Biomarker discovery for the early diagnosis of cervical cancer

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the property of quercetin to change the spectral properties upon binding to specific target proteins. The protein autofluorescence induced by excitation at 285 nm is quenched by the quercetin ligand and in the visible spectrum fluorescence may be induced (Ex485nm, Em545nm). These effects were studied in detail using bovine serum albumin and insulin as model proteins. The induced fluorescence can be exploited to localize target proteins in living cells by fluorescence microscopy. A high concentration of target proteins was found to be present in nuclei. By making use of the induced spectral changes we identified some major target proteins of quercetin in nuclear extracts of human leukaemia cells (HL-60). We have fractionated nuclear proteins by column chromatography, probed the fractions with quercetin, analysed the spectra in a fluorescence spectrophotometer, and finally separated promising protein fractions on SDS-PAGE. Single bands were cut out and the proteins identified by MALDI-MS. In this way we identified, amongst others, actin as a quercetin binding protein. The interaction was confirmed using purified actin. This protein has recently been shown to play an essential role in transcription. This experimental approach opens up new ways in interpreting and predicting biological and pharmacological effects of drugs of interest.

L1-015P
Immunoproteomic approach identifies novel proteins of *Aspergillus fumigatus* with specific IgE immunoreactivity

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Allergic bronchopulmonary aspergillosis (ABPA), a severe respiratory disease in humans, is caused by allergenic/antigenic proteins of *Aspergillus fumigatus* inducing type I and type III hypersensitivity reactions. Since a number of secretory proteins are reported to be allergenic/antigenic/virulent factors which are in direct contact to the host tissue mediating important host–pathogen interactions, an immunoproteomic studies have been undertaken to map secretory allergenic proteins by modern proteomic approach to identify novel allergens with specific IgE immunoreactivity. Comparative analysis of 2-DE gels (coomassie-stained) and specific IgE immunoblots of *A. fumigatus* culture filtrate proteins at different time intervals was performed. We observed a total of 159 proteins in culture filtrate of *A. fumigatus* out of which 75 proteins showed specific IgE immunoreactivity. Third week culture filtrate had maximum number of proteins and specific IgE immunoreactive proteins. MALDI-TOF analysis of 33 spots showing specific IgE immunoreactivity led to the identification of 25 proteins, 19 of which are new to *A. fumigatus* proteome database and six are known allergens of *A. fumigatus*. Out of 19 proteins function is known for eight proteins in other fungi: NADPH dependent alcohol dehydrogenase, mitochondrial matrix acyl carrier protein, DST, SEC5, two putative GSTs, 60S ribosomal subunit, cytochrome P450, acid proteinase precursor and eleven were hypothetical proteins. For eight proteins conclusive match could not be obtained. Availability of allergenic proteome and 19 novel allergens/antigens would facilitate a sensitive and specific diagnosis, immunotherapy and further understanding of the biology of the fungus.

L1-016P
Binding of IgM antibodies to bovine serum albumin as a biomarker for type 1 diabetes mellitus

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The aim of this study was to investigate the humoral immune response to bovine serum albumin (BSA) and their relation to the pathophysiology of type 1 diabetes mellitus. Serum immunoglobulin M (IgM) concentration was measured by enzyme-linked immunosorbent assay (ELISA) in 25 adult patients with newly diagnosed type 1 diabetes mellitus, and 25 matched normal adult individuals. Binding of BSA to diabetic serum immunoglobulins led to an over-estimation in the levels of IgM in human diabetic sera. The increase detected by ELISA and turbidimetric assay varied between 10% and 109%. If anti-BSA antibodies were present in serum, they might interfere with the ELISA assay, thus a suitable method was employed to minimize such interference. Initial results before purification from the interfering anti-BSA antibodies suggested that diabetic patients had incremented levels of IgM in their sera. It was found that normal individuals had a mean IgM level of 1.67 mg/ml and diabetic individuals had a mean IgM level of 2.30 mg/ml ($P < 0.0003$). However, the mean level of IgM in diabetic sera after purification from anti-BSA antibodies was 1.69 mg/ml. Therefore, there was no significant difference in IgM level in patients with type 1 diabetes mellitus purified from anti-BSA antibodies, as compared to normal individuals ($P < 0.84$). In conclusion, a high level of heterophile antibodies reactive with BSA commonly associates with type 1 diabetes and may well play a role in the complex immunopathogenetic interactions. However, the demonstration of the binding of IgM antibodies to BSA in patients with newly diagnosed type 1 diabetes initiated a controversial debate on the utility of BSA antibodies as a disease marker.

L1-017P
Biomarker discovery for the early diagnosis of cervical cancer

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Aim: Development of an analytical method for the comparative analysis of serum samples in the benefit of biomarker discovery for cervical cancer diagnosis.

Introduction: Cervical carcinoma is the second most frequent carcinoma in women worldwide, while in the developing countries cervical carcinoma is the most frequent carcinoma in women. In an approach to perform comparative analyses of samples from a serum bank from patients in a longitudinal and cross-sectional manner, we have developed a methodology for the comparative analysis of depleted, trypsin digested serum samples by LC-MS followed by data pre-processing and multivariate statistics.

