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Sessie 1 Analytische onderwerpen

09.00 - 09.15 uur

Analytische evaluatie van de Spermalite (SQA-v) ten behoeve van oriënterend fertiliteitsonderzoek

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Inleiding: Semenanalyses conform de WHO-richtlijnen, ten behoeve van oriënterend fertiliteitsonderzoek (OFO), zijn arbeidsintensief. De analytische spreiding tussen analisten en laboratoria is groot. Geautomatiseerde uitvoering van semenanalyses zou uitkomst kunnen bieden. In deze studie is de Spermalite (SQA-v) voor dit doel onderzocht.
Methode: Simultaan aan de semenanalyses, die conform de WHO-criteria werden uitgevoerd, zijn verschillende semenparameters met behulp van de Spermalite vastgesteld in 50 ejaculaten, die ten behoeve van een OFO waren ingeleverd. De reproduceerbaarheid van de analyses werd vastgesteld door monsters in vijfvoud te bepalen.
Resultaat: De reproduceerbaarheid (CV) van de bepaling van semenparameters, die bepaald zijn met de Spermalite, is goed. De CV van klinisch relevante parameters zijn: Total sperm concentration (TSC (M/ml) = WHO-concentratie (WHOconc)): CV= 7,6 %, progressive motility (PM (%) = WHO A+B-motiliteit (WHOpm)) CV= 10,0 % en progressive motile sperm concentration (PMSC (M/ml) = WHO-motiele spermaconcentratie (WHOpmsc)): CV = 7,1 %. De correlatie tussen bovenstaande Spermalite- en de WHO-parameters is goed: TSC (M/ml)= 1,10x WHOconc + 2,4; r = 0,97, PM (%) = 1,02xWHOpm +5,4; r = 0,93 en PMSC (M/ml) =1,02x WHOpmsc - 1,8; r = 0,98.
Conclusie: Met de Spermalite kunnen eenvoudig en betrouwbaar klinisch relevante (WHO-) semenparameters zoals de TSC, PM en PMCS worden vastgesteld. De reproduceerbaarheid is beter dan met de conventionele WHO-semenanalyse. De Spermalite biedt een aanzienlijk expoitatievoordeel t.o.v. de WHO-methode. Naar schatting kunnen circa 60 % van de OFO's worden uitgevoerd met behulp de Spermalite, zonder additioneel microscopisch onderzoek. Om bovenstaande redenen zal binnen onze instelling de Spermalite routinematig voor OFO worden ingezet.

09.15 - 09.30 uur

Results of an international round robin for the quantification of serum Non Transferrin-Bound Iron

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Introduction: Non transferrin-bound iron (NTBI) in the circulation is a pathological phenomenon, which generally occurs in patients with iron-overload. The significance of NTBI as a potentially toxic agent is attracting increased attention.

Methods: An international, interlaboratory comparison study was conducted to assess differences in the various methods used to quantify NTBI: 7 chelation methods (varying in scavenging molecules for NTBI, blocking of apotransferrin and detection methods)

and a bleomycine assay for the quantification of redox active iron. For each method NTBI results were reported for serum samples of 12 patients with iron overload, in a 3-day analysis, of 4 determinations per day for a total of 12 measurements. Means, SDs and CVs were calculated for each method, each patient sample, within methods and across samples. Furthermore, Pearson's correlations between methods means were calculated.
Results: Results reported for serum NTBI levels demonstrated across sample, within method means and CVs that ranged from 0.13 to 4.32 µmol/L and 6.7 % to 255 %, respectively, for the 8 methods. There were significant mutual correlations between the means of the NTBI obtained by the various chelation methods ($p<0.01$, correlation coefficients R ranging from 0.77 to 0.99), but these correlations were of lower significance ($p<0.05$) or even non-existent when the chelating methods were compared with the method for redox active iron (R range 0.50-0.60)
Conclusion: Sample means and CVs for the various NTBI methods differed considerably. However, with the exception of the method for redox active iron, sample means of NTBI methods highly correlated. These results form the first step towards standardization of the NTBI-assay and increase our insight in the pathophysiological meaning of NTBI.

09.30 - 09.45 uur

The Cel I mutation detection technique with 100 % sensitivity for detecting disease causing mutations

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Introduction: In the past several enzymes and techniques have been described and used for mutation detection. Despite extensive efforts, no enzyme or technique, showed high sensitivity in screening DNA for the presence of a mutation. Now there is a new endonuclease, Cel I extracted from celery, that can be used for mutation detection. Cel I is unique in its ability to cut DNA at the site of a mismatch in heteroduplexes and Cel I can detect 100% of the sequence variants present, including deletions, insertions and base-substitutions.
Methods: In the Cel I mutation detection assay, the first PCR of the target is followed by a second PCR with primers labeled with a fluorescent dy. After heteroduplex formation, the PCR products are incubated with Cel I endonuclease, that preferentially cleaves the mismatches. The cleavage products (=mutations or polymorphisms) are easily detected by electrophoresis on a sequencer. The mutation can be detected as an enlarged or additional band.
Results: We have tested more than 20 identified mutations in several hundreds of PCR products. In addition, we screened many exons in DNA of patients with inherited diseases, like Wilson, FMF and FNDI. All of the previously known mutations were detected. Also, novel mutations were identified by sequencing of the suspected DNA fragments. The Cel I mutation detection technique has detected all of the mutations with 100 % sensitivity and 100 % specificity.
Conclusion: Our results demonstrate that the Cel I mutation detection technique is a higly sensitive and rapid method for detecting disease-causing mutations.

Literature: Oleykowsky CA, et al. Nucleic Acids Res 1998; 26: 4597-4602.

