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S1 Genomic variants of PAX4: possible link to beta cell regenerative capacity

Lauber-Biason A., Rosa S., Meyer M., Schoenle E. J. (Zurich)

Purpose: Loss of pancreatic beta cells is the crucial event in the development of type1 diabetes mellitus and the result of an imbalance between autoimmune destruction and insufficient regeneration of islet cells. To study the role of islet cell regeneration in the pathogenesis of type 1 diabetes we focused on PAX4, a paired homeodomain transcriptional repressor that determines exclusively islet cell growth.
Methods: 379 diabetic children and 1070 controls from two distinct populations and a cohort of children who never developed type 1 diabetes despite the presence of islet cell antibodies were enrolled in the study. Genomic DNA analysis of the PAX4 gene was carried out via direct sequencing of polymerase chain reaction amplified fragments and allelic discrimination. We compared transactivation capability of the PAX4 variants in TC3 cells and analyzed their influence on beta-cells growth.
Results: Two variants of PAX4 are differentially distributed among normal individuals and type 1 diabetes children. The PAX4C variant is frequent in type 1 diabetes children (73%) and rare in the control population (32%), whereas the heterozygote PAX4A/C combination is prevalent in controls (62%) and in type 1 diabetes-free antibody-positive children (73.6%) but uncommon in type 1 diabetes (17.5%). PAX4A/C is functionally more active than the PAX4C "diabetic" variant. Beta cells expressing the PAX4A/C set-up, but not those expressing the PAXC variant, efficiently proliferate under glucose stimulation.
Conclusions: We identified a link between beta cell regenerative capacity and susceptibility to type 1 diabetes, and could provide one possible explanation for the fact that only a part of the individuals who develop autoimmunity against beta cells actually contract the disease. PAX4 C can be seen as a predisposition marker that can help to detect individuals prone to develop type 1 diabetes.

S2 Promoter methylation of tumor suppressor genes in circulating plasma DNA quantified by pyrosequencing: potential as tumour marker

Kern I., Deutsch S., Yasrebinejad H., Chappuis P. O., McKee T. A., Rossier M. F., Hochstrasser D. F. (Geneva)

Epigenetic silencing of tumor suppressor genes through promoter methylation has been recognized as an early and common mechanism underlying carcinogenesis. Development of the highly sensitive Methylation Sensitive PCR (MSP) has allowed methylation profiling not only in tumour tissues, but also in body fluids, opening the perspective of using MSP on a tumour suppressor gene panel as a tumour marker. However, MSP is limited by its incapacity to identify partially methylated promoters. This shortcoming can be overcome through pyrosequencing, a novel quantitative DNA sequencing technique, that we have used to evaluate the methylation status of tumour cell lines, tumour tissues and circulating plasma DNA.
Genomic DNA was extracted, treated with bisulfite, which converts unmethylated cytosines to uracil while leaving methylated cytosines intact, and amplified by PCR. Specific primers were designed to amplify 65–240 bp fragments in CpG rich regions of the promoters of Rassf1a, p16 (CDKN2A), DAP-kinase, BRCA1 and hMLH1. Methylation status of 3 to 5 CpG sites per gene was determined by pyrosequencing.
Distinct hypermethylation profiles were found in various tumour cell lines and tissues. Differences in degree of methylation of individual CpG sites were noted within and between genes. Preliminary results of methylation analysis of circulating plasma DNA by pyrosequencing, an application that has not yet been reported in the literature, are shown. The sensitivity of the method is high, allowing analysis of as few as 50–100 pg of DNA per screened gene.
The next step will be the technical validation of this method on plasma DNA samples taken from cancer-affected patients. After that, we should be able to determine whether quantitative promoter methylation analysis by pyrosequencing of a tumor suppressor gene panel is indeed a promising tool for early cancer diagnosis and therapeutic follow-up.

S3 Two different genetic macular dystrophies in one family: Age-related macular dystrophy and Sjorsby fundus disease

Hergersberg M., Suter C., Gallati S., Wolf S., Wolf U., Huber A., Sarra G. M. (Aarau, Berne)

Background: Macular degeneration (MD) is a common form of visual impairment originating through different pathogenetic pathways. A pathologic feature of several MD is the accumulation of extracellular "drusen" between the retina pigment epithelium and the choroid. By far most common and therefore most important MD is age-related macular degeneration (AMD), which is a complex disease, resulting from different combinations of life style and inherited factors. Several alleles in genes encoding different factors of the complement system, as well as in the gene LOC387715 of unknown function confer susceptibility to or protection against development of AMD. Several genes cause rare inherited forms of MD when mutated. One of them is the gene encoding tissue inhibitor of metalloproteinases-3 (TIMP3), which is mutated in Sorsby fundus dystrophy (SFD). SFD is inherited in an autosomal dominant mode and shows broad phenotypical variation, suggesting the effect of modifier gene(s). We tested the hypothesis, if the AMD susceptibility allele Y402H of the complement regulatory factor (HF1/CFH) is modifier allele for the phenotype in a multigenerational SFD family.
Probands and methods: DNA of 59 family members was available for analysis after informed consent. Ophthalmologic analysis of 33 family members included funduscopy, autofluorescence and optical coherence tomography (OCT). Mutation analysis of the TIMP3 gene was performed by single strand conformation polymorphism analysis (SSCA), followed by DNA sequencing (ABI sequencing automate). The genotype of the allele Y402H of HF1/CFH was determined by real-time PCR (Lightcycler).
Results: Several of the affected family

members have been found with an unusual phenotype, which includes choroidal neovascularisation. The mutation Glu139Lys was identified in exon 5 of the TIMP 3 gene. The genotype of allele Y402H of the HF1/CFH was determined in 59 family members. 15 family members were homozygous for the wildtype allele 402Tyr, 15 family members were homozygous for the AMD susceptibility allele 402His, and 29 family members were 402Tyr/402His heterozygous.

Conclusions: There was no identifiable evidence that a more severe phenotype (ie early onset of choroidal neovascularization) of TIMP3 gene mutation carriers is associated with homozygosity or heterozygosity for the AMD susceptibility allele Y402H. The identification of susceptibility alleles for AMD is relevant, because AMD treatment is possible by injection of humanized monoclonal antibodies against VEGF.

