Universität, Frankfurt am Main.

Introduction: The serum levels of the brain protein S-100B are becoming an established method for determination of the severity and the course of brain damage. Therefore, stability of the sample is important for the assessment of the clinical state and therapeutic procedures.
Probands and Methods: Protein S-100B concentrations were assayed in serum of 29 healthy persons [male: 17, female: 12; age: 49 ± 14 y ($\bar{x} \pm$ SD)] with specific monoclonal antibodies.
Results: The initial concentration of protein S100B of the volunteers amounted to 73 ± 35 ng·l^{-1} ($\bar{x} \pm$ SD, n=29). The mean concentrations were 73±38 ng·l^{-1}, 68±36 ng·l^{-1}, 72±36 ng·l^{-1} and 85±41 ng·l^{-1} ($\bar{x} \pm$ SD, n = 29) after 4, 8, 12 and 24 hours, respectively, if the blood was stored at room temperature. The means differ 1.00-, 0.93-, 0.99- and 1.16-fold after these intervals compared to the initial level. At

4 °C the mean concentrations of protein S100B were 70 ± 36 ng·l^{-1}, 73 ± 42 ng·l^{-1}, 81 ± 43 ng·l^{-1} and 80 ± 35 ng·l^{-1} ($\bar{x}$ ± SD, n = 29) after 4, 8, 12 and 24 hours of storage, respectively. At this temperature the means differ 0.96-, 1.00-, 1.11- and 1.10-fold from the initial value.
Conclusion: The blood concentration of protein S100B is stable up to 24 hours at ambient temperature as well as in a refrigerator. Thus, such samples may be stored for one day at the one or the other temperature until the determination of this indicator of brain damage.
(This study was supported by the Adolf Messer foundation)
Keywords: S-100B, concentration, storage, temperature.

Q16
Evaluation and Comparison of Different Reference Systems for Application in Continuos On-line Measurement Devices for Determination of Na^+, K^+ Ca^{2+}, Mg^{2+} and pH

F.H. Boguschewski, J.P. Heidrich and M. Seyfarth

Zentrallabor/Klinische Chemie, Universität zu Lübeck

Ion-selective electrodes (ISE) were applied for real time monitoring of various processes by means of on-line measurement devices. Continuous measurements in those systems have already been successfully applied in the process-control of hemodialysis, transplantation and for research. Those devices allows to evaluate electrodes and reference systems independently from their routine analyzer of the manufacturer's. Most reference systems for completion of the ion-selective galvanic cell works with the reference solution KCl with a concentration of two or three mol per liter. Furthermore, we tested in this study the contamination of samples after measurement by the reference electrolyte KCl for research application in a rat model for continuos extracorporal circulation of whole blood without a dialysis chamber. KONE (KoneLab®, Espoo, Finland) reference systems consists of semi-permeable membranes, whereby the 2 molar reference solution KCl diffuses. The contamination of the sample during passage through the flow-through channel was significant with 0.07 mmol/l, determined for continuos measurement devices with a flow rate of 0.2 ml/min. For exactly measurements the reference system should placed after ISE in continuous or discontinuous hosts, because of the contamination of sample with K^+ and Cl^-. However, this K^+-increase will be tolerate and compensate by an animal with a normal function of the kidneys in the research model. But, we suggest for exact measurements or monitoring of various processes for several hours the application of a dialysis chamber.

Q17
Mathematical Algorithms and Correction Factors for Calibration of Mg^{2+}-Selective Electrodes in Flow-Through Measurement Devices

F.H. Boguschewski, J.P. Heidrich and M. Seyfarth

Zentrallabor/Klinische Chemie, Universität zu Lübeck

Magnesium (Mg) is the second most abounded cations in living cells, but only 1 % of the total body Mg is present in the blood. In clinical laboratories only total Mg is determined by atomic absorption spectrometry (AAS) or by Xylidyl blue spectrophotometry for routine methods. However, ionized Mg (Mg^{2+}), the physiological and active form of Mg, can be determined by ion-selective electrodes (ISE). But those Mg^{2+}-ISE showed in past disadvantages regarding electrode characteristics, e.g. sensitivity, selectivity, slope and imprecision. Mathematical algorithms and correction factors for calibration for elimination of the calcium interference, the most important factor of faulty Mg^{2+} and the high bias and imprecision are necessary. Mg^{2+}-ISE's of two analyzers, KONE® Microlyte 6 (KONE® Instruments, Espoo, Finland) and NOVA® CRT (NOVA® Biomedical, Waltham, MA, USA), were placed into a special on-line measurement device. This flow-through device allows to compare different ISE-types during continuos on-line measurement independently from the host of the manufacturer's analyzers.

