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Voordrachten

Voordrachten tijdens het 55e congres van de Nederlandse Vereniging voor Klinische Chemie
in twee parallele sessies op vrijdag 26 april 2002 te Lunteren

Sessie I

11.00 – 11.15 uur

Identification of mutations in *WFS1* in Dutch patients with Wolfram syndrome

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Introduction: Wolfram syndrome is a rare autosomal recessive neurodegenerative disorder characterized by juvenile-onset diabetes mellitus (DM) and optic atrophy (OA). It is also known by the acronym DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy & deafness). A gene responsible for Wolfram syndrome (WFS1) has recently been identified on chromosome 4p16.1 and subsequently mutations in WFS1 have been described.
Methods: We have screened five patients with Wolfram syndrome from three Dutch families for mutations in the WFS1 coding region by SSCP analysis and direct sequencing. Furthermore, we analyzed the mitochondrial genome for gross abnormalities and the A3243G point mutation in the leucyl tRNA gene, as Wolfram syndrome shows phenotypic similarity with mitochondrial disease.
Results: We identified two previously described mutations in the WFS1 gene; a homozygous intronic G to A transition in the splice donor site of exon 4 (460+1G->A) in both affected sibs from one family and a homozygous 1515-1530del15nt in both sibs from another family. In a third family a novel heterozygous C insertion in exon 8 was detected (1751insC). A mutation in promoter and/or intronic sequences in the other allele of this patient cannot be ruled out. MtDNA analysis did not reveal the presence of major re-arrangements or the leucyl tRNA A3243G point mutation in any of the Wolfram patients.
Conclusion: The current study confirms the association of WFS1 with Wolfram syndrome. It expands the spectrum of mutations in WFS1 and represents the first molecular characterization of Dutch patients with Wolfram syndrome.

11.15- 11.30 uur

Molecular diagnostics of Familiar Mediterranean Fever

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Introduction: Hereditary periodic fever (HPF) syndromes are characterized by self-limited episodes of fever and inflammation of serosa or synovia, without infectious etiology. Because it is difficult to exclude an infectious origin of fever, the diagnosis of HPF may be complicated. Familiar Mediterranean Fever (FMF) was the first recognized HPF syndrome and is seen most often in populations of eastern Mediterranean ancestry. Recently, the FMF gene (MEFV) was identified and a number of mutations described. In this study, we screened parts of the FMF gene for possible mutations in two patients suspected of FMF.
Methods: Genomic DNA was isolated from peripheral leucocytes. MEFV mutations in exons 2, 5 and 10 were screened by PCR and direct sequencing.
Results: In both patients, two mutations in MEFV were identified. In patient A, we detected a phenylalanine to leucine mutation at codon 479 in exon 5 (F479L) and a valine to alanine mutation at codon 726 in exon 10 (V726A). In patient B, we detected a methionine to isoleucine mutation at codon 680 (M680I) and a methionine to valine mutation at codon 694 (M694V), both in exon 10.
Conclusions: All four mutations detected in our patients have previously been identified in patients with FMF, confirming the suspected diagnosis. Particularly the combination of mutations in patient B, M680I and M694V, may determine the severity of presentation and course of FMF, consistent with the clinical presentation of this patient. Our molecular approach thus proves to be an excellent diagnostic tool to confirm a suspected diagnosis of FMF.

11.30 – 11.45 uur

Effect of genetic polymorphisms in cobalamin and folate metabolism on plasma cobalamin and homocysteine levels