S4 Relationship between glomerular filtration rate and the adipokines adiponectin, resistin and leptin in coronary patients with predominantly normal or mildly impaired renal function

Risch L., Saely C. H., Hoefle G., Berchtold S., Weber M., Langer P., Aczel S., Drexel H. (Liebefeld, Feldkirch, Berne, Schaan)

Background: The adipokines adiponectin, resistin and leptin are low molecular weight proteins involved in the pathogenesis of metabolic disorders conferring a cardiovascular risk. Due to their molecular weight it might be possible that these proteins might accumulate, when glomerular filtration rate (GFR) is decreased. In reduced renal clearance, altered concentrations of these proteins might affect cardiovascular risk.

Methods: The association between GFR and the adipokines adiponectin, resistin and leptin was assessed in a cross-sectional study done in a cohort of coronary patients (n = 538; 363 male, 165 female). GFR was determined by means of the abbreviated MDRD equation. After calculation of correlations between GFR and adipokine concentrations the association was further assessed by analysis of covariance following adjustment for age, gender, BMI, presence of type II diabetes, presence of hypertension, history of smoking as well as for serum lipid concentrations.

Results: Mean GFR was 68.74 + 15.27 ml/min/1.73 m^2. 74.3% of the patients had a GFR >60 ml/min/1.73 m^2, 24% of the patients had a GFR between 30 and 60 ml/min/1.73 m^2, 1.7% of the patients had a GFR <30 ml/min/1.73 m^2. There were significant inverse correlations between adiponectin, resistin and leptin concentrations and GFR. After adjustment, the association remained significant for adiponectin and resistin. Subgroup analysis in the group with GFR >60 ml/min/1.73 m^2 showed a significant correlation between GFR and adiponectin and leptin concentrations. However, after adjustment the association has lost its significance.

Conclusions: There is an independent association between GFR and the concentrations of adiponectin and resistin. However, this association is not present at GFR >60 ml/min/1.73 m^2. This finding suggests that adipokine concentrations in mildly impaired and normal renal function are influenced by factors other than GFR.

P1 Therapeutic correction of PKU in a mouse model by ectopic expression of PAH and its BH4-cofactor genes in skeletal muscle by a recombinant triple-cistronic AAV2–based pseudotype 1 vector

Ding Z., Harding C. O., Rebuffat A., Elzaouk L., Wolff J. A., Thöny B. (Zurich, Portland, Madison)

Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism with a deficiency of the hepatic phenylalanine hydroxylase (PAH) leading to toxic accumu-

lation of circulating phenylalanine (Phe) in blood, resulting in growth failure, microcephaly, seizures, and mental retardation. PAH catalyzes the hydroxylation of Phe to tyrosine and needs oxygen and tetrahydrobiopterin (BH4) as cofactor. BH4 biosynthesis requires the consecutive action of the enzymes GTPCH and PTPS, with dihydroneopterin triphosphate as the first intermediate. Here we aimed at expressing the PAH system in skeletal muscle to degrade serum Phe in PKU patients, as this tissue is abundant, easy accessible, and persistent due to its post-mitotic nuclei. However, BH4 is abundant in liver but scarce in skeletal muscle, as the cofactor-synthesizing enzyme GTPCH is absent in muscle tissue, and PTPS is expressed at low levels. We first demonstrated that transgenic PKU mice that had no liver PAH and expressed coordinately PAH along with GTPCH in skeletal muscle tissue accumulated dihydroneopterin triphosphate and remained hyperphenylalaninemic unless synthetic BH4-cofactor was supplied by intraperitoneal injections. Thus, PTPS activity is definitely limiting in skeletal muscle to synthesize sufficient BH4 and to support Phe hydroxylation. A recombinant triple-cistronic AAV2-based pseudotype 1 vector expressing PAH along with the two cDNA-genes for BH4 biosynthesis, GTPCH and PTPS, was then generated. Upon single injections of at least 3.5×10^{12} recombinant triple-cistronic AAV2/1 vector particles into each of the gastrocnemius muscles of the hind legs of the PKU mouse model Pah-enu2 resulted in long-term clearance of blood Phe, including complete phenotypic reversion. A similar therapeutic effect was achieved when a combination of two AAV2/1 vectors expressing individually PAH and GTPCH-PTPS were co-injected into the same hind leg muscles. As a control, an AAV2/1-vector expressing only PAH with GTPCH was not therapeutic. This non-invasive application is the basis to develop an efficient therapy for PKU using a triple-cistronic gene transfer into skeletal muscle.

P2 CScqi, An Internal Quality Control Software for Medical and Clinical Laboratories

Grouzmann E., Vallotton P., Albe X., Thiébaud A., Deom A. (Vevey, La Tour de Peilz, Chêne-Bourg, Liebefeld)

Internal Quality Control (IQC) provides much of the information needed in the evaluation of reproducibility and precision of laboratory tests. To that purpose, internal quality control samples are included in the assays performed in the laboratory and results are compared to predetermined limits, in order to validate or invalidate patient test results.

We have developed a software (CScqi) specifically designed for internal quality control in medical and clinical laboratories. This software proposes a list of more than 200 analytes. The user first enters the experimental conditions of the analyte evaluation (target value, sample ID, analytical system, …). The tolerance limits are calculated from the data indicated by the sample provider or from the QUALAB specifications (Swiss committee for QA in medical laboratories) if more restrictive. Measurements are then entered and stored into a database. Results are dynamically plotted on a graph including the warning and alarm limits (± 2 SD and ± 3 SD respectively), the mean, coefficient of variation and SD of the IQC measurements. Thus an easy and instantaneous visual evaluation of the reproducibility of the measurements can be performed. The coherence of measurements is evaluated using the main Westgard rules and a message displayed when a rule violation occurs. The most interesting feature is that every modification of experimental conditions (change of reagent, new calibration, …) is traced into the database and displayed on the graph. At last, every set of measurements can be archived and retrieved very easily.

In conclusion, the CScqi software is inexpensive, easy to install and to use. In addition to external quality assessment programs, it is a valuable tool to evaluate and monitor the quality of medical and clinical laboratories analysis.

P3 Proteomics as key technology in biomarkers discovery

Hochstrasser D., Lescuyer P., Moll S., Vezzadel A., Sanchez J. C., Zimmermann C. (Geneva)

The development of proteomic last decade provides now powerful tools to unravel part of the tremendous complexity of patient samples. Although, the number of specific biomarkers available in routine clinical practice is relatively small, it is anticipated from available transcriptomic data that many single specific protein biomarkers exist in multiple diseases conditions. To discover and establish them as reliable biomarkers in clinical practice, two investigation phases should implemented: a discovery phase and a validation phase. Liquid chromatography and mass spectrometry are essential tools in the discovery phase. For the validation phase, the most practical approach is the development of immunoassays.