The NOVA-electrode showed superior characteristics for calibration and doesn't need some mathematical algorithms or factors for correction of calibration. In comparison, the KONE-sensor determined 62 % of the Ca^{2+} in samples and needs a mathematical correction for the Ca^{2+}-interference. This study should help to demonstrate calibration of different sensor types and different ways for correction of interference effects in flow-trough measurement devices.

Q18
Evaluation of an Automated Nephelometric Immunoassay for Quantification of Free Light Chains in Serum

D. Hermsen[1], A.R. Bradwell[2], H. Reinauer[1]

[1] Institut für Klinische Chemie und Laboratoriumsdiagnostik, Heinrich-Heine-Universität Düsseldorf,
[2] Department of Immunology, The Medical School, University of Birmingham, UK.

Objective: Monoclonal free light chains (flc, Bence-Jones Proteins, BJP) are typically found in B-cell lineage tumors (e. g. multiple myeloma). The routine laboratory procedure for diagnosis of BJP is to perform urine immunofixation (IFE). The aim of this study was to compare results of the urine IFE with a new assay for determination of κ and λ flc in serum.
Methods and Results: The new assay specific for κ/λ flc is a latex-enhanced nephelometric immunoassay (The Binding Site, TBS) which is performed on the Dade Behring Nephelometer II™. Urine and serum samples were analyzed from 165 patients with suspected flc monoclonal gammopathies. Results of urine IFE (Helena) were compared with those obtained in serum using the TBS flc assay. Results of urine IFE were divided in four groups: 23 urine samples containing BJP, 16 urine samples containing whole paraproteins and BJP, 6 urine samples containing whole paraproteins without BJP and 120 urine samples without monoclonal proteins. In all but one serum of the first group elevated flc concentrations or abnormal κ/λ ratios were measured. All detected BJP of the second group were confirmed by increased serum flc concentrations or abnormal κ/λ ratios. Although in urine IFE of the third group no flc were detected, elevated serum concentrations of flc or abnormal κ/λ ratios were measured in all cases. In 61 sera of the last group increased flc concentrations or abnormal κ/λ ratios were analyzed.
Summary: In 38/39 cases BJP were confirmed by serum measurements. In 67/126 cases without detected BJP increased serum flc concentrations or abnormal κ/λ ratios were measured by the TBS flc assay.
Conclusion: The TBS flc assay is a useful and reliable tool for detection of free κ and λ light chains in serum and could reduce the need of urine testing.
Keywords: Monoclonal free light chains, nephelometry, evaluation

Q19
Determination of Urinary Enzyme Alanine Aminopeptidase Activity Using an Automated Method at 37 °C

B. Holdt-Lehmann, E. Peters, H.-R. Nagel, K.P. Schneider, P. Schuff-Werner

Universität Rostock, Institut für Klinische Chemie und Pathobiochemie

Urinary enzymes, mainly the lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAG) and the brush border enzyme alanine aminopeptidase (AAP), are reliable biomarkers of nephrotoxicity and are useful in the early diagnosis of tubular damage. We have previously shown that AAP indicates very early tubular impairment. In addition, in some cases AAP is elevated in the presence of normal NAG and ?1-microglobulin in urine.

With the aim of developing an automated enzyme assay for routine use, we adapted the photometric assay established at 37 °C (Clin Chem 1980;26:1251), using the substrate L-alanine-4-nitroanilide on a dedicated clinical chemistry analyser (SYNCHRON LX20, Beckman Coulter). Due to the lack of reference material with defined AAP activity, we first determined AAP activity in a commercially available control material (Liquicheck, Unassayed Chemistry Control, 16121, Bio-Rad) using the manual method. This material was subsequently used for calibration purposes of the automated method. Quality assurance was performed with commercial control material (Multiqual 1, Liquid Assayed, Bio-Rad). To avoid analytical interference, a fast gel filtration of urine samples with MicroSpinTM tubes containing Sephadex G50 (Amersham Pharmacia Biotech) was performed before AAP analysis.

Comparison of the newly established automated assay with a manual method employing the substrate L-alanine-β-naphthylamide at 25 °C demonstrated highly significant correlation ($r = 0.98$, $n = 50$, Passing Bablok analysis). We conclude that the AAP assay adapted to an automated analytical system at 37 °C represents an approach to determine urinary AAP activity in the setting of a busy clinical laboratory.

Schlüsselwörter: Urin-Enzymanalyse, Automatenmethode