09.45 - 10.00 uur

DNA sequencing of the ß-globin gene in the identification of ß-globin chain associated hemoglobin disorders

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Introduction: Hemoglobin disorders are a heterogenous group of autosomal recessive disorders mainly seen in the immigrant population and characterized by either the reduced synthesis of one or more globin chains (the thalassemias) or the synthesis of a structural abnormal globin chain (the hemoglobin variants). Potential carriers of a ß-globin chain associated hemoglobin disorder are identified in our anemia protocol, based on having microcytic hypochromic erythrocytes without iron deficiency, by family screening or in chromatographic HbA1c-analysis in diabetic patients.
Methods: When hemoglobin IEF electrophoresis of a patient reveals either an increased % HbA2 (> 2,8 %) or a variant hemoglobin, the promotor region (nt –307 to nt +50 relative to the cap site), the 5' region (nt –103 to nt 144 in IVS-II) and/or the 3' region (nt 603 in IVS-II to nt +62 relative to the 3' UTR) of the ß-globin gene is amplified and sequenced in order to identify the mutation present.
Results: In 303 consecutive cases presented for ß-globin gene sequence analysis, 175 with a hemoglobin variant (mainly HbS, HbC, HbE and HbD) were observed, 73 revealed ß-thalassemias differentiated in ß+ and ß0 variants and 15 showed compound heterozygosity for mutations in the ß-globin gene (e.g. HbS and ß+ thalassemia).

Conclusion: The described procedure offers an elegant method for identifying unknown carriers of ß-globin chain associated hemoglobin disorders and screening of family members of known carriers. In addition, compound heterozygosity of mutations in the ß-globin gene can easily be identified.

10.00 - 10.15 uur

Een nieuwe klinische functie-/screeningstest voor het integrale stollingssysteem?

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Inleiding: Het stollingssysteem in vivo is zo complex dat zijn functie niet goed wordt weerspiegeld door een of meer stollingsfactorconcentraties of bloedplaatjeseigenschappen.
Methode: De trombinegeneratiecurve ('trombogram') werd vanouds gebruikt om de stolbaarheid van bloed als geïntegreerd systeem te bestuderen. Verhoging (verlaging) van trombinegeneratie (TG) met >10% (>70%) duidt op een verhoging van het trombose-(bloedings)risico. De klassieke TG-bepaling is echter te arbeidsintensief voor routinegebruik. Met de Calibrated Automated Thrombin Generation (1) kunnen zowel in plaatjesarm als plaatjesrijk plasma, 48 curves gelijktijdig bepaald worden, hetgeen toepassing in het klinisch-hematologisch laboratorium mogelijk maakt. Door variatie van de weefselfactor- (TF) en de trombomodulineconcentratie (TM) kunnen afwijkingen nader gelokaliseerd worden.
Resultaat: Hyperprotrombinemie (G20210A), remmerdeficiënties (antitrombines, proteïnes S,C) en APC-resistentie verhogen TG. Lupus-anticoagulans veroorzaakt een late start (stollingsremming) en verhoogde trombinevorming (tromboseneiging). Deficiënties van alle stollingsfactoren (incl. von-Willebrand-factor en fibrinogeen), trombocytopenie, trombocytopathie (Glanzmann, Bernard-Soulier) zowel als antistollingsbehandeling (heparines (ook LMWH!), orale antistolling, directe trombineremmers) en plaatjesremmers (reopro, clopidogrel) verlagen het trombogram. Effectieve orale antistollingstherapie remt het trombogram 60-80%. Ook combinatietherapie kan vervolgd worden (bv. heparine plus OAC, OAC en antiplaatjesmedicatie).
Conclusie: Met het trombogram kan men dus A: hyper- en hypocoagulabiliteit opsporen, onafhankelijk van de oorzaak en B: bepalen in welk areaal van het stollingsproces een gevonden afwijking gelokaliseerd moet worden. Dit vermindert de noodzaak om een groot aantal specifieke bepalingen uit te voeren.

Literatuur: 1. Hemker, et al. Pathophysiol Haemost Thromb 2003; 33: 4-15.

10.15 - 10.30 uur

Automatische celtelling in liquor met de ADVIA 120

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Inleiding: Het tellen van leukocyten (WBC) en erytrocyten (RBC) in liquor gebeurt in de meeste laboratoria microscopisch met behulp van telkamer en eventueel cytospin. Dit is tijdrovend, vereist praktische ervaring en moet snel gebeuren in verband met instabiliteit van het monster. In dit onderzoek is nagegaan of de celtelling en leukocytendifferentiatie in liquor betrouwbaar is uit te voeren m.b.v. de ADVIA 120 CSF-methode.
Methode: Liquormonsters (n=77) werden geteld in een Fuchs-Rosenthal-telkamer en gedifferentieerd, waarbij gebruik is gemaakt van een cytospinpreparaat (referentiemethode). Tevens werden monsters geprepareerd met ADVIA 120 CSF reagens en binnen 4 uur geanalyseerd m.b.v. de CSF-module van de ADVIA 120. Monsters met RBC en/of WBC hoger dan de bovengrens van de ADVIA 120 CSF methode (RBC: 1500 x 10^6/l; WBC: 5000 x 10^6/l) werden voorafgaand aan de analyse verdund. De functionele ondergrens (VC=20%) van de ADVIA 120 CSF methode werd in dit onderzoek bepaald voor zowel WBC als leukocytendifferentiatie.
Resultaat: Er was een goede overeenkomst tussen de automatische RBC-telling en de manuele methode (r^2 = 0,99). De functionele ondergrens voor WBC bedroeg 7 x 10^6/l, terwijl een betrouwbare automatische differentiatie kon worden geleverd indien WBC > 17 x 10^6/l. Voor zowel WBC-telling als leukocytendifferentiatie was de overeenkomst tussen de ADVIA 120 CSF methode en de referentiemethode goed (WBC: r^2 = 0.99; %monocyten: r^2 = 0.88; %lymfocyten: r^2 = 0.92; %neutrofiele granulocyten: r^2 = 0.96).
Conclusie: De ADVIA 120 CSF methode geeft een snelle geautomatiseerde analyse van leukocyten en erytrocyten in liquor. Fixatie van de cellen in CSF reagens maakt analyse tot 4 uur na monsterafname mogelijk.

