Jahrestagung der Österreichischen Gesellschaft für Laboratoriumsmedizin

veranstaltet gemeinsam mit

der Österreichischen Gesellschaft für Klinische Chemie
der Österreichischen Gesellschaft für Gute Analysen und Laborpraxis
der Österreichischen Gesellschaft für Qualitätssicherung und Standardisierung im Medizinischen Laboratorium
dem Bundesverband der diplomierten medizinisch-technischen Analytiker/innen Österreichs
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Autorenverzeichnis

Posterpräsentation

Freitag, 1. 12. 2000, 17.00 bis 18.00 Uhr
Vorsitz: M. Truschnig-Wilders, W. Woloszczuk

Inverse Relationship Between Neopterin and Immunoglobulin E

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Polarized human T helper (Th) cells play a key role in the network of the specific immune system. Cell-mediated immune response depends on activation of Th1-type cells, typically producing interferon-γ (IFN-γ) and interleukin (IL)-2, whereas activation of Th2-type cells and production of cytokines such as IL-4, -5 and -10 are involved in humoral immune response and the production of immunoglobulins. Increased amounts of neopterin are produced during Th1-type immune response by human monocytes/macrophages upon stimulation with the Th1-derived cytokine IFN-γ, and thus the determination of neopterin concentrations allows to monitor Th1-type immune response.

In order to examine the relationship between Th1 and Th2 cell system stimulation we compared serum concentrations of neopterin with those of immunoglobulin E (IgE), a typical product of Th2-type immune response, in 709 otherwise healthy outpatients, who visited the physician's office for a medical health check-up.

Eleven percent of the patients presented with elevated serum concentrations (>8.7 nmol/L) of neopterin, 26% had increased serum concentrations (>100 kIU/L) of IgE. There existed an inverse correlation between serum neopterin and IgE concentrations (Spearman's rank correlation coefficient: $r_s = -0.100$; $p<0.01$) which was stronger when excluding data within the reference ranges (n=246; $r_s= -0.519$; $p<0.0001$). None of the individuals with increased IgE concentrations presented with increased neopterin levels and vice versa.

Data indicate that increased serum neopterin concentrations are associated with low serum IgE and increased serum IgE with low serum neopterin concentrations. This finding fully agrees with the current understanding that in humans the activations of Th1- and Th2-cell mediated immune responses are down-regulating each other.

Pränatale Diagnose einer transienten abnormen Myelopoiese bei Trisomie 21

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Die transiente abnorme Myelopoiese (TAM) stellt ein seltenes Syndrom dar, welches fast ausschließlich bei Kindern mit Anomalien am Chromosom 21 beobachtet wird. Die TAM ist durch eine passagere Blastenproliferation, zumeist in der Neonatalperiode, charakterisiert, die innerhalb von 1-3 Monaten ohne therapeutische Intervention spontan ausheilt. Wir berichten über die Diagnose einer TAM in der 33 SSW bei einem Fetus mit nicht-immunologisch bedingtem Hydrops fetalis (Pleuraergüsse, Perikarderguß, Aszites) und Hepatomeglie mit sonografisch echodichten Arealen. Aus dem Nabelschnurblut wurden folgende Laborbefunde erhoben:

Blutbild: WBC 9,4 G/l, Ery 3,4 T/l, Htk 42%, Hb 12,8 g/dl, Plt 209 G/l

Differential-BB: Seg 43%, Eo 2%, Baso 2%, Mono 5%, Lympho 24%, Stab5%, Meta 1%, Myelo 3%, Promyelo 3%, Blasten 12%, NB 32%

Immunphänotypisierung: Myeloische Blastenpopulation: CD33+ CD117+ MPO^{dim} $CD7^{dim}$ $CD45^{dim}$ (Mikromethode)

Zytogenetik: Trisomie 21

Infektionsserologie: negativ (TORCH, Parvoviren).

Das Kind wurde in der 36+2 SSW spontan entbunden.