The presentation will show available transcriptomic data that demonstrates the potential existence of multiple single specific biomarkers. It will highlight successful biomarker discovery and application in a clinical chemistry setting using multiple protocols.

P4 New Aspects of the Serine-Palmitoyltransferase – a key enzyme in the sphingolipid metabolism

Hornemann T., Richard S., Rütti M. (Zurich)

Sphingolipids and their metabolites, like ceramides or gangliosides, are ubiquitous constituents of membrane lipids in eukaryotic cells and involved in various cellular functions like apoptosis, signal transduction and membrane trafficking. The serine-palmitoyltransferase (SPT) is the key regulatory enzyme in the cellular de novo sphingolipid synthesis pathway.

Three individual point mutations (C133W/Y, V144D) in the SPTLC1 subunit of SPT were identified to cause an inherited sensory neuropathy (HSN1). As already reported earlier, those three mutations lead to a reduced cellular SPT activity.

In our work we characterised a recently reported, forth HSN1 mutation which leads, in contrast to the other mutations, to a two-fold increase in SPT activity. This indicates that both, a reduced and enhanced SPT activity, can result in the same pathological disorder.

Interestingly, although SPT is ubiquitously expressed, the identified HSN1 mutations do not show a negative effect on the function of other sphingolipid rich tissues like brain, kidney or lung. The pathological disorders in HSN1 patients are restricted to PNS tissue only. By an EST database screen we could identify a second, previously unknown, isoform of SPT that could serve as a backup in those non-PNS tissues. Interestingly this new isoform shows a very high level of expression in placenta (200–300-fold higher than in other tissues) which indicates a special function of this isoform during placenta formation and pregnancy.

We could further demonstrate that the functional enzymatic SPT complex is, in contrast to earlier reports, not a dimer but forms a high molecular weight holoenzyme with a molecular weight of 460 kDa. The functional SPT complex contains the newly identified but also the previously reported SPT subunits. A functional characterisation of the new isoform and its role in the cellular sphingolipid metabolism is currently under investigation.

P5 Iron Metabolism – News from an Oldie (Der Eisenstoffwechsel – eine Übersicht)

Huber A. R. (Aarau)

Für die Abklärung von Eisenstoffwechselstörungen wurde in jüngster der Zeit eine Vielzahl von neueren Untersuchungsverfahren zusätzlich zu den herkömmlichen, mehrheitlich morphologischen Abklärungen angefügt. Diese betreffen vor allem den Bereich der automatisierten Analyse der

Blutzellen sowie die verlässliche Differenzierung neuer Zellsubpopulationen (myeloisch und erythrocytär). Interessant sind die neuen Retikulozyten Indizes wie CHR, RET-He, Hypo%, die alle vor allem in der Differenzierung von mikrozytären Anämien einen wesentlichen Beitrag leisten. Die klassischen chemischen Messgrössen des Eisenhaushalts wie Ferritin, Transferrinsättigung und neuere Messgrössen für die Erfassung eines funktionellen Eisenmangels (Zinkprotoporphyrin und löslicher Transferrinrezeptor) helfen in der oft nicht einfachen Differenzialdiagnose zwischen Eisenmangelanämie, Anämie bei chronischen Erkrankungen und Thalassämien.

Bei den Thalassämien sind es vor allem molekularbiologische Abklärungen, während sich bei der Anämie von chronischen Erkrankungen ein neuer Parameter herauskristallisiert, nämlich das Hepcidin. Im grundlegenden Verständnis des Eisenstoffwechsel hat sich in den letzten Jahren Einiges getan. So konnten wichtige Regulatoren wie Ferroportin, Hämojuvelin und Hepcidin beschrieben werden. Es kann davon ausgegangen werden, dass ein besseres Verständnis von Eisenüberladungssyndromen, Verwertungsstörungen, chronische Entzündungen, aber auch Sideroblastosen die Diagnostik und die Therapie verbessern wird.

Im Übersichtsreferat werden wir die neuen pathologischen Erkenntnisse, vor allem aber auch den klinischen Einsatz der neuen Parameter, darstellen. In den Folgereferaten wird im Speziellen auf die Eisenüberladung, die Eiseneinbaustörungen und die Differenzialdiagnose von mikrozytären Anämien eingegangen werden.

P6 Using heparinized blood greatly facilitates cyclosporine determination in a routine setting

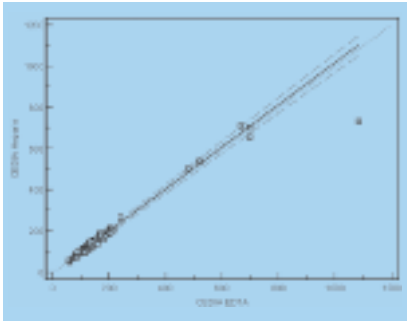
Korte W., Engler H., Aldebert E., Riesen W. (St. Gallen)

Introduction: Cyclospsporine (CsA) measurement is routine in daily hospital practice, primarily to prevent overdosing and related side effects.

Only few publications examined potential differences in CsA levels determined from heparin or EDTA anticoagulated blood (Prasad R et al., Transplantation. 1985, 39,667; Poller JM et al., Ther Drug Monit. 1986,8,122), where significant differences between CsA levels determined from samples with different anticoagulants were described. We hypothesized that this might no longer be a problem with new assay technologies: given that most routine analytes in clinical chemistry can be performed from heparinized blood, the possibilty of using it for CsA determination would be of great practical advantage. The CEDIA Cyclosporin Plus Assay assay seems very robust due to recombinant DNA technology rendering this system very homogeneous.

