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Posterabstracts

Samenvattingen van de posterpresentaties tijdens het 54e Congres van de Nederlandse Vereniging voor Klinische Chemie op 11 en 12 april 2001 te Lunteren

Lipiden

1. Evaluation of lipoprotein integrity in commercial and home-made NCCLS C37-P like calibrators

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Introduction: The calibration 2000 project aimed at meeting the NCEP performance goals for lipids (and eventually apolipoproteins A-I and B) in 75% of the Dutch clinical laboratories. Hitherto, commutable calibrators with intact lipoproteins are a prerequisite. Potential calibrators were either home-brewed, or purchased from industry. Home-made calibrators were prepared according to NCCLS C37-P protocol; besides, modifications that make future logistics practical, were made (i.e. less stringent pooling, intermittent freezing, addition of 20 (g/v) % sucrose and lyophilization). In the end, C37-P calibrator material and 15 variations upon it were obtained. Commercial, liquid stabilized or lyophilized calibrators of human origin, which are stated to be commutable, were purchased from different manufacturers (N = 18).
Methods: Lipoprotein integrity of the calibrators was investigated by their physico-chemical characteristics: turbidity at 710 nm, lipoprotein pattern upon agarose gel electrophoresis, and presence or absence of flakes at the LDL layer after ultracentrifugation.
Results and discussion: In the home-brewed series, all lyophilized materials massively displayed flakes, irrespective of the addition of sucrose; if sucrose was present, only tiny flakes were present. The home-made frozen materials did not contain flakes at all, had the lowest intrinsic absorbance at 710 nm and did not precipitate at the application site upon agarose gel electrophoresis. Commercial calibrators all displayed huge amounts of flakes at the LDL layer after ultracentrifugation.
Conclusion: It is concluded that home-made calibrators have superior physico-chemical characteristics compared to commercial calibrators. Second, lyophilization detoriates lipoproteins, even in the presence of sucrose. Third, frozen calibrators prepared according to NCCLS C37-P or with a minor modification, have potential as universal standards in case they are proven to be commutable.

2. Ultieme lipid en apolipoproteïne standaardisatie in Nederland binnen handbereik

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Inleiding: De commuteerbaarheid van NCCLS C37-P bereide kalibratoren is getest aan de hand van een tweelingstudie. De tweelingstudie bood tevens de gelegenheid om de state-of-the-art bias van de lipidenmetingen in Nederland te berekenen. Naderhand werd er, in twee SKZL kwaliteitscontrolerondes en aan de hand van 8 controlemonsters, gekeken naar het effect van standaardisatie middels NCCLS C37-P bereide materialen op de interlaboratoriumspreiding van de metingen in Nederland.
Methoden: De statistische analyse gebeurde in EXCEL. Kalibratoren werden beschouwd als commuteerbaar indien de genormaliseerde residuele afwijkingen < 3 SD. De gemiddelde absolute bias voor lipiden werd beoordeeld t.o.v. de criteria van het National Cholesterol Education Program; de gemiddelde absolute bias van de apo's diende < 6% te zijn. In de effectmetingen werd standaardisatie gesimuleerd door de NCCLS C37-P kalibratoren mee te nemen in dezelfde runs als de SKZL kwaliteitscontrolemonsters. De controlemeetresultaten werden vervolgens herrekend naar de referentiewaarde van de kalibratoren. De daaropvolgende afname in interlaboratoriumspreiding op de meetresultaten is een maat voor biasreductie door calibratie.
Resultaten en discussie: Mediane residuele afwijkingen van de NCCLS C37-P bereide kalibratoren waren voor alle lipid parameters < 0,92 SD en voor de apo's < 1,60 SD, dus ver beneden de gestelde 3 SD. Het aantal labs in Nederland dat niet aan de gestelde bias criteria voldeed op het moment van de tweelingstudie varieerde van 28%-68%, naargelang de parameter. De SKZL kwaliteitscontrolerondes toonden aan dat calibratie op één of meerdere NCCLS C37-P kalibratoren een biasreductie tussen laboratoria van circa 40% meebrengt.
Conclusie: De NCCLS C37-P bereide materialen zijn commuteerbaar gebleken niet alleen voor cholesterol, maar ook voor HDL-c, LDL-c, TG, apo A-I en apo B. Standaardisatie in Nederland is met deze kalibratoren binnen handbereik aangezien de spreiding in meetresultaten tussen laboratoria door ijking met 40% kan worden teruggebracht.

3. Association between the composition of LDL and its susceptibility to in vitro oxidation in Type II diabetic patients

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Introduction: Cardiovascular disease due to atherosclerosis is the leading cause of death in diabetes. There is increasing evidence that oxidation of LDL in the vascularwall plays an important role in the development of atherosclerosis. This study was undertaken to characterize how different constituents of LDL contribute to the in vitro oxidisability of LDL in Type II diabetes.
Methods: LDL composition, i.e. lipids, antioxidants, fatty acids, plasmenylcholines, and baseline level of conjugated

dienes of 94 well controlled and normolipidaemic Type II diabetic patients was measured. Two oxidisability indices were determined: the lag time, reflecting the resistance of LDL to copper induced oxidation, and the amount of conjugated dienes formed during oxidation of LDL.
Results and discussion: The cholesterol, phospholipid and triglyceride content of LDL was not related to LDL oxidisability. Lag time of LDL was not related to the total level of saturated, monounsaturated, and polyunsaturated fatty acids, but a strong inverse relationship was observed with fatty acids with 3 or more double bonds ($r=-0.56$, $p<0.001$). In addition, an inverse relation was observed between the lag time and LDL-plasmenylcholine ($r=-0.35$, $p<0.001$). A multiple linear regression model with LDL polyunsaturated fatty acids with 3 or more double bonds, alpha-tocopherol, monounsaturated fatty acids, and plasmenylcholines as determinants explained 47% of the variation in lag time. Conjugated diene production was negatively correlated to oleic acid and positively to linoleic acid, ($r=-0.45$ and $r=0.73$, respectively; $p<0.001$).
Conclusion: Fatty acids are major determinants of the susceptibility of LDL to in vitro oxidation. Linoleic acid and arachidonic acid are associated with an increased LDL oxidisability because linoleic acid was positively correlated to conjugated diene production and arachidonic acid was negatively related to lag time. Fatty acids with 3 or more double bonds were the most important determinant of LDL lag time. Although not related to lag time in univariate analysis, alpha-tocopherol was a significant determinant in multiple regression analysis.

4. Estimated biological variation of the mature milk fatty acid composition

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Introduction: There is lack of reliable data showing the ranges of the biologically important fatty acid species in human milk. We estimated the inter-individual biological variation (CV_{biol}) for 28 fatty acids in 465 mature samples collected in The Netherlands (n=222), Jerusalem (n=63), Tanzania (n=11), Pakistan (n=10) and the 'Caribbean Region', i.e. Antigua (n=23), Belize (n=10), Curaçao (n=47), Dominica (n=17), St. Lucia (n=12), St. Vincent (n=30) and Surinam (n=20). The samples were over the last 25 years analyzed in one laboratory with a single capillary gas chromatographic method.
Methods: CV_{biol} was calculated from the observed variation (CV_{obs}) and between-series analytical variation (CV_{anal}), using $CV_{biol} = r(CV^2_{obs} - CV^2_{anal})$.
Results and discussion: CV_{anal} was low compared with CV_{biol}. The CV_{biol} of the various regions were remarkably similar. The average CV_{biol} of 455 samples, Pakistan excluded, ranged from 12.7% for 16:0 and 18.9% for 18:1ω9 to 68% for 22:6ω3 and about 100% for 20:5ω3. Twenty of the 28 fatty acids had CV_{biol} below 40%. Those of 20:4ω6, 18:2ω6 and 18:3ω3 were 28.0, 33.0 and 37.3%, respectively.
Conclusion: Because of the large CV_{biol} and the many dietary changes in recent history it seems impossible to consider the present human milk fatty acid composition as the 'gold standard' for infant formula manufacturing. The optimal human milk fatty acid composition should rather derive from populations that consume traditional diets consistent with our genetic background, or from scientific data that show the function of the individual fatty acids in neonatal development.

5. Analysis of phosphatidylcholines and sphingomyelins in CSF using electrospray tandem-mass spectro-metry

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Introduction: Phospholipids play a central role in cellulase functioning not only as essential constituents of lipid bilayers but also bacause they play a major role in signaling. Because of our interest in the consequences of an impairment in phospholipid biosynthesis in several human diseases, we have developed methods to analyse phosphatidylcholine and sphingomyelin in CSF. The results are described here.
Methods: Electrospray tandem-mass spectrometry was used for the individual analysis of phosphatidylcholines and sphingomyelins.
Results and discussion: We developed a sensitive semi-quantitative analysis of individual phosphatidylcholines (PC) and sphingomyelins (SM) using electrospray tandem-mass spectrometry. In normal CSF samples over 30 individual molecular species could be identified, including PC's with C22:6n-3 or C20:4n-6 attached to their sn-2 position and SM's conjugated with very long chain fatty acids. The structures of these lipids were verified using daughter-ion analysis. Quantification of 13 PC species and 7 SM species correlated well with total lipid phosphorus concentrations, implicating that the species quantified represent > 98 % of the phospholipids present in CSF. Analysis of CSF samples from patients with SLO syndrome revealed very low concentrations of PC and SM. Analysis of a sample from a patient with Krabbe's disease revealed a high concentration of C16:0-SM, and a very low concentrations of C18:0-SM. Finally, samples from two patients suffering from a serine biosynthesis defect (3-phosphoglycerate dehydrogenase deficiency) showed reduced concentrations of SM species and low-normal concentrations of PC species. The phosphatidylserine concentration was too low to be measured in the amount of sample available.
Conclusion: The method developed can be extented by measuring ceramides, cerebrosides and gangliosides and might be useful in the screening or therapy control of patients with neurological disorders.

Enzymen / Eiwitten

6. Identification and characterization of the human mitochondrial trimethyllysine hydroxylase, a key enzyme of carnitine biosynthesis

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Introduction: Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is a vital compound, which plays an indispensable role in the transport of activated fatty acids across the inner mitochondrial membrane into the matrix, where beta-oxidation takes place. Furthermore, carnitine is involved in the transfer of the products of peroxisomal beta-oxidation, including acetyl-CoA, to the mitochondria for oxidation to CO_2 and H_2O in the Krebs cycle. Apart from the dietary intake of carnitine, most eukaryotes are able to synthesize this compound from trimethyllysine in four steps.
Our goal is to identify all four enzymes of the carnitine biosynthesis at the molecular level to investigate whether a defect in carnitine biosynthesis can be disease-causing. So far we have identified two of the four enzymes, trimethylaminobutyraldehyde dehydrogenase and butyrobetaine hydroxylase, catalyzing the penultimate and ultimate step, respectively.
Methods: The enzyme trimethyllysine hydroxylase was purnified from rat kidney using liquid chromatography.
Results and discussion: We here present the identification and characterization of the first enzyme in the carnitine biosynthesis, trimethyllysine hydroxylase. To this end, an enzyme assay was developed to measure trimethyllysine hydroxylase activity that was used for the purification of the protein. The monomeric protein has an apparent molecular weight of 41 kDa and gelfitration experiments showed that the native conformation is dimeric. Subcellular localization studies showed that the protein is localized in mitochondria. This is in contrast with the three other enzymes of the carnitine biosynthesis, which are cytosolic. The purified protein was digested with trypsin and the resulting peptide fragments were sequenced using Q-TOF tandem mass spectrometry. With these peptide sequences the cDNA could be identified using the online databases, and the gene was localized on Chromosome X.
Conclusion: We have characterized the enzyme trimethyllysine hydroxylase at the enzyme and molecular level and we are currently investigating whether defects in this gene may be responsible for some candidate X-linked diseases.

7. Consensus referentiewaarden Regio Rijnmond

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Inleiding: De regio Rijnmond omvat een 17 tal ziekenhuislocaties. De laboratoriumondersteuning voor de (poli)klinische patiënten vindt in de ziekenhuizen plaats en de laboratoriumondersteuning voor de patiënten van de huisarts vindt deels in de ziekenhuizen plaats en deels in het huisartsenlaboratorium (STAR). Dit betekent voor de specialist bij verwijzing door de huisarts of een ander ziekenhuis dat hij met verschillende referentiewaarden geconfronteerd wordt. Omgekeerd geldt dit ook voor de huisarts die informatie van de specialist krijgt. De klinisch chemici in de Regio Rijnmond hebben zich in 2000 ten doel gesteld consensus te bereiken over de te hanteren referentiewaarden binnen de regio.
Methoden: Een selecte groep klinisch chemici is begonnen een inventarisatie uit te voeren van de nu gehanteerde referentiewaarden met betrekking tot een uitgebreid pakket klinisch chemische en hematologische routinebepalingen. Op basis van de verkregen gegevens is aan de hand van een aantal regels getracht te komen tot een voorstel voor regionale referentiewaarden:

1. Wanneer meer dan 70% van de ziekenhuizen dezelfde waarden hanteren, wordt deze waarde opgenomen in het voorstel.
2. Bij 40-70% overeenstemming, wordt die referentiewaarde overgenomen wanneer dit door de literatuur (o.a. Tietz 1999, Wintrobe 1999) wordt ondersteund.
3. Bij 40-70% overeenstemming zonder steun uit de literatuur wordt een gemiddelde van de gehanteerde waarden genomen. Dit gemiddelde is vergeleken met de literatuur en onderling besproken.
4. Bij minder dan 40% overeenstemming is onderzocht of er een methode-afhankelijkheid is. Zo niet, dan wordt regel 3 gevolgd.
5. Wanneer bovenstaande punten niet tot een duidelijke referentiewaarde leiden, wordt er geen voorstel geformuleerd
6. Alle voorgestelde referentiewaarden zijn vergeleken met de waarden uit het Diagnostisch Kompas.

Resultaten en discussie: In 5 plenaire regiobijeenkomsten zijn de data gepresenteerd en besproken. Het voorstel is naar aanleiding van argumenten die op deze vergaderingen naar voren kwamen op enkele punten aangepast. Voor meer dan 75 klinisch chemische en hematologische bepalingen werd consensus bereikt over de te hanteren regionale referentiewaarden. Alle klinisch chemici hebben ingestemd om in 2001 deze regionale referentiewaarden bij hun aanvragers bekend te maken.
Voor de enzymen is een andere werkwijze gevolgd. Aangezien de verschillen in referentiewaarden van de enzymen erg groot bleken, werd een regionale standaardisatie uitgevoerd. Er werd gekozen voor een ijkpunt binnen het AZR-Dijkzigt. Vervolgens zijn via een viertal rondzendingen de waarden voor de verschillende laboratoria vergeleken met het referentielaboratorium. Dit leverde voor elk laboratorium per enzym een correctiefactor op, waardoor de resultaten van de laboratoria binnen de regio nu gelijk getrokken zijn. Middels periodieke rondzendingen wordt gecontroleerd of alle analyzers nog afgestemd zijn op de referentieanalyzer. Door deze standaardisatie kunnen de landelijk voorgestelde referentiewaarden (NTKC 24; 1999: 260) gehanteerd worden.
Conclusie: Voor meer dan 75 klinisch chemische (inclusief enzymen) en hematologische bepalingen werd consensus bereikt over de referentiewaarden binnen de Regio Rijnmond.

8. Calibration 2000: results of a twin study for ten serum proteins

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Introduction: The aim of the Calibration 2000 project is to define and distribute calibrators in various fields of laboratory diagnostics that are commutable with patient sera. These calibrators will be used as device to check for drifting and to reduce interlaboratory variation. In the twin study performed by the task force for serum protein the best commutable calibrator for ten serum proteins will be selected.
Methods: In a twin study of 39 laboratory couples 3 commercial calibrators (all liquid) and 2 calibrators produced by the SKZL section MCA (1 liquid, 1 lyophilised, both containing 10% sucrose) and 10 patient sera (5 sera of each lab) were measured for maximally 10 serum proteins (IgG, IgA, IgM, C3, C4, Albumin, Transferrin, Haptoglobin,a1-Antitrypsin, CRP). For each laboratory couple a regression line was constructed from the measured patient data. The distance of the location of the tested calibrators to this regression line (normalised residuals) reflects the commutability of the calibrator with patient sera. The best commutable calibrator has the lowest residuals.
Results and discussion: Analysis of the data shows that one of the commercial calibrators has the lowest normalised residuals and therefore has the best commutability with patient sera. However both calibrators prepared by the SKZL section MCA have comparable commutability. Selection of the final calibrator will take place on additional criteria such as stability, availability, batch to batch variation and prize. Consequently, values will be assigned according to the international standard CRM470 (1).

Conclusion: The promissing results of the first phase of the Calibration 2000 project on serum proteins show that selection of a commutable calibrator is feasible.

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Elektrolieten

9. Can the Cockcroft formula be used as method to estimate the creatinine clearance? The measurement of the creatinine clearance in a large group of students. Comparison of the formula of Cockcroft and Gault and the classical method

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Introduction: First year medical students have been asked to take part in an experiment to compare the results of the measurement of the clearance of creatinine in the classical manner using creatinine measurements in bloodsamples and in 24 hrs-collections of urine to those using the formula of Cockcroft and Gault (CG). A total of 126 students (39 male, 87 female) took part (89 % of the whole group). No criteria for in- or exclusion have been used.
Methods: The clearance was measured and calculated as "Clearance of creatinine (ml/min)= (number of mmol creat excreted per 24 hours in urine)*1.000.000/ (concentration of creat in serum(micromol/l)*1440)". The Cockcroft and Gault formula consists of: "Clearance (ml/min)= (140-age(yrs)) *weight(kg)*1.26/serumcreat(micromol/l). For females 1.26 is replaced by 0.99."
Results and discussion: The CG-clearances correlate weakly with the measured clearance (R= 0.6). The best correlation of the CG-clearances was a weak correlation (R=0,7) with the weights, while the measured clearances correlate strongest with the amounts of creatinine excreted in 24 hrs (R=0,9). These results explain the poor correlation between the CG-results and the measured creatinine clearances. The levels of serum creatinine only correlate weakly with the creatinine clearance. Analyzing the results according to Bland-Altman a mean difference +/- SD was found between CG-results and the measured clearance of 0 +/- 40. The differences show a negative correlation with the mean values.
Conclusion: 1. the Cockcroft formula is not valid for estimation of the creatinine clearance within this group of students of approximately the same age; 2. the measured creatinine clearance correlates strongly with the excretion of creatinine in urine; 3. the use of the Cockcroft formula to illustrate the effect of age on the clearance will be influenced by the weight of the patient.