10.30 - 10.45 uur

Hemocytometrische analyse van vochten met de Sysmex XE-2100

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Inleiding: In toenemende mate wordt het klinisch-chemisch laboratorium geconfronteerd met aanvragen voor celtellingen in vochten zoals CAPD, ascites en pleura. De referentiemethode voor het tellen van cellen in dit soort vochten (telkamer/cytospin/microscoop) is onderhevig aan een grote binnen- en tussenlaboratoriumimprecisie en is bewerkelijk. Dit onderzoek ging daarom na of het mogelijk is om hematologische analyses in CAPD-, ascites- en pleuravochten betrouwbaar te meten met een standaard-hematologieanalyzer (Sysmex XE-2100).
Methode: Vochten werden opgevangen in EDTA. Het aantal leukocyten (WBC) werd geteld met behulp van een standaard-telkamer (Bürker), waarna de microscopische differentiatie werd gedaan op een cytospin-preparaat (referentiemethode). Alle monsters werden tevens geanalyseerd m.b.v. de Sysmex XE-2100 (WBC/BASO kanaal + DIFF kanaal). De functionele ondergrens (VC=20%) werd bepaald door CAPD-vloeistof serieel te verdunnen en elke verdunning in 10-voud te meten op de XE-2100.
Resultaat: De functionele ondergrens bedroeg ~0,04* 10^9/l voor WBC (WBC/BASO en DIFF kanaal) en ~0,06*10^9/l voor het aantal neutrofielen (NEUT; DIFF-kanaal). Automatische WBC-telling in ascites (n=23; WBC/BASO-kanaal) kwam goed overeen met de referentiemethode (WBC-analyzer = 1,07*WBC-referentiemethode – 0,005; r = 0,99). De overeenstemming tussen de referentiemethode en de automatische DIFF was goed voor het %NEUT (NEUT-analyzer = 0,94*NEUT-referentiemethode + 1,64; r = 0,97), het %MONO (MONO-analyzer = 0,87*MONO-referentiemethode + 2,03; r = 0,91) en het %LYMPH (LYMPH-analyzer = 0,95*LYMPH-referentiemethode + 1,73; r = 0,94). Voor CAPD (n=14) en pleura (n=15) werden vergelijkbare resultaten gevonden.
Conclusie: Automatische telling en differentiatie van leukocyten in verschillende vochten met de Sysmex XE-2100 komt goed overeen met de handmatige referentiemethode. Voor vochten met weinig cellen (<0,04*10^9/l) zoals liquor is geautomatiseerde analyse met de XE-2100 minder betrouwbaar.

10.45 - 11.00 uur

Direct measurement of lithium in whole blood using a glass chip with integrated conductivity detection for capillary electrophoresis

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Introduction: At the present state of micro fluidic chip technology, it is now possible to combine sample treatment steps with separation methods on a single device. However, still few examples have been presented, which fully exploit combining multiple functionalities. We demonstrate here that the measurement of alkali metals in a drop of whole blood can be performed on a capillary electrophoresis (CE) chip with defined sample loading applying the principles of column coupling. Using this approach specifically the analysis of lithium in whole blood was examined.
Methods: Whole blood collected from a finger prick was mixed with anticoagulant and transferred directly onto the chip without diluting, extracting or removal of components. The electrokinetic transport of red blood cells inside the channels was studied to find sample loading conditions suitable for analysis of lithium, but that did not inject cells into the separation channel. Both bare glass chips and CE chips coated with polyacrylamide were used showing the behaviour of the cells under different electroosmotic flow conditions.
Results: Lithium could be determined in whole blood diluted only with anticoagulant and spiked with lithium. In a matrix of 150 mmol/L sodium the detection limit for lithium is with 0.4 mmol/L on the high side as a quantitation limit of 0.2 mmol/L is desirable for clinical use. Chips with untreated surfaces quickly fouled with proteins, but devices with coating gave reproducible electropherograms. In addition potassium and sodium can also be detected in the same separation run.
Conclusion: The presented experiments demonstrate that it is possible to measure alkali metals in a sample as complex as whole blood with a micro fluidic glass chip.

Sessie 2 Bedrijfsvoering

09.00 - 09.15 uur

De implementatie van een kwaliteitssysteem tijdens een reorganisatietraject met behulp van een projectorganisatie