Blutbild Tag 1: WBC 6,3 G/l, Ery 3,9 T/l, Htk 45%, Hb 14,4 g/dl, Plt 86G/l

Differential-BB: Seg 16%, Eo 21%, Baso 4%, Mono 4%, Lympho 50%,Stab2%, Meta 1%, Myelo 1%, Blasten 1%, NB 20%

Blutbild 8. Woche (Entlassung): WBC 6,3 G/l, Ery 3,6 T/l, Htk 35%, Hb 11,1 g/dl, Plt 348 G/l;

Differential-BB: Seg 26%, Eo 4%, Baso 1%, Mono 5%, Lympho 59%, Stab2%, Meta 2%, Myelo 1%.

Neuerliche Infektionsserologie: negativ.

Im klinischer Verlauf mußte unmittelbar postpartal die Punktion und Drainage des die Atemmechanik störenden Aszites durchgeführt werden, passager trat eine starke Cholestase auf und in der 6. Lebenswoche war eine Pylorotomie (inklusive offener Leberbiopsie) erforderlich. Die fortlaufenden Kontrollen in unserer Frühgeborenenambulanz zeigen seither eine gute Entwicklung bei Down-Syndrom, unverändertem Lebersonografiebefund und normalen Blutbildwerten.

Serum-CTX and Osteocalcin Reference Range in a Croatian Peri- and Postmenopausal Population Sample

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Objective: We measured the biochemical markers of bone turnover osteocalcin and serum c-telopeptide (CTX) in a Croatian peri- and postmenopausal female population.

Methods: The population comprised 116 healthy female subjects aged 42-87 years.

For determination of osteocalcin we used the N-MID hOsteocalcin ELISA™ (Osteometer A/S, Denmark). The serum c-telopeptide (CTX) was measured with the Cross-Laps-S ELISA™ (Osteometer A/S, Denmark). All measuring steps were performed as previously described by an ELISA autoanalyzer (PersonalLab, IASON Labormedizin, Graz, Austria) [1, 2].

Results: The mean concentrations were 17.6 ng/ml (SD±12,9) for osteocalcin and 3146.2 pmol/l (SD±2238,6) for the serum-CrossLaps. Normal range was calculated as mean±1.96 SD. Conclusion: Osteocalcin and serum CrossLaps concentrations show comparable values to the Austrian population, although we observed in accordance to previous reports higher values for both markers [3, 4].

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Anti-RA33-Antikörper: ELISA nur als Suchtest verwendbar, IBLOT zur Diagnose erforderlich

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Isoliert auftretende anti-RA33-Antikörper gelten als spezifischer Marker für die chronische Polyarthritis (cP) und stellen zusammen mit den Antikörpern gegen Filaggrin (AKA) eine wertvolle serologische Entscheidungshilfe für die Frühdiagnose der cP dar.

569 Patienten der Rheumaambulanz mit Verdacht auf cP und eine Serie von 111 Patienten mit Morbus Bechterew (MB) wurden mit einem seit kurzem kommerziell erhältlichen ELISA (Fa. IMTEC, Berlin, Deutschland) auf Antikörper gegen RA33 untersucht.

Die Seren der Patienten mit MB zeigten in 28 von 111 Proben (25%) einen positiven Befund, bei der Kontrolle von 20 positiven Seren mittels Immunoblot (IBLOT) konnten aber keine Ak gegen RA33 gefunden werden. Eine retrospektive Auswertung der aus der Rheumaambulanz zugewiesenen Patientenseren ergab ebenso eine unerwartet hohe Frequenz an positiven Proben von Patienten mit der Diagnose einer rheumatischen Erkrankungen, die nicht der cP entsprach.

RA33-Antikörper ohne begleitende U1-nRNP Antikörper sind ein hochspezifischer Marker der chronischen Polyarthritis. Die vorliegenden Ergebnisse lassen vermuten, daß der ELISA in einem relativ hohen Prozentsatz falsch positive Werte ergibt und eine diagnostische Verwertbarkeit erst nach Bestätigung durch einen kommerziell derzeit noch nicht erhältlichen Immunoblot sinnvoll ist.