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Introduction: It has been established that moderately increased plasma concentrations of total homocysteine (tHcy) represent an important risk factor for vascular disease. Deficiency of cobalamin (Cbl, vitamin B_{12}), a cofactor involved in the remethylation of homocysteine, may result in elevated total plasma homocysteine (tHcy) levels. Therefore, we investigated the impact of polymorphisms in genes involved in cobalamin transport and folate metabolism on Cbl and tHcy levels. *Methods:* Venous blood was drawn from women with a history of pre-eclampsia (n = 165, mean age 31±4) for determination of Cbl, folate, vitamin B_6 and tHcy levels; tHcy was determined in EDTA-blood after 12 hours fasting and 3 and 6 hours after an oral methionine load (0.1 g/kg body weight) using HPLC. From isolated DNA, polymorphisms in methylenetetrahydrofolate reductase (*MTHFR* C677T and *MTHFR* A1298C), methionine synthase (*MS* A2756G), methionine synthase reductase (*MTRR* A66G), and transcobalamin (*TC* C779G) were determined by PCR-RFLP. *Results and Discussion:* Multiple regression analysis identified *MTHFR* A1298C (P=0.06) and *TC* (P=0.06) as possible independent determinants of Cbl. Furthermore, *MTHFR* C677T, *TC*, folate, and Cbl were independent determinants of fasting tHcy ($P \leq 0.05$); *MTHFR* C677T ($P<0.01$) and *TC* ($P<0.05$) were independent determinants of post-load tHcy. The homozygous (GG) variant of *TC* increased fasting and post-load tHcy levels by 11% and 17%, respectively, while the heterozygous (CG) *TC* variant decreased Cbl levels by 20 %. *Conclusion:* This study shows that the C779G polymorphism in the TC gene significantly affects Cbl and tHcy levels.

11.45 – 12.00 uur

MDR-1 genetische polymorfismen C3435T en G2677T correleren niet met P-glycoproteïne expressie en functie in acute myeloïde leukemie

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Inleiding: Een belangrijke oorzaak voor het falen van therapie bij acute myeloïde leukemie is het optreden van resistentie, welke geassocieerd is met de expressie van multidrug resistentie eiwitten, waaronder het efflux-eiwit P-glycoproteïne (P-gp) dat gecodeerd wordt door het MDR-1 gen. Het G2677T polymorfisme in exon 21 van het MDR-1 veroorzaakt een aminozuursubstitutie in P-gp (Ala>Ser). Het C3435T polymorfisme in exon 26 veranderd welliswaar geen aminozuur, maar bleek wel gecorreleerd met verminderde P-gp expressie en functie (PNAS 97, 3473-3478).
Methoden: Beenmerg mononucleaire cellen (>85% blasten) van 23 AML patiënten werden geïsoleerd en genetisch gekarakteriseerd. Monsters met een gehele of gedeeltelijke deletie van chromosoom 7 werden niet opgenomen in de studie. Op basis van de DNA sequentie werd een specifieke PCR-RFLP opgezet en gevalideerd voor de detectie van de C3435T mutatie van MDR-1. Het G2677T polymorfisme werd gedetecteerd met behulp van oligonucleotide hybridisatie met specifieke probes voor de G en T variant. Voor functionele P-gp expressie werd een rhodamine retentie assay toegepast, met de P-gp modifyer PSC833. De hoeveelheid P-gp eiwit werd bepaald met behulp van monoclonale antilichamen UIC2 en MRK16. P-gp mRNA-nivo's werd bepaald middels kwantitatieve RT-PCR (TaqMan).
Resultaten: Van de 23 AML patiënten bleken er 8 heterozygoot en 6 homozygoot te zijn voor de C3435T mutatie. Voor het G2677T polymorfisme werden 12 heterozygoten en 5 homozygoten gevonden. Statistische analyse liet geen verschil zien tussen de groep wild typen, heterozygoten of homozygoten voor beide polymorfismen, wanneer mRNA nivo's, eiwit expressie of functionele activiteit werden vergeleken.
Conclusie: In tegenstelling tot wat op basis van de literatuur werd verwacht, werd in onze studie geen correlatie gevonden tussen expressie/functie van P-gp en de aanwezigheid van de C3435T en G2677T polymorfismen in MDR-1.