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Inleiding: In de gezondheidszorg vinden op dit moment vele reoganisaties plaats, zoals fusies en bezuinigingsoperaties. Deze activiteiten hebben hun weerslag op de slagvaardigheid van het laboratorium. Veel vernieuwingen komen tot stilstand. Regelmatig wordt eerst gereorganiseerd en volgt daarna pas de implementatie van een kwaliteistsysteem. Dit duurt enige jaren. Het is echter mogelijk dit traject aanzienlijk te versnellen (1).
Methode: Als onderdeel van een fusietraject tussen 2 middelgrote laboratoria werd een projectorganisatie ingericht bestaande uit een zogenaamde kerngroep met medewerkers uit ieder niveau van de organisatie over 2 lokaties en diverse projectgroepen, bestaande uit ter zake kundige medewerkers en 1 lid van de kerngroep. De projectgroepen waren verantwoordelijk voor de uniformering en protocollering van voorschriften en afspraken voor de beide locaties. De resultaten van de projectgroepen werden geevalueerd in de kerngroep. Bij accoordbevinding werden de nieuwe afspraken uitgewerkt in SOP's.
Resultaat: Na de benoeming van de leden van de kerngroep werden de projectgroepen ingesteld. De resulaten van de projecten werden in de kerngroep beoordeeld op samenhang met de overige projecten en het tempo van de reorganisatie. Aldus onstond er binnen een periode van een half jaar een dynamische veranderingsgezinde organisatie. Parallel aan het reorganisatietraject werd het kwaliteitssysteem ontwikkeld. Na een jaar was de reorganisatie zo ver gevorderd dat de projectorganisatie afgebouwd kon worden. De verwachting is binnen 2 jaar na de fusie accreditatiewaardig te zijn.
Conclusie: Reorganiseren legt een groot beslag op mens en middelen in een organisatie. Door de inrichting van een projectorganisatie is het mogelijk gebleken om tijdens een reorganisatietraject slagvaardigheid te behouden. Dit levert een enorme tijdwinst op in de orde van enkele jaren.

Literatuur: 1. Senge P. De vijfde discipline praktijkboek. Academic service; 1995.

09.15 - 09.30 uur

Kwaliteit met minder moeite; een evaluatie van kwaliteitscriteria op basis van biologische variatie

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Inleiding: Het analyseren van controlemonsters is een essentiële, maar kostbare en vooral arbeidsintensieve aangelegenheid. In het verleden zijn diverse beoordelingscriteria ontwikkeld om een juiste balans te vinden tussen het ten onrechte af- en goedkeuren van bepalingenseries.
Methode: In lijn met de Westgard-criteria, hebben we binnen ons laboratorium in 2001 nieuwe criteria ontwikkeld voor de bepalingen op de Roche Modular. Als basis werd echter gekozen voor de intra- en interindividuele biologische variatie.
Resultaat: Als gevolg hiervan zijn de beoordelingscriteria van een zevental bepalingen verruimd naar een 3, 4 of 5-s regel. Deze studie laat retrospectief zien dat deze verruiming resulteert in een significante reductie van het aantal controlemonsters met 7%. Bovendien bleek het percentage controlemonsters dat moest worden herbepaald zelfs met 42% af te nemen.
Conclusie: De gekozen methodiek op basis van biologische variatie leidt tot een grotere efficiëntie, zonder dat de kwaliteit van de bepalingen volgens externe kwaliteitscontroles van de SKZL afnam.

09.30 - 09.45 uur

Clinical Chemistry and Intellectual Property: should we care?

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Introduction: Intellectual Property (IP) is developed within hospitals on a daily basis. Part of this will have little commercial value, and will be best utilised through the normal routes of dissimination e.g. publishing. However, some IP will need further investment to reach its full potential, and this will only be obtained through proper protection of the IP for the investor. This presentation will explain various types of IP and its relevance within Clinical Chemistry.
Methods: The presentation will use some practical examples of successful (and unsuccessful) exploitation mainly from the healthcare and universities in the UK and the process involved, including audits and protection mechanisms. Where the technology has a high development and acceptance costs (for example in clinical chemistry), IP management is of paramount importance.
Results: The presentation explains the relevance of IP in institutes working in the Clinical Chemistry arena. It highlights the risk of unsuccessful IP management. Using a management tool that is adapted to the special needs of the institute(s), IP management will be beneficial to the instite(s), the individual(s) involved and society as a whole.
Conclusion: IP management can lead to successful development of technologies that would otherwise not been able to reach their full potential. The US has realised this over a decade ago and is now benefiting from their activities. The UK has followed the trend recently. The Dutch health care sector, in particular areas where commercially strong IP with a long route to market is being developed should make an informed decision how to engage in IP management.

Sessie 2 Klinische onderwerpen I

09.45 - 10.00 uur

Biological variation of NT-proBNP in patients with heart failure and its impact on NT-proBNP monitoring for tailored treatment

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Introduction: N-terminal brain natriuretic peptide (NT-proBNP) may be useful for the diagnosis of heart failure, tailored treatment, and prognosis assessment. Monitoring for tailored treatment necessitates insight into the 'reference change value' (RCV). We established within-day analytical variation (CVa), within-day, day-to-day and week-to-week total variation (CVt) and calculated individual variation (CVi) and RCV for 45 patients with 'steady state' heart failure living in Curaçao.
Methods: The patients had stage I (n=3), II (n=31) and III (n=11) heart failure according to the NYHA classification. EDTA-blood samples were collected in their homes within one day (n=6, every 2 h, starting at 8.00h), within one week (5 consecutive days between 8.00 and 10.00) and within 6 weeks (6 consecutive weeks at the same day of the week between 8.00 and 10.00h). Plasma samples were stored at -80°C. NT-proBNP (Roche) was measured within 6 months after collection within a single series on two consecutive days.
Results: The mean CVa of 5 quality control samples (NT-proBNP means: 17, 23, 287, 595, 1147 pmol/L) was 2.9% and proved independent of the NT-proBNP level. The mean within-day, day-to-day and week-to-week CVi values were 9, 24 and 35%, respectively. Within-day, day-to-day and week-to-week RCV values for single measurements before and after therapy adjustment amount to 26, 67 and 97%, respectively. RCV for the means of 3 assays before treatment and 3 assays after treatment amount to 15, 39 and 56%, respectively.
Conclusion: We conclude that the CVi of NT-proBNP highly exceeds CVa and that RCV is high. Insight into the determinants of CVi in patients with heart failure is needed. Only reduction of CVi might save the applicability of NT-proBNP for tailored treatment in individual patients.