Literatur

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Specificity and Distribution of ERE-Binding Proteins in Human Reproductive Cancers, and Their Relationship to Known Prognostic Factors

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While screening extracts of human reproductive cancers for estrogen receptors using [^{32}P]labeled vitellogenin A2 estrogen response elements (ERE) in gel mobility shift and super-shift assays, we observed additional macromolecules associating with ERE. We conducted a comprehensive evaluation of these ERE-binding proteins in extracts of human breast, endometrial and ovarian carcinomas using gel-mobility shift and supershift assays with several domain-specific monoclonal antibodies to human estrogen receptor (hER). Using recombinant hER as a control, it was shown that the ERE-binding proteins were not estrogen receptors. One of these ERE-binding proteins appeared to recognize response element sequences in a manner similar to a known orphan receptor, COUP-TFI, in the steroid hormone/thyroid hormone receptor super-family, when compared with COUP-TF1 controls (lysates of Jurkat and K562 cells). However, certain polyclonal antibodies to COUP-TF1 (Santa Cruz Biotechnology) did not react with the ERE-binding proteins. The proteins recognized four other ERE sequences (cathepsin D, h-fos, jun & pS2) with different affinities than those of hER. Neither patient age nor sex influenced the expression pattern of ERE-binding components. Furthermore, no relationship to carcinoma type nor to estrogen and progestin receptor status of the tissue biopsy was observed. Our discovery of orphan receptor-like molecules (i.e., COUP-TFI) in reproductive cancer suggests further study to determine their role in receptor-mediated signaling.

B.V. is a Visiting Medical Research Fellow, University of Vienna. Research supported in part by a grant from Phi Beta Psi Sorority, the Summer Research Scholarship Program (ACF) & the NCI Dental Research Fellowships (AP).

Molecular Dissection of Estrogen Receptor-initiated Cascades for Drug Discovery

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Sex-hormone receptor proteins are members of a family of ligand-activated transcriptional factors that form both homo- and hetero-dimers with their cognate hormone response elements (HRE) in the region of promotor sequences. Nuclear receptors recruit accessory proteins (coactivators, corepressors) to gene sequences involved in transactivation altering gene expression. To dissect steps in estrogen signaling pathways, recombinant human estrogen receptors (rhER) and progestin receptors (PR) from calf uterus were used in radio-

ligand binding assays in titration and competition modes to directly assess influence of certain hormone mimics and combinatorial peptide libraries. We developed miniaturized, double-isotope assays using [^{125}I]- and [^{3}H]-labeled ligands. These techniques measure alterations in both ligand binding capacities and affinities inferred by candidate inhibitors. To determine influence of candidate drugs on HRE recognition, either rhER or PR was incubated with a candidate compound; receptors were reacted with [^{32}P]HRE and separated with gel-mobility shift (GMSA) and super-shift assays (SSA). These assays may be modified by use of different HRE sequences known to exist in sex-hormone target organs. GMSA and high performance liquid chromatography in size exclusion and ion-exchange modes are used to assess influence of a candidate drug on receptor dimerization as well as association with accessory proteins. Sensitivities and reproducibilities of these techniques were well within limits accepted for detection of a candidate drug's influence on reactions in the estrogen-initiated cascade.

E.Z. is a Research Scholar from the University of Innsbruck, Austria. Supported in part by a grant from Phi Beta Psi Sorority.