10. Calcium gecorrigeerd voor albumine is niet geschikt voor de diagnose hyper- of hypocalciëmie bij intensive care patiënten

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Inleiding: Calcium heeft een belangrijke rol in de fysiologie van het menselijk lichaam. De klinische consequenties van een veranderde calciumstatus zijn zeer divers en kunnen variëren van geen symptomen tot een hartstilstand. Een frequente controle van de calciumspiegels is bij IC patiënten noodzakelijk omdat door vele interventies als bloedtransfusies, infusen en niervervangende therapie, de calciumspiegel snel verandert. Op basis van de totaal calcium gecorrigeerd voor albumine volgens de formule: Ca gecorrigeerd = totaal Ca - (0,025 x albumine) + 1 [1], vonden wij frequent een onverklaarbare hypercalciëmie. Dit was aanleiding om het totaal calcium gecorrigeerd voor albumine te vergelijken met de geïoniseerde calcium bij IC-patiënten

Methoden: In 53 bloedafnamens bij 36 patienten (22 mannen) met een gemiddelde leeftijd van 66,2 jr (25-86), een APACHE II score van 21,9 (0-39), en een SAPS II score van 44,5 (12-82) werden totaal calcium, albumine en totaal eiwit gemeten met een Hitachi 717 analyser en geïoniseerd calcium m.b.v. ISE op een AVL bloedgas-analyser.
Het gecorrigeerde calcium werd berekend m.b.v. de bovenstaande formule en vergeleken met het geïoniseerde calcium (gouden standaard). Statistische evaluatie vond plaats m.b.v. een t-toets voor gepaarde waarnemingen
Resultaten en discussie: De correlatie tussen het gecorrigeerde en geïoniseerde calcium leverde de relatie: Ca gecorrigeerd = 1,211 x Ca geïoniseerd + 0,382 (r= 0,255). Het gecorrigeerde calcium heeft voor de diagnose hypercalciëmie bij IC patienten een sensitiviteit van 83% en een specificiteit is 30%. De positief voorspellende waarde (PVW) is 50% en de negatief voorspellende waarde (NVW) 92%. Voor de diagnose hypocalciëmie is de sensitiviteit 0% en de specificiteit 100%. De PVW is 0% en de NVW 74%. Voor de diagnostiek van hyper- en hypocalciëmie zijn de gecorrigeerde calcium en de geïoniseerde calcium zijn (gecorrigeerd voor het concentratieverschil) significant verschillend (P< 0,001).
Conclusie: De gecorrigeerde calcium is niet geschikt voor de controle van de calciumspiegel bij IC-patienten. Alle hypocalciëmien worden gemist en in ruim 20% wordt een fout-positieve hypercalciëmie gevonden. Voor een juiste interpretatie van de calciumstatus bij IC-patiënten is een geïoniseerd calcium noodzakelijk

Literatuur

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11. Hyperkaliëmie door pre-analytische factoren: een wolf in schaapskleren ?

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Inleiding: In een monster van een extern afnamepunt werd een kalium uitslag van 7,9 mmol/l gemeten. Een aantal voor de hand liggende oorzaken van een vals-verhoogde kalium uitslag werden onderzocht. Er bleek geen sprake te zijn van in vitro hemolyse, afname in K3-EDTA buis, K-infuus, stuwing bij afname, of een analytische fout. Tevens waren fosfaat en LD concentraties normaal waardoor vertraging in het pre-analytisch traject niet waarschijnlijk werd geacht. Wel was er sprake van het gebruik van K-sparende diuretica. Controle van de patiënt op de EH leverde een kalium uitslag van 4,6 mmol/l op !! Uiteindelijk bleek dat bij deze patiënte op de vóórgaande dag van analyse bloed was afgenomen, waarna het monster in de koelkast was gezet !
Methoden: n.v.t.
Resultaten en discussie: Volgens de leerboeken zijn in een monster van 24 uur oud, waarbij de cellen en het plasma niet van elkaar zijn gescheiden, de concentraties van o.a. K, fosfaat en LD in het plasma verhoogd. Dit bewaareffect werd door ons in een aantal experimenten bevestigd. Andere experimenten toonde aan dat als bloedmonsters (óók afgedraaide monsters zonder gel !) bij 4ºC werden bewaard, er géén significante verhoging van fosfaat en LD werd waargenomen. De K concentratie daarentegen is veel sterker verhoogd in vergelijking met monsters bewaard bij kamertemperatuur. Dit fenomeen zou verklaard kunnen worden doordat K in de erytrocyt wordt gehouden door een energie-afhankelijke Na/K-ATP-ase pomp. Bij 4ºC wordt dit metabolisme stopgezet, waardoor deze pomp niet meer actief de K concentratie in de erytrocyt hoog kan houden. Fosfaat en LD worden via een ander mechanisme in de erytrocyt gehouden, waarbij celintegriteit waarschijnlijk een grote rol speelt.invullen
Conclusie: Deze casus leert dat hyperkaliëmie door vertraging in het pre-analytische traject niet altijd op het laboratorium te onderkennen is, indien gebruik gemaakt wordt van Li-heparine buizen zonder gel.

Endocrinologie

12. Marked depletion of DHEAS in acute and chronic critical illness

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Introduction: Dehydroepiandrosteron (DHEA) and its sulphate ester (DHEAS) are pleiotropic adrenal hormones with putative immunostimulating, antiglucocorticoid and neuropsychological effects (1). In critically ill patients adrenal androgen production declines, whereas adrenal cortisol production is maintained at a high level (2). The aim of this study was to evaluate DHEAS levels in acute and chronic severe illness and to explore their relation with the pituitary-adrenal axis and the acute phase response.
Methods: During 14 days or until discharge or death, we serially measured blood concentrations of DHEAS, cortisol, TNF-alpha, IL-6, procalcitonin (PCT), lipoprotein-binding protein (LBP), and ACTH immunoreactivity. We also recorded heamodynamic parameters, haematology, biochemistry, APACHE II and SOFA scores, the use of dopamine, and ICU-mortality. We included 30 patients with septic shock , 8 patients with severe multitrauma and 40 healthy control subjects.
Results and discussion: On admission, DHEAS was extremely low in sepstic shock (1.2 ± 0.8 µmol/l) in comparison with multitrauma (2.4 ± 0.5 µmol/l; $p < 0.05$) and controls (4.2 ± 2.1; $p < 0.01$). Hypercortisolism was present in both patient groups. DHEAS had a significant negative correlation with age ($r = -0.55$, $p < 0.01$) and IL-6 ($r = -0.61$, $p < 0.01$) in both patient groups, but no relation was found with gender, use of dopamine, disease severity or markers of the acute phase response (PCT, LBP). Non-survivors of septic shock (n = 12) had even lower DHEAS levels (0.4 ± 0.3 µmol/l) than survivors (1.7 ± 1.1 µmol/l, $p < 0.01$). The time course of DHEAS showed a persistent depletion during follow-up, whereas cortisol levels were increased at the same time points.
Conclusion: We found a marked DHEAS depletion in both the acute and chronic phase of septic shock and to a lesser degree in multitrauma patients. At the same time hypercortisolism was present in both patient groups. Nonsurvivors had the lowest DHEAS levels, suggesting that DHEAS might be a prognostic marker in septic shock. The negative correlation of DHEAS with IL-6 indicates a regulatory role for IL-6, suppressing adrenal DHEAS production. Whether or not to substitute DHEAS in these DHEAS-deficient disease states is still unknown, but of great interest (1).

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13. Circulating CEA in a patient with hypothyroidism: a benign cause for elevated CEA concentrations due to a reduced clearance

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Introduction: Carcinoembryonic antigen (CEA) is a member of a family of cell surface glycoproteins which appear in the blood in association with a wide variety of cancers, including colon, breast and lung. CEA in the patient's blood however may not only be increased due to these cancers but also due to benign conditions such as cirrhosis or biliary obstruction. Here we present a patient with an elevated CEA concentration in it's serum caused by hypothyroidism and a hypothesis to explain for this phenomenon, respectively. After a literature search only 2 references were found describing this phenomenon however, no explanation was offered.
Methods: Serum CEA, TSH and free T4 concentrations were assayed on the Immulite 2000 (DPC, LA,CA,USA).
Results and discussion: A 53-year old woman was referred to the outpatient clinic internal medicine because of an elevated concentration of CEA in it's serum requested for by a clinician for diagnostic purposes. This patient complained of general malaise, easy fatigability and coldness. Physical findings include a cool dry skin and a hoarse husky voice. Laboratory results in the patient's serum: TSH, 45.8 mIU/l (reference interval:0.4-4.0); freeT4, <2.5 pmol/l (reference interval:11.0-23.5); CEA, 21.5 µg/l (reference interval:<3.5). The clinical symptoms and laboratory results are characteristic for primary hypothyroidsm. After replacement therapy with levothyroxine the CEA concentration in the patient's serum decreased to normal values. CEA is cleared by the liver. Elevated CEA concentrations in serum of patients with benign liver diseases are believed to be due to a reduced number of surface receptors on the Kupffer cel. It's clearance rate is strongly influenced by the content of carbohydrates, a higher content causes a slower clearance rate. In hypothyroidism other tumormarkers such as CA 125 and CA 15-3 are also increased. We hypothesize that during hypothyroidism the carbohydrate content of CEA is increased leading to elevated concentrations of CEA in a patient's serum due to a decreased clearance rate by the liver.
Conclusion: The elevated CEA in the serum of patients with hypothyroidism is probably due to a decreased clearance rate by the liver caused by an increased carbohydrate content of the CEA molecule. This case report once again illustrates that the routine assessment of CEA is not suitable for diagnostic purposes.

14. Evaluatie testosteron bepaling op de Architect i2000

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Inleiding: Recent is op ons laboratorium de Architect i2000 (Abbott Diagnostics) in gebruik genomen. Om te onderzoeken of we voor de testosteron bepaling over konden gaan van onze huidige RIA-methode naar de methode op de Architect i2000, is een uitgebreide evaluatie van deze test gestart.
Methoden: : Voor de testosteron bepaling is gebruik gemaakt van twee methoden. Een in-house RIA methode (antilichaam J. Pratt, met chloroform/ether-extractie) en de methode op de Architect i2000 (een-staps CMIA). Methode vergelijking is uitgevoerd volgens Passing-Bablok.
Resultaten en discussie: De Architect i2000 testosteron bepaling voldeed aan de acceptatievoorwaarden zoals gesteld in het NCCLS EP-10 protocol, welke gedurende 5 dagen werd uitgevoerd met drie verschillende concentraties in triplo gemeten. Een precisie-dosis curve werd uitgevoerd gedurende 10 dagen. De variatiecoëfficient varieerde van 4,0% (8 nmol/l) - 9,4% (0,8 nmol/l) - 16,0% (0,5 nmol/l). Een correlatiestudie werd uitgevoerd met de Architect i2000 testosteron assay en de RIA-extractiemethode. Correlatie met 61 monsters (41 mannen, 20 vrouwen) resulteerde in een regressie vergelijking van y = 0,06 + 0,90x (r=0,97). De regressievergelijking bij mannen was nagenoeg identiek, met een goede correlatie tot 0,3 nmol/l . Echter, de testosteron bepaling in monsters afkomstig van 8 van de 20 vrouwen resulteerde in significant discrepante uitslagen, waarbij de uitslagen van de Architect methode hogere waarden lieten zien dan de RIA-methode. Status onderzoek toonde dat bij deze vrouwen sprake was van PCO, hirsutisme of climacterium precox. Monsters met een goede correlatie waren afkomstig van vrouwen zonder klinische symptomen van androgeen exces.
Conclusie: De testosteron bepaling op de Architect i2000 bepaling lijkt analytisch gezien geschikt voor klinische diagnostiek bij mannen. Nader onderzoek zal moeten uitwijzen of deze bepaling ook voor de klinische diagnostiek bij vrouwen gebruikt kan worden.

15. De bepaling van cortisol in urine: Een verbetering van de inter-laboratorium spreiding als gevolg van een gestandaardiseerde extractie methode

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Inleiding: De bepaling van cortisol in urine speelt een belangrijke rol bij de diagnostiek van de syndroom van Cushing. Op grond van een eerder onderzoek is gebleken dat de inter-laboratorium spreiding van deze bepaling groot is (ca 50 %) en onafhankelijk van het gebruikte analytische systeem. In een rondzending waarbij 26 Immulite gebruikers betrokken waren werd onderzocht waardoor de grote inter-laboratorium spreiding wordt veroorzaakt.
Methoden: De deelnemers moesten de cortisolconcentratie in een rondgestuurd urinemonster bepalen met behulp van de in-huis extractie methode en een eenvoudige gedetailleerd beschreven gestandaardiseerde dichloormethaan extractiemethode. Daarnaast moest de cortisol concentratie bepaald worden in een dichloormethaan-extract van de rondgestuurde urine dat in de "Immulite-multidiluent" was opgenomen. Op basis van de rondzending zijn gemiddelde waarden voor de gevonden cortisol-concentraties en de inter-laboratorium spreiding (VC) vastgesteld.
Resultaten en discussie: Tabel 1 toont de resultaten van de rondzending.
Op grond van tabel 1 kunnen enkele conclusies worden getrokken met betrekking tot de oorzaak van de totale inter-laboratorium spreiding van 45,6 %, die is verkregen met de in-huis extractiemethoden. Op grond van de vergelijking van de inter-laboratorium VC van 15,2 % met de intra-laboratorium VC van 7,6 %, dat met het extract is verkregen, kan worden geconcludeerd dat het aandeel van de onderlinge standaardisatie van gebruikte Immulite op de totale VC beperkt is. Het effect

van de toegepaste extractiemethode is aanzienlijk indien de resultaten van de "in huis" en de standaard "Antonius" worden vergeleken.
Conclusie: Het is aannemelijk dat de aanzienlijke inter-laboratorium CV van de bepaling van cortisol in urine kan worden verbeterd indien door de laboratoria een gestandaardiseerde dichloormethaan-extractie methode wordt toegepast.

Tabel 1. Resultaten rondzending cortisol in urine

Methode	Gem. (nmol/l)	Inter-lab VC (%)
Extract	307,7	5,2
In-huis methode	212,1	45,6
Antonius methode	269,6	22,8
Ratio (Antonius/extract) (%)	86,8	14,7

Tumordiagnostiek /Maligniteiten

16. Progesteron Receptor, Bcl-2 and Bax Expression in Meningioma Cytosol

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Introduction: Meningiomas are common benign central nervous system neoplasias and expressing the progesterone receptor (PR) in the virtual absence of the estrogen receptor (ER). Knowledge of proteins involved in tumor growth control, and their relationship to PR, may be important for the development of endocrine anti cancer therapy. In this study ER, PR, bcl-2 and Bax expression levels were determined in meningioma cytosols. As a reference for our experimental conditions we also determined these proteins in breast cancer cytosols.
Methods: PR and ER were determined with a ligand binding assay and scatchard plot analysis. The expression levels of the anti- and pro- apoptotic proteins, bcl-2 and Bax respectively, were determined with Western immunoblot.
Results and discussion: In meningioma, we found a significant negative association between PR and bcl-2 ($p<0.01$). In breast cancer, bcl-2 was significantly positively associated with both ER and PR ($p<0.0001$). In both tissues, meningioma and breast cancer, Bax seems to be constitutively expressed. Bax expression levels in meningioma were twice as high compared to Bax expression levels in breast cancer cytosols.
Conclusion: Meningiomas express both bcl-2 and Bax. Bcl-2 is negatively associated with the progesterone receptor, suggesting a role for PR in the apoptotic cascade. The relation between PR and apoptotic proteins might have clinical significance.

17. PR isoform expression in human meningioma cytosol

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Introduction: The majority of meningiomas expresses the progesterone receptor (PR) and shows progesterone responsiveness. Also an association has been reported between PR and prognosis. At least two PR isoforms exist, PR-B (116-120 kDa) and PR-A (81 kDa), each with most likely different biological functions. Knowledge of the differential expression of both isoforms is necessary to understand the effects of progesterone on meningioma growth.
Methods: PR-A and PR-B expression levels were determined in 61 human meningiomas with immunoblot analysis. Total PR expression levels were determined with a ligand binding assay (total PR (LBA)).
Results and discussion: This study shows that both PR isoforms and an additional PR 78kDa protein (PR-78) are expressed in meningiomas. Meningiomas expressing more PR-A than PR-B have significantly higher total PR (LBA) levels ($p<0.001$). PR-78, of which the band intensity is negatively associated with that of PR-B ($rs=-0.76$, $p<0.0001$), may represent an endogenous degradation product, but a similar regulation pathway in the biogenesis of both PR-B and PR-78 is not excluded.
Conclusion: Meningiomas contain both PR isoforms, but in highly variable ratios and this variability may have some biological significance. Most meningiomas express more PR-A than PR-B. Therefore in meningioma, as opposed to assumptions made before, progesterone responsiveness could be based on trans-repression rather than on trans-activation of target genes.

18. Cyclopentenyl cytosine induces S-phase accumulation and differentiation in neuroblastoma cell lines

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Objectives: Cyclopentenyl cytosine (CPEC) is a cytidine analog with strong cytostatic properties. In its triphosphate form CPEC inhibits CTP synthetase and causes a depletion of cytidine nucleotides. We studied the effect of CPEC on cell cycle-distribution and morphological differentiation in two neuroblastoma cell lines.
Methods: The cell-lines used were SK-N-BE(2)c and SK-N-SH. SK-N-BE(2)c is MYCN amplified and 1p-deleted. Cell cycle-distributions were determined by measuring fluorescence of propidium iodide stained nuclei. Differentiation was quantified by microscopically examining cells on outgrowth of neurites. Cells with neurites that were at least twice the diameter of the cell body were marked as differentiated, as were cells that were fused by means of neurites.
Results and discussion: We observed accumulation in the S-phase of the cell cycle in both cell lines after 2 days incubation with cytostatic concentrations of CPEC. This was probably caused by the depletion of cytidine nucleotides. After 7 days, both cell lines showed accumulation in the G0/G1-phase. Apparently, the cells CPEC were able to complete the cell cycle and finally arrest in the G0/G1-phase. A novel effect of CPEC is that it strongly induced differentiation in SK-N-SH. While not more than 26 % of the cell population was differentiated in SK-N-BE(2)c after 7 days of incubation with 250 nM CPEC, in SK-N-SH already 90% was differentiated after 48 hours.
Conclusion: CPEC caused an initial S-phase accumulation in both cell lines followed by a G0/G1-phase arrest. Induction of differentiation of neuroblastoma by CPEC may be a useful asset in a possible future clinical setting.