12.00 – 12.15 uur

Familiaire diabetes insipidus: van klein tot groot

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Inleiding: Familiaire Neurohypofysaire Diabetes Insipidus (FNDI) is een autosomaal dominant overervende aandoening die gekarakteriseerd wordt door polyurie en polydipsie. De oorzaak hiervoor ligt in een tekort aan het anti-diuretisch hormoon.
Methoden: Vier jaar geleden is in dit ziekenhuis de moleculair biologische oorzaak van FNDI bij een tweejarig jongetje en zijn vader opgespoord: een T?G mutatie in exon 3 van het AVP-NP II gen, waardoor in codon 116 het aminozuur Cysteine wordt vervangen door Glycine. Wat begon met een klein groepje van twee patiënten, is uitgegroeid tot een FNDI familie, bestaande uit ca. 200 personen. In een samenwerkingsverband met het Rudolf Magnus Instituut in Utrecht, zijn studies verricht naar het effect van de Cys116Gly mutatie op het AVP-NP II eiwit. Hierbij is het gemuteerde DNA van de patiënt ingebracht in een cellijn, die in staat is het AVP-NP II eiwit tot expressie te brengen.
Resultaten: Uit deze studie is gebleken dat het gemuteerde AVP-NP II eiwit niet geprocessed wordt en zich opstapelt in het endoplasmatisch reticulum (ER) van de AVP-NP II producerende cellen.
Conclusie: De tengevolge van de Cys116Gly veranderde morfologie van het ER kan de functie van de cellen verstoren en mogelijk schade toebrengen, waardoor het normale AVP-NP II eiwit uiteindelijk ook niet meer in de bloedbaan terecht kan komen. Dit verklaart het autosomaal dominante karakter en de delayed onset (enkele maanden tot jaren) van de ziekte. De Cys116Gly mutatie was nog niet eerder beschreven en binnen Nederland de tweede mutatie. Verder onderzoek en diverse publicaties hebben er toe geleid dat er zich ondertussen acht Nederlandse FNDI families bij ons hebben gemeld voor mutatieanalyse. Bij deze acht families zijn vijf verschillende mutaties gevonden. Inmiddels is ook bij een aantal FNDI-families uit diverse Europese landen door ons een mutatie in het AVP-NP II gen vastgesteld. Een neuropsychologisch onderzoek staat inmiddels in de steigers. Hierbij worden de cognitieve functies (concentratie, korte termijn geheugen) bestudeerd voor en na therapie met Minrin™ van FNDI patiënten t.o.v. een controlegroep.

12.15 – 12.30 uur

The clinical and genetic characteristics of congenital plasma dopamine beta-hydroxylase deficiency: a severe orthostatic syndrome or not?

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Introduction: Dopamine β-hydroxylase (DβH) catalyses the conversion of dopamine into norepinephrine. Plasma DβH deficiency has been associated with a congenital severe orthostatic syndrome with absent plasma (nor)epinephrine. Low plasma DβH, however, also occurs in 5% of normal subjects having normal (nor)epinephrine. The genetic basis of symptomatic DβH deficiency has never been investigated.
Methods: We sequenced the DβH gene in two unrelated patients with DβH deficiency and an orthostatic syndrome. We determined plasma DβH in 49 healthy blood donors to identify asymptomatic individuals with low plasma DβH activity to sequence their DβH gene.
Results: Two mutations uniquely associated with plasma DβH deficiency and absent plasma (nor)epinephrine were found in the patients with the orthostatic syndrome. One patient was homozygous for a splice site mutation (IVS1+2 T>C), and the other was compound heterozygote for this splice site variant and a deletion of base 575. In blood donors with low plasma DβH activity we found a mutation at -1021, immediately upstream of the transcription initiation site. Individuals homozygous for –1021T had almost absent plasma DβH activity.
Conclusions: Our study is the first to describe pathogenic mutations in the DβH gene. It defines the genetic basis of DβH deficiency with absent plasma (nor)epinephrine leading to an orthostatic syndrome. It also explains the genetic background of asymptomatic DβH deficiency. The concurrence of homozygosity for the DβH –1021 T-allele with low plasma DβH in healthy individuals suggests that the–1021-locus determines secretion of DβH into the blood without interfering with normal catecholamine synthesis.