Diagnostic BioChip *Array* for the Detection of *Ki-ras* Mutations in Stool

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A BioChip *array* for the detection of *Ki-ras* mutations in codons 12 and 13 was developed. To achieve the required analytical performance of a BioChip *array* for diagnostic applications, many of the currently used components and techniques such as substrate material, coupling chemistry, arraying, probe design, hybridization and high resolution scanning were reevaluated and replaced by appropriate new approaches. Capture probes (CP) for 10 most common mutations (incidence > 98%) in codons 12 and 13 and controls were designed using a new nearest neighborhood algorithm. 1,5 nl of each CP, consisting of photolabel, spacer and oligonucleotide were spotted in duplicates using a piezo pump onto a novel plastic surface with subsequent UV-illumination for covalent coupling. DNA was extracted in triplicates from 200 mg stool followed by a mutation enrichment PCR. Cy5-labeled PCR-products were denatured and hybridized for 1 hour at 37°C. Without stringent washing hybridization signals were detected by high resolution fluorescence scanning or by chemiluminescence detection. The performance of the BioChip fulfilled all necessary criteria for diagnostic applications (intra-assay CV < 7%, inter-assay CV <14%, match/mismatch ratio for all mutations >1:15). In a pilot investigation 31 stool samples of patients at risk for colon cancer were investigated. 9 mutations could be detected and confirmed by sequencing of the PCR-products. Mutation detection on the Chip was superior (sensitivity and specificity) to the reference methods (mutation enrichment PCR, PCR-RFLP).

Independent Prognostication, Drug Targeting and Therapy Monitoring of Breast Cancer Patients by DNA/RNA Typing of Minimal Residual Cancer Cells (MRCC)

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A novel analytical technology for purification and multiparametric molecular characterization of MRCC was developed and clinically evaluated in blood and samples of n=768 breast cancer patients. This technique fulfils all prerequisites of an IVD product for cancer analytics: (1) ≤100% sensitivity and specificity, (2) independent prognostication, (3) therapy monitoring leading to the identification of responder patients prior to clinical endpoints, (4) applicable for parmacogenomics, (drug targeting; detection of splice variants, SNP's, effectors and therapy resistance), (5) early detection of tumors and (6) improvement of clinical results. MRCC are purified from blood or bone marrow by a new antigen-independent filtration process. DNA-typing of MRCC is performed with a set of 18 genomic imbalances (GI) (LOH, amplifications, mutations). For mRNA typing quantitative real time RT-PCR tests have been developed for analytes dealing with drug targeting, (pre)apoptosis, metastatic potential, angiogenesis, cell cycle, cell dormancy, therapy resistance, others. Complete medical files of n=534 patients were evaluated for time to relapse with respect to lymph node and menopausal status, grading and tumor size showing GI to be an independent prognostic marker (risk ratio 1.66, p=0.005). Genotyped cells have successfully been applied for risk evaluation, therapy monitoring and drug targeting (including alternative splicing and silencing of targets).

Structure-Based Drug Design of Z-DNA Intercalators

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There is intense interest in the stabilization of novel DNA structures for the development of new anticancer agents. Recently it has been shown by equilibrium dialysis, fluorescence spectroscopy, and circular dichroism that (-)-daunomycin (WP900), an enantiomer of (+)-daumomycin, binds preferentially to left-handed DNA. Furthermore, WP900 was found to be an allosteric converter of right-handed DNA (B-form) to left-handed DNA (Z-form). This was the first report of an intercalator, or any other DNA–binding ligand, to possess a high conformational preference and allosteric effect.

Computer simulations have rationalized the Z-DNA preference for WP900 using fully solvated molecular dynamics calculations. We have extended this work to consider sequence effects and the inclusion of multiple intercalation sites and have designed new Z-DNA intercalation ligands.

Wissenschaftliche Sitzung

Samstag, 2. 12. 2000, 10.00 bis 12.00 Uhr – Ausgewählte Freie Vorträge – Posterpräsentationen
Vorsitz: A. v. Eckardstein, J. Hofmann

BNP is Superior to NT-proBNP or NT-proANP in Diagnosing Impaired Left Ventricular Dysfunction

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Introduction: Natriuretic peptides seem to be useful markers for diagnosing asymptomatic left ventricular dysfunction (LVD).

Methods: From 63 patients (45-80 years) who underwent 3D echocardiography and radionuclid ventriculography, EDTA-blood was drawn. Patients were divided into two groups with normal and impaired resting left ventricular ejection fraction (LVEF) and in a second analysis in patients with resting LVEF <40% or >40%. Brain natriuretic peptide (BNP), NT-proBNP and NT-proANP (atrial natriuretic peptide) were compared for diagnosing impaired LVEF. Enzyme immunoassays (Biomedica) and immunoradiometric assays (Shionogi) were used.