Methods and results: In a first step, we compared results from 187 samples obtained from heparinized blood in two different assays. The samples were run on a BC DXC system using the CEDIA assay as well as on an Abbott Axsym using the FPI assay; the correlation obtained is described by y = 6.0584 + 0.9784x, r = 0.989, p <0.0001. In a second step, we compared results in 81 patients; CsA levels were measured in samples obtained from EDTA and heparinized blood drawn at the same time, using the CEDIA assay on a BC DXC; the correlation obtained is described by y = –2.4524 + 1.0210x, r = 0.9720, p <0.0001. If only results <500 µg/l were evaluated, the correlation obtained is described by y = –2.0087 + 1.0159x, r = 0.9732, p <0.0001. For a typical 6 months period at our institution (1030 requests for CsA and 4 minutes additional handling time per request if a specific EDTA tube was needed), an overall decrease in handling time of 69 hours results from the use of heparinized blood. This equals 138 hours per year or more than 3 weeks of work for a fully employed laboratory technician. If additional centrifugation (which might be necessary according to the work flow established) was also considered, the overall decrease would even be considerably greater.

Conclusions: CsA determination from heparinized blood is well feasible and accurate with the two assays evaluated. Using heparinized blood reduces handling time as well as hands on time. We suggest that this methodology be formally evaluated by the manufacturers for inclusion into CE labelling of their products.



CsA from heparinized vs. EDTA blood.

P7 Mutation Screening of the Ornithine Transcarbamylase (OTC) and the Medium-Chain Acyl-CoA Dehydrogenase gene by Multiplex PCR Amplification and Sequencing

Largiadèr C. R., Mäder-Heinemann G., Zürcher G. (Berne)

Multiplex PCR is a variant of PCR enabling simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers. Although the advantage of this technique is obvious in optimizing sequencing based mutation screening assays of genes encompassing a large number of exons, only very few such assays have been developed so far. This is mainly because multiplex assays have generally been tedious and time-consuming to establish, requiring lengthy optimization procedures. Here, we propose the use of the Qiagen Multiplex PCR kit to generate multiplexed PCR templates for subsequent DNA sequencing. Applying a standard PCR protocol, the 10 exons of the OTC gene and the 12 exons of the MCAD gene were amplified in two and three multiplex PCRs, respectively. On the basis of confidence scores (KB base caller software; ABI) assigned to each base call in the chromatogram files, we compared the quality of the DNA sequencing data derived from multiplex templates with those obtained by established protocols, which involve PCR amplifications of single exons followed by DNA sequencing. We found qualitatively comparable results for the two approaches. Because we have not made any specific re-design of the PCR-primers for multiplexing and because we could successfully apply a standard PCR protocol without any optimization steps, we suggest that many existing sequencing based mutation analysis protocols could be easily optimized with the proposed multiplex kit.

P8 Effects of continuous positive airway pressure on N-terminal-pro-B-type-natriuretic peptide and functional capacity in obstructive sleep apnea

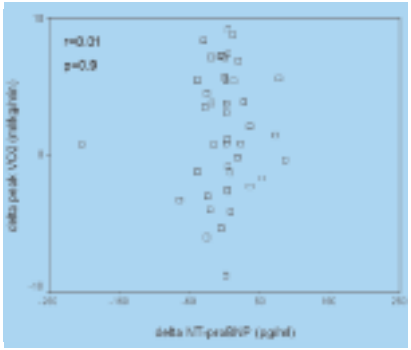
Maeder M. T., Ammann P., Muenzer T., Schoch O. D., Korte W., Huerny C., Rickli H. (St. Gallen)

Background: The obstructive sleep apnea syndrome (OSAS) is a risk factor for the development and progression of congestive heart failure (CHF). OSAS shares many similarities with CHF including sympathetic hyperactivity, myocardial dysfunction and exercise intolerance. In patients with OSAS, the effects of continuous positive airway pressure (CPAP) therapy on neurohumoral activation and functional capacity, both prognostic predictors in patients with CHF, are poorly characterized.

Aim: To determine the impact of CPAP therapy on and circulating levels of N-terminal-pro-B-type natriuretic peptide (NT-proBNP) and peak oxygen consumption (peak VO_2).

Methods: Forty-four patients with newly diagnosed OSAS [age 50±9 years, apnea-hypopnea-index 36 (17–65) h-1] underwent NT-proBNP measurement and cardiopulmonary exercise testing at baseline (BL) and at follow-up (FU) after 7.8 ± 1.3 months of effective CPAP therapy (nightly use 5.8± 1.3 hours, CPAP pressure 8.6±1.2 cmH_2O). As NT-proBNP levels require interpretation in the light of renal function, serum creati-

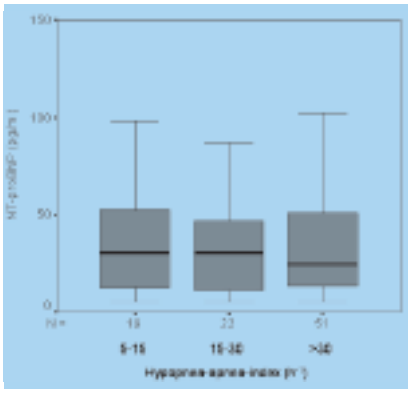
nine was determined at BL and at FU, and glomerular filtration rate (GFR) was estimated.
Results: CPAP therapy led to a significant improvement in nightly oxygen saturation [% time with oxygen saturation <90% at BL: 3.5 (0.7–12.9) vs. FU: 0 (0–0.4); p <0.001]. However, there were no changes in body-mass-index (BL: 30.8 ± 4.8 vs. 30.5 ± 4.5 kg/m²; p = 0.3) and NT-proBNP levels [BL: 23 (11–48) vs. 27 (6–60) pg/ml; p = 0.83]. In contrast, peak VO_2 increased from BL (31.0±9.5 ml/kg/min) to FU (32.9 ± 9.2; p = 0.009). Changes in peak VO_2 and NT-proBNP were not related (r = 0.01; Figure 1). NT-proBNP and GFR were not related at BL (r = –0.17) and at FU (r = –0.17). However, there was a slight but statistically significant increase in mol/l; p = 0.003) and a decrease in serum creatinine (BL: 80±12 vs. FU: 84±13 estimated GFR (BL: 93 ± 13 vs. FU: 88 ± 13 ml/min/1.73 m²; p = 0.003) respectively over time.
Conclusions: Despite improvement in functional capacity and unchanged body-mass-index, CPAP therapy during several months is not associated with a change in circulating daytime NT-proBNP levels. Failure to achieve the expected decrease in NT-proBNP might have been due to changes in body composition (possibly responsible for the previously observed inverse relationship between NT-proBNP and BMI). The slight decrease in GFR is unlikely to have blunted the expected decrease in NT-proBNP, but is remarkable and warrants further investigation.