10.00 - 10.15 uur

Plasma fatty acid-binding protein outperforms troponin as a sensitive marker of myocardial injury in heart failure

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CARIM, Dept. of Molecur Genetics[1], Maastricht University; Dept. of Clinical Chemistry[2] and Cardiology[3], University Hospital, Maastricht, The Netherlands

Introduction: Elevated plasma concentrations of cardiac troponin T (cTnT) have been shown in a marked proportion of patients with congestive heart failure (CHF), indicating that progressive deterioration of ventricular function in CHF patients is associated with ongoing myocardial injury. We compared (heart-type) fatty acid-binding protein (FABP) and cTnT as markers for myocardial injury in CHF.
Methods: Plasma samples were obtained upon admission from 58 consecutive patients with CHF (NYHA class III or IV). All patients had elevated plasma NT-pro-BNP concentrations (> / = 62 pmol/L) and no skeletal muscle injury (plasma myoglobin/FABP ratio <7). Kidney malfunction was excluded by creatinine levels <120 µmol/L. FABP was measured using a specific immunoassay, and TnT, NT-proBNP and myoglobin were measured with an ECLIA method (Elecsys®).
Results: In the total CHF group, plasma cTnT was elevated (>0.02 µg/L) in 31 patients; median level was 0.083 µg/L (25-75 percentiles 0.036-0.269 µg/L). FABP was elevated (>6 µg/L) in 47 patients; median level 13.3 µg/L (25-75 percentiles 9.1-20.7 µg/L). Patients could be subdivided into four groups according to plasma concentrations of FABP and cTnT. In 28 patients, cTnT and FABP levels were elevated together, while in 15 patients cTnT and FABP levels were normal. Only 3 patients had a normal FABP level together with an elevated cTnT level, while 19 patients (29%) showed increased plasma FABP despite a normal plasma cTnT concentration.
Conclusion: (i) Minimal myocyte injury is detectable in a significant proportion of patients with CHF. (ii) FABP is a more sensitive marker for detection of minimal myocyte injury than cTnT.

10.15 - 10.30 uur

Tissue specific types of fatty acid-binding protein, B- and H-FABP, as novel plasma markers of human brain injury

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Introduction: Sensitive biochemical markers are important for early detection of acute brain injury. Brain- and heart-type fatty acid-binding protein (B- and H-FABP) are expected to be released rapidly from damaged brain cells. Tissue content and clinical utility of B-and H-FABP were evaluated in this study.
Methods: Tissue contents of B-FABP and H-FABP were determined in human brain samples from respectively frontal-, temporal- and occipital lobe, striatum and cerebellum. For investigation of clinical utility, serum B- and H- FABP, S-100B and neuron-specific enolase (NSE) were measured in 130 patients with mild traumatic brain injury (MTBI). All markers were determined via specific immunoassays (ELISA).
Results: Human brain tissue contents of H-FABP were found to be >10 fold higher than those of B-FABP. Frontal-, temporal- and occipital lobe, striatum and cerebellum contained 26.3 ± 1.8, 31.9 ± 4.8, 21.2 ± 3.0, 30.6 ± 5.1, 39.5 ± 4.0 and 16.2 ± 2.2 µg/g ww H-FABP respectively (mean ± sd). The contents of B-FABP, were 3.2 ± 0.19, 2.2 ± 0.03, 1.01 ± 0.05, 0.755 ± 0.03, 2.01 ± 0.07 and 2.80 ± 0.18 µg/g ww, respectively. In the MTBI study, S-100B serum levels were above reference value (> 0.3 µg/L) in 45,4% of the cases, and NSE in 50.8% (>10 µg/L). However, B-FABP and H-FABP were elevated in 66.9% and 70.0% respectively.
Conclusion: This is the first study to document tissue contents of both B-FABP and H-FABP in various areas of human brain. Moreover, in patients with mild traumatic injury, B- and H-FABP were elevated in more cases than were the currently used markers S-100B and NSE, suggesting higher sensitivity for detection of brain injury.

10.30 - 10.45 uur

Essential fatty acid and B-vitamin status in schizophrenia

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Introduction: The 'membrane phospholipid hypothesis' of schizophrenia postulates a relation between schizophrenia, low status of arachidonic (20:4ω6) and docosahexaenoic (22:6ω3) acids, and impaired methylation of phoshatidylethanolamine to phosphatidylcholine by S-adenosylmethionine. Metabolic clearance of the homocysteine, formed in the latter process, makes use of B-vitamins (i.e. folate, vitamin B12 and vitamin B6) as cofactors. We investigated whether patients with schizophrenia in our hospital have low status of 20:4_6, 22:6ω3 and B-vitamins.
Methods: Blood samples were collected from 61 schizophrenics [61% men, median age 30 years (19-53), median duration of psychiatric disease 5 years (1-20)]. We performed the following assays: fatty acids (FAs) in erythrocytes (RBC), folate and vitamin B12 (serum), vitamin B6 (whole blood) and homocysteine (EDTA-plasma). Data were evaluated by comparison with reference values of healthy controls (RBC-FAs, n=69), local reference values (folate, vitamin B12, vitamin B6, homocysteine) or a vitamin-optimized reference value (homocysteine).
Results: Patients had significantly lower RBC polyunsaturated FAs, ω6FAs, 20:4ω6, 22:4ω6, ω3FAs and 22:6ω3, and higher saturated and monounsaturated FAs, 20:3ω6 and 22:5ω6/22:6ω3. The percentage patients with above normal 22:5ω6/22:6ω3 (an indicator of low 22:6ω3 status) and 20:3ω9 (an indicator of essential fatty acid deficiency) amounted to 15 and 3%, respectively. Patient percentages with below-reference values for folate, vitamin B12 and vitamin B6 were: 3,28 and 5%, respectively. Homocysteine was increased in 26% (cut-off 15 µmol/L) and 70% (cut-off 10 µmol/L) of patients, with 4 showing extremely high values (range: 57.5-74.8 µmol/L).
Conclusion: Subgroups of schizophrenic patients have low status of 20:4ω6, 22:6ω3 and B-vitamins (notably vitamin B12). More attention should be paid to their diet, since at least low 20:4ω6 and 22:6ω3 may be causally related to their symptoms.