Hematologie / Allergie

19. Snelle detectie van de translocatie t(8;21) bij acute myeloide leukemie op de LightCycler met behulp van AML1-ETO specifieke hybridisatie probes

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Inleiding: De translocatie t(8;21) is een van de meest voorkomende chromosomale afwijkingen in acute myeloïde leukemie (AML). Van alle AML patiënten vertoont 40% het AML1-ETO fusiegen [1]. Met een conventionele nested RT-PCR zijn we in staat semi-kwantitatief het fusiegen aan te tonen met een gevoeligheid van 10^{-4}. De PCR primers omvatten het breekpunt met een productgrootte van 338 bp in de eerste PCR en 185 bp in de nested PCR. De vraag naar detectie en kwantificering van minimal residual disease (MRD) bij de behandeling van leukemiëen wordt steeds groter. Daarom wordt getracht een kwantitatieve real-time RT-PCR op te zetten met behulp van de LightCycler-technology.
Methoden: De conventionele nested RT-PCR is overgezet op de LightCycler door gebruik te maken van de eerste primerset en de positieve cellijn Kasumi-1. Het PCR product (338 bp) wordt gedetecteerd met een set probes die sequentie-specifiek is. De detectie van de probes is gebaseerd op het principe van fluorescentie resonantie energie transfer (FRET). Er wordt gebruik gemaakt van een fluoresceïne gelabelde donorprobe en een LC-Red gelabelde acceptorprobe. Wanneer de probes beide gehecht zijn aan het specifieke product treedt er energieoverdracht op van de donor- naar de acceptor-probe. Hierbij komt fluorescentie vrij die evenredig is met de hoeveelheid gevormd product.
Resultaten en discussie: Met real-time RT-PCR en met behulp van specifieke probes kan het AML1-ETO fusiegen, semi-kwantitatief, snel en specifiek aangetoond worden. Hiermee is één afwijkende cel op 10^{3}-10^{4} negatieve cellen detecteerbaar. Er wordt gestreefd naar een kwantitatieve methode met behulp van een z.g. exogene interne homologe standaard.
Conclusie: Het overzetten van een goed lopende conventionele RT-PCR naar een real-time assay op de LightCycler blijkt relatief eenvoudig. De gevoeligheid die met de enkelstaps PCR bereikt wordt is vergelijkbaar met de conventionele nested PCR. Dit houdt in dat de real-time RT-PCR een gevoelige en snelle methode is om de translocatie t(8;21) te detecteren. De gevoeligheid zou verder verhoogd kunnen worden door toepassing van real-time nested PCR.

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20. Cycline D1 expressie bij Mantel Cel Lymfoom

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Inleiding: Het mantelcel lymfoom(MCL) is een B-cel lymfoom met een slechte prognose in tegenstelling tot de chronisch verlopende maligne B-cel lymfoproliferatieve aandoening zoals de chronische lymfocytaire B-cel leukemie(B-CLL) en het kleincellig lymfocytair lymfoom(SLL). Het MCL toont als kenmerkende eigenschap een overexpressie van cycline D1(cD1) als uiting van een t(11:14) gen herrangschikking. cD1 is een eiwit dat de voortgang van de celcyclus regelt op de overgang van de G1 naar de S-fase. Aangezien het onderscheid tussen MCL, CLL en SLL op morfologische en immunofenotypische kenmerken vaak moeilijk is, kan meting van cD1 expressie bijdragen aan de diagnostiek van MCL. Meting van de cD1 expressie wordt meestal verricht op paraffine coupes met immuunblotting of met fluorescentie in situ hybridisatie van cD1-mRNA. De vraag was of cD1 expressie van lymfocyten gemeten kan worden middels flowcytometrie met gebruik van fluorescerende monoklonale antilichamen(M0A1). Getest werden MoA1.G124-326(Pharmingen) en MoA1.DCS-6(Dako)
Methoden: Na een isolatie van de mononucleaire cellen mbv de vacutainer CPT buizen(Beckton Dickinson)werden de cellen gefixeerd mbv metanol bij –20 °C. De cel-kern membramen werden gepermeabiliseerd mbv triton. De cellen werden gelabeld met CD79a-PE als B-celmarker en met een anti cycline D1-FITC(of isotype controle) Meting vond plaats mbv een Coulter EPICs flowcytometer invullen
Resultaten en discussie: Clone DCS-6: Er was geen fluorescentiesignaal verschil aantoonbaar tussen negatieve controle en anti-cycline D1.Het MoAl G124-326gaf een positief fluorescentie signaal zowel bij de JVM2 cellen als bij de MCL cellen(2X) CLLcellen (2X), Normale B cellen(3X).
Conclusie: Het anticycline D1(clone DCS-6) is niet geschikt voor de flowcytometrische bepaling van cycline D1. Het anti cycline D1 (clone G124-326) is wel geschikt voor de flowcytometrie maar is volgens deze methode niet geschikt om onderscheid te kunnen maken tussen normale B cellen, CLL cellen en MCL cellen.

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21. Bepaling van de soluble transferrinereceptor op de Cobas Mira

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*Inleiding:*De laatste tijd verschijnen er diverse artikelen die het nut van de soluble transferrinereceptor als parameter bij de diagnose van ijzergebreksanemie beschrijven (1,2). Veranderingen in de sTfR zijn waargenomen bij veranderingen in de erythropoïese en/of ijzerstatus van een patiënt. De belangrijkste toepassing lijkt vooral te liggen bij de diagnose van ijzergebrek bij inflammatoire aandoeningen. Tot voor kort werd de sTfR bepaald met arbeidsintensieve IRMA’s en ELISA’s, recent zijn er een aantal geautomatiseerde bepalingen beschikbaar gekomen. Wij hebben de bepaling van sTfR met de IDeA sTfR-IT kit van Orion Diagnostica op de Cobas Mira geëvalueerd.

Methoden: De analyses werden verricht met een Cobas Mira (Abx Diagnostica) met een immunoturbidimetrische assay bij een golflengte van 600 nm. Er werd gebruik gemaakt van geanonimiseerd materiaal en voor het bepalen van de referentiewaarde werd gebruik gemaakt van serum van 72 vrijwilligers.
Resultaten en discussie: De volgende parameters werden bepaald: detectiegrens, lineariteit, reproduceerbaarheid, bewaarcondities, gebruik van diverse materiaalsoorten en de referentiewaarde. Tevens werd als pilot bij een aantal patiënten met reumatoïde artritis de sTfR bepaald. De resultaten worden hier kort weergegeven: detectiegrens is 0,03 mg/l ; lineariteit tot 13,0 mg/l ; alle inter- en intra-dag-variaties < 4,2 %; monsters kunnen gedurende een week dagelijks een vries/dooi cyclus ondergaan zonder dat dit invloed heeft; de waarde gevonden bij plasmamonsters liggen iets hoger (ca 0,3 mg/l) dan in serum. De referentiewaarde in serum bedraagt: 1,10-2,41 mg/l . Een pilot studie bij een aantal reumatoïde artritis patiënten geven nog geen eenduidige resultaten en zal uitgebreid dienen te worden.
Conclusie: De sTfR is met de IDeA sTfR-IT kit van Orion Diagnostica op de Cobas Mira te bepalen. Een vervolg studie zal dienen uit te wijzen of de bepaling ook klinische relevantie heeft.

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22. A case of EDTA-induced pseudo-neutropenia resolved with kanamycin

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Introduction: Pseudo-neutropenia due to in vitro agglutination of neutrophils is a very rare phenomenon. The literature contains only 45 cases and EDTA-dependent antibodies cause only part of these. This contrasts considerably with EDTA-induced platelet agglutination, with an estimated incidence as high as 0.1%. The usual way to circumvent spurious low neutrophil counts is using an alternative anticoagulant or prewarming the sample. Recently, Sakurai demonstrated that EDTA-dependent platelet aggregation could successfully be dissociated by addition of amino glycosides (1). We found a case of EDTA-induced pseudo-neutropenia and tried to resolve it using kanamycin.
Methods: Blood was collected into standard tubes containing K2-EDTA and trisodium citrate, respectively. We used a CELL-DYN® 4000 instrument (Abbott Diagnostics Division, Santa Clara, USA). After finding neutrophil clumps, we added kanamycin to EDTA blood (final concentration 20 mg/ml).
Results and discussion: A 49-year-old male patient was investigated routinely. His WBC was $4.1x10^9$/l , but the result was flagged and this prompted further investigation. The blood smear showed many aggregates consisting of 5-15 neutrophils and an occasional lymphocyte or monocyte, but no platelets. The WBC count gradually decreased to $2.7x10^9$/l when the sample was kept at ambient temperature. Pre-heating to 37°C had no effect on the WBC count and neither had cooling to 4°C. After addition of kanamycin to the blood sample, the WBC count increased to $6.0x10^9$/l , which was in excellent agreement with the WBC count in citrated blood: 6.2 10^9/l . In a smear prepared from the blood with kanamycin, no neutrophil clumps were observed, so the agglutination could be resolved completely.
Conclusion: This case demonstrates that kanamycin cannot only resolve EDTA-induced pseudo-thrombocytopenia, but also at least some cases of EDTA-induced pseudo-neutropenia.

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23. Genotyping of hemochromatosis-associated mutations in the *HFE* gene by PCR RFLP and a novel reverse hybridization method

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Introduction: Specific C282Y-, H63D- and S65C-mutations in the human HFE-gene are associated with hemochromatosis. In this study the performance of two different PCR RFLP methods and a novel reverse hybridization method to detect these mutations was evaluated.
Methods: A total of 51 patients were studied, using three different methods. Two methods comprised PCR RFLP. One PCR RFLP method was based on the use of general primers and the other employed mutation-specific mismatched primers. The third method was a newly developed reverse hybridization line probe assay (LiPA), comprising DNA amplification by general primers followed by a single step reverse hybridization to wild-type and mutant-specific probes that are immobilized on a nitrocellulose strip.
Results and discussion: A total of 48 (94%) of the 51 samples yielded identical results by all three methods. Three discrepant results were obtained. These were all due to polymorphisms in the primer binding region, resulting in no amplification or selective amplification, leading to misinterpretation of the HFE genotype by PCR-RFLP.
Conclusion: Primer binding problems can have a serious impact on the reliability of PCR-based HFE genotyping methods, since they can result in erroneous genotyping results. Assays, employing primers that contain specific mismatches are especially more prone to produce erroneous typing results, due to selective amplification. Methods based on highly conserved primer binding regions are therefore preferable. The novel PCR-reverse hybridization assay, using highly conserved primers and type-specific probes offers an easy and reliable alternative to currently used conventional methods.

24. De waarde van de serum-transferrinereceptor bepaling bij dialysepatiënten

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Inleiding: In eerdere publicaties (1) is aangetoond dat de serum-transferrinereceptor (sTfR) een geschikte parameter is voor de diagnose van ijzergebrek. Over de klinische toepasbaarheid van deze marker bestaat echter nog discussie.
Hemodialysepatiënten hebben doorgaans een multifactoriëel bepaalde anemie op basis van erytropoëtine-deficiëntie/-resistentie, bloedverlies, ijzergebrek en/of anemia of chronic disorders. het effect van r-HuEPO (recombinant humaan erytropoëtine) therapie kan bij deze patiënten sterk variëren. Niet zelden kan een verminderd effect van r-HuEPO verklaard worden door een tekort aan functioneel beschikbaar ijzer. Uit een eerdere pilotstudie (2) bleek dat de sTfR-bepaling - in tegenstelling tot de gangbare ijzerparameters - van nut kan zijn bij het vaststellen van dit functionele ijzergebrek. Het huidige onderzoek wil inzicht verschaffen in de indicatie en frequentie van sTfR-bepalingen bij dialysepatiënten.
Methoden: Gedurende 12 weken zijn bij 17 dialysepatiënten vóór elke dialysebehandeling (2-3 x per week) een aantal laboratoriumonderzoeken verricht (anemie-, ontstekings-, nierfunctie-, ijzerstatusparameters en sTfR). Venofer- (i.v. ijzerpreparaat) en r-HuEPO-toedieningen werden systematisch geregistreerd. De sTfR bepaling werd verricht met een IDeAs-TfR-kit van Orion op een Beckman Immage analyser.
Resultaten en discussie: De sTfR-bepaling blijkt een in de tijd stabiele bepaling die functioneel ijzergebrek - ook in aanwezigheid van een (chronisch) ontstekingsproces - detecteert. De ferritineconcentratie daarentegen fluctueert onder invloed van ontstekingsprocessen (acute fase reactie) en ijzertherapie. In een klinisch stabiele situatie is bepaling van sTfR bij dialysepatiënten slechts beperkt nodig. Wel kan met sTfR een (latent) functioneel ijzertekort worden aangetoond en zodoende een mogelijk suboptimale respons op r-HuEPO worden voorkomen. Uiteraard is sTfR ook een snelle en gevoelige parameter bij een manifest ijzergebrek.
Conclusie: De sTfR-bepaling blijkt een bruikbare parameter om bij dialysepatiënten functioneel ijzertekort en (relatieve) r-HuEPO-resistentie op basis van ijzergebrek aan te tonen.

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25. Aantonen van acute porfyrie: evaluatie van een porfobilinogeen sneltest in urine

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Inleiding: Acute porfyrieën zijn een groep van zeldzame erfelijke aandoeningen waarbij er problemen optreden bij de synthese van haem. Acute porfyrieën kunnen zich presenteren met symptomen als abdominale pijn en neurologische aandoeningen. Voor een snelle diagnose worden er vaak lastige en onnauwkeurige kwalitatieve methodes gehanteerd. Voor avond-, nacht- en weekenddiensten is een PBG-sneltest een ideale uitkomst welke binnen ons laboratorium is uitgetest. Uit onderzoek van een andere groep is gebleken dat de PBG-sneltest een welkome aanvulling is binnen de acute diagnostiek van porfyrien (1).
Methoden: De PBG screeningtest is gebaseerd op de Watson-Schwartz test (2), waarbij PBG uit urine geabsorbeerd wordt door hars dat zich in een spuit bevindt. Na een wasstap met water wordt het PBG geelueerd van de hars en wordt PBG gecondenseerd met p-dimethylaminobenzaldehyde in zure oplossing tot een rose/violet gekleurd product. Middels een kleurenkaart kan de PBG-concentratie semikwantitatief worden afgelezen.
Resultaten en discussie: Aan de hand van een verdunningsexperiment met PBG (25-200 µM) uit verse urine is gebleken dat de verschillende concentraties PBG redelijk overeenkomen met de kleurenkaart zoals die door de firma wordt geleverd, waarbij aangetekend dat de kleurintensiteit bij hoge PBG-concentraties (>75 µM) enigszins achterblijft bij de kleurreferentie. Beoordeling van het eindproduct van een tweetal verse urine monsters (15 en 30 µM) door 5 analisten gaf een eensgezinde beoordeling van de monsters. Uit de zelfanalyse van een tweetal monsters (15 en 75 µM) door dienstdoende analisten (n=15 per monster) bleek dat de lage PBG-concentratie een eenduidig resultaat gaf, i.t.t. de hoge concentratie, waarbij de resultaten uiteen liepen van 40 µM tot 75 µM. Een mogelijke oorzaak voor deze spreiding kan zijn de interpersoonlijke variatie in het beoordelen van kleuren, daarbij vanuitgaande dat de test op identieke wijze is uitgevoerd door de verschillende analisten.
Conclusie: Een handzame, maar vrij dure semikwantitatieve test voor een cito PBG in urine bij de verdenking op acute porfyrie.

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26. Correlations of 25 years of pollen counts (1975-1999) of Betula, Poaceae, Urtica and Artemisia with local meteorological parameters of temperature and rain

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Introduction: Daily numbers of airborne pollens from Betula, Poaceae, Urtica and Artemisia, as counted during the last 25 years, were correlated with the daily mean temperature and rain volume during their individual seasons.
Methods: Sampling of airborne pollens was done continuously since 1975 at a single location, 15 meters height on the hospital roof with a volumetric pollen trap, filtering 1 cubic meter of air every 24 hours. Local meteorological parameters, notably daily temperature and rain averages, were obtained from the Royal Dutch Meteorological Institute (KNMI, De Bilt, The Netherlands). The season start was defined as the day at which 1% of the season-total was reached. Total season counts for Betula, Poaceae, Urtica and Artemisia were correlated with the season temperature and rain averages. To reduce the

meteorological influence on pollen counts, total season counts were multiplied with the quotient of temperature and rain. Total season counts of the last 25 years were analysed for trends.
Results and discussion: Average season-totals varied widely (Betula: 1112-22462 pollens counted between 16/3-31/5; Poaceae: 2947-7919 pollens counted between 1/5-31/7; Urtica: 3212-16831 pollens counted between 15/5-10/9; Artemisia: 43-393 pollens counted between 1/7-30/8). No significant correlations could be detected between total season pollen counts and temperature or rain averages or the quotient of temperature and rain, with the exception of Poaceae. Its season counts showed a weak, but significant, correlation (r=0.58) with temperature. No trends could be detected in the pollen counts of Betula and Poaceae in the period from 1975-1999. The counts of Urtica and Artemisia exhibited significant increasing trends with time, with Urtica increasing by 500% (r=0.81) and Artemisia increasing by 600% (r=0.86).
Conclusion: There is no correlation between meteorological conditions and polen counts, except for Poaceae. Pollen counts of Urtica and Artemisia increase with time.