Results: Optimization of the analytical method from sample preparation (clotting time, various depletion methods of the most abundant proteins) to the final LC-MS analysis were performed to lower the within sample variability. Further improvement of the reproducibility of the overall procedure was achieved by the use of horse heart Cytochrom C as internal

standard added to the sample prior to sample preparation. The obtained LC-MS data were pre-processed prior to statistical analysis by alignment of retention time and the normalization of intensity of the chromatograms by using specific internal standard peaks prior to selecting the most ''information rich'' m/z traces. The selected chromatographic traces containing a significantly decreased level of spikes and noise, were subjected to unsupervised multivariate statistics (principal component analysis). In an effort to validate the methodology, we spiked various amounts of horse heart Cytochrom C into the original serum and found that samples containing the internal standard were discriminated from the non-spiked samples down to a level of 1 pmol in 20 μl serum.

Perspectives: In order to further improve the sensitivity of the overall method, we performed comparative studies using an on-line nanoLC-MALDI spotter set-up combined with MALDI-TOF/(TOF)-MS*. Using the described methodology, we are presently performing a comparative, longitudinal study with samples from early and late stage cervical cancer patients prior to and after treatment. Furthermore, we are comparing samples from patients with a positive or negative prognosis in order to define new discriminatory biomarkers or biomarker patterns.

*Collaboration with Dr. Theo Luider (Erasmus Medical Centre, Rotterdam).

L1-018P
Assessment of urinary and serum cystatin C in determination of renal function in children with renal scar

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Urinary tract infection (UTI) is a frequently encountered problem in childhood, leading to scar development in renal tissue with sometimes impaired renal functions. The existing routine laboratory renal function tests might not reflect slight changes or have some other limitations due to age, body mass, etc. dependence in the follow-up of children with pyelonephritis. The aim of this study was to evaluate changes in plasma and urine concentrations of cystatin C, a novel glomerular filtration rate marker; creatinine, sodium, phosphorus, as well as urine N-acetyl-beta-D-glucosaminidase (NAG) activity, a sensitive and specific tubulary damage marker and microalbumin level in children with renal scar. The study group comprised children with pyelonephritis ($n = 18$) with renal scar and ($n = 10$) without scar both groups diagnosed with dimercaptosuccinic acid (DMSA) renal scans. Cystatin C in urine and serum was determined using ELISA. There were no significant differences between renal scar positive and negative patients regarding age, gender, body weight and length, serum cystatin C, serum creatinine, creatinine clearance, tubulary phosphate reabsorption, fractional sodium excretion, microalbuminuria and urinary cystatin C and NAG levels. However, there was a significant correlation between 1/serum cystatin C and both endogen creatinine clearance ($r = 0.385$, $P = 0.047$) and glomerular filtration rate (GFR) calculated with Schwartz formula ($r = 0.396$, $P = 0.041$) while the same correlation could not be found with 1/serum creatinine. This data suggest that children with renal scar diagnosed with DMSA does not show any change in serum, urine cystatin C and other renal function tests. However, cystatin C-based GFR estimate is better than creatinine in those patients.

L1-019P
Developmental expression of neogenin protein in human brain

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Neogenin (NGN) protein was first identified in a chicken as developmentally highly regulated protein in neuronal tissue, suggesting a role in the generation of the fully functional nervous system. To address possible role of NGN in a critical period for human brain development we used an affinity-purified antibody raised against NGN of human origin in Western blot, immunoperoxidaze histochemistry and immunofluorescent multilabeling analyzing NGN cell-origin and its spatio-temporal expression, on postmortem human fetal brains, staged from 10th week of gestation (w.g.) to newborn. The most prominent feature was perinuclear and extracellular expression in subventricular subcallosal zone below anterior extent of corpus callosum (cc) in cells with low neuron-specific nuclei protein (NeuN) expression or activated caspase-3 and on the surfaces of growing fibers in ventral cc at midgestation. Furthermore, strong cellular NGN expression was displayed where fibers of fornix diverge from cc, as well as in the fornix fibers on the insertion of plexus chorioideus. At about 30 w.g. the most prominent was expression confined to bushy astroglial like cell-population in the developing putamen. From 35 w.g. to newborn we could only observe a very low level of NGN expression in cells in subventricular zone laterally to and in cc.

In conclusion, NGN is expressed during important gestational developmental window showing topographically very specific localization confined to a small number of differentiating cells or cells undergoing apoptosis and to some midline growing fibers. In developing human brain NGN expression decreases and disappears during latest fetal stage.

L1-020P
The investigation of serum N-acetyl-β-D-glucosaminidase and its isoenzymes as markers of the progression of diabetic complications in IDDM

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Significantly increased of serum N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30) activity in diabetic patients, especially in diabetics with secondary complications was found. However, the results obtained for total NAG and its relationship with development of the secondary diabetic complications are often contradictory and unexplained. Consequently, we have attempted to establish whether total NAG and/or NAG isoenzymes can provide additional diagnostic information regarding diabetic status and the complications of diabetes. The serum NAG isoenzymes in control ($n = 18$) and in four groups of IDDM patients (1st – without complications, $n = 20$; 2nd – with retinopathy, $n = 6$; 3rd – with retinopathy and neuropathy, $n = 11$; 4th – with retinopathy, neuropathy and nephropathy, $n = 12$) were separated by ion-exchange chromatography on DEAE cellulose. In all diabetic groups there were a statistically significantly increase ($P < 0.001$; $P < 0.01$) of total NAG activity compared to the control. Analysis of isoenzyme profiles in all diabetic groups showed significantly decreased ($P < 0.001$) contribution of the B form to total NAG activity (15.1 ± 4.5%; 16.3 ± 3.4%; 18.3 ± 6.0%;