10.45 - 11.00 uur

Elevated concentration of cerebrospinal fluid tissue transglutaminase in Parkinson's disease indicating apoptosis

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Introduction: Tissue transglutaminase (t.TG) is a transamidating enzyme, that catalyses the cross-linking of intracellular proteins, thus assembling a protein scaffold that prevents leakage of intracellular components. T.TG is activated during the apoptotic cell death cascade and plays a key role in the formation of apoptotic bodies. Apoptotic cell death has been postulated to be among the mechanisms responsible for the loss of the dopaminergic nigrostriatal neurons in Parkinson's disease (PD). This view is based on histochemical studies performed in brain tissue of autopsied patients with PD where there is no way to distinguish between neuronal cell death in vivo and post mortem.
Methods: We collected cerebrospinal fluid (CSF) samples of 54 patients with PD and 34 control subject without signs of neurodegenerative diseases. The diagnosis of PD was based on clinical signs (tremor, rigidity, bradykinesia and postural disturbance), cognitive functions and sleep disturbances. T.TG measurements of CSF samples were performed by using a sensitive and specific ELISA. The sensitivity of the assay was 0.5 pg/ml and inter- and intra-assay precisions were <5 %.
Results: We found in CSF samples of PD patients a highly significant elevation of t.TG concentration compared to CSF t.TG concentration measured in control subjects. (70.3 vs. 7.6 pg/ml, $p < 0.001$). We did not found correlation between CSF t.TG concentrations and the cognitive functions, sleep-disturbances and the duration of the disease.
Conclusion: To our knowledge, this is the first direct ex vivo observation that shows increased neural cell apoptosis in patients with PD. T.TG is a rather robust enzyme which may be an ideal in vivo indicator of apoptosis associated with neurodegenerative diseases a stage where therapy may still be feasible.

Sessie 3 Klinische onderwerpen II

09.00 - 09.15 uur

Aangeboren afwijkingen: rol van een verstoord cobalamine- en homocysteïnemetabolisme

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Inleiding: Hyperhomocysteïnemie speelt mogelijk een belangrijke rol bij het ontstaan van aangeboren afwijkingen. Een tekort aan cobalamine (Cbl) met als gevolg een defect in de remethylering van homocysteïne tot methionine kan leiden tot hyperhomocysteïnemie. Deze reactie wordt gekatalyseerd door de enzymen methioninesynthase (MS) en methioninesynthasereductase (MTRR). In deze studie wordt de associatie van verstoorde remethylering en daarbij betrokken polymorfismen met het ontstaan van aangeboren afwijkingen bepaald.
Methode: Van 45 zwangerschappen van foetussen met een aangeboren afwijking: spina bifida (n = 11), encephalocele (n = 3), ex-(an)encephaly (n = 15), congenitale hartafwijking (n = 10), omphalocele (n = 5) en orofaciale schisis (n = 1), en 97 controlezwangerschappen werd amnionvloeistof verzameld bij een zwangerschapsduur van 14 tot 20 weken. De cobalaminestatus in amnionvloeistof werd bepaald door het meten van cobalamine-, apo-transcobalamine-, apo-haptocorrine- en homocysteïneconcentraties. Genomisch DNA werd geïsoleerd uit foetale cellen en het genotype ten aanzien van MS 2756A>G, MTRR 66A>G en TC 779C>G werd bepaald. De verschillende groepen werden statistisch vergeleken met de Mann-Whitney-test.
Resultaat: Amnionvloeistof van foetussen met een aangeboren afwijking bevat significant verlaagde Cbl- (mediaan (range), 95 (22-963) vs. 262 (22-1480) pmol/L, p = 0,002), en significant verhoogde apotranscobalamine- (82 (2-788) vs. 29 (1-488) pmol/L, p < 0,001), apo-haptocorrine- (508 (96-2893) vs. 248 (8-1572) pmol/L, p < 0,001) en homocysteïneconcentraties (2,9 (1,0-9,2) vs. 2,4 (1,0-7,5) µmol/L, p = 0,003). De odds ratios (95% CI) voor de aanwezigheid van tenminste een variant allel van MTRR 66A>G en TC 779C>G zijn respectievelijk 2,3 (1,0-5,3) en 2,2 (1,1-4,3).
Conclusie: Deze resultaten suggereren dat een verstoorde cobalaminestatus van de foetus mogelijk een rol speelt in de pathofysiologie van aangeboren afwijkingen.