27. Specialisme afhankelijke scores en herhaaltermijnen voor allergiediagnostiek

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Inleiding: Het Elkerliek ziekenhuis is een algemeen ziekenhuis met 524 erkende bedden en een poliklinische adherentie van 145.000 patiënten. Het Algemeen Klinisch Laboratorium verricht ook de diagnostiek voor 93 huisartsen. Onderzocht werd of het gevonden percentage positieve resultaten van allergie-onderzoek verschilt per specialisme en gedurende welke termijn het herhalen van allergie-onderzoek op medisch verantwoorde wijze kan worden ontraden.
Methoden: Uit de Laboratorium database werden alle resultaten geselecteerd die verkregen zijn tussen 01.01.1998 en 01.09.1999 voor de specifiek IgE testen Phadiatop, huisstofmijt, katten-epitheel, honden-roos, graspollenmix en ruwe berk. Vervolgens werd per aanvragend specialisme het gevonden percentage positieve resultaten vastgesteld. Verder werd onderzocht hoe vaak onderzoek herhaald was en hoe vaak dit resulteerde in klinisch relevant veranderde resultaten, hetgeen gedefinieerd werd als een toe- of afname met tenminste 1 klasse. Tenslotte werd bepaald wat de gemiddelde tijd was tussen alle herhalingen en die tussen de herhalingen met veranderde resultaten.
Resultaten en discussie: Het percentage positieve resultaten lag voor alle onderzochte specialismen tussen de 37 en 46%, waarbij de huisartsen opvallend hoog scoorden met 45%. Het percentage positieve resultaten was het hoogst voor huisstofmijt: 64% (n=893) en het laagst voor katten-epitheel: 28% (n=877). In de betreffende periode werden 242 testen (4% van totaal) herhaald met een gemiddelde herhaaltijd van 184 dagen. In 23 gevallen was sprake van een klinisch relevante verandering. Het gemiddelde van de herhaaltijd van deze gevallen was 217 dagen.
*Conclusie:*De aanvragende specialismen voor allergie-onderzoek scoren vergelijkbare percentages positieve resultaten. De herhaaltermijn voor allergie-onderzoek kan op medisch verantwoorde wijze worden ingesteld op een half jaar. Het te verwachten effect van deze maatregel zal voor het Elkerliek ziekenhuis echter klein zijn.

28. White blood cell suspect flagging of the Beckman Coulter HmX haematology analyzer

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Introduction: The HmX haematology analyzer has a througput of 75 samples/hour. 26 parameters are measured in each sample. It is equipped with the Coulter VCS technology for the differentiation of leucocytes. Microscopic review of samples is warranted when morphological abnormalities are suspected i.e. when the HmX generates suspect flaggings or when absolute numbers of cells exceed user defined limits. In this study the suspect flagging of the HmX is evaluated.
Methods: We measured 282 unselected blood samples on two days (one month apart) on the HmX and Bayer H3 haematology analyzer. Furthermore 172 selected samples with abnormal blood cell counts and/or morphology flagging on the H3 were measured on the HmX. Two slides were made of all the samples for microscopic examination (gold standard) according to the recommendations of the VHL, which is considered as reference method in the Netherlands. Positivity for microscopic abnormalities was defined as follows: left shift > 4 points (band form 1 point, meta 2, myelo 3, promyelo 4), blast >= 1%, atypical lyphocytes, NRBC >= 1 %.
Results and discussion: Sensitivity and specificity for microscopic white blood cell abnormalities were calculated using the results of the 282 unselected samples. The sensitivity for both analyzers was 77 % and the specificity was 77 % (HmX) and 87 % (H3). The suspect flaggings of the HmX were evaluated using all the 454 samples. No microscopic abnormalities were found in 373 samples. A left shift was detected with the HmX in 29 of the 38 positive samples (all false negatives had a minor left shift i.e. less than 10 points). Atypical lymphocytes were detected in 9 of the 19 positive samples. Blast flagging was generated in 9 of the 12 positive samples (false positives < 3 % blasts) and a NRBC flagging in 4 of the 12 positive samples. All the atypical lymhocytes except one were flagged by the H3.
Conclusion: Specificity of the HmX suspect flagging for microscopic white blood cell abnormalities is 77 % and comparable with the performance of the H3. About half of the samples with atypical lymphocytes are not flagged. Left shift and blasts are detected well.

29. Hb-Groene Hart. Mild alpha-thalassemia phenotype induced by a new abnormal hemoglobin.

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Introduction: Alpha-thalassemia is generally considered to be an expression defect caused mostly by deletions silencing the expression of one or more alpha globin genes. Although non-deletion mutation alpha-thalassemia is considered rare, we frequently observe in our laboratory alpha-thalassemia phenotypes induced by points mutation by various mechanisms.

Methods: A family of recent Moroccan origin living in The Netherlands was studied because of the persisting microcytic hypochromic anemia observed in a non-iron depleted young son. No abnormal hemoglobin components were visible on electrophoresis or HPLC but the expression unbalance of beta/alpha = 1.27 measured in absence of HbH inclusion bodies, indicating the depletion of one alpha gene. No deletion defects were detected by Southern Blot analysis or by brake point PCR. Amplification and direct sequencing was applied for point mutation analysis.
Results and discussion: A normal sequence of both the alpha2 genes and the heterozygous pattern for a C->T transition at position 119 of the alpha1 genes was observed. Over 200 single amino acid substitutions have been described affecting the homologous alpha1 and alpha2 globin chains, mainly inducing detectable abnormal hemoglobin tetramers. The CCT triplet at position 119 of the alpha gene codes for a proline which at the beginning the "H helix" of the alpha chain and therefore a fundamental residue in the formation of the secondary structure of the alpha-globin chain. The CCT->TCT mutation, changing proline 119 to a serine, is the first mutant ever described at this particular position, suggesting that residue 119 might be fundamental for dimers and tetramers formation. Indeed no abnormal hemoglobin fraction was detectable in the lysate probably indicating the absence of tetramers containing the anomalous chain and an early proteolytic degradation of the monomer resulting in the unbalanced beta/alpha ratio and in the mild alpha-thalassemia phenotype observed in the carriers of this mutation.
Conclusion: In spite of the mild character, this mutation could generate an intermediate hemolytic anemia (HbH disease) in the progeny of parents with a combination of this point mutation with one of the frequent alpha zero deletion defects.

30. Alpha$^+$-thalassemia induced by a novel deletion

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Introduction: Alpha-Thalassemia is most frequently the result of deletions involving one or both alpha-globin genes on the short arm of chromosome 16. These deletions involve homologous or non-homologous recombinations based upon the presence or absence, respectively, of homology between parental sequences at the site of recombination. One of the most common mechanisms leading to alpha+-thalassemia involves misalignment between the two highly homologous 4-kb duplicated units in which the alpha-globin genes are embedded. Crossing-over between the misaligned homology boxes (X, Y and Z) gives rise to the frequently occurring -alpha 3.7 (Rightward) and -alpha 4.2 (Leftward) deletion. However, several other alpha+-thalassemia determinants have been described for which the mechanisms are either still unknown or involve a non-homologous recombination event.
Methods: Southern blotting was performed using the restriction enzymes EcoRI, BglII, HindIII, XbaI, BamHI and AccI. The following probes were used for hybridization; the 1.7 kb fragment from the p-zetaBR, the 1.5 kb PstI fragment containing the alpha1 globin gene and the 4.0kb HinfI fragment from p-alpha3'HVR .64 containing the 3'HVR. The molecular characterization of the deletion was done by break point PCR analysis using specific primers.
Results and discussion: Southern blot analysis revealed abnormal fragments after hybridization with the alpha- and zeta-probes, due to the presence of a novel deletion. The deletion breakpoint was characterized by direct sequencing of the PCR breakpoint fragment. The deletion was 7.9 kb in length involving the alpha2 globin gene. The breakpoint fragment revealed neither involvement of Alu-repeat sequences nor the presence of homologous regions prone to recombination, suggesting a non-homologous recombination event. This alpha+-thalassemia deletion was found to give rise to an atypical HbH-disease characterized by a non-transfusion dependent moderate microcytic hypochromic anemia, in combination with a poly-adenylation signal mutation of the alpha-globin gene (alpha2 -AA).
Conclusion: We report the characterization of a novel 7.9 kb deletion taking away one of the duplicated alpha-globin genes and causing an alpha+-thalassemia phenotype in two independent carriers of Surinam-Indian origin. Characterization of alpha+-thalassemia defects is, in spite of the mild phenotype, important for preventing obsolete and deleterious iron therapy in the carrier and severe HbH disease in the progeny.

31. Een patiënt met hereditaire elliptocytose. Oorzaak: duplicatie van een leucine-codon in exon 4 van het α-spectrine gen

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Inleiding: Inleiding: Een 31 jarige vrouw is snel vermoeid en heeft last van duizeligheid, hartkloppingen en hoofdpijn. Zij wordt gezien door de internist-hematoloog. Het lichamelijk onderzoek brengt een anemie zonder icterus en zonder een vergrote milt naar voren. Microscopisch onderzoek laat een bloedbeeld zien met microcyten maar ook veel elliptocyten. De osmotische resistentie van de erytrocyten is verlaagd, evenals het serum ferritine. Op grond van deze resultaten is geconcludeerd, dat het hier gaat om een patiënt met een ijzergebreks anemie, maar tevens een vermoeden van een hereditaire elliptocytose.
Methoden: Meting van de vervormbaarheid en osmotische fragiliteit met behulp van een Ektacytometer (Technicon).
PCR en DNA sequencing met behulp van respectievelijk GeneAMP PCR system 2400 en ABI PRISM 310 Genetic Analyser (beiden Applied Biosystems).
Resultaten en discussie: Uit ektacytometrisch onderzoek blijkt dat de erytrocyten van de patiënt een elliptocytair vormveranderings patroon laat zien. De ervaring leert, dat het mutatie-onderzoek zich dan moet richten op het 5'-gedeelte van het α-spectrine gen, wat een belangrijke structuurbepalende component is van het membraanskelet van de erytrocyt. Bij de bepaling van de nucleotide volgorde werd in exon 4 van dit gen een dubbel signaal op heterozygote wijze gevonden. Het gemuteerde gen codeert voor een dubbele leucine op positie 154 van het α-spectrine eiwit.
Tevens werd in exon 40 van het α-spectrine gen de Low Expression LYon (LELY) mutatie aangetroffen. Het allel dat deze mutatie bevat komt verminderd (50%) tot expressie.
Conclusie: Aan de hand van ektacymetrisch en DNA onderzoek werd een erfelijke elliptocytose gediagnosticeerd met een bijzondere mutatie in exon 4 van α-spectrine bij een patiënt met tevens een ijzergebreksanemie. Aangezien de onderzochte patiënt een sterke elliptocytose vertoont, mag worden aangenomen, dat de exon 4 en de LELY mutatie in trans gelegen zijn.

32. Analysis of different folate forms by HPLC and electrochemical detection

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Introduction: Vital cellular processes depend on folate-mediated-one-carbon metabolism, i. e. the transfer of a carbon group. Folate is involved in nucleic acid biosynthesis, protein biosynthesis, amino acid metabolism, methyl group biogenesis and vitamin metabolism.
Disturbances in folate metabolism play a role in disorders like neural tube defects, cardiovascular disease, recurrent early pregnancy loss and pre-eclampsia, but its precise role is not always clear. Unravelling the mechanisms leading to these disorders requires not only the measurement of total folate concentration but also the estimation of the multiple forms of folate which differ in pteridine ring structure and number of glutamate residues.
Methods: A two column HPLC system with four channel coulometric electrochemical detection (1) was used to analyse folate standards and folates extracted from blood. Purified folates were eluted from the affinity column onto a phenyl analytical column utilizing a switching valve, and folate forms were separated using an acetonitrile gradient.
Results and discussion: Folates extracted from erythrocytes, as well as different (poly)glutamate derivatives of pteroylglutamate and 5-methyl tetrahydrofolate (THF) were identified by retention time and characteristic response across the channels of the detector. Folates were quantified by comparison to an external calibrator. Limits for detection ranged from 1 - 10 pmol for pteroyl glutamate and 5-methyl THF. Synthesis and analysis of other forms of folate are in preparation.
Conclusion: This automated HPLC system allows the simultaneous and sensitive determination of polyglutamyl forms of folates from biological samples such as erythrocytes and lymphocytes. This method is of potential interest to unravel the pathogenesis of several disorders in folate metabolism and for folate standardisation purposes.

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33. Analytical performance and clinical use of four soluble transferrin receptor (sTfR) assays: is sTfR really a new diagnostic tool?

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Introduction: It has been shown that sTfR assays are useful in the diagnosis of iron deficiency anaemia; on the other hand these assays have not been internationally standardized and information on analytical performance is scarce.
Methods: We compared characteristics of four sTfR kits (latex-particle enhanced: Dade-Behring and Imphos; ELISA: DPC and ITK). Morover, we measured sTfR levels in a group of 156 healthy blood donors and in a group of 50 patients with both serum ferritin $\leq$ 10 µg/l and Total Iron Binding Capacity > 75 µmol/l .
Results and discussion: As measured with a normal poolserum, the inter-assay precision of both particle-enhanced assays (below 5%) is better compared to that of the ELISA assays (14.6% resp. 8.8%). As the within subject analytical variation is reported to be 13.6%, the inter-assay precision has to be 6.8% or lower. For the ELISA assays this criterium is not met.
Recoveries and linearity of the assays are generally acceptable, except for the DPC kit at low levels. The Orion kit shows suboptimal linearity.
In the group of blood donors several persons had elevated levels of sTfR in the different assays, leading tot a specificity of 98%, 97%, 100% resp. 96% (Dade, Imphos, DPC resp. ITK). No persons had elevated sTfR levels in all four assays. On the contrary, sensitivity was low: 72%, 64%, 60% resp 68%. Ten patients had normal sTfR levels in all assays. Statistical evaluation on the comparison of results of one kit with those of another did not assess agreement in any of the combinations.
Conclusion: Both latex-particle enhanced assays are analytically sufficient. These automated tests are not time consuming and are easily implemented in the daily laboratory routine. In our hands all four assays lack sensitivity and on the basis of this study we question the measurement of sTfR instead of serum ferritin in the diagnosis of iron deficiency anaemia.

34. Het tellen van erytroblasten m.b.v. hematologie-analyzers: een vergelijkend onderzoek

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Inleiding: De aanwezigheid van erytroblasten kan de automatische leukocytentelling in bloedmonsters beïnvloeden. De blasten kunnen interfereren met lymfocyten of met segmentkernige granulocyten en met name bij (premature) kinderen kan dit vergaande gevolgen hebben.
Methoden: In deze evaluatie zijn van 140 en 156 monsters het aantal erytroblasten op respectievelijk de Cell-Dyn 4000 en de Sysmex XE-2100 gemeten. Beide apparaten maken bij de bepaling van de hoeveelheid erytroblasten gebruik van de scattersignalen in combinatie met de fluorescentie-activiteit. De verkregen aantallen zijn vergeleken met de microscopische telling van erytroblasten per 200 leukocyten. Daarnaast is geprobeerd is om de uitslagen van beide analyzers te vergelijken met een erytroblastentelling op de flowcytometer waarbij gebruik gemaakt werd van de monoclonalen CD45 en CD36.
Resultaten en discussie: Geen significant verschil (gepaarde t-toets) werd gevonden tussen de microscopische telling enerzijds en de Cell-Dyn 4000 of de Sysmex XE-2100 anderzijds. Ook tussen beide analyzers kan geen significant verschil worden aangetoond. Bij een positieve microscopische bevinding van aanwezigheid van erytroblasten geven de Cell-Dyn 4000 en de Sysmex XE-2100 in respectievelijk 94% en 89% aan dat er erytroblasten inzitten. In de gevallen dat er geen erytroblasten worden gedetecteerd worden wel andere vlaggen gegenereerd die een microscopische beoordeling nodig maken.
De resultaten van de flowcytometer waren te afwijkend ten opzichte van de drie andere methodes en konden dus niet meegenomen worden in de verwerking van de resultaten.
Conclusie: Zowel de Sysmex XE-2100 als de Cell Dyn 4000 blijken goed in staat te zijn erytroblasten aan te tonen en te kwantificeren. Een flowcytometrische methode met behulp van CD45 en CD36 geeft minder goede resultaten.

35. Calibration 2000 project. Hemocytometry

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Introduction: The Task Force hemocytometry of the calibration 2000 project tries to accomplish further harmonization of the determination of hemoglobin, erythrocytes, MCV, leucocytes and thrombocytes in patient samples.
Methods: On the basis of prelimanary results four commercially available controls/calibrators were selected and tested for possible use as calibrator on all common hematology analyzers: Testpoint (Bayer), Instrublood Plus (Instruchemie), SCS-1000 (Sysmex TOA) and Hemcal (Abbott). These materials were tested on commutability with 20 fresh patient samples in twin studies i.e. a simultaneous measurement of exchanged patient samples and test material on two different analyzers. A linear regression line was calculated with patient samples (y'=ax+b). Tested materials are regarded as commutable with patient samples when the normalized residual y value ((y' - y)/Sy,x) is less than 3. Eight blood samples distributed for the EQA (fresh blood samples analyzed within one day after donation) were also tested for commutability with the patient samples. Results from 18 twins with analyzers from Bayer, Coulter, Abbott and Sysmex were studied.
Results and discussion: Commutability between the controls/calibrators and patient samples could be demonstrated for Hb and erythrocytes except for SCS-1000 on a Sysmex K-4500. Commutability was not found for MCV. The SCS-1000 calibrator was commutable with patient samples for leucocytes and thrombocytes on all the analyzers studied. The results with Hemcal were also quite good for these parameters, whereas the results with Testpoint on Abbott analyzers were far out of range. Most EQA samples were commutable with patient samples for all parameters and analyzers.
Conclusion: None of the selected commercially available materials can be used as a calibrator for all parameters on all of the common hematology analyzers. The results with SCS-1000 and Hemcal deserve further study. Most EQA samples are commutable with fresh patient samples, however their use as a calibrator is limited, because it concerns fresh human blood.

36. Mutations in the human pyruvate kinase gene leading to pyruvate kinase-deficiency in The Netherlands

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Introduction: Mutations in the liver (L) and red cell (R) type pyruvate kinase gene (PKLR) are associated with pyruvate kinase deficiency, a disorder causing hereditary nonspherocytic hemolytic anemia. The phenotypic expression of the disease is variable. In this study, 23 Dutch families with pyruvate kinase deficiency were characterized at the molecular level. Three dimensional computer modelling was used in order to obtain an insight in the effect of the mutations on structure and allosteric interactions of the molecule. In addition we evaluated the phenotypic expression of the disease in relation to the detected mutations in these patients.
Methods: Polymerase Chain Reaction (PCR) amplification and DNA sequence analysis was used for mutational analysis of the promoter, introns and 12 exons of the PK gene. Three dimensional computer-modelling was used to predict the effect of mutations on the molecule.
Results and discussion: In total, 22 different mutations were found, thirteen of which were specific for the Dutch population. In addition, a novel polymorphic C to T substitution at nt –109 in the PKR promoter was identified. Three mutations were nonsense and frameshift mutants, three mutations resulted in aberrant RNA splicing and sixteen were missense mutations. Of the total number of mutations, 59% was located in exons 7, 9 and 11 (worldwide 43%). Strikingly, in contrast to the worldwide spectrum of mutations, no mutations were found in exon 8. The most common mutations were G1529A (24%) and C1456T (13%), in agreement with the international population, and one exclusively Dutch mutation (G331A) with a prevalance of 13%. Two unrelated patients were homozygous (G1529A). All other clinically affected patients were compound heterozygous. Using computer modelling of the missense mutants, we were able to divide the mutants into three categories. Those which resulted in disrupting the monomeric structure, those influencing interface and domain interactions and those with a possible effect on allosteric behavior of the enzyme. Correlation studies of the molecular defect with the phenotypic expression are currently underway.
Conclusion: Identifying the molecular basis of mutations underlying this rare disorder will help to obtain more insight into the mechanisms involved in the variable clinical phenotype of PK deficient patients.