P9 Does N-terminal-pro-B-type-natriuretic peptide predict disease severity and functional capacity in patients with the obstructive sleep apnea syndrome?

Maeder M. T., Ammann P., Rickli H., Schoch O. D., Korte W., Huerny C., Muenzer T. (St. Gallen)

Background: The obstructive sleep apnea syndrome (OSAS) is associated with sympathetic hyperactivity and cardiac dysfunction, and thus is thought to contribute to the development and progression of congestive heart failure (CHF). Whereas in CHF, N-terminal-pro-B-type natriuretic peptide (NT-proBNP) is a marker of disease severity and functional capacity, its role in patients with OSAS is ill defined. In particular, it is unknown whether NT-proBNP is related to the severity of OSAS expressed as the apnea-hypopnea-index (AHI), and whether the recently established inverse relationship between NT-proBNP and body-mass-index (BMI) also applies for the most often overweight OSAS patients.
Aim: To investigate the relationship between NT-proBNP, functional capacity, AHI, and BMI in patients with OSAS.
Methods: Ninety-two patients with untreated OSAS underwent cardiopulmonary exercise testing and NT-proBNP measurement.
Results: Median (interquartile range) NT-proBNP levels in patients with AHI 5–15 (n = 19), 15–30 (n = 22), and >30 h-1 (n = 51) were 30 (10–57), 30 (11–51), and 24 (13–52) pg/ml, respectively (p>0.05, Figure 1). NT-proBNP was related to age (r = 0.33; p = 0.001), but not AHI (r = 0.01) and BMI (r = 0.05). Peak oxygen consumption (peak VO_2) was not related to the AHI either (r = 0.01), but to BMI (r = –0.48, p <0.001) and NT-proBNP (r = –0.31, p = 0.003). Multivariate linear regression identified BMI, gender, age, resting heart rate, and NT-proBNP as independent predictors of peak VO_2. Peak VO_2 levels in patients with BMI <25, 25–30, 30–35, and >35 kg/m² were 38.8±9.7, 32.4±8.6, 29.0±8.4, and 20.8±7.0 ml/kg/min, respectively (p <0.001).
Conclusions: NT-proBNP does not identify patients with more severe OSAS, and in contrast to previous findings is not related to BMI. Although NT-proBNP is an independent predictor of peak VO_2 in patients with OSAS, its usefulness in predicting functional impairment is limited by the fact that BMI is a much stronger and easily available determinant of peak VO_2.



P10 Einfluss von Trenngel und Heparin auf verschiedene Analysenbestimmungen

Nusbaumer C., Hodzic E., Scholer A. (Basel)

Einführung: Schnellere turn around Zeiten (TAT) bei der Routineanalytik sowie die Vereinfachung der Probenaufbereitung kann durch Reduktion der Anzahl Probenentnahmegefässe und saubere Trennung von Blutkuchen und Serum oder Plasma erreicht werden. In der vorliegenden Studie wurden 26 klinisch chemische Parameter und 14 Hormone/Vitamine nach paralleler Blutentnahme mittels Gefässen ohne Zusatz (Nativblut = Serum), Lithium Heparinat Zusatz (Plasma) und Gefässen mit Lithium Heparinat und Trenngel als Zentrifugationshilfe, bestimmt. Die Analysen wurden mittels gängiger Methoden (Roche oder Wako) auf den Analysengeräten Hitachi 917 und auf einem Elecsys 2010, sowie die Osmolalität mittels Kryoskopie, analysiert. Neben der Prüfung auf Abweichungen wurde auch die Stabilität der Parameter in den einzelnen Gefässen geprüft.
Vorgehen: Über 10 Tage wurden pro Tag bei 5 Personen je die 3 oben erwähnten Proben abgenommen und analysiert. Serie A Vergleich Lithium Heparinat Zusatz ohne/mit Trenngel: Analysenparameter: Albumin, ALAT, AP, ASAT, Gesamt Bilirubin, Gesamt Calcium, Chlorid, Cholesterin, CRP, Gamma-GT, Harnsäure, HDL-Cholesterin, Harnstoff, Kalium, Kreatinin, LDH, Natrium, Anorg. Phosphat, P-Amylase, Gesamt Protein, Triglyceride; Magnesium, Eisen, Ferritin, Transferrin, Transferrinsättigung, lösl. Transferrin Rezeptor, CK, Gesamt Amylase. Serie B Vergleich Serum/Lithium Heparinat Zusatz ohne/mit Trenngel: TSH, FT4, T4, TUP, T3 F2, Progesteron, Folsäure, Vitamin B_{12}: FSH, CH, Prolaktin, Testosteron; Osmolalität
Resultate und Schlussfolgerungen: Die geforderte Toleranz (Regressionsgerade slope 1,0 plus minus 10%) wurde beim Vergleich der 26 klinisch chemischen Parametern zwischen Analysen im Lithium Heparinat Plasma mit und ohne Gel für alle Analysen erfüllt. Die Bewertung der Achsenabschnitte (Toleranz 0 + 2s) wurde ebenfalls für alle diese Parameter erfüllt.
Hormone/Vitamine: Mit Ausnahme von 2 Analyten (Folsäure, T4 total) wurden die obgenannten Bedingungen in allen 3 Probenmaterialien erfüllt. Folsäure darf gemäss Firmenangabe nur aus Serum analysiert werden. Für T4 total ist die Abweichung des slope (>10%) klinisch irrelevant. Die Reproduzierbarkeit der Bestimmungen der meisten Parameter in den verschiedenen Probenmaterialien ist kurz (meist nur 1 Tag). Extreme: Triglyceride, Cholesterin und LDH. Die Ursachen sind falsche Aufbewahrung der analysierten Proben (offen

in Originalgefässen-Konzentrierung), die Durchmischung der Proben für Nachbestimmungen fast nicht möglich. (richtige Aufbewahrung: Serum/Plasma Proben abgegossen in geschlossenen Behältern). Fette in Flüssigkeiten floaten (Triglyceride, Cholesterin), Thrombozyten werden möglicherweise durch Trenngel nicht abgetrennt (LDH steigt).