09.15 - 09.30 uur

Effect of polymorphisms in folate-related genes on methotrexate sensitivity in pediatric acute lymphoblastic leukemia (ALL)

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Introduction: Sensitivity to the anti-folate methotrexate (MTX) is dependent on the patient's cellular folate status. Therefore, we studied whether common polymorphisms in genes involved in folate metabolism affect MTX sensitivity.
Methods: Ex vivo MTX sensitivity of lymphoblasts obtained from pediatric ALL patients (n=125) was determined by the thymidylate synthase inhibition assay with continuous (21-h) MTX exposure (TSI50,cont). DNA was isolated from leukocytes obtained from cytospin slides. Common gene mutations in methylenetetrahydrofolate reductase (MTHFR 677C>T and MTHFR 1298A>C), methionine synthase (MS 2756A>G), methionine synthase reductase (MTRR 66A>G), cystathionine beta-synthase (CBS 833T>C/844ins68) and serine hydroxymethyl transferase (SHMT1 1420C>T) were detected by PCR-RFLP.
Results: Univariate regression analysis revealed MTHFR 1298 AC (P=0.01), MTRR 66 AG (P=0.08) and MTRR 66 GG (P=0.09) as potential determinants of TSI50,cont. Similarly, multiple regression analysis identified MTHFR 1298 AC (st.beta=0.30, P=0.02), MTRR 66 AG (st.beta=0.26, P=0.06), MTRR 66 GG (st.beta=0.27, P=0.08), and SHMT1 1420 TT (st.beta=0.22, P=0.05) as determinants of MTX sensitivity.
Conclusion: Polymorphisms in the MTHFR, MTRR and SHMT1 genes are related to MTX resistance in pediatric ALL patients.

Literature: Rots MG, et al. Blood 1999; 93: 1067-1074.

09.30 - 09.45 uur

The 894 G>T variant of endothelial nitric oxide synthase (eNOS) increases the risk of recurrent venous thrombosis through interaction with elevated homocysteine levels

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Introduction: Venous thrombosis is a multicausal disease involving both genetic as well as acquired risk factors. Hyperhomocysteinemia is associated with a 2-fold increased risk of recurrent venous thrombosis. Recently, the 894 G>T variant of endothelial nitric oxide synthase (eNOS) was postulated to be associated with hyperhomocysteinemia. We hypothesized an interrelation of hyperhomocysteinemia, the eNOS 894 G>T variant and recurrent venous thrombosis risk.
Methods: The eNOS 894 G>T variant was studied in 170 cases with a history of recurrent venous thrombosis and 433 controls from the general population.
Results: The eNOS 894 TT genotype slightly increased recurrent venous thrombosis risk (OR 1.3 [0.7-2.6]), but no association of the eNOS 894 G>T variant with elevated homocysteine was found in controls. Interestingly, in RVT cases the coexistence of both the 894 TT genotype and elevated tHcy levels (>90th percentile) was more frequently present than in controls, which leads to a substantially increased risk of recurrent venous thrombosis (fasting tHcy OR 5.3 [1.1-24.1], post-load tHcy OR 6.5 [1.6-29.5]).
Conclusion: The results of the present study demonstrate that the eNOS 894 G>T variation interacts with elevated tHcy levels leading to an increased risk of recurrent thrombotic events. This interaction points to the direction of S-nitrosation as a mechanism by which homocysteine exerts its detrimental effects on thrombosis.

09.45 - 10.00 uur

Ex vivo analysis of aberrant splicing induced by two donor site mutations in PKLR of a patient with severe pyruvate kinase deficiency

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Introduction: Pyruvate kinase deficiency is a hereditary cause of hemolytic anemia. In order to better understand the genotype to phenotype relations, we studied the effect on RNA processing, subcellulair localisation of transcripts and protein expression of two mutations identified in a PK-deficient patient.
Methods: RNA was obtained from ex vivo produced nucleated erythroid cells from the patient. The effects of the mutations on PKLR pre-mRNA processing were studied, using RT-PCR, cloning, fragment analysis, polysome analysis, and Western blotting.
Results: One novel mutation abolished the GT dinucleotide at the donor splice site in intron 5: IVS5+1GtoA. The other mutation, c.1436GtoA, altered nt –1 of the donor splice site of intron 10 and, moreover, predicted the substitution of arginine by histidine at residue 479. Abolishment of the intron 5 donor splice site initiated two events: either skipping of exon 5 or simultaneous skipping of exon 5 and 6. Polysome analysis suggested that no functional protein was produced by the IVS5+1A allele.The other mutation caused skipping of exon 10 but was mainly associated with a severe reduction in (normally processed) transcripts. Accordingly, low amounts of PK were detected in the nucleated erythroid cells of the patient by Western Blot analysis.
Conclusion: The transcript lacking exon 5 and 6 is very unusual. We propose that efficient inclusion of exon 6 in wild-type PKLR mRNA, depends on the presence of, yet undefined, splice enhancing elements in exon 5. The low amounts of PK in the nucleated erythroid cells correlate with the PK-deficient phenotype of the patient. Several low-abundant transcripts were detected that either reflected the minor consequences of the mutations or represented the first examples of 'leaky-splicing' events in the human PKLR gene.

10.00 - 10.15 uur

Central nervous system functions in a large dutch family with Familial Neurohypophysial Diabetes Insipidus (FNDI)

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Introduction: FNDI is caused by a deficiency of the hormone vasopressin as a result of a heterozygous mutation in the vasopressin prohormone gene and is characterized by polydipsia and polyuria. Besides these peripheral endocrine effects, vasopressin also regulates numerous brain functions, like learning and memory processes, aggression, behavior, the regulation of the autonomic nervous system, the cardiovascular system and body temperature. These effects are probably mediated by the parvocellular vasopressin containing nerve fibres, which project to midbrain limbic structures and could be affected in FNDI. Our lab identified a novel mutation previously. Now we studied this large family, carrying a Cys116Gly mutation of the vasopressin gene, to compare cognitive and other CNS effects in FNDI positive and FNDI negative members.
Methods: A total of 37 adult volunteers (23 positive and 14 negative) of the same FNDI family and 11 non-family members were neuropsychologically tested. Neuropsychological examinations were performed in two-hour sessions by the same neuropsychologist. Exclusion criteria were brain damage or related traumas. For neuropsychological assessment, well-known, internationally published and validated tests were used. The subjects were tested for motivation, premorbid intelligence, attention and executive functions, organization and planning and memory functions.
Results: The FNDI positive subjects were moderately, however not significantly, inferior in the performance of an auditory verbal learning test. No marked differences were found except a significant retrieval failure and being inferior in sustained attention in the FNDI positive subjects.
Conclusion: We were able for the first time to perform a neuropsycological study in an large FNDI family. Our results indicate moderate, albeit statistically significant differences between neuropsychological measures of the FNDI positive and control subjects, especially in those related to memory retrieval and sustained attention.