12.30 – 12.45 uur

Quantification of mixed chimerism after allogenic bone marrow transplantation by VNTR analysis; a comparison with in situ hybridization using X and Y chromosome specific probes

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Introduction: Patients who develop mixed hematopoietic chimerism after an allogenic bone marrow transplantation might be at increased risk for graft failure and relapse. Therefore, techniques that can distinguish patient cells from donor cells are very useful in the follow up of patients post transplantation.
Methods: In our laboratory we use highly polymorphic VNTR/STR loci for the detection of mixed chimerism. Before transplantation six VNTR/STR containing loci from donor and patient are amplified by PCR and analyzed by capillary electrophoresis. After transplantation only the informative loci are amplified again to detect mixed chimerism. In the present study we have established the sensitivity of the VNTR/STR analysis by comparison with FISH analysis. Hereto, a series of mixed chimerisms was generated by mixing patient and donor cells at different ratios. These cell mixtures were analyzed by FISH using X- and Y- chromosome specific probes. From the same cell mixtures DNA was isolated and the degree of mixed chimerism was determined by VNTR/STR analysis.
Results: The results of the VNTR/STR analysis correlate well with the FISH analysis and the theoretical percentages of the mixed patient and donor cells. At least 5% of patient DNA in a background of donor DNA and visa versa can be detected by VNTR/STR analysis.
Discussion: The use of polymorphic VNTR/STR analysis by PCR and capillary electrophoresis is an easy and powerful method to monitor engraftment of the donor bone marrow and to detect an early relapse.

12.45 – 13.00 uur

A novel single amino acid substitution in the human hexokinase gene is associated with hexokinase deficiency and severe nonspherocytic hemolytic anemia *in vivo*

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Introduction: HK deficiency is a rare autosomal recessive disease characterized by nonspherocytic hemolytic anemia. Little is known about the molecular basis underlying this disease and to date only one patient has been characterized at the DNA level.
Methods: DNA from the affected proband of a Dutch family with HK deficiency previously described by Rijksen et al. was used to characterize the promoter, entire coding region and flanking intronic sequences of the HK gene by PCR and subsequent DNA sequence analysis. A 3D diagram of Thr680 was generated using computer programs Molscript and Raster3D.
Results and Discussion: A homozygous C to G base substitution was found in exon 15 that predicted a serine at residue 680 rather than a threonine. The parents, who were first cousins, were found to be heterozygous for this novel missense mutation and displayed reduced HK activity in their red blood cells. Thr680 is located in a putative ATP binding domain and is highly conserved. Recently, Thr680 was predicted to interact with the γ-phosphoryl of ATP.[2] Expression of a recombinant HK in which Thr680 was mutated to Ser showed a decrease in k_{cat} and K_m for glucose. Furthermore, K_m for MgATP and K_i for inhibitor 1,5-An-G6P were normal. These results contrast with enzyme properties of partially purified HK derived from the patient which was found to have a decreased affinity of the mutant enzyme for phosphate-containing ligands, illustrated by increased K_m for MgATP and increased K_i for inhibitor Glc-1,6-P_2. These observations indicate different *in vivo* enzyme properties of the Thr680Ser HK variant as compared to the recombinant one, possibly as a result of post-translational processing of (mutant) HK *in vivo*.

Sessie 2

11.00 – 11.15 uur

The clinical and molecular basis of a novel neurometabolic disease with severe white matter involvement discovered with body fluid NMR spectroscopy

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Introduction: Body fluid NMR spectroscopy provides an overview of proton-containing metabolites. The technique may show abnormal metabolites that cannot be shown with conventional techniques used to screen for metabolic diseases.
Patients: Two unrelated female patients were investigated suffering from a severe connatal neurometabolic disease. The disease is clinically characterized by microcephaly, failure to thrive, muscular hypotonia, severe mental retardation, epilepsy and abnormal eye-movements. The brain MRI showed severe white matter disease with almost complete absence of myelin. Extensive routine metabolic investigations were unremarkable.
Results: 600 MHz NMR spectroscopy of cerebrospinal fluid (=CSF) revealed the presence of N-acetylaspartylglutamate (=NAAG) in both patients (90-197 μmol/L; reference <18 μmol/L). It could not be demonstrated in urine of the patients. Canavan's disease and X-linked Pelizaeus Merzbacher disease could be ruled out by appropriate methods in both patients (organic acid analysis and full sequencing of the proteolipid protein (=PLP) gene respectively). NAAG is synthesized and released by neurons and catabolized by astrocytes. This metabolic compartmentalization suggests a role in signaling between these brain cell types.
Conclusion: Our data define a novel inborn error of brain N-acetyl aspartate metabolism. The primary defect in our patients remains unknown. The working hypothesis is a defect at the level of brain N-acetylated-α-linked acidic dipeptidase (=NAALADase: EC 3.4.17.21). The disease is the fourth novel inborn error of metabolism discovered by body fluid NMR spectroscopy. It is an example of an inherited metabolic disease that can only be diagnosed in CSF and will be missed with all conventional metabolic screening techniques.