P11 Dépistage et suivi thérapeutique de patients autistes et hyperactifs ou intolérants au gluten et à la caséine par HPLC

Rey J., Roduit C. (Geneva)

L'excrétion urinaire de fragments protéiques appelés peptides résulte pour beaucoup de la dégradation enzymatique incomplète de protéines provenant de la nourriture le long du tractus gastro-intestinal. Il a été montré que certains peptides provenant de céréales (gliadines et gluten exorphine) ou de produits laitiers (casomorphines) pouvaient franchir les barrières intestinale, rénale et même hémato-encéphalique pour y déclencher divers troubles du comportement tels que schizophrénie et hyperactivité ou avoir des manifestations dermatologiques.

Par ailleurs, d'autres peptides ne sont pas directement issus de fragments protéiques d'origine alimentaire. Ainsi, le peptide indolylacryloylglycine (IAG) est issu de la conjugaison hépatique des acides aminés tryptophane et glycine. Sa détection en forte concentration dans les urines semble corrélée à une perméabilité intestinale accrue et apparaît comme la signature métabolique d'un grand nombre de troubles envahissants du développement (TED). Les peptides dermorphine et deltorphine sont quant à eux produits par l'organisme à partir d'acides aminés. Tous ces peptides ont été montrés comme ayant une très forte affinité pour les récepteurs opioïdes du cerveau.

Les peptides énumérés ci-dessus sont aujourd'hui identifiables et quantifiables par chromatographie liquide à haute pression (HPLC). Cette analyse est particulièrement intéressante pour mettre en évidence des carences enzymatiques. Un régime alimentaire dépourvu en source de peptides non-métabolisables pourra ainsi être préconisé. Des améliorations spectaculaires du comportement peuvent être observées à la condition de suivre un régime alimentaire strict complété parfois d`un traitement médicamenteux.

A partir d`un collectif de plus de vingt enfants ou adolescents suivis par des médecins pédiatres pour des troubles comportementaux souvent complexes, nous avons évalué la contribution apportée au diagnostic et au suivi thérapeutique des patients par l'identification et le dosage de peptides urinaires par HPLC. Grâce à une excellente collaboration entre médecins et laboratoire, notre étude illustre les différentes peptiduries identifiables à ce jour en corrélation avec un bilan médical complet des patients.

P12 Evaluation of two fully automated novel enzyme linked immunosorbent assays for the determination of human adiponectin in serum

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Background: In contrast to RIA, recently available ELISA's provide the potential for fully automated analysis of adiponectin. To date, studies reporting on the diagnostic characteristics of ELISA's and investigating on the relationship between ELISA- and RIA-based methods are rare. Methods: Thus, we established and evaluated a fully automated platform (BEP 2000; Dade-Behring, Switzerland) for determination of adiponectin levels in serum by two different ELISA methods (competitive human adiponectin ELISA; high sensitivity human adiponectin sandwich ELISA; both Biovendor, Czech Republic). Further, as a reference method, we also employed a human adiponectin RIA (Linco Research, USA). Samples from 150 patients routinely presenting to our cardiology unit were tested.
Results: ELISA measurements could be accomplished in less than 3 hours, measurement of RIA had a duration of 24 hours. The ELISA's were evaluated for precision, analytical sensitivity & specificity, linearity on dilution and spiking recovery. In the investigated patients, type 2 diabetes, higher age and male gender were significantly associated with lower serum adiponectin concentrations. Correlations between the ELISA methods and the RIA were strong (competitive ELISA, r = 0.82; sandwich ELISA, r = 0.92; both p <0.001). However, Deming regression and Bland-Altman analysis indicated lack of agreement of the 3 methods preventing direct comparison of results. The equations of the regression lines are: Competitive ELISA = 1.48 × RIA – 0.88; High sensitivity sandwich ELISA = 0.77 × RIA + 1.01.
Conclusions: Fully automated measurement of adiponectin by ELISA is feasible and substantially more rapid than RIA. The investigated ELISA test systems seem to exhibit analytical characteristics allowing for clinical application. In addition, there is a strong correlation between the ELISA methods and RIA. These findings might promote a more widespread use of adiponectin measurements in clinical research.

P13 Quantification Method of Free Plasma Metanephrine, Normetanephrine and Methoxytyramine by Liquid Chromatography-Mass Spectrometry and Comparison with Electrochemical Detection for Biochemical Diagnosis of pheochromocytoma

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Pheochromocytomas are rare tumours making necessary the use of a very sensitive and specific test to reliably exclude or confirm the disease. Several studies support the determination of free metanephrines in plasma as the "gold standard" for the diagnosis of pheochromocytoma. Quantification of plasma free metanephrines is usually accomplished by HPLC with electrochemical detection, but diet or kidney failure may generate interfering substances that sometimes obscure the relevant peaks. To circumvent analytical artefacts, we report a new method using liquid chromatography coupled to an electrospray source and triple quadrupole mass spectrometer. Metanephrine (MN), normetanephrine (NMN) and methoxytyramine (MTX) and their corresponding deuterated compounds (D-MNs), used as internal standard were extracted from 1 mL of plasma using solid phase extraction. Dried residues were dissolved in the mobile phase and directly injected onto an analytical c18 column (2.1 × 50 mm). Compounds were resolved using hydrophilic interaction chromatography and detected by electrospray ionization in positive mode. Total run time was 14 min. No carry over was observed on column. Extraction yield were independent from spiked concentrations and were 100%, 53% and 91% for MN, NMN and MT, respectively. A plasma MN value greater than 77 pg/ml or a plasma NMN value of at least 160 pg/ml is considered positive. MT concentrations are usually below 20 pg/ml. Lower limit of quantification were 20 pg/mL for MN and MT and 30 pg/mL for NMN. The calibration curve ranged between the LLOQ and 640 pg/mL. Interassay imprecision values (CV; n = 9) for MN at 55, 85, and 325 pg/ml were 6.2%, 8.1%, and 4.1%, respectively. Interassay imprecision values (CV; n = 9) for NMN at 80, 110, and 350 pg/ml were 10.9%, 8.6%, and 4.8%, respectively. Interassay imprecision values (CV; n = 9) for MT at 30, 60, and 300 pg/ml were 6.7%, 9.8%, and 5.8%, respectively. Intraassay precision values ranged between 1.9 and 12.9% for NMN, MN and MT at 30, 60 and 300 pg/ml concentrations. Passing-Bablock regression of HPLC and LC-MS/MS results (n = 49) yielded the following equations (amounts in pg/ml): NMN-MS = 1.0523 × NMN-HPLC –19.7, MN-MS = 0.8226 × MN-HPLC –3.3.