37. Evaluatie van de Beckman-Coulter® HMX, een nieuwe mid-range hematologie analyser

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Inleiding: De Beckman-Coulter HmX is een nieuwe mid-range hematologie analyser, die 24 hematologische parameters bepaalt respectievelijk berekent. De five-part differentiatie vindt plaats met behulp van de reeds lang toegepaste Coulter VCS technologie.
Methoden: Evaluatie heeft plaatsgevonden door het uitvoeren van de NCCLS (1) en ICSH (2) protocollen voor hematologische analysers met electronische differentiatie.
Resultaten en discussie: Bepaling van intern en extern controle materiaal met zowel bekende als onbekende resultaten gaf aan dat de precisie en reproduceerbaarheid goed zijn. De VC in % within batch lag voor de tellingen in bloed tussen de 0,61 (hemoglobine) en 2,14 (trombocyten) en between batch tussen de 0,65 (hemoglobine) en 2,16 (trombocyten). Voor de electronische differentiatie werd voor de sensitiviteit, gedefiniëerd als positieve screening van abnormale monsters, 100 % gescoord evenals voor de specificiteit, (negatieve screening van normale monsters). De carry-over bleek laag te zijn, maxi-

maal 1 % voor hemoglobine, voor de andere parameters <0,5 %. De analyser bleek in staat te zijn monsters tot 48 uur oud nog goed te kunnen analyseren, inclusief de electronische differentiatie. Omdat de analyser permanent bij te laden is, is de doorvoersnelheid van 75 monsters per uur voor middelgrote laboratoria geen probleem.
Conclusie: De HmX is een robuuste, goed functionerende analyser met goede tot zeer goede analytische prestaties. Het bedieningsgemak is beter ten opzichte van zijn voorgangers. De analyser is geschikt voor middelgrote laboratoria of als back-up voor grotere.

Literatuur

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Stolling

38. Orale antistolling vermindert het aantal geactiveerde trombocyten bij patiënten die een percutane coronaire angioplastiek ondergaan

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Inleiding: Geactiveerde trombocyten spelen een belangrijke rol in acute vaatafsluiting na percutane coronaire angioplastiek. In de Balloon Angioplasty and Anticoagulation Study (1) werden 1058 patiënten gerandomiseerd naar gebruik van alleen aspirine® of orale anti-stolling naast aspirine® wat resulteerde tot een afname van 47% in acute complicaties (dood, myocardinfarct of revascularisatie). Een direct effect van orale anti-stolling op de trombocytenfunctie wordt hierdoor gesuggereerd. In deze studie hebben wij geprobeerd het effect van orale anti-stolling op activatie en functie van trombocyten bij patiënten die een percutane coronaire angioplastiek ondergaan nader te beschrijven.
Methode: Patiënten die een angioplastiek ondergaan zijn gerandomiseerd naar alleen aspirine® (groep A, n=26) of aspirine® plus orale anti-stolling (groep B, n=26). De studiemedicatie werd een week voor de ingreep gestart. Metingen aan trombocyten werden 1 uur voor, 1 uur na en 1 en 7 dagen na de ingreep uitgevoerd. Geactiveerde trombocyten werden bepaald m.b.v. de monoklonalen CD62p en CD63 op de flowcytometer. De trombocytenfunctie is bepaald m.b.v. de PFA-100® analyzer.
R*esultaten:* Voor de ingreep was het aantal geactiveerde trombocyten in groep A significant hoger dan in groep B ($p<0,05$). Na de ingreep was er een significante afname in de hoeveelheid geactiveerde trombocyten in beide groepen. De afname in groep A (van 54 naar $24/10^4$ trombocyten, $p=0,01$) was significant groter dan voor groep B (van 28 naar $13/10^4$ trombocyten, $p<0,05$). Tijdens dag 1 en 7 zijn er geen significante veranderingen in het aantal geactiveerde trombocyten in beide groepen waargenomen. De trombocytenfunctie, gemeten met de PFA-100® analyzer, werd niet beïnvloed door orale antistolling.
Conclusie: Behandeling met orale antistolling resulteert in een significante afname van geactiveerde trombocyten voor en na de percutane coronaire angioplastiek wat volledig in overeenstemming is met het klinisch effect n.l. een afname van complicaties ten gevolge van de procedure.

Literatuur

1. Ten Berg JM, Kelder JC, Suttorp MJ et al. Effect of coumarins started before coronary angioplasty on acute complications and long-term follow-up: a randomized trial. Circulation 1998; 98: 2829-35

39. Een eenmalige hoge dosis aspirine®, naast een dagelijkse lage dosis leidt tot een vermindering van geactiveerde trombocyten bij patiënten die een percutane coronaire angioplastiek ondergaan

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Inleiding: Trombocyten activatie speelt een belangrijke rol bij acute vaatafsluiting na percutane coronaire angioplastiek. Chronisch aspirinegebruik lijkt minder effectief te werken dan aspirine-inname vlak voor de ingreep. In gezonde individuen leidt een eenmalige hoge dosis aspirine® tot een verminderde activatie van trombocyten. Voor patiënten die een percutane coronaire angioplastiek ondergaan is dit effect nog niet nader bekeken.
Methode: Er zijn 51 patiënten geïncludeerd en gerandomiseerd naar twee groepen nl. groep A: 24 patiënten die dagelijks een dosis van 100 mg aspirine® gestart minimaal een maand voor de ingreep innamen en groep B: 27 patiënten die naast de dagelijkse dosis van 100 mg eenmalig 1000 mg aspirine® innamen de dag voor de ingreep. Vlak voor en 1 uur na de percutane coronaire angioplastiek werden geactiveerde trombocyten m.b.v. flowcytometrie en de trombocytenfunctie m.b.v. de PFA-100® analyzer gemeten.
Resultaten: Groep A had significant meer geactiveerde trombocyten voor de ingreep dan groep B. De eenmalige hoge dosis aspirine® leidde zelfs tot het vrijwel kompleet afwezig zijn van geactiveerde trombocyten in groep B. Na de ingreep was er een significante vermindering van het aantal geactiveerde trombocyten in groep A terwijl er geen verandering was in het aantal geactiveerde trombocyten voor groep B. Een eenmalig hoge dosis aspirine® had geen invloed op de trombocytenfunctie gemeten op de PFA-100® analyzer.
Conclusie: Een eenmalige hoge dosis aspirine® een dag voor de ingreep naast de dagelijkse dosis van 100 mg leidt tot een significante afname van het aantal geactiveerde trombocyten voor en na de percutane coronaire angioplastiek terwijl de trombocytenfunctie onveranderd blijft. Deze gegevens suggereren dat een eenmalig hoge dosis aspirine® acute complicaties kan verminderen zonder kans op een toename van bloedingen.

40. Invloed van full draw vs partial draw en buizenposttransport op closure times gemeten met de PFA-100

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Inleiding: De closure time (CT) vastgesteld met de Platelet Function Analyzer 100 (PFA-100, Dade Behring) vormt een alternatief voor de bloedingstijd (BT) (1) om preoperatief een inschatting te maken van het bloedingrisico bij patiënten die spinaal anesthesie moeten ondergaan bij behandeling met cyclooxygenase remmers, (2) als (grove) screening op afwijkingen in de primaire hemostase bij episodes van onverklaard bloedverlies (bv. o.i.v. von Willebrand ziekte (vWD)).
Wij hebben de invloed van een tweetal preanalytische variabelen nl. (1) afname van bloed in full draw (4.5 ml BD buis) vs. partial draw (2,7 ml BD buis, uit de handel vanaf 1/10/2000) 3,2% citraatbuizen, en (2) buizenposttransport op collageen/ADP (CADP) en collageen/epinephrine (CEPI) CT waarden onderzocht.
Methoden: Bij 46 vrijwilligers (23 mannen/23 vrouwen) werd middels een vragenlijst leeftijd/rookgedrag/ gezondheidsklachten/ geneesmiddelgebruik/ bloedingproblemen in kaart gebracht en gelijktijdig een 4,5 en 2,7 ml citraatbuis bloed afgenomen. Gebruikers van acetyl salicylzuur (ASA) werden geëxcludeerd. In volbloed werd de RBC/hematocriet, WBC, plaatjescount/MPV gemeten; geen abnormaliteiten werden aangetroffen.
Resultaten en discussie: Noch hematologische parameters noch items in vragenlijst vertoonden een significante samenhang met CT-waarden. De CEPI CT lagen voor 4,5 ml buizen tussen 71-174 s (gemiddelde +/- 2* SD) en voor de 2,7 ml buizen tussen 56-163 s (matched paired t-test, $p<0,01$). CT gemeten met CADP cartridge waren resp. 55-108 en 54-121 (niet significant).
Invloed van buizenposttransport werd (gepaard) onderzocht bij patiënten van de afdeling neurologie die preventief een lage dosis aspirine gebruiken (verlengde CEPI CTs worden verwacht die optimaal gevoelig zijn voor transportcondities). Buizenposttransport gaf geen significante verschillen in CT voor CEPI en CADP d.w.z. de beoordeling van CT (normaal vs. verlengd) was gelijk bij hand gebrachte en buistransport vervoerd bloed.
Conclusie: Concluderend kunnen wij stellen dat het afnamesysteem (partial draw vs. full draw) invloed heeft op CEPI CT maar niet op CADP CT. Buizenposttransport (interne post) heeft daarnaast geen significante invloed op de CT.

41. Kwaliteit CoaguChek (Roche) controles

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Inleiding: Binnen het Deventer Ziekenhuis is begin 2000 het CoaguChek (Roche) project gestart voor thuismonitoring van orale antistolling. Hierin werden 46 patiënten met een langdurige indicatie voor antistolling opgeleid zelf de INR te bepalen.
Methoden: Tijdens de opleidingssessies en steeds na drie maanden werd de INR bepaald. Dit gebeurde op de CoaguChek van de patiënt, op een CoaguChek van de trombosedienst en aan de hand van een venapunctie (ACL Futura, IL).
De INR van de CoaguChek van de patiënt ten opzichte van de veneuze INR werd uitgezet tegen de INR van de venapunctie. Wij gebruikten de teststrips batches 171 en 196. Tevens werden de CoaguChek van de patiënt en de Coaguchek van de trombosedienst vergeleken.
Resultaten en discussie: De resultaten van de verschillende batches werden over een interval van 0,5 INR gemiddeld en uitgezet. Uitgangspunt was de waarde van de INR op de Coaguchek. Wat we daarbij wilden weten was of je aan de hand van de gemeten INR een voorspelling kan doen over de veneuze INR. Het blijkt dat er ten eerste een verschil is in voorspellende waarde tussen batch 171 en 196. Batch 171 vertoont grote afwijkingen boven een INR van 4,0, batch 196 wijkt met name af onder een INR van 2,5. Bij een verschil van 0,5 INR als grens voor een acceptabel verschil tussen de veneuze INR en de CoaguChek INR zijn de INR gebieden waarbinnen dit optreedt per batch verschillend. De correlatie tussen de verschillende CoaguCheks is redelijk: r=0,91. Desondanks kan er, ook binnen het streefgebied een INR verschil optreden dat 1 INR is of groter.
Conclusie: 1) Controle van de kwaliteit van de CoaguChek moet geschieden ten opzichte van een venapunctie en niet ten opzichte van een andere CoaguChek. 2) Verschillende batches van de CoaguChek geven een verschillende afwijking ten opzichte van de venapunctie. Binnen het therapeutisch gebied zijn de uitslagen van de gezamenlijke batches nog redelijk betrouwbaar, daarbuiten nemen de afwijkingen sterk toe.

42. Factor XII bij patiënten op orale antistollingsmedicatie

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Inleiding: Patienten met een tekort aan FXII kunnen een verhoogde stollingsneiging hebben ten gevolge van een verminderde fibrinolyse. Het doel van deze studie was om bij patienten met orale antistollingsmedicatie, die een bewezen episode van verhoogde stollingsneiging hebben doorgemaakt, na te gaan of een tekort aan FXII hieraan mede ten grondslag kan liggen. Tevens werd bestudeerd of de activiteit van FXII gerelateerd is aan FVIIIc en wat de mate van FXII activatie (FXIIa) is onder orale antistolling.
Methoden: Op de trombosedienst werd plasma verzameld van 461 patienten met orale antistollingsmedicatie vanwege verschillende aandoeningen (hartinfarct, klepvervanging, diepe veneuze trombose, perifeer arterieel vaatlijden, longembolie, coronaire arteriele bypass) en van 130 patienten met profylactisch orale antistollingsmedicatie vanwege heup- of knieprothese (controlegroep 1). 50 patienten zonder antistollingsmedicatie werden geincludeerd als controlegroep 2. Voor de bepaling van de activiteit van FXII en FVIIIc werd een coagulometrische methode gehanteerd. Factor XIIa werd gemeten middels een directe immuno-assay.
Resultaten en discussie: FXII activiteit was in alle patienten met orale antistollingsmedicatie (inclusief controlegroep 1; gemiddelde activiteit = 81 %, sd = 20%) significant ($P< 0,01$) lager dan in controlegroep 2 (gemiddelde activiteit = 115 %, sd = 27 %). In 10 % van de patienten lag de FXII activiteit lager dan de referentiewaarden (60-120 %). FXII activiteit correleerde slecht met FXIIa (r= 0,20) en FVIIIc (r=0,32). FVIIIc was verhoogd ($p<0,05$) in patienten met hartinfarct (gemid-

deld: 150 %), diepe veneuze trombose (144 %), longembolie (152 %) en coronaire arteriele bypass (145 %) ten opzichte van controlegroep 2 (133 %).
Conclusie: FXII is gemiddeld lager bij patienten met antistollingsmedicatie, die een bewezen episode van verhoogde stollingsneiging hebben doorgemaakt, maar ook bij patienten die profylactisch antistollingsmedicatie ontvangen (knie- of heupprothese). Dit lijkt erop te wijzen dat een verlaagde FXII activiteit niet de oorzaak is van een verhoogde stollingsneiging, maar het gevolg is van de antistollingsmedicatie. Daarentegen lijkt een verhoogde activiteit van FVIIIc betrokken bij de aandoeningen met een verhoogde stollingsneiging.

43. Effect van acetylsalicylzuur op de trombocytenfunctie

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Inleiding: Acetylsalicylzuur (ASA, aspirine) wordt in de cardiologie en de neurologie voorgeschreven als tromboprofylacticum. ASA acetyleert irreversibel cyclo-oxygenase-1 in de trombocyt waardoor blokkade van de tromboxaan A2 (TXA2) productie optreedt. Meer dan 90% remming van TXA2 is noodzakelijk voor een remming van de trombocytenfunctie. Echter, niet alle personen vertonen een geremde plaatjesfunctie na ASA inname (de zgn. non-responders). Het percentage non-responders is afhankelijk van de methode waarmee de plaatjesfunctie wordt gemeten.
Methoden: De trombocytenfunctie van 9 CVA patienten, die gedurende 10 dagen ASA (30 of 80 mg daags) hebben ingenomen, is bepaald. Hierbij is gekeken naar de PFA-100 sluitingstijd en de plaatjesreactiviteitsindex (PRI). PRI = E/F, waarbij E staat voor het aantal trombocyten in EDTA-bloed (trombocytaggregaten desintegreren in aanwezigheid van EDTA) en F voor het aantal trombocyten in bloed gefixeerd in formaline (trombocytaggregaten worden gefixeerd in formaline). Bij een PRI van 1 zijn geen trombocytaggregaten aanwezig terwijl dit wel het geval is bij PRI>1.
Resultaten en discussie: Acht patiënten hebben een normale PRI (1 ASA non-responder) terwijl slechts 1 patiënt een verlengde PFA-100 sluitingstijd vertoont (8 ASA non-responders). Gebruik van de twee methoden geeft tegenstrijdige resultaten in deze patientengroep.
Conclusie: De resultaten van dit onderzoek geven aanleiding voorlopig dit onderzoek bij CVA patiënten te staken. Om de bovenbeschreven tegenstrijdige resultaten te kunnen verklaren en om de waarde van een aantal trombocytenfunctie-parameters te bepalen, dient bij 100 gezonde vrijwilligers gekeken te worden wat de invloed van een therapeutische dosis ASA is op PRI, PFA sluitingstijd, TXA2 en 12-HETE productie.

44. Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation

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Introduction. We determined the numbers, cellular origin and thrombin-generating properties of microparticles in healthy individuals (n=15).
Methods. Microparticles were isolated from fresh blood samples by differential centrifugation and identified by flow cytometry.
Results and discussion. Microparticles originated from platelets (237 x 10^6/l (median; range 116-565)), erythrocytes (28 x 10^6/l ; 13-46), granulocytes (46 x 10^6/l ; 16 - 94) and -possibly- endothelial cells (64 x 10^6/l ; 16-136). They bound annexin V, indicating exposure of phosphatidylserine, and initiated and supported thrombin formation in normal plasma. This thrombin generation occurred via tissue factor (TF)-independent pathways, because antibodies against TF or factor (F)VII, that completely inhibited a commercial thromboplastin-induced but not a kaolin-induced thrombin generation, were ineffective. In contrast, microparticle-induced thrombin generation was partially inhibited by antibodies against FXII (12%, p=0.006), FXI (36%, p<0.001), FIX (28%, p<0.001) or FVIII (32%, p<0.001). Both the number of annexin V positive microparticles and the thrombin-generating capacity inversely correlated to the plasma concentrations of thrombin-antithrombin (r=-0.49, p=0.072 and r=-0.77, p=0.001, respectively), but did not correlate to prothrombin fragment F_{1+2} (r=-0.002, p=0.99). The inverse correlation between the number of microparticles and their thrombin forming capacity and the levels of thrombin-antithrombin complex in plasma may indicate that microparticles present in the circulation of healthy individuals have an anticoagulant function by promoting the generation of low amounts of thrombin that activate protein C.
Conclusion. We conclude that microparticles in blood from healthy individuals support coagulation via TF- and FVII-independent pathways, and which may have an anticoagulant function.