11.15 – 11.30 uur

The differentiation of Multiple System Atrophy from Idiopathic Parkinson's Disease by cerebrospinal-fluid analysis

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Introduction. Multiple system atrophy (MSA) is an adult onset sporadic neurodegenerative disorder clinically characterized by extrapyramidal, cerebellar, pyramidal, and autonomic dysfunction signs and symptoms. Idiopathic Parkinson's disease (IPD) is a common adult onset neurodegenerative disease characterized by extrapyramidal symptoms and neuronal cell loss in the nigrostriatal system. The diagnostic accuracy in both diseases is limited because of the overlap in the clinical presentation. To date, an accurate diagnostic test that differentiates between these two disorders is lacking.
Aim of the study. We investigated if cerebrospinal fluid (CSF) analysis could be a valuable tool in discriminating between MSA and IPD patients.
Methods. CSF from 37 MSA and 42 IPD patients were analysed for: monoamine metabolites (HVA, 5-HIAA, and MHPG), brain specific proteins (NSE, MBP, S-100, and GFAP), tau and A?$_{42}$, pyruvate, lactate, Q albumin, and total protein. Additionally, IBZM-SPECT analyses were performed. Statistical analysis of the data was performed by using either Student's t-test or Mann-Whitney U-test. Binary logistic regression models were used in order to evaluate the value of specific combinations of CSF parameters and IBZM-SPECT in discriminating between MSA and IPD.
Results. CSF levels of tau and other brain specific proteins were significantly higher in MSA patients than in IPD patients, whereas the levels of monoamine metabolites were significantly lower in this patient group. Combinations of these CSF parameters, either together with IBZM-analysis or alone, separated MSA from IPD patients with very high sensitivity and specificity (both >80%).
Conclusions. CSF analysis may be very valuable additional tool in the clinicial work-up of MSA and IPD patients.

11.30 – 12.45 uur

S-100 protein and neuron specific enolase: Biochemical markers for spinal cord dysfunction after thoraco(abdominal) aortic aneurysm surgery

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Introduction: Paraplegia is still a devastating neurologic complication following thoraco(abdominal) aortic aneurysm (TAA(A)) surgery. The aim of this study is to explore whether serum S-100 protein and neuron specific enolase (NSE) could be prognostic factors for the occurrence of paraplegia after TAA(A) repair.
Methods: Forty patients undergoing TAA(A) repair were included in this prospective study. Serum samples were drawn after the induction of anaesthesia and haemodynamic stabilisation, during the cross-clamp period of the critical aortic segment, 5 minutes, 2, 4, 6, 8, and 19 hours respectively after reperfusion. Determinations of S-100 and NSE in serum were performed using a LIAISON® Random Access Analyser. In all patients recording of myogenic motor evoked potentials (MEPs) following transcranial electric stimulation was carried out to monitor the integrity of the motor pathways.
Results: All patients showed increasing serum concentrations of S-100 and NSE until 2 hours after reperfusion. Thereafter, most of the neurological healthy patients (MEP loss ? 50%) demonstrated a decreasing trend in S-100 concentrations whereas the patients with spinal cord dysfunction (MEP loss > 50%) showed more variable patterns. At 19 hours after reperfusion the mean of both S-100 and NSE serum concentrations was higher in paraplegic patients (MEP loss > 50%) than in non-paraplegic patients (MEP loss > 50%).
Conclusions: Our results suggest that the time course of S-100 concentrations in serum might be a prognostic factor for spinal cord dysfunction after TAA(A) repair. In addition, both S-100 and NSE serum concentrations could play a role in the diagnosis of paraplegia.