Results: For the detection of impaired LVEF areas under curve (AUC) were 0.75±0.06 (BNP), 0.67±0.07 (NT-proBNP) and 0.69±0.08 (NT-proANP; differences not significant). For detection of resting LVEF <40% AUC were 0.83±0.06 (BNP), 0.79±0.07 (NT-proBNP) and 0.65±0,08 (NT-proANP, difference between BNP and NT-proANP was significant). In contrast to NT-proANP, BNP and NT-proBNP correlated inversively with the resting LVEF (BNP: r=-0.472, p<0.01; NT-proBNP: r=-0.306, p<0.05).

Conclusion: BNP was tendentiously the best marker to detect patients with impaired LVEF. The differences between BNP and NT-proBNP were not significant and therefore, NT-proBNP is an interesting new marker.

Relations of Mean Cellular Volume to Serum Homocysteine, Vitamin B12 and Folate

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Objective: Vitamin B12 (B12) and folate deficiency is related to both, increased mean cellular volume (MCV) and raised serum total homocysteine (Hcy) values. But there are indications that B12 and folate values do not represent the tissue status of the two vitamins exactly. It was therefore of interest, whether there is a direct relationship between MCV and Hcy, which would support the hypothesis, that Hcy may be a better indicator for the cited vitamin status.

Methods: We studied MCV, gamma glutamyl transferase (GGT), B12, folate and Hcy values in 200 hospitalized patients. MCV was determined by impedance method, GGT by optimized standard method according to IFCC, B12 by microparticle-enzyme-immuno-assay, folate by ion-capture-assay and Hcy by HPLC. Independant variables were logarithmically transformed to ensure normal distribution and linear association with MCV.

Results: Bivariate comparisons showed a positive correlation of MCV with GGT ($r = 0.266$, $p < 0.001$) and with Hcy ($r = 0.248$, $p < 0.001$), but not with B12 and folate. Stepwise multiple linear regression with MCV as dependent and GGT, B12, folate and tHcy as independant variables revealed significant associations of MCV with GGT (B = 2.18, 95% CI 0.95 - 3.42, p = 0.001) and Hcy (B = 3.33, 95% CI 1.26 - 5.39, p = 0.002). When removing Hcy from this model B12 became a significant predictor of MCV (B = -1.70, 95% CI -3.25 - -0.15, p = 0.032). Folate was not significantly associated with MCV in multivariate analysis.

Conclusion: There is evidence that Hcy enables clinicians to identify patients with B12 or folate deficiency even if B12 and folate values are within the reference intervall. The present study confirms these indications by showing MCV was better associated to Hcy, than to B12 or folate. Such observations demonstrate Hcy may be useful in diagnosing patients with B12 or folate deficiency.

Quantification of Minimal Residual Disease in Patients with Acute Promyelocytic Leukemia (APL) by Real Time Quantitative RT-PCR with Specific Fluorescent Hybridization Probes

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The majority of APL cases are associated with the translocation t(15;17)(q21;q12) which results in fusion of the retinoic acid alpha nuclear hormone receptor *RARα* to the *PML* gene. We have developed an automated real-time RT-PCR assay based on LightCycler technology for reliable and sensitive detection and quantification of *PML-RARα* fusion transcripts. The method allows the reproducible amplification and quantification of 10 molecules per reaction, and the detection of 1 blast cell among 10^4 (bcr1-/L-form) to 10^5 (bcr3-/S-form) normal cells. Intra- and inter-assay variation coefficients were lower than 19% and 10%, respectively. In all samples, *ABL* transcripts were quantified as internal control to determine the amount of amplifiable mRNA/cDNA. The percentage of *PML-RARα* molecules/*ABL* molecules was calculated. A total of 568 consecutive bone marrow and peripheral blood samples from 15 patients (median, 35 samples/patient; range, 9-81 samples) collected at diagnosis and at various times after conventional chemotherapy and/or bone marrow transplantation were first analyzed by 2-step RT-PCR. Then, all *PML-RARα*-positive samples were quantified by LightCycler-PCR. In 5 patients, increasing levels