Moleculaire Biologie

45. Two non-isotopic and sensitive methods for diagnosis of the MIDD diabetic subtype, which is characterized by maternal inheritance and associated deafness

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Introduction: Maternally inherited diabetes and deafness (MIDD) is a distinct subtype of non-autoimmune diabetes that is associated with a single mutation (A3243G) in the mitochondrial leucyl (UUR) tRNA gene at np3243 (1). Its estimated prevalence is 1.3% of the insulin-depending diabetic patients in The Netherlands. This mutation is present only in a fraction of all mitochondrial DNA (mtDNA) molecules and shows variation among tissues in a single individual, with very low levels of mutated mtDNA in peripheral leukocytes. Current methods for mutation detection are based on isotopic PCR-RFLP, which is unpracticle for routine screening. We have screened for the presence of the A3243G mutation by

means of two newly developed non-isotopic detection techniques in a cohort of diabetic patients in the Zwolle region.
Methods: DNA was extracted from peripheral leukocytes from 289 type I diabetics and 1000 type II diabetics. Mutation detection was performed using A) a non-radioactive PCR-RFLP strategy, based on amplification of a mtDNA fragment encompassing the mutated site, ApaI digestion (cuts only when mutation is present), fragments separation by electrophoresis on PAAGE gel, and detection by silver staining and B) a single tube quantitative PCR assay using molecular beacons as probes eliminating any post-PCR detection step (2).
Results and discussion: The A3243G mutation was detected in one of the 289 type I diabetics, but not in any of the type II diabetics using either methodology. Both methods were highly reproducible and very sensitive (mutant mtDNA levels as low as 1% could easily be detected) minimizing the number of false-negative results without the use of radio-active tracers. The prevalence of the A3243G mutation in this cohort of diabetic patients is lower than was reported in literature. The advantage of the molecular beacons methodology over PCR-RFLP based technique is that it is a closed, single tube assay eliminating any post-PCR detection steps. In addition, it generates a quantitative result. If no quantitative PCR system is available then PCR-RFLP is a good alternative.
Conclusion: Both non-isotopic methods permit accurate detection of the mtDNA A3243G mutation for diagnosis of the MIDD diabetic subtype

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46. Rapid single-tube genotyping of FV Leiden and Prothrombin mutations by real-time PCR using dual-color detection

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Introduction: Venous thromboembolism is a common cardiovascular disease affecting 1 out of 1000 Caucasian individuals annually. Genetic factors play a crucial role, the mutations G1690A in the Factor V gene and G20210A in the prothrombin gene being the most prominent. Because of the high prevalence of these two mutations there is growing demand for rapid, reliable and simple methods for combined detection of both point mutations.
Methods: We developed a novel method for the combined, single-tube detection of the Factor V G1690A and the Factor II G20210A mutations in peripheral blood. Our method is based on rapid-cycle multiplex PCR with real-time monitoring of the amplification process and melting curve profiling on the LightCycler using dual color detection by means of two fluorescent hybridization probes. The use of two fluorescent dyes, each reporting at a different wavelength, allows the measurement of two independent target sequences in a single capillary. Forty-seven blood samples, representative for both mutations in wild type, heterozygotic and homozygotic state, were subjected to this single-tube assay and compared with our conventional PCR genotyping procedures.
Results and discussion: the results of all 47 individuals screened (31 wild types, 11 heterozygotes and 5 homozygotes for factor V Leiden, as well as 38 wild-types, 6 heterozygotes and 3 homozygotes for the prothrombin mutation were 100% concordant with the conventional genotyping.
Conclusion: We have shown that the LightCycler mutation detection system with the use of two different fluorescent reporter dyes allows the simultaneous genotyping of the factor V Leiden and the prothrombine mutations in a single capillary. The new technique is simple, reliable and requires only 45 minutes run-time and minimal reagent costs. The dual-color detection demonstrates its broad utility for the fast, simultaneous genotyping of independant target sequences.

47. Applying molecular beacons immobilised on micro-arrays to determine single nucleotide polymorphisms in the homocysteine metabolism

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Introduction: Over the past few years several new technologies to perform DNA mutation analysis have been developed to increase sample throughput. Tyagi et al. described a technology using hairpin shaped oligonucleotide probes that only fluoresce upon hybridisation, so called molecular beacons (1). The hairpin structure of these probes results in an enhanced specificity compared to linear probes, making molecular beacons excellently suitable for the detection of single nucleotide polymorphisms (SNPs). In this study we want to demonstrate the feasibility to link molecular beacons to a solid surface (microscopic glass slide) and to demonstrate their enhanced specificity compared to linear probes after immobilisation.
Methods: We used a set of molecular beacons to analyse the 1298 A to C mutation in the methylenetetrahydrofolate reductase (MTHFR) gene to test the immobilisation of molecular beacons to the microscopic glass slide. MTHFR is one of the key enzymes in the homocysteine metabolism, playing an important role in the remethylation of homocysteine to methionin. Both the wildtype-recognising and the mutant recognising molecular beacon were 5'-CY3 and 3'-DABCYL labelled. To link the molecular beacon to the streptavidin coated glass slide, the molecular beacons were modified by attaching a Biotin-TEG-linker (Glen Research) to the base one position from the 3'-end to minimise interaction between the linker and the fluorophore on the 5'-end. At the moment of abstract submission, we are carrying out experiments to immobilise the molecular beacons to the glass slide, which will be followed by incubation with succesively the wildtype and mutant complementary oligonucleotides at different hybridisation temperatures. We will measure fluorescence of both the wildtype- and mutant-recognising molecular beacon at all temperatures. The ratio of these fluorescent signals is an index for the specificity at that temperature. The same experiment will be repeated by immobilising linear probes to the glass slide,

followed by incubation with CY3-labelled complementary oligos. A comparison will be made between the specificity of immobilised molecular beacons and linear probes.
Results and discussion: Primary results show a succesfull linking of the molecular beacons to the solid surface. Hybridisation experiments are being conducted at this moment and results will be shown at the time of the poster exhibition.
Conclusion: Immobilisation of molecular beacons to a solid surface will result in micro-arrays that are more suitable for the detection of SNPs than arrays with linear probes. Moreover, no sample labelling will be necessary since molecular beacons are probes that only fluoresce upon hybridisation. Finally, this will lead to the development of a micro-array for reliable and high-throughput analysis of all known SNPs in the homocysteine metabolism.

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48. Direct PCR op cellysaten verkregen uit vol bloed maakt DNA-isolatie overbodig

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Inleiding: Huidig uitgangsmateriaal voor de PCR is meestal opgezuiverd genomisch DNA. Isolatie van genomisch DNA, veelal uit volbloed, is tijdrovend en daarmee kostbaar. Verschillende procedures voor direct gebruik van volbloed als uitgangsmateriaal voor de PCR zijn dan ook beschreven. Deze hebben echter als nadeel dat extra handelingen nodig zijn om substanties in volbloed, welke PCR remmen, te verwijderen. Eveneens moeten de PCR-condities opnieuw geoptimaliseerd worden en zijn post-PCR handelingen, zoals restrictie-enzym analyses, niet altijd mogelijk. Dit abstract beschrijft een protocol dat bovengenoemde bezwaren ondervangt.
Methoden: Volbloed (200 µl) wordt 51x verdund in een NH4/HCO3 buffer voor selectieve lysis van rode bloedcellen. Na centrifugatie wordt de witte bloedcel pellet geresuspendeerd in 200 µl Tris-EDTA buffer en bewaard bij -20 °C. Na ontdooien is het verkregen cellysaat geschikt als uitgangsmateriaal voor de PCR.
Resultaten en discussie: De opbrengst van het PCR-product met de apoE primerset onder condities als eerder beschreven (1), was met cellysaat minder dan met opgezuiverd genomisch DNA. Additie van 5 extra amplificatiecycli was voldoende om dit verschil te overbruggen. Het type anti-coagulans (heparine, oxalaat, citraat of EDTA) gebruikt voor het ontstollen van vol bloed had geen invloed op de opbrengst van het apoE-PCR-product. Tevens was restrictie-enzymanalyse van apoE-PCR producten geen probleem. Drie andere primersets voor amplificatie van respectievelijk stollingsfactor V-gen, COL4A5-exon 29 en COL4A5-exon 35 gaven met cellysaten opbrengsten vergelijkbaar met opgezuiverd genomisch, onder PCR-condities als eerder gebruikt voor opgezuiverd genomisch DNA. Cellysaten zijn minimaal een half jaar houdbaar bij -20 °C zonder achteruitgang in de opbrengst van het PCR-product en meerdere vries-dooi cycli zijn niet nadelig voor de kwaliteit van cellysaten in PCR.
Conclusie: Met de bovenbeschreven methode is het mogelijk om vol bloed te gebruiken in plaats van opgezuiverd genomisch DNA als uitgangsmateriaal voor PCR.

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49. CYP3A4*3: een zeldzaam allel?

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Inleiding: Cytochroom P450 3A4 (CYP3A4) is het belangrijkste cytochroom in de lever dat betrokken is bij de afbraak van geneesmiddelen. Er bestaat een grote interindividuele variatie in de farmacokinetiek van geneesmiddelen die via CYP3A4 worden gemetaboliseerd. Genetische polymorfismen in het CYP3A4 allel kunnen hier deels verantwoordelijk voor zijn. Tot nu toe zijn er twee genetische polymorfismen beschreven: het CYP3A4*1B allel (A(-290)G mutatie in de promoterregio) en het CYP3A4*2 allel (Ser222Pro). Van beide polymorfismen is bekend dat zij de CYP3A4 enzymactiviteit beïnvloeden. Een derde variant van het allel, het CYP3A4*3, betreft een T1437C mutatie, wat correspondeert met een Met445Thr verandering (1). Dit allel is tot nu toe slechts bij één individu (Chinees) aangetoond, en wordt daarom beschouwd als een zeldzaam allel.
Methoden: Een polymerase ketting reactie-restrictie fragment lengte polymorfisme (PCR-RFLP) methode werd opgezet om het CYP3A4*3 allel aan te kunnen tonen. Het verkregen PCR product van een vijftal monsters werd gesequenced om te controleren of er geen pseudogenen van CYP3A4 werden geamplificeerd. Vervolgens werden 499 DNA monsters, geïsoleerd uit bloed van Caucasische donoren, gescreened op het CYP3A4*3 allel met behulp van PCR-RFLP. Heterozygote en homozygote monsters werden herhaald met PCR-RFLP (duplo). Tenslotte werd de nucleotideverandering bevestigd met behulp van sequencing.
Resultaten en discussie: Sequencing van het PCR product toonde aan dat alleen het CYP3A4 allel werd geamplificeerd. Toepassing van de PCR-RFLP op het DNA van 499 Caucasiers resulteerde in de detectie van 11 heterozygoten (2,2%) en geen homozygoten voor CYP3A4*3. Hiermee komt de allelfrequentie van CYP3A4*3 in de Caucasische populatie op 1,1%.
*Conclusie:*Het CYP3A4*3 allel is geen zeldzaam allel, maar is aanwezig in een aanzienlijk deel van de Caucasische bevolking. Het CYP3A4*3 is derhalve een genetisch polymorfisme waarbij verder onderzoek naar klinische implicaties noodzakelijk is.

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50. Genotypering of fenotypering? Een alfa-1-antitrypsine deficiëntie door het gelijktijdig voorkomen van het Z-allel en het M Heerlen allel

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Inleiding: Een deficiëntie van het alfa-1-antitrypsine (AAT) gaat gepaard met een verhoogd risico op longemfyseem en levercirrose. De meest voorkomende oorzaken zijn mutaties in het AAT gen (Z-allel en S-allel), die leiden tot een verminderde AAT secretie vanuit de hepatocyten in het plasma. Vele andere mutaties die leiden tot een AAT deficiëntie komen weinig voor. De AAT eiwitvarianten worden aangetoond met electroforetische technieken, veelal IEF, die een behoorlijke expertise vereisen (1). De laatste tijd is ook een veelheid aan snelle specifieke moleculair biologische technieken ontwikkeld om de meest voorkomende varianten te detecteren.
Methoden: Genotypering van S- en Z-mutaties werd uitgevoerd volgens Braun et al. (2). Sequentie analyse werd uitgevoerd met de BigDye Terminator Cycle Seqencing kit op een ABI-Prism 3700 DNA Analyzer System (PE Biosystems). IEF werd uitgevoerd in het CLB volgens de daar gebruikte procedure.
Resultaten en discussie: Bij een 40 jarige vrouw met een AAT van 0,3 g/l en dyspnoe werd met IEF alleen de Z isovorm aangetoond. Zij werd beschouwd als ZZ in overeenstemming met het beginnende emfyseem. Haar zuster (AAT 0,4 g/l) had eveneens een verminderde longfunctie met alleen de Z isovorm. Bij haar twee kinderen werd een normaal fenotype gevonden (M), terwijl tenminste heterozygotie (MZ) werd verwacht. Bij genotypering wordt gezocht naar de aanwezigheid van de Z of S mutatie. Bij deze vrouw en haar zuster werd genptype MZ gevonden en bij haar twee kinderen MM. Deze onverwachte bevinding bleek te worden veroorzaakt door de aanwegheid van het M Heerlen allel, dat nauwelijks bijdraagt aan de in plasma uitgescheiden AAT en met IEF niet kan worden aangetoond. Genotype van moeder en zus bleek bij nadere analyse ZMheerlen en van kinderen MMheerlen.
Conclusie: Met moleculair biologische technieken worden bijzondere mutanten niet opgemerkt. Met IEF wordt de niet secreterende Heerlen eveneens variant gemist.

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51. Five novel mutations in the gene for human blood coagulation factor V associated with type I factor V deficiency

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Introduction: Coagulation factor V (FV) plays an important role in maintaining the hemostatic balance in both the formation of thrombin in the procoagulant pathway as well as in the protein C anticoagulant pathway. FV deficiency is a rare bleeding disorder with variable phenotypic expression. Little is known about the molecular basis underlying this disease due to its low frequency in the population combined with the complexity of the gene itself.
Methods: Polymerase Chain Reaction (PCR) amplification and DNA sequence analysis was used for mutational analysis of the promoter and the 25 exons of the FV gene. Three dimensional computer-modelling was used to predict the effect of mutations on the molecule.
Results and discussion: In this study we have investigated three patients with severe factor V deficiency, but different clinical expression, and two unaffected carriers from five different families. We identified six mutations associated with a FV null allele. Five of these mutations have not been described before. Four mutations lead to a premature termination codon either by a nonsense mutation: A1102T, Lys310Term. (FV Amersfoort) and C2491T, Gln773Term. (FV Casablanca) or a frameshift: an 8 basepair deletion between nucleotides 1130 and 1139 (FV Seoul$_1$) and a 1 basepair deletion between nucleotides 4291 and 4294 (FV Utrecht). One novel mutation was a missense mutation (T1927C) that resulted in the replacement of a cysteine by an arginie at residue 585 (FV Nijkerk). This mutation was associated with absence of mutant protein despite normal transcription to RNA. Most likely, as judged from 3D molecular modelling, an arginine at this position disrupts the hydrophobic interior of the factor V A2 domain. The sixth detected mutation was a previously reported missense mutation: A5279G, Tyr1702Cys (FV Seoul$_2$). In all cases, the presence of the mutation was associated with type I factor V deficiency.
Conclusion: Identifying the molecular basis underlying this rare coagulation disorder will help to obtain more insight into the mechanisms involved in the variable clinical phenotype of FV deficient patients.

Neurologie en Psychiatrie

52. Increased serum concentrations of the S-100 protein and neuron specific enolase in two patients who developed paraplegia after thoraco(abdominal) aortic aneurysm surgery

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Introduction: Paraplegia is a serious neurologic complication of thoraco(abdominal) aortic aneurysm (TAA(A)) operations with prosthetic replacement. The aim of this study was to explore whether serum concentrations of the S-100 protein and neuron specific enolase (NSE) could be of value in the diagnosis of paraplegia after TAA(A) surgery.

Methods: Twenty patients undergoing TAA(A) surgery 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 (AXC), 5 minutes, 2, 4, 6, 8, and 19 hours respectively after reperfusion. Determinations of the S-100 protein and NSE were performed using a LIAISON® Random Access Analyser. In all patients recording of somatosensory and motor evoked potentials was carried out to monitor the function of the spinal cord. Neurological examination was performed according to the protocol of the American Spinal Injury Association (ASIA).
Results: Eighteen patients undergoing elective surgery showed increasing serum concentrations of the S-100 protein and NSE during the procedure. The highest concentrations of S-100 and NSE were found 2 and 4 hours after reperfusion, respectively. Thereafter, the concentrations showed a decreasing trend but 19 hours after reperfusion the levels did not reach baseline values. Two patients undergoing emergency surgery developed postoperative paraplegia. These patients showed the same trend in concentrations of the S-100 protein and NSE with one remarkable exception: 19 hours after reperfusion the serum concentrations of the S-100 protein and NSE were increased. In these particular patients, the motor evoked potentials were reduced as well. The somatosensory evoked potentials, however, remained unaffected.
Conclusion: The obtained results suggest a role of serum concentrations of the S-100 protein and NSE in the diagnosis of paraplegia after TAA(A) surgery.

53. Increased intraoperative cerebrospinal fluid concentrations of xanthine and hypoxanthine may predict neurologic deficits after thoracoabdominal aortic aneurysm surgery

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Introduction: Paraplegia, as a result of spinal cord ischaemia, is still a devastating neurologic complication following thoracoabdominal aortic aneurysm surgery. The aim of this pilot study was to explore whether cerebrospinal fluid concentrations of xanthine and hypoxanthine could detect intraoperative spinal cord ischaemia in patients developing postoperative paraplegia.
Methods: Ten patients undergoing thoracoabdominal aortic aneurysm surgery were included in this pilot study. Cerebrospinal fluid samples were drawn after induction of anaesthesia and haemodynamic stabilisation, during the cross-clamp period of the critical aortic segment (AXC), 5 minutes, 2, 4, 6, 8, and 19 hours respectively after reperfusion. Determinations of xanthine and hypoxanthine were performed using high performance liquid chromatography with photodiode-array detection. In all patients recording of myogenic motor evoked potentials following transcranial electric stimulation was carried out to monitor the integrity of the motor pathways. Neurological examination was performed according to the protocol of the American Spinal Injury Association (ASIA).
Results: One patient undergoing emergency surgery developed postoperative paraplegia. Two hours after reperfusion she showed increased cerebrospinal fluid concentrations of xanthine and hypoxanthine compared to nine other patients undergoing elective surgery. In this particular patient, the areas under the curves of the motor evoked potentials were decreased as well.
Conclusion: These findings suggest that increased cerebrospinal fluid concentrations of xanthine and hypoxanthine may detect spinal cord ischaemia and predict paraplegia after thoracoabdominal aortic aneurysm surgery.