A non-linear relationship was observed between the HPLC and LC-MS/MS methods for MT due to low concentrations of this analyte. In conclusion, LC-MS/MS is an attractive alternative to electrochemical detection for the diagnosis of pheochromocytoma.

P14 Reliability of M-protein quantification on Paragon CZE 2000 and Capillarys 2

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Introduction: Densitometric quantification of monoclonal immunoglobulins (M-proteins) is important in the clinical follow-up of patients with monoclonal gammopathies of unknown significance (MGUS), multiple myeloma, Morbus Waldenström and plasmocytoma. In this study we compared the M-protein levels determined by two different capillary zone electrophoresis systems and different peak integration methods.
Methods: Electophoresis was performed on a Paragon CZE 2000 (Beckman Coulter) and a Capillarys 2 (Sebia). On both systems the peaks were integrated by the perpendicular drop method. In addition, a tangent integration method was examined on Capillarys 2. Linearity of M-protein measurement was determined by dilution series of patient samples containing a monoclonal IgG or a monoclonal IgA.
Results and Discussion: M-Protein concentrations measured by the two systems were very similar at concentrations above 10 to 15 g/l. In the range below 15 g/l, quantification of M-Proteins by the perpendicular drop method tended to overestimate M-protein concentration. In addition this method was found to be susceptible to interference by polyclonal immunoglobulin. In contrast, the tangent integration method on capillarys 2 proved to be accurate down to the range of about 3 g/l in the experiments with dilution series.

P15 Study of possible Interferences by Additives and Gel Separators in blood sampling tubes on Chromatographic Methods

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Introduction: Additives are necessary in collection tubes to ensure the preservation of certain qualities of the sample placed inside. These additives can either be applied directly onto the tube wall or placed inside the tube in liquid or solid form. Gels might also be added to the sample tube to allow for the separation of blood samples. These additives may contain substances which can interfere with chromatographic separation and therefore it is important to know possible interferences of the additives and separation gels prior to performing HPLC and/or LCMSMS analysis.
Materials and methods: Six different types of tubes from two different manufactures were chosen to be tested (Sarstedt, Becton Dickinson (BD) The tubes used were: plasma separation tube with gel as a separator and lithium heparin as an anticoagulant 4.5 ml BD vacutainer, plasma collection tube with lithium heparin 2 ml BD vacutainer, serum gel tube 4.7 ml Sarstedt monovette, lithium heparin tube 7.5 ml Sarstedt monovette, lithium heparin gel tube 5.5 ml Sarstedt monovette, serum separator tube with gel 5 ml BD vacutainer.
The procedure for interference testing was started by addition of three milliliters of physiologic sodium chloride (NaCl 0.9%) to each tube and in a second part by addition of 3 ml serum. The tubes were manually inverted eight times and then agitated for thirty minutes. It was unknown at this point if there would be significant mixing between the gel and the sodium chloride and or the serum. After mixing the tubes had been run on a Remedi (Biorad) HPLC system for General Unknown Screening (GUS) analysis. The same procedure was then used for LCMS/MS and HPLC methods. Sodium chloride was used as the baseline marker; Serum is the material used for chromatographic routine analysis.

Manufacturer/ tube/additive	Remedi GUS	HPLC
Sarstedt Li-Hep	no interference	small interference
Sarstedt Li-Hep Gel	no interference	small interference
Sarstedt Se tube Gel	no interference	small interference
BD Li Hep	no interference	several small peaks
BD Li Hep Gel	interference	small interference
BD Se tube Gel	interference	several small peaks

Conclusions: Remedi method: The interference that was found on this machine may be misleading and waste valuable time in the GUS if not known. HPLC methods: The interfering peaks which were found may contribute to false calculations of concentrations for several substances with the same retention times.

P16 Evaluation of HPLC methods for the measurement of carbohydrate deficient transferring

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Background: Carbohydrate deficient transferrin (CDT) is currently the most specific biomarker for the diagnosis of sustained alcohol abuse. CDT levels are elevated in individuals with high daily alcohol intake (more than 50–80 g per day) over a period of at least two weeks.
Analytically, HPLC may have advantages over CDT detection using classical immunoassay methods as it can clearly separate individual transferrin isoforms. We evaluated the diagnostic performance of two new HPLC methods and compared the results to an established immunoassay.
Methods: CDT was measured in 74 individual samples in parallel with two HPLC methods (ClinRep, Recipe and %CDT-HPLC, BioRad) and the routinely used immunoassay method (%CDT-TIA, BioRad). For the CDT-TIA the initial iron saturation step is followed by microcolumn separation of CDT and non-CDT isoforms and detection using a turbidimetric immunoasaay (TIA) and the result is given as %CDT of total transferrin measured in parallel. In contrast, HPLC separates transferrin into 5 transferrin isoforms carrying 0 and 2 to 5 sialic acid residues after iron saturation. Results are expressed as %CDT, a ratio of peak areas of asialo- and disialotransferrin over the sum of all 5 transferrin peaks.
Results: Intra-run and inter-run precision was 11.8% and 16.6%, respectively, for the Recipe HPLC and 3.1–14.5% and 16.8%, respectively, for the BioRad HPLC. The CDT values were slightly lower when using the Recipe HPLC compared to the BioRad HPLC. However, the results of both chromatographic assays showed an excellent correlation ($R^2 = 0.96$). Although the correlations of CDT values between each individual HPLC method and the TIA were rather good as well, the associations were less strong ($R^2 = 0.87$ for the BioRad HPLC and $R^2 = 0.88$ for the Recipe HPLC compared to %CDT-TIA). Two BC but no CD heterocygotes were found among the tested samples. The largest differences in CDT values between the immunoassay and the HPLC methods were found for patients with large trisialotransferrin fractions.
Conclusions: HPLC is a reliable method for the measurement of CDT and the results are well correlated to the established immunoassay method. Since rare genetic variants and high amounts of trisialotransferrin are readily identified by HPLC, CDT measurement using HPLC is able to sort out false positive and false negative values arising from such variants, which is not the case for the classical immunological detection after column separation.

P17 B-type natriuretic peptide levels are associated with the severity of renal impairment and predict progression of chronic kidney disease

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Introduction: Earlier observations show, that cardiac malfunction and chronic kidney disease (CKD) are epidemiologically and patho-physiologically linked. BNP and NT-proBNP, established markers of congestive heart failure (CHF), are also elevated in CKD. In a multicenter prospective study we analyzed whether these parameters are associated with the severity of renal impairment and whether they are able to predict the progression rate of CKD.