of *PML-RARα* fusion transcripts were detectable in complete remission. All 5 patients experienced a hematologic relapse within 0.6-3.2 months. Our data demonstrate that quantification of *PML-RARα* mRNA allows the assessment of the dynamics of the leukemic clone and the early detection of a continuous increase. Thus, quantification is valuable for the evaluation of minimal residual disease following various therapies and for early detection of relapse.

A Frequent C→T Polymorphism in the Promoter Region of Coagulation Factor XII (FXII) Is a Protective Factor for the Development of the Acute Coronary Syndrome

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Background: In vitro, in the presence of negatively charged surfaces factor XII is activated to FXIIa, which can activate the intrinsic coagulation pathway as well as plasminogen and the fibrinolytic system. The biological role of FXII is still discussed controversially. Increased serum antigen levels of FXIIa were found to represent an independent risk factor for the development of myocardial infarction. Recently, a frequent C→T polymorphism at nucleotide –46 in the promotor region of the FXII gene was identified. The sequence change destroys a Kosak consensus sequence, leads to a decrease in transcription and significantly lower levels of FXII plasma activity and antigen. To evaluate the clinical significance of this polymorphism, we developed a new mutagenic-separated PCR-assay for rapid and economic analyses in large groups of patients.

Patients: A total of 504 unselected hospitalized patients from the division of cardiology, Derpartment of Internal Medicine II of the General Hospital of Vienna were included. Three groups were formed: no coronary artery disease (CAD, n=193), stable CAD (NYHA II-III, n=142) and CAD with acute coronary syndrome (ACS, n=169). All patients with ACS underwent coronary angiography for confirmation of the clinical diagnosis.

Results: Patients with CAD and with CAD and ACS were compared to patients without CAD

Genotype	No CAD	Stable CAD	CAD and ACS
Wildtype	62.2%	54.2%	55.6%
FXII -46C		n.s.	n.s.
Heterozygotes	29.0%	38.7%	41.4%
FXII C-46T		n.s.	(p<0.05; OR=1.2, 95%CI: 1.0-1.4)
Homozygotes	8.8%	7.0%	3.0%
FXII -46T		n.s.	(p<0,05; OR=0.3, 95%CI: 0.1-0.9)

Conclusion: Our data indicate that homozygosity for FXII –46T may represent a protective factor for the development of ACS (odds ratio=0.3, 95%CI: 0.1-0.9).

Persistierende polyklonale B-Zell-Lymphozytose mit zweikernigen Lymphozyten

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Die persistierende polyklonale B-Zell-Lymphozytose (PPBL) ist ein seltenes Syndrom, welches zytologisch durch das Auftreten zweikerniger Lymphozyten im peripheren Blutausstrich, immunophänotypisch durch den Nachweis einer absoluten B-Zell-Lymphozytose ohne klonale Leichtkettenrestriktion und serologisch durch eine polyklonale IgM Erhöhung gekennzeichnet ist. Die PPBL tritt vor allem bei jüngeren Frauen mit Nikotinabusus auf; genetisch besteht eine Assoziation zu einer HLA-DR7 Expression. Die Lymphozytose bleibt über Jahre stabil; das Krankheitsbild nimmt meist einen gutartigen klinischen Verlauf.