54. Hypoglycorrhachia: a simple clue, simply missed (tevens voordracht)

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Introduction: The central nervous system (CNS) depends upon glucose as the fuel for its energy requirements. Persistent hypoglycorrhachia, i.e. low glucose concentrations in cerebrospinal fluid (CSF), in normoglycemic patients, is expected to lead to neurological abnormalities. At the blood-brain barrier, transport of glucose is facilitated by the type 1 glucose transporter (GLUT1), which is also present in the erythrocyte membrane. In 1991, two patients with developmental delay, seizures, and persistent hypoglycorrhachia, were shown to suffer from GLUT1-deficiency. The molecular basis for the disorder was unraveled in 1998. So far, approximately 20 cases have been reported in the literature. We postulated that GLUT1-deficiency might be more common than is currently recognized because unexplained hypoglycorrhachia might be overlooked. Therefore, we performed a retrospective study for the years 1994 to 1999, using our computerized laboratory database.
Methods: Patients included satisfied the following criteria: [1] ages 0 to 16 years, [2] CSF glucose concentrations < 2.0 mmol/l or ratio of CSF glucose to blood glucose < 0.5, and [3] CSF leukocytes < 10/µl. The medical files of all selected patients (n=65) were studied. We rejected patients with reasonable explanations for the hypoglycorrhachia, such as hypoglycemia (8%), ventriculoperitoneal shunt dysfunction (20%), (posthemorrhagic) hydrocephalus without shunt (40%), CNS infection (14%), and malignancy with CNS involvement (9%). Six patients (9%) remained and were studied for GLUT1 function by analyzing the erythrocyte uptake of 3-O-methyl-D-glucose using the methods reported previously.
Results and discussion: Three patients (from three different families) were diagnosed as GLUT1-deficient. They all suffered from developmental delay, behavioral disturbance, and intractable epilepsy. Neurological examination revealed ataxia and pyramidal signs. This neurological picture had led to extensive diagnostic procedures in the past, including a lumbar puncture showing hypoglycorrhachia that did not alert the clinician at that time.
Conclusion: In conclusion, our findings show that in day-to-day practice, a low glucose level in CSF might not be given the weight it deserves or might simply be overlooked rather than recognized as the laboratory sign that points to GLUT1-deficiency. Diagnosing GLUT1-deficiency protects the patient against ongoing diagnostic procedures and opens the way to a rational treatment strategy. Moreover, early diagnosis should be sought for in view of the benefit of the ketogenic dietary treatment for this disorder. Finally, our findings suggest that the true prevalence of GLUT1-deficiency might be much higher than expected.

55. Elevated CSF levels of brain specific proteins in hydrocephalus

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Introduction: The brain specific proteins NSE, S-100b, MBP and GFAP may serve as markers of brain damage in various neurological disorders. In studies on patients with head trauma and cerebrovascular disorders a significant correlation has been observed between concentrations of these proteins in cerebrospinal fluid (CSF) and blood with the severity of brain damage and clinical outcome. The objective of our study was to investigate concentrations of brain specific proteins in CSF and serum in patients with hydrocephalus and to evaluate the value of these proteins as markers of brain damage.
Methods: Patients with a newly developed hydrocephalus and with a drain dysfunction were investigated. The severity of the hydrocephalus was classified into three categories on clinical grounds. During the operation blood samples were taken at the induction of anesthesia and CSF was sampled after puncture of the ventricular system. CSF and serum concentrations of S-100b, NSE, MBP and GFAP were analyzed by using ELISA.
Results and discussion: Concentrations of S-100b and GFAP in CSF were significantly elevated in patients with a hydrocephalus, but we did not observe a correlation with the clinical grades 1 and 2. In grade 3 patients however, extremily high values of S-100b and GFAP in CSF were observed. NSE and MBP values were normal in CSF of grade 1 and 2 patients, but were strongly elevated in grade 3 patients. These data suggest that neurons are probably relatively resistant to elevated intracranial pressure, whereas astrocytes seem to be more sensitive to this condition, reflected by their elevated secretion of S-100b and GFAP. Furthermore, our data suggest that, when only S-100b and GFAP are increased in CSF, but not NSE, a hydrocephalus is not associated with neurological damage. In contrast, grade 3 patients revalidated longer than grade 1 or 2 patients, suggesting that elevated NSE and MBP levels are associated with neurologic damage. We did not observe elevated serum levels of all proteins, except for the grade 3 patients, suggesting that the blood-brain barrier remains intact in this kind of neurological disorder unless the intracranial pressure reaches very high levels.
Conclusion: CSF concentrations of S-100b, GFAP and NSE seem to be good markers to assess (transient) brain damage in hydrocephalic patients, but an increase in CSF levels of these proteins is usually not reflected by a parallel increase in serum levels in these patients.

56. A single intrathecal “oligoclonal” IgG band in CSF: indication for Multiple Sclerosis?

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Introduction: The analysis of cerebrospinal fluid (CSF) for the presence of oligoclonal IgG bands is a very helpful diagnostic parameter to assess the diagnosis of Multiple Sclerosis (MS) and of other immune-mediated neurological diseases. In 90% of MS patients a oligoclonal IgG reaction in CSF is observed. A varying number of IgG bands unique to the CSF, up to 20, can be identified. Furthermore, it has been suggested that the analysis of free kappa chains in the CSF may be an even stronger parameter in the diagnosis of MS. In this preliminary study we analysed the detection limit of the technique of combined isoelectric focussing with immunoblotting with regard to the diagnosis of MS. We investigated if a single, but clearly present, IgG band unique to the CSF may support the diagnosis of MS or other neuro-auto-immune diseases and analysed in these cases the presence of free kappa chains in both CSF and serum.
Methods: Paired CSF and serum samples were analysed for the presence of oligoclonal IgG bands by using iso-electric focussing combined with immunoblotting. Results from the past two years were retrospectively analysed. In case we observed a single unique IgG band in CSF, free kappa chains were also analysed by using the same technique and, furthermore, the definite diagnosis was retrieved from the patient’s medical file by a neurologist.
Results and discussion: 2087 cases (analysed in the years 1999 and 2000) were re-evaluated for the presence of a unique IgG band in CSF. This resulted in a selection of 50 patients. Of 30 patients of this group a definite diagnosis could be retrieved from the patient files. Three patients were diagnosed with Multiple Sclerosis, two patients with SLE and or vasculitis. Many other diagnoses were reported only once. The presence of free kappa chains was analysed in CSF and serum from 20 patients.
Conclusion: Although typical oligoclonal IgG bands (two or more) unique to the CSF have been used to support the diagnosis of MS, these preliminary data indicate that the presence of a single intrathecal IgG band - together with the usual clinical work-up - does not exclude, or may even support, the diagnosis of MS. This study will be extended in close cooperation with dr. C.J. Sindic (Brussels, Belgium).

57. Neurotransmitter profiles in CSF and urine as a diagnostic aid in conditions with secondary dopamine β-hydroxylase deficiency

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Introduction: Menkes disease (MD) is a neurodegenerative disorder of intracellular copper transport leading to secondary deficiencies of copper enzymes, a.o. dopamine beta-hydroxylase (DBH). Though the contribution of this secondary DBH deficiency in the clinical symptoms is still unclear and presumably minor, it can be used as an additional parameter in diagnosis and monitoring of (e.g. copper) treatment. Measurement of serum DBH enzyme activity however is unsatisfactory because of the wide normal range with a lower level of almost zero, particularly for young children. Neurotransmitter (NT) metabolite profiles in CSF and to a lesser degree in urine, much better reflect the functional in-vivo DBH activity. A comparable situation exists for Riley Day Familial Dysautonomia and other hereditary sensory and autonomic neuropathies (HSAN). Here we describe examples of the role of NT profile analysis in diagnosis and therapy monitoring of cases of MD and a case of HSAN type IV.
Methods: Biogenic amine neurotransmitter metabolites were

measured with HPLC and fluorometric and electrochemical detection.
Results and discussion: Examples are given of NT profiles in CSF and/or urine samples of MD patients and a patient with HSAN type IV. NT profiles were abnormal in CSF of all MD patients, while this was less pronounced in their urine; in the HSAN patient the abnormalities were confined to the urine only.
Conclusion: NT profiles in CSF and/or urine can be a valuable contribution to diagnosis and therapy monitoring of patients with MD and HSAN.

Hart- en Vaatziekten

58. Curaąao CAD patients have higher HFE Cys 282Tyr prevalence

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Introduction: Atherosclerosis is a multifactorial disease, in which oxidation of low density lipoproteins (LDL) plays a central role. Results from epidemiological studies suggest that low iron status, established as low serum ferritin, is associated with lower coronary artery disease (CAD) risk (1). Iron is possibly involved in LDL oxidation and myocardial reperfusion damage. Heterozygous carriers of the HFE Cys282Tyr mutation have slightly higher serum iron and ferritin concentrations, reduced iron-binding capacity and increased CAD risk (2).
Methods: We investigated, in a case-control design, whether HFE Cys282Tyr constitutes a CAD risk factor in Curaçao. For this we compared the frequencies of this mutation in 52 CAD patients and 84 apparently healthy, age and sex matched controls.
Results and discussion: Five patients (9.6 %) and 1 control (1.2 %) were heterozygous for the HFE Cys282Tyr mutation [p<0.03; Odd's Ratio 8.8 (95% CI 1.001-77.8)]. Curaçao CAD patients have significantly higher HFE Cys282Tyr frequency. The prevalence of the HFE Cys282Tyr mutation in Curaçao is nevertheless low. This is in accordance with its low carrier frequency in Africans (0%), Brazilian Africans (1.1%), American Africans (3.0%), and blacks from Ghana (0%), as compared with a high frequency in Caucasians (3-15%). The Curaçao population is considered to derive mainly from Ghana, suggesting that the encountered HFE Cys282Tyr heterozygotes derive from Caucasian admixture.
Conclusion: Curaçao CAD patients have significantly higher HFE Cys282Tyr frequency. The consequence of our finding is uncertain, since the prevalence of the mutation in Curaçao is low and there is as yet no proof that iron-lowering in HFE Cys282Tyr heterozygotes reduces CAD risk.

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59. Fasting and 6h-postload plasma homocysteine as cardiovascular risk factors. No indications for ordering these tests with use of vitamin-optimized cut-off values (tevens voordracht)

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Introduction: Hyperhomocysteinemia (HHcy) is a cardiovascular disease (CVD) risk factor. HHcy is defined as an increased fasting and/or 6h post-load homocysteine (Hcy) after an oral methionine tolerance test. Future demonstration of a beneficial effect of Hcy lowering on CVD risk would justify the use of vitamin-optimized cut-off values. We determined plasma Hcy cut-off values at optimized vitamin status and investigated their influence on HHcy prevalence in healthy adults and patients.
Methods: 101 healthy adults received folate (5 mg/day) and vitamin B_{12} (1 mg/day) during 2 weeks, and the same dosages folate and vitamin B_{12} plus vitamin B_6 (1 mg/kg/day) during the following 2 weeks. Vitamin-optimized cut-off values (i.e. 97.5th percentiles) for fasting and 6h-postload Hcy were calculated at the study end. The consequences of the vitamin-optimized cut-off values for HHcy prevalence were studied in: 1) the group of 101 healthy adults at baseline, and 2) a retrospective cohort of 3,477 suspected HHcy patients at first attendance.
Results and discussion: The vitamin-optimized cut-off values were 9.3 (fasting Hcy) and 35.1 µmol/l (6h-postload Hcy). HHcy prevalences in healthy subjects were: 58 (all), 58 (men), 76 (premenopausal women) and 89% (postmenopausal women). For patients these values were: 86, 85, 88 and 86%, respectively. Of the healthy HHcy subjects, 3 (all), 3 (men), 0 (premenopausal women) and 6% (postmenopausal women) could only be diagnosed on the basis of an increased 6h-postload Hcy. For HHcy patients, these figures were 3, 4, 3 and 2%, respectively.

60. Intensive nutritional education in groups does not affect plasma homocysteine

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Introduction: Hyperhomocysteinemia is an independent cardiovascular disease (CVD) risk factor. Folic acid status is the main determinant of plasma homocysteine (Hcy) in the general population. Vegetables and fruits are the principal sources of dietary folic acid. A controlled trial indicated 10% Hcy lowering upon a 4 weeks augmented fruit and vegetable con-

sumption from 150 to 500 g/day. We investigated whether intensive dietary health education results in lower plasma Hcy.
Methods: The study was part of the MARGARIN project, which is a prospective placebo-controlled intervention trial in a high CVD risk group, characterized by hypercholesterolemia (6.0-8.0 mmol/l) and at least two other CVD risk factors. A total of 102 subjects (38 men, 64 women; mean age 55 years) received intensive nutritional education (INE) in groups regarding a Mediterranean-type of diet. A control group of 161 subjects (78 men, 83 women; mean age 54 years) received a posted leaflet (PL) with the standard Dutch dietary guidelines. Vegetable and fruit intakes were established by validated questionnaires and plasma Hcy was determined at baseline (t=0) and after 16 and 52 weeks.
Results and discussion: Vegetable intake increased from 141± 55 (baseline) to 166±60 (t=16) g/day (women, INE) and from 133±52 (baseline) to 156±63 (t=16) g/day (women, PL). Fruit intake increased from 256±195 (baseline) to 327±176 (t=16) g/day (men, INE) and from 302±134 (t=16) to 342±180 (t=52) g/day (women, INE). There were no significant changes in plasma Hcy. The concentrations (t=0, 16, 52 weeks; in µmol/l) were: 12.1±3.5, 11.8±3.3, 12.1±3.2 (men, INE), 12.5±4.9, 12.1±3.3, 12.8±3.3 (men, PL), 11.6±3.1, 11.2±3.2, 11.8±3.3 (women, INE) and 12.3±4.1, 12.0±4.4, 12.4±4.7 (women, PL).
Conclusion: INE caused moderate increases in vegetable and fruit intakes, notably in women, but neither INE, nor PL, affected plasma Hcy. It seems that administration of B vitamin supplements or food fortification are the only effective ways to lower plasma Hcy.

61. Beneficial effects of C1-inhibitor treatment in patients with acute myocardial infarction

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Introduction: C1-esterase inhibitor (C1-inh) inhibits the classical pathway of the complement activation cascade and has been shown to reduce acute myocardial ischemia / reperfusion injury in animal models with rats, cats, pigs and dogs. This is the first pilot study that reports of the effects of C1-inh treatment in human patients with acute myocardial infarction.
Methods: At 6 hours after the onset of symptoms 22 patients received an initial bolus of 50 or 100 Units C1-inh/kg body weight, followed by a continuous infusion of 1.25 or 2.0 U/kg/hr during the next 48 hours. Efficacy of complement inhibition was estimated from C4 activation fragments. To quantify possible differences in the extent of myocardial injury, release patterns of four myocardial markers (troponine T (TnT), creatine kinase isoenzyme B (CK-MB), creatine kinase (CK) and alpha-hydroxybutyrate dehydrogenase (HBDH)) were compared to the release patterns of 18 control subjects.
Results and discussion: C4 activation products were significantly reduced (p=0.005) in a dose-dependent way, indicating successful complement inhibition. Cardioprotection was shown by a reduction of 36%, 57% (p=0.001), 38% (p=0.022) and 18% of the area under the plasma curves of TnT, CK-MB, CK and HBDH respectively.
Conclusion: The present pilot study indicates that 48 hours of continuous C1-inh treatment, given in addition to reperfusion therapy, provides effective inhibition of complement activation and may significantly reduce myocardial injury.

62. Troponine en myoglobine: vergelijking van 3 assays

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Inleiding: Voor het vaststellen van hartspierschade wordt door de IFCC aanbevolen om het hartspecifieke eiwit troponine te meten ter vervanging van de aspecifiekere creatine kinase activiteit. Er kan Troponine T (TnT) of Troponine I (TnI) bepaald worden. Aangezien troponine pas 4-6 uur na hartspierschade verhoogd wordt aangetroffen in het bloed, wordt myoglobine (na 0,5-2 uur verhoogd) als vroege marker aanbevolen. Drie assays voor troponine en myoglobine zijn met elkaar vergeleken.
Methoden: Van 400 monsters van patiënten met pijn op de borst werd troponine en myoglobine bepaald met een Cardiac Reader (TnT, striptest, Roche; referentiewaarde TnT <0,1 µg/l, myoglobine <76 µg/l), Triage (TnI, striptest, Merck; referentiewaarde TnI <0.19 µg/l , myoglobine <107 µg/l) en Immulite (TnI, DPC; referentiewaarde TnI <1,0 µg/l , myoglobine <70 µg/l). Vergelijking tussen Cardiac Reader en Elecsys (TnT, Roche) werd uitgevoerd op 157 monsters.
Resultaten en discussie: Er is gekeken of een marker al dan niet verhoogd is ten opzichte van de referentiewaarde. De drie assays geven voor troponine 339x (85%) een gelijke laboratoriumdiagnose. Cardiac Reader en Immulite geven in 91% van de gevallen een kwalitatief gelijke uitkomst, Cardiac Reader en Triage in 89% en Triage en Immulite in 90%. Cardiac Reader en Elecsys gaven 149x (95%) eenzelfde laboratoriumdiagnose, waarbij voor 0-2 µg/l geldt: y=0,91+0,02 (R(2)= 0,89). Voor myoglobine werd 277x (69%) een kwalitatief vergelijkbare uitkomst in alle assays gevonden, waarbij Cardiac Reader met Immulite in 92%, Cardiac Reader met Triage in 70% en Triage met Immulite in 76% overeen komen. Met de Triage werd 36x >700 µg/l (bovengrens myoglobine-assay) gemeten tegen Immulite 8x en Cardiac Reader 4x.
Conclusie: Het vaststellen van hartspierschade blijkt mede afhankelijk van de gebruikte assay. Voor troponine kan in 10% van de patiënten een afwijking in de laboratoriumdiagnose worden verwacht bij gebruik van verschillende assays. Bij myoglobine geldt dit ook voor de Cardiac Reader en de Immulite. Met de Triage werd aanzienlijk vaker een verhoogd myoglobine gemeten. Meting van TnT op Cardiac Reader of Elecsys geeft vergelijkbare resultaten.