11.45 – 12.00 uur

Creatine transporter defect: a new disorder with a relatively high incidence?

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Introduction: We identified a new inborn error of metabolism caused by a defect in the X-linked creatine transporter/CRTR mapped at Xq28 (MIM 300036) (1). An X-linked creatine transporter was hypothesized because of 1) the absence of creatine in the brain of the index patient as indicated by H-MRS 2) the presence of marked creatine levels in urine and plasma ruling out a creatine biosynthesis defect 3) the absence of an improvement on creatine supplementation, and 4) the fact that the pedigree suggested an X-linked disease. The CRTR1 gene was mapped at Xq28.
Methods: Creatine levels in body fluids and in cultured fibroblasts were determined by stable isotope-dilution GCMS. DNA sequence analysis was performed by standard molecular biology techniques using bigdye terminators and an ABI 310 machine.
Results: Our hypothesis was proven by the presence of 4 different mutations in the CRTR1 gene (in five unrelated families) and by the impaired creatine uptake in fibroblasts of male patients. Three families were encountered in one institute (CHMCC)
Conclusion: This newly discovered X-linked disorder might account for a considerable fraction of mental retardation observed in males. The expressive speech and language delay, autistic behavior, the absence of creatine in the H-MRS of the brain and the increased creatine levels in body fluids are hallmarks of this disorder. It might prove worthwhile to screen males with mental retardation in association with significant expressive speech and language delay or autism for creatine in urine.

Literature

1. Salomons et. al ., Am J Hum Genet, 2001; 68: 1497-1500.

12.00 – 12.15 uur

Combined cholesteryl ester transfer protein (CETP) and hepatic lipase (HL) gene variants associate with coronary artery disease (CAD) despite increased HDL cholesterol

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Introduction: HDL cholesterol is considered an antiatherogenic lipoprotein due to its role in reverse cholesterol transport. HDL cholesterol concentration/metabolism is affected by HL and CETP, two proteins active in the reverse cholesterol transport pathway.
Methods: We assessed the impact of combined presence of HL and CETP lowering variants (HL –480C>T substitution and CETP TaqIB polymorphism) on CAD risk, comparing 792 men with CAD and 718 nonsymptomatic controls.
Results: Cases and controls had similar allele frequency distributions of the separate gene variants. However, in CAD patients but not in controls, the observed distribution of the combined HL and CETP gene variants was significantly different from the expected distribution had the variants occurred independently ($P<0.05$). More CAD patients than expected had the combined genotype B2B2-TT (10 versus 6), despite relatively high HDL cholesterol (30% higher than in patients with other genotype combinations ($P<0.001$)).
Conclusion and discussion: The combined presence of the CETP and HL lowering variants is increased in CAD patients despite relatively high HDL cholesterol. These high HDL levels probably indicate a compromised reverse cholesterol transport and do not represent an antiatherogenic risk. We propose that the analysis of CETP and HL gene variants may help to ascertain coronary risk in individual patients, with possible therapeutic consequences.

12.15 – 12.30 uur

Elevated troponin T concentrations in critically ill patients

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Introduction: Damage of myocardial cells results in release of cardiac-specific proteins, such as troponin T (cTnT). Elevated cTnT levels are observed in acute myocardial infarction (AMI), unstable angina, myocarditis, cardiac trauma and perioperative cardiac complications. In addition, elevated cTnT levels have also been observed in critically ill patients suffering from sepsis or septic shock. In this study we selected a group of critically ill patients to determine the incidence of cTnT elevations.
Methods: Thirty-four critically ill patients who were admitted to the intensive care unit were included. Inclusion criteria were mechanical ventilation, thoracic or vascular surgery. Bloodsamples were collected at admission, the next morning and 24 hours after the second bloodsampling. These samples were used for cTnT determination. Electrocardiographs (ECG) were made when cTnT levels were elevated (>0,1 µg/L).
Results: Eleven patients (34%) had elevated cTnT levels, which were already present upon inclusion in 8 out of 11 positive patients (73%). Based on ECG, only 2 of cTnT-positive patients (18%) had an AMI. Five cTnT-positive patients (45%) showed ischemic ECG changes. Mean age of cTnT-positive patients was higher (73 years) compared to cTnT-negative patients (59 years). No differences in mortality rates between positive and negative patients were observed.
Conclusion: Among our selected group of critically ill patients an unexpected high percentage (32%) has elevated cTnT concentrations. Only 18% of cTnT-positive patients suffered from AMI based on ECG results suggesting that a high percentage of critically ill patients suffer from clinically unrecognized (minor) myocardial damage.