Im Donauspital wurden bisher drei Patientinnen mit PPBL diagnostiziert. In allen Fällen wurden die labordiagnostischen Charakteristika der PPBL (Durchflußzytometrie: B-Lymphozyten 1.2-2.7x10^9/l, Ig-Rearrangements negativ, keine Progression: Beobachtungszeitraum 16-40 Monate) mit morphologischen Atypien (große Lymphozyten mit zartbasophilem, weitem Zytoplasmasaum und den charakteristischen zweikernigen Lymphozyten) und polyklonaler IgM Erhöhung (6.3-15.7 g/l, Immunfixationen neg) gefunden. Bei der HLA-Typisierung konnte in allen Fällen HLA-DR7 nachgewiesen und anamnestisch ein langjähriger Nikotinabusus erhoben werden . Klinisch bestand bei keiner der Patientinnen eine Lymphadenopathie oder Splenomegalie. Die in der Literatur bei PPBL häufig beschriebenen Chromosomenanomalien (+i(3)(q10)) und/oder frühzeitige Chromosomen-Kondensation wurden in keinem der Fälle gefunden.

Zusammenfassung: Erkennen, Abklärung und Monitoring bei PPBL sind wichtig zur

1. Vermeidung invasiver diagnostischer und/oder therapeutischer Maßnahmen;
2. Beantwortung der Frage, ob die PPBL eine benigne oder prämaligne Veränderung darstellt, da die bisher in der Literatur beschriebenen Fälle für eine diesbezügliche Stellungnahme nicht ausreichen.

Fluorescence Rapid-Cycle PCR in Temporarily Compartmentalized LightCycler™ Capillaries. Part I: Two-round Nested Primer PCR in a Single Closed Capillary

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For many diagnostic applications the specificity and sensitivity of PCR analysis is markedly enhanced by applying two rounds of PCR with nested pairs of primers. In rapid-cycle PCR using the LightCycler™ instrument nested PCR suffers from the capillaries' inaccessibility to retrieve ampli-

cons by pipetting. Amplicons are usually collected from the capillaries by centrifugation, a procedure particularly prone to product carry-over. To address this problem a general two-round PCR method performed in a single closed capillary was developed.

We demonstrate the feasibility of the method using nested primer PCR for the detection of the MBR/JH chromosomal translocation in cells of the human lymphoma cell line DOHH2. The 1st round PCR reaction mixture including 1st round primers and DNA was applied to the capillary and covered by a layer of silicone oil. Then, the 2nd round PCR reaction mixture containing the 2nd round nested primers was applied on top of it. PCR was run in two rounds. A centrifugation step after the 1st round PCR combined the second-round PCR mixture with the 1st round products. Amplicons were analyzed by fluorescence melting curve analysis using SYBR Green I.

The single capillary two-round nested PCR detected the MBR/JH fusion gene within the range of 50 ng to 0.05 ng DNA from DOHH2 cells. In some experiments the MBR/JH specific amplicon was also detected from 0.01 ng DNA. Compared to a single-round PCR performed in parallel a 10–50 fold increased sensitivity was achieved. Furthermore, we show that the oil layer sufficiently separates the 1st from 2nd round reaction mixture.

In conclusion, we have developed a general method to perform two-round rapid-cycle PCR in single closed LightCyclerTM capillaries. Since the capillaries remain closed during the procedure, amplicon carry-over is prevented. The described method is simple and can easily be applied for numerous PCR applications.

Fluorescence Rapid-Cycle PCR in Temporarily Compartmentalized LightCycler™ Capillaries. Part II: Amplicon Detection by SYBR® Green I Followed by FRET-hybridization in a Single Closed Capillary

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The analysis of amplicons during or after rapid-cycle PCR has been described to using SYBR® Green I, FRET-hybridization probes, Taqman probes or molecular beacons. When FRET-hybridization probes are chosen the PCR is performed in their presence. Albeit carefully designed these probes may sometimes cause the PCR to run inefficiently, or occasionally inhibit the PCR. When SYBR Green I is used for amplicon detection, it is assumed that the melting temperature is to be specific for a given amplicon. However, in some diagnostic or analytical applications it is desirable to additionally analyze parts of the sequence of the amplicon by e.g. hybridization. Combining two amplicon detection formats within one capillary would, therefore, contribute to greater confidence about the nature of the amplicon(s) obtained, and thus, would enhance the reliability of the results obtained. Also, in those cases which hybridization probes hamper the PCR it is desirable to separate the amplification reaction from the detection by hybridization.