10.15 - 10.30 uur

Cog7 deficiency as the cause of a new lethal glycosylation disorder: CDG IIIa

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Introduction: Two siblings are presented with multiple congenital defects. The patients were the 4th and 6th child of healthy consanguineous parents. Both were born with perinatal asphyxia and showed several dysmorphic features. There was generalized hypotonia and severe liver involvement. Both died within 10 weeks after recurrent infections and cardiac insuffiency.
Methods: Extensive metabolic workup in the first patient showed increased lysosomal activities in plasma, mucolipidosis II was excluded. Serum IEF of transferrines showed an abnormal pattern not associated with one of the earlier described CDG types: there were increased bands of tri-, di-, mono- and asialotransferrines.
Results: Further experiments in the patients fibroblasts in search for glycosylation defects showed a modest decrease in sialylation of N- and O-glycans. There were normal to slightly elevated CMP-sialic acid levels and a decrease of CMP-SA and UDP-Gal transport into the Golgi. Immunolocalization studies in intact fibroblasts showed absence of Cog7 and mislocalization of Gog5, Cog6 and Cog8. In both patients the amount of Cog7 mRNA and protein was low. Analysis of Cog7 gene revealed a point mutation at the boundary of the first exon and intron, leading to a new splice site introducing a 19-base pair deletion in the mRNA sequention. This introduced a premature stopcodon and a truncated Cog7 protein. The abnormalities in the expression of Cog7 and the glycosylation defects were corrected by transfection with a Cog 7 construct.
Conclusion: These patients are the first reported with a form of CDG not caused by a single enzyme defect, but due to a defect of a protein of the COG complex, which is involved in the trafficking of the components of the glycosylation apparatus in the Golgi.

10.30 - 10.45 uur

Creatine transporter deficiency a frequent cause of X-linked mental retardation

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Introduction: We identified a new inborn error of metabolism caused by a defect in the X-linked creatine transporter SLC6A8 gene mapped at Xq28 (SLC6A8 deficiency, OMIM 300352). The hallmarks of the disorder are XLMR, expressive speech and language delay, epilepsy, developmental delay and autistic behavior. In approximately 50% of the female carriers, learning disabilities of varying degrees have been noted. Treatment with creatine supplementation is unsuccesful for affected males.
Methods: We have tested by molecular and biochemical analysis 10 unrelated families (17 male patients and 10 carriers) in whom 1) creatine deficiency was diagnosed in the brain of the index patient by H-magnetic resonance spectroscopy (n=7) or 2) linkage analysis in a large X linked mental retardation family suggested that the defect was located at Xq28 (n=1) or just presumed XLMR(n=1). Creatine and guanidinoacetate was measured in urine and plasma by stable isotope dilution GC MS.
Results: In all male cases tested the creatine/creatinine ratio in urine was increased. Creatine uptake was impaired in cultured fibroblasts of the affected males. DNA sequence analysis of the coding region of the SLC6A8 gene revealed one-amino acid deletions (n=3), nonsense mutations (n=2), one base insertion (n=1), a transversion that resulted in a missense mutation and alternative splicing (n=1), splice-site mutation (n=1) and large genomic deletion (n=2). Five families originate from one metropolitan area. This suggests that SLC6A8 deficiency may have a relatively high incidence.
Conclusion: H-MRS of brain, creatine/creatinine measurement in urine, creatine uptake assay in fibroblasts and SLC6A8 sequence analysis appear all to be valuable primary diagnostic tests for the evaluation of SLC6A8 deficiency and complement each other.

10.45 - 11.00 uur

Apolipoprotein E3-Utrecht. A new variant of human apolipoprotein E

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Introduction: Apolipoprotein E (apo E) is carried in plasma by chylomicrones, very low density lipoproteins (VLDL) and high density lipoproteins (HDL). Apo E serves as the ligand for receptors for the LDL receptor. In humans three common apo E isoforms have been described designated E2, E3 and E4. This genetic variation seems to be a major determinant of plasma lipid concentrations and of the interindividual variants in susceptibility to coronary artery disease. A new variant in Apo E3, denoted als E3-Utrecht has been identified in a family associated with dyslipidemia.
Methods: DNA was isolated from leucocytes, amplified by PCR and subjected to restriction enzyme digestion. Two different endonucleases were used (BstAPI and CfoI). For further confirmation of the mutation 4028G>A, we also performed DNA sequence analysis and compared these results with the reference sequence. Thirteen family members were analysed. Cholesterol, triglyceride and Apo E concentrations were measured in a routinely way in our laboratory.
Results: The 4028G>A substitution in the Apo E gen predicted a Arg142His substitution in Apo E. Seven family members were heterozygous for 4028G>A. All these affected persons had elevated body mass indexes (> 25 kg/m2), or elevated triglycerides (>1,3 mmol/L) or elevated cholesterol levels (>5,5 mmol/L). Apo E concentrations were significantly higher in carriers of the mutation.
Conclusion: 4028G>A encodes a novel Apo E variant: Apo E-Utrecht. Heterozygous persons all showed dyslipidemia or obesitas, indicating the relevance of Apo E-Utrecht in lipid metabolism.