Methods: 227 patients with non-diabetic chronic kidney disease and mild to moderate renal failure were included in the study and underwent baseline evaluation.177 patients were followed up in a prospective study for a period of up to 7 years or until the progression to one of the predefined endpoints (doubling of baseline plasma creatinine or terminal renal failure).

Results: Both, BNP and NT-proBNP plasma levels were significantly correlated with the GFR. When patients were stratified into groups according to the CKD classification of the National Kidney Foundation (NKF) the increase of NT-proBNP was more pronounced than that of BNP: median BNP plasma levels increased from 34 ng/L in patients with a GFR ≥90 ml/min/1.73 m^2 to 57 ng/L in those with GFR <30 ml/min/1.73 m^2, p = 0.019 , median NT-proBNP from 39 ng/L in patients with GFR ≥ 90 ml/min/1.73 m^2 to 456 ng/L in those with GFR <30 ml/min/1.73 m^2, p <0.001.

Both parameters were associated with the rate of progression of chronic kidney disease. An increase by 1 SD of log transformed BNP was associated with a 1.41-fold increased risk of progressing to one of the predefined renal endpoints (95% CI 1.09–1.81). For NT-proBNP the risk was even higher: for each increment of 1SD the progression risk increased 2.36-fold (95% CI 1.80–3.08). After adjustment for other covariates known to be associated with progression of CKD, the association remained significant for NT-proBNP.

Conclusion: BNP and NT-proBNP, established diagnostic and prognostic markers of heart failure, are also associated with severity and progression rate of CKD and may therefore evolve as relevant markers of the cardiorenal syndrome.

P18 Methodenvergleich VEDALAB PSA-CHECK-1 vs. Immulite 2000® von DPC

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Einleitung: VEDALAB PSA-CHECK-1 Test ist ein immunometrischer Einzeltest Assay zur Bestimmung des Prostatspezifischen Antigens (PSA). Endpunkt ist eine Farbreaktion, welche mit Hilfe einer hochauflösenden Kamera registriert und quantitativ ausgewertet wird. Die Richtigkeit und die Präzision des Tests wurden evaluiert und mit der PSA-Messung am Immulite 2000® verglichen.

Methoden und Resultate: Präzision und Richtigkeit von VEDALAB PSA-CHECK-1 wurden mit einem CLSI Protokoll EP5-A2 konformen Versuchsdesign evaluiert und mit auf dem Immulite 2000® ermittelten Streuungsparametern in Beziehung gesetzt. Dabei wurden mit n = 20 folgende Resultate gefunden: Sollwert 1.13 ng/mL, m = 1.86 (+65%), Total VK = 85.5%, Intraday VK = 82.2%, Sollwert 3.8 ng/mL, m = 5.3 (+40%), Total VK = 74.7%, Intraday VK = 66.5%, Sollwert 20.0 ng/mL, m = 53.2 (+166%), Total VK = 144.3%, Intraday VK = 177.3%.

Mit dem Immulite 2000® werden routinemässig VK zwischen 3% und 9% erreicht, der Mittelwert weicht maximal ± 10% vom Sollwert ab.

Durch parallele Bestimmung von PSA aus 39 Serumproben auf dem VEDALAB PSA-CHECK-1 und dem Immulite wurden die Ergebnisse der beiden Geräte verglichen. Dabei wurde eine Korrelation (Pearson) von r^2 = 0.904 erreicht. y = 1.4412x + 5.0178, wobei y = PSA-Wert mit dem VEDALAB PSA-CHECK-1 und x = PSA-Wert auf dem Immulite 2000® bedeuten.

Schlussfolgerung: VEDALAB PSA-CHECK-1 misst das PSA auf den untersuchten Levels weniger präzise und weniger richtig als der Immulite 2000® von DPC. Im Methodenvergleich zeigt sich, dass eine einfache Korrelation der Ergebnisse nicht gegeben ist. Gegenüber dem Analyzer-basierten Immunoassay ist zu bemängeln, dass der deklarierte Messbereich >4.0 ng/mL den kritischen und für das Screening von Prostatakarzinomen relevanten Messbereich bis 2.5 ng/mL nur teilweise abdeckt.

P19 Comparison of 5 onsite screening tests for drugs of abuse with CEDIA drugs of abuse tests and Chromatography

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Introduction: Onsite tests for drugs of abuse are used as screening tests in drug substitution programs, workplace screening tests and screening for substances in case of intoxication (urgency laboratory tests). The antibodies in the tests and the methods of detection differ from manufacturer to manufacturer what includes the possibility of incomparable results. There are few information in the literature about possible interferences produced by therapeutic drugs in high concentrations. In the comparison trial 40 urines (positive and negative) were analysed with different methods. In a second part of the trial analysis of aqueous solutions of several drugs supposed to interfere with different drug of abuse tests were analysed in order to get information about the performance of the methods.

Materials and methods: Fife different types of on site test sets were evaluated by determination of 40 urine samples from the daily routine and comparing the results with those of CEDIA drug of abuse tests (Microgenics) on a Hitachi 917 analyser (Roche). All positive results were confirmed by chromatographic methods (HPLC, LCMS LCMSMS). The specificity of the on site tests was evaluated by analysing 32 different aqueous solutions of therapeutic drugs in toxic concentrations. The chosen substances are often prescribed and supposed to interfere with the drugs of abuse tests.

Results and conclusions: The following results show some examples of the evaluation: The agreement between the tests was in most cases better than 95% for all test results of Benzodiazepines, Benzoylecgonin, Methadon, Opiates, THC-Carbonic acid Opiates, TCA (Tricyclic Antidepressives. But each onsite test had some divergent results in one or two of the performed analysis. The analysis of amphetamine derivatives and other stimulating substances (Amphetamine, Methamphetamine), Ecstasy like acting or structural similar compounds (MDMA, MDA, MDEA, PMA, PMMA, MBDB, BDB and others) as one test was impossible because of missing cross reactivity in one test zone. This urged the manufacturers to produce on site tests with different test zones for one class of similar acting substances. This leads to complicated constructions and misinterpretation when performed by none trained personal.

The interference testing showed similar influences of substances as none from whet chemical drug of abuse tests on clinical chemical analysers. Most of the interferences were found with Ibuprofen, Tramadol and Mefenamic acid.