To address these points we have developed a simple general method to perform rapid-cycle PCR followed by amplicon analysis using SYBR® Green I and FRET-hybridization probes. This is accomplished by temporarily compartmentalizing the LightCycler's capillary using silicone oil. As a result the PCR mixture is separated from the hybridization probes for the duration of the PCR. Thereafter, by a brief centrifugation of the capillary the hybridization probes are combined with the presumed amplicons to initiate hybridization.

We demonstrate that this method permits within a single closed LightCycler capillary (i) the monitoring of amplicon generation in real-time fashion during PCR, (ii) the analysis of amplicons generated by the assessment of their melting temperatures, and (iii) the analysis of amplicons generated using hybridization probes. This method is simple and convenient. It should especially prove useful to all applications which sequence constraints prevent ideal probe and primer design, and amplicon analysis by e.g. FRET-hybridization is still wanted.

Detection of Specific ERE-binding Proteins in Colon Diseases

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As a complement of a larger investigation of estrogens, their environmental mimics and their receptor proteins in extracts of tissue biopsies from endocrine related carcinomas (e.g., breast, endometrial and ovarian), we sought tissues accepted as estrogen unrelated for our controls. Colon carcinoma, various non-malignant colon diseases, such as chronic ulcerative colitis, Crohn's disease and familial adenomatous polyposis and normal colon were selected because of the virtual absence of estrogen receptors (ER). Using gel-mobility shift assays, four different proteins were detected binding specifically to wild-type vitellogenin A2 estrogen response elements, which are DNA sequences positioned in 5'-flanking regions of estrogen responsive genes. ERE-binding components detected were not estrogen receptors *per se* as demonstrated by ER monoclonal antibody-based super-shift assays. At least one of these ERE binding proteins appeared to recognize ERE sequences and behave in a manner similar to a known orphan receptor, COUP-TFI, in the steroid hormone/thyroid hormone receptor super-family, when characterized by Western blotting. There did not appear to be a tissue or disease dependent specificity to appearance of ERE-binding proteins and neither patient age nor sex influenced the pattern of ERE-binding components. To our knowledge this represents the first report of orphan receptor-like molecules (i.e., COUP-TFI) in normal colon, colon carcinoma or the non-malignant colon diseases, chronic ulcerative colitis, familial adenomatous polyposis or Crohn's disease. Collectively, these results and the growing evidence for a contribution by sex-hormones in colon diseases warrant further study.

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Evaluierung des Kapillarelektrophoresensystems Paragon CZE 2000 (Beckman) für das Routinelabor

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Das Kapillarelektrophoresensystem Paragon CZE 2000 (Beckman) wurde in unserem Labor für die Erstellung der Serumelektrophorese und für die Detektion monoklonaler Serum-Immunglobuline (Immunsubtraktionselektrophorese) verwendet.

Die Ergebnisse der Serum-Kapillarelektrophoresen und der Immunsubtraktionselektrophoresen wurden mit den Ergebnissen des Olympus Hite 310 (CA-Folien) bzw. der Gel-Immunfixations-Methode (Paragon SPE Agarose Gel) verglichen. Ziel der Arbeit war ein Vergleich der Sensitivität, Spezifität aber auch eine Evaluierung der Wirtschaftlichkeit (Zeitstudie) der Methoden.

Die technischen Einrichtungen des Paragon CZE 2000 (Befundung über Bildschirm, Vergrößerung und Überlagerung abweichender Kurvenverläufe) erleichtern das Erkennen auch schwach ausgeprägter pathologischer Veränderungen der Elektrophorese. Mit einer nachfolgenden Immunsubtraktionselektrophorese läßt sich ein monklonales Protein auch bei kleinen Konzentrationen nachweisen (insbesondere IgA). Die Zeitstudie am voll automatisierten Paragon CZE 2000 ergab Einsparungen bei Personalkosten gegenüber den oben erwähnten traditionellen Methoden.