12.30 – 12.45 uur

In vivo thrombogenicity of human cell-derived microparticles

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Introduction: We previously reported that microparticles isolated from the pericardial cavity of patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) promote thrombin generation in vitro via tissue factor (TF). Here, we investigated their thrombogenic effect in vivo.
Methods: Microparticles were isolated from pericardial plasma of patients (n=5) or venous plasma of healthy controls (n=3), characterized by flow cytometry, and studied in a venous stasis thrombosis model in rats.
Results: Pericardial microparticles were highly thrombogenic, whereas control microparticles, at 3-fold higher concentrations, were not (median thrombus weights 24.8 versus 0 mg; p=0.001). Preincubation of pericardial microparticles with anti-human TF abolished their thrombogenicity (median 0 mg; $p<0.01$) while anti-human factor XII had no effect (median 19.6 mg; $p>0.05$). The antibodies were specific for the respective human proteins, so anti-TF only inhibited TF present on the surface of human microparticles without any effect on rat TF, and anti-factor XII only served as a control antibody, not as an inhibitor of rat contact activation.
Conclusions: Pericardial microparticles promote thrombus formation in vivo via TF. Their reinfusion may contribute to the thromboembolic and adverse neurologic sequelae in patients after cardiac surgery with CPB. Also, these results substantiate our previous findings of a pathophysiological role of TF-exposing microparticles in disseminated intravascular coagulation.

12.45 – 13.00 uur

Antistoffen tegen transglutaminase bij autoimmuunziekten: nieuwe gegevens

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Inleiding: Antistoffen tegen weefseltransglutaminase (tTG) van het IgG-type worden aangetroffen bij diverse autoimmuunziekten en zijn sterk geassocieerd met de aanwezigheid van anti-ds-DNA- en SSA/SSB antistoffen. (1). De theorie is dat bij sommige autoimmuunziekten het opruimingsproces van apoptotische lichaampjes, die tTG bevatten, gestoord is waardoor het door het immuunsysteem kan worden gedetecteerd.
Nader onderzoek werd verricht naar het gebruik van tTG uit diverse bronnen. Verder werd de klinische betekenis van de antistoffen bestudeerd.
Methoden: Voor het meten van IgG anti-tTG werd een time-resolved fluoroimmunoassay gebruikt. Als antigeen werden resp. gebruikt cavia-levert-tTG, recombinant tTG (baculovirussysteem), recombinant tTG (humane cellen).
Voor de klinische betekenis werd statusonderzoek verricht naar patiënten met anti-tTG Tevens werden sera van zeer goed gedocumenteerde patiënten met beginnende RA afkomstig uit de z.g. COBRA-trial onderzocht op de aanwezigheid van anti-tTG.
Resultaten en discussie: De bron van het in de assay gebruikte antigeen is van groot belang. Recombinant tTG blijkt hiervoor niet bruikbaar te zijn. Kennelijk zijn het complexen van het enzym tTG en zijn substraat die neoantigenen presenteren. De relatie tussen anti-tTG en micropartikels, waarschijnlijk bestaande uit apoptotische lichaampjes kon worden aangetoond. Anti-tTG kwam voor bij patiënten met SLE, MCTD, M. Sjögren.
*Conclusie:*Het verband tussen apoptose, apototototisch lichaampjes en hun immunogeniteit wordt door de waarnemingen bevestigd.

Literatuur

1. Van der Sluijs Veer G, Vermes I. IgG autoantibodies to tissue transglutaminase in relation to antinuclear antibodies. Clin Chem 2001; 47: 952-954.