63. De waarde van troponine en myoglobine ten opzichte van CK bij het vaststellen van hartspierschade (tevens voordracht)

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Inleiding: Voor het vaststellen van hartspierschade wordt door de IFCC aanbevolen om het hartspecifieke eiwit troponine te meten ter vervanging van de aspecifiekere creatine kinase activiteit. Troponine stijgt 4-6 uur na hartspierschade; myoglobine (verhoogd na 0,5-2 uur) wordt derhalve als vroege marker aanbevolen. De creatine kinase (CK) meting is vergeleken met de troponine (TnT) en myoglobine bepaling.
Methoden: Van 245 patiënten, binnengebracht op de SEH met pijn op de borst, werd direct (t=0) en zo mogelijk na 4, 8 en 12 uur bloed afgenomen voor het meten van troponine T (TnT; Cardiac Reader, striptest, Roche), myoglobine (Cardiac Reader, striptest, Roche), creatine kinase (CK) en CKMBactiviteit.
Resultaten en discussie: Bij 32 patiënten (13%) was de TnT bij binnenkomst verhoogd en bij 50 (20%) de CK; bij 22 (9%) waren beiden verhoogd. Als sensitiviteit van CK, berekend ten opzichte van TnT, werd 69% en als specificiteit 87% gevonden. Op basis van CKMB/CK werd de sensitiviteit 32% en de specificiteit 98% ten opzichte van TnT. Er werden 63 patiënten (26%) vervolgd met één of meerdere troponine-aanvragen, waarbij 53 patiënten een negatief TnT hadden bij binnenkomst. In 10 gevallen (19%) was de troponine alsnog verhoogd na 4 uur en in 1 geval (2%) na 8 uur. Een normaal myoglobine op t=0 voorspelde in 70% van de gevallen correct een negatief troponine op latere tijdstippen. Van 182 patiënten werd slechts één monster ingestuurd: bij 12% was TnT verhoogd en bij 16% CK. Bij 11 van deze patiënten (6%) was myoglobine verhoogd bij een normaal TnT en CK.
Conclusie: Het gebruik van TnT in plaats van CK en CKMB leverde bij 17% van de patiënten een andere laboratoriumdiagnose op bij binnenkomst. Eén op de vijf patiënten die ter observatie werden vervolgd vertoonde pas een positief troponine 4 uur na opname. Het gebruik van myoglobine als vroege marker ter uitsluiting van hartspierschade is mogelijk, maar lijkt in deze studie achter te blijven bij de gegevens uit de literatuur.

64. The ultra-sensitive CRP in peripheral arterial disease

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Introduction: Moderate elevation of C-reactive protein (CRP) has emerged as a strong independent pedictor of cardiovascular complications (CVC) in healthy individuals and in patients with coronary artery disease. In light of the association of moderately elevated CRP with CVC we wanted (i) to assess the correlation between a CRP assay used routinely in clinical practice (Beckman Coulter Synchron CX-7 turbidimetric test) and 2 ultrasensitive CRP-assays (the IMMULITE two-site chemiluminescent enzyme immunometric assay from Diagnostic Product Corporation, and the BN II high sensitive CRP assay from Dade-Behring), for the measurement of CRP in patients with peripheral arterial disease (PAD), and (ii) to explore the association between CRP and severity of disease in PAD.
Methods: CRP of 292 PAD-patients were assessed with the Synchron CX-7, the IMMULITE and the BN-II assay. and related to ankle-brachial pressure index (ABPI) and clinical diagnosis as a marker of severity of disease.
Results and discussion: The 2 ultrasensitive tests (IMMULITE and BN-II) correlated excellently with each other ($r=0.986$, $p<0.0001$). The correlation between the Beckman Synchron CX-7-assay and the 2 ultrasensitive tests was weaker but significant ($r=0.546$ and $r=0.572$ for Synchron CX-7 versus IMMULITE and for Synchron CX-7 versus BN-II respectively). Patients with elevated CRP measured with the ultrasensitive CRP-assays, were more likely to have a low ABPI (OD:1.96, p=0,02) and critical ischemia (OD:2.4, p=0.02). When CRP was measured with the Synchron CX-7-assay, the association between high CRP and low ABPI persisted, but the association with critical ischemia lost it's significance.
Conclusion: Peripheral arterial disease should be regarded as an inflammatory process characterized by elevated CRP. Patients with elevated CRP are more likely to have severe PAD. The conventional CRP assay is not sensitive enough, in the low range of CRP values, to study the association of cardiovascular disease with markers of inflammation.

65. Determinants of fasting and post-methionine homocysteine levels

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Introduction: Hyperhomocysteinemia is an independent risk factor for cardiovascular disease. Elevated plasma homocysteine results from inadequate removal through transmethylation and/or transsulfuration reactions. It is assumed that an elevated fasting homocysteine level reflects defective remethylation whereas a high homocysteine level after oral methionine loading indicates defective transsulfuration. This study investigates whether both tests overlap or are determined by different factors.
Methods: Venous blood was drawn from women with preeclampsia (n=118, mean age 33.5 ± 5.5 years) for determination of creatinine and vitamins B6, B12, and folic acid. Plasma homocysteine was determined after 12 hours fasting and 6 hours after an oral methionine load (0.1 g/kg body weight). From isolated DNA, the C677T polymorphism in the methylenetetrahydrofolaat reductase (MTHFR) gene was determined using PCR-RFLP.
Results and discussion: Of 118 patients, 42% (n=49) was classified as hyperhomocysteinemic based on an elevated fasting homocysteine (>15 μM) and/or elevated post-load homocysteine (>45 μM). Of these patients classified as hyperhomocysteinemic, 31% showed both elevated fasting and postload homocysteine, 14% had solely elevated fasting homocysteine and 55% was classified as hyperhomocysteinemic based on elevated postload homocysteine only. In univariate regression analysis, potential determinants of fasting homocysteine were

folic acid (P<0.001), vitamin B12 (P=0.06) and vitamin B6 (P=0.07), while for postload homocysteine, the only potential determinant was the MTHFR TT genotype (P=0.09).
Conclusion: Diagnosis of hyperhomocysteinemia based on fasting homocysteine levels alone fails to identify a large percentage (55%) of patients with hyperhomocysteinemia. Furthermore, fasting and postload homocysteine are not synonymous to remethylation and transsulfuration, respectively.

66. Active tissue factor on cell-derived microparticles in the pericardial cavity of patients undergoing cardiopulmonary bypass

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Introduction. Increased coagulation activation products and soluble tissue factor (TF) concentrations are found in pericardial cavity samples of patients undergoing cardiopulmonary bypass (CPB). Previously, we reported microparticles in such samples and –more recently- localised TF on microparticles in other clinical conditions. The so called 'soluble' TF may therefore be partly microparticle-associated in pericardial samples.
Methods. Samples, both systemic blood and pericardial cavity material, were collected before and during CPB from six patients. Concentrations of prothrombin fragment F_{1+2} and thrombin-antithrombin complexes (TAT), as estimates of in vivo coagulation status, and 'soluble' TF were determined by ELISA. Exposure of TF on microparticles was determined by flow cytometry. The ability of microparticle-associated TF to trigger thrombin formation was assessed in normal plasma (thrombin generation assay), without and with inhibitory antibodies to coagulation factors VII, XII or XI.
Results and discussion. Concentrations of F_{1+2}, TAT and soluble TF were all elevated in pericardial cavity samples compared to systemic blood samples. 'Soluble' TF was up to 50% microparticle-associated in the pericardial cavity samples, and these microparticles strongly triggered factor VII-mediated thrombin formation. In contrast, microparticles from systemic samples triggered thrombin generation independent from factor VII, except at the end of bypass ($p<0.05$). TF became only visible on the pericardial microparticles –in the flow cytometry assessment- in the presence of Triton X-100. Moreover, concentrations of soluble tissue factor, as assessed by ELISA, increased 2.5-3 fold in pericardial- but not systemic-samples in the presence of Triton-X-100, indicating that in the absence of Triton-X-100 a major part of (soluble) TF is antigenetically cryptic despite its biological activity.
Conclusion. Taken together, TF is substantially microparticle-associated in samples from the pericardial cavity, and TF enables them to generate thrombin via factor VII. Evidently, this pericardial cavity material may be capable of the observed ongoing coagulation activation upon its return into the systemic circulation at the end of the CPB procedure. Since in a variety of diseases an increased risk for thromboembolic events is paralleled by elevated concentrations of soluble TF, the present finding that a major fraction of this 'soluble' TF is microparticle-associated and biologically active may offer new insights into the pathophysiology of such events.

Interne Geneeskunde

67. The pathogenesis of urinary concentration defects can be unraveled by assessment of urinary excretion of aquaporin-2

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Introduction: Urinary concentration is governed by water transport in the collecting ducts, which is critically dependent on vasopressin, the water channel aquaporin-2 (AQP2), and a medullary osmolar gradient. Nephrogenic diabetes insipidus (NDI) may be caused by a defect in any of these determinants. We hypothesized that the measurement of urinary AQP2 (UAQP2) may be helpful in defining the mechanism of a urinary concentration defect. In a previous study in healthy volunteers we demonstrated that UAQP2 increased upon administration of the vasopressin analogue dDAVP. Pretreatment with furosemide, which will dissipate the medullary osmolar gradient, attenuated urinary concentration but did not affect UAQP2.
Methods: In the present study we have measured urine osmolality and UAQP2 after 40 µg dDAVP intranasally in two groups of patients with NDI i.e patients treated with lithium (n=5) and patients with homozygous sickle cell disease (n=6). In this latter group of patients the urinary concentration defect generally is ascribed to a loss of the medullary gradient.
Results: Creatinine levels were within the reference rate in all patients. In lithium-treated patients urine osmolality increased modestly after dDAVP (maximal values 580 ± 77 vs 860 ± 30 mosmol/kg H_2O in healthy volunteers). Likewise, UAQP2 increased less than in the controls. Urinary concentration was more severely disturbed in patients with sickle cell disease, maximal urine osmolality averaging 304 ± 37 mosmol/kg H_2O. Notably, in these patients urinary AQP2 levels remained undetectable.
Conclusion: our findings suggest that the defects in urinary concentration in lithium-treated patients as well as in patients with sickle cell disease are related injury of collecting duct cells with a consequent reduced expression of the water channel AQP2. Dissipation of the medullary osmolar gradient is not the sole determinant of the urinary concentration defect in patients with sickle cell disease.

68. Antistoffen tegen transglutaminase van het IgG type: een nieuwe (apoptose-)marker bij auto-immuunziekten

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Inleiding: Antistoffen (van het IgA-type) tegen weefseltransglutaminase (tTG) zijn diagnostisch voor coeliakie. Ons bleek dat sommige patiënten met (andere) auto-immuunziekten sterk verhoogde waarden van het IgG-type anti-tTG vertonen. Wij besloten systematisch onderzoek naar het diagnostische nut van deze antistoffen.
Methoden: Voor het meten van het anti-tTG werd een time-resolved fluoroimmunoassay ontwikkeld met dierlijk transglutaminase als antigeen. F(ab')2 fragmenten van konijnen-antistoffen tegen menselijk IgG, gelabeld met Eu^{3+} diende als tracer.Als calibrator werd een menselijk serum met een zeer hoge anti-tTG-concentratie gekozen. De referentiewaarden werden vastgesteld op < 20 (arbitraire) U/l . Patiëntensera werden o.a. geselecteerd uit een serumbank op grond van de aanwezigheid van andere auto-antistoffen
Resultaten en discussie: Bij patiënten met antistoffen tegen anti-ds-DNA of SSA en/of SSB werd vrijwel steeds een (sterk) verhoogde anti-tTG gevonden. Bij patiënten met andere antistoffen was het anti-tTG veel minder prominent aanwezig.
Een mogelijke verklaring: het enzym transglutaminase (tTG) katalyseert de polymerisatie van proteinen. Bij apoptose wordt de inhoud van de cel in pakketjes verpakt, de z.g. apoptotische lichaampjes. Deze worden door de werking van het tTG gestabiliseerd. Een theorie over het ontstaansmechanisme van antistoffen bij de ziekte Systemische Lupus Erythematosus is dat de opruiming van apoptotische lichaampjes gestoord is (1), waardoor deze door het immuunsysteem worden gedetecteerd en er auto-antistoffen worden gevormd.Vaak zijn deze gericht tegen enzym-substraatcomplexen, die immers neoantigenen kunnen presenteren. In de lichaampjes zijn zowel tTG als tTG-substraten aanwezig. Onze bevindingen passen dus in genoemde theorie.
Conclusie: De aanwezigheid van antistoffen tegen transglutaminase kan klinisch mogelijk van belang zijn en past in de theorie dat een stoornis in de opruiming van de celbestanddelen na apoptose de oorzaak is van het ontstaan van auto-immuunziekten

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69. Marked depletion of DHEAS in acute and chronic critical illness

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Introduction: Dehydroepiandrosteron (DHEA) and its sulphate ester (DHEAS) are pleiotropic adrenal hormones with putative immunostimulating, antiglucocorticoid and neuropsychological effects (1). In critically ill patients adrenal androgen production declines, whereas adrenal cortisol production is maintained at a high level (2). The aim of this study was to evaluate DHEAS levels in acute and chronic severe illness and to explore their relation with the pituitary-adrenal axis and the acute phase response.
Methods: During 14 days or until discharge or death, we serially measured blood concentrations of DHEAS, cortisol, TNF-alpha, IL-6, procalcitonin (PCT), lipoprotein-binding protein (LBP), and ACTH immunoreactivity. We also recorded heamodynamic parameters, haematology, biochemistry, APACHE II and SOFA scores, the use of dopamine, and ICU-mortality. We included 30 patients with septic shock , 8 patients with severe multitrauma and 40 healthy control subjects.
Results and discussion: On admission, DHEAS was extremely low in sepstic shock (1.2 ± 0.8 µmol/l) in comparison with multitrauma (2.4 ± 0.5 µmol/l ; $p < 0.05$) and controls (4.2 ± 2.1; $p < 0.01$). Hypercortisolism was present in both patient groups. DHEAS had a significant negative correlation with age ($r = -0.55$, $p < 0.01$) and IL-6 ($r = -0.61$, $p < 0.01$) in both patient groups, but no relation was found with gender, use of dopamine, disease severity or markers of the acute phase response (PCT, LBP). Non-survivors of septic shock (n = 12) had even lower DHEAS levels (0.4 ± 0.3 µmol/l) than survivors (1.7 ± 1.1 µmol/l , $p < 0.01$). The time course of DHEAS showed a persistent depletion during follow-up, whereas cortisol levels were increased at the same time points.
Conclusion: We found a marked DHEAS depletion in both the acute and chronic phase of septic shock and to a lesser degree in multitrauma patients. At the same time hypercortisolism was present in both patient groups. Nonsurvivors had the lowest DHEAS levels, suggesting that DHEAS might be a prognostic marker in septic shock. The negative correlation of DHEAS with IL-6 indicates a regulatory role for IL–6, suppressing adrenal DHEAS production. Whether or not to substitute DHEAS in these DHEAS-deficient disease states is still unknown, but of great interest (1).

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70. Evaluatie van de nierfunctie en de mate van oxidatieve stress bij on en off-pomp hartchirurgie (tevens voordracht)

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Inleiding: Oxidatieve stress is veelvuldig beschreven voor patiënten die een open hart operatie met extra-corporele circulatie ondergaan. De oorzaak voor oxidatieve stress is multifactorieel. Het gebruik van extra- corporele circulatie gaat vergezeld met systemische ontstekingsreacties zoals activatie van complement en leukocyten, verhoogde productie van vrije radicalen en micro-emboliën naast trauma veroorzaakt door de operatie. Het doel van deze studie is de vergelijking van oxidatieve stress en de nierfunctie bij patiënten met open hart chirurgie zonder en met extra-corporele circulatie.
Methode: Urine en bloed monsters worden op vastgezette tijdstippen van 40 patiënten verzameld, gerandomiseerd naar twee groepen van ieder 20 patiënten. Naast ischemie/reperfusie parameters en malondialdehyde als maat voor oxidatieve stress

werden er ook routine laboratoriumbepalingen uitgevoerd voor beide groepen.
Resultaten: Biochemisch waren er geen tekenen van een myocard infarct noch van nierfalen gedurende of na de ingreep in beide groepen. De nierfunctie bepaald aan de hand van kreatinine in urine was tijdens de operatie significant verlaagd van gemiddeld 7,6 met een standaard fout van het gemiddelde van (1,1) mmol/l preoperatief naar 3,1 (0, 3) mmol/l bij aankomst op de intensive care unit ($p<0,001$) waarna deze toenam tot 5,1 (0,7) mmol/l , 24 uur na de ingreep voor de groep patiënten met extracorporele circulatie terwijl in de groep patiënten zonder extracorporele circulatie de kreatinine in urine constant bleef. De concentratie aan hypoxanthine, xanthine en urinezuur in urine was significant verhoogd in beide groepen. Voor malondialdehyde was er een significant verschil in de ratio bij aankomst op de intensive care unit voor beide groepen patiënten waarbij de concentratie van malondialdehyde voor de groep patiënten zonder extracorporele circulatie 0,23 (0,04) mmol/mol kreatinine fors lager was ten opzichte van de groep patiënten met extracorporele circulatie 0,57 (0,07) mmol/mol kreatinine ($p<0,001$).
Conclusie: De resultaten bevestigen dat bij open hart chirurgie zonder extra corporele circulatie slechts minimale tekenen van oxidatieve stress aanwezig zijn en dat de nierfunctie onveranderd blijft in tegenstelling tot patiënten die open hart chirurgie met extra corporele circulatie ondergaan.

71. Low specificity of INNO-LIA-ANA test for Scl-70

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Introduction: Scleroderma is a serious but rare disease. Its clinical picture, especially the diffuse form is very striking, characterized by abnormally increased collagen deposition in the skin. The disease is slowly progressive and disabling, but due to the involvement of the internal organs it can rapidly progress and become fatal. Scleroderma usually starts in the third to fourth decade of life and has an incidence of 4-12 cases per million.
Methods: In our laboratory we use the INNO-LIA ANA kit of INNOGENETICS, a line-blot immunoassay to identify autoantibodies in human sera directed against Scl-70/topoisomerase I, SmB, RNP-70k, RNP-A, RNP-C, Ro52, SSB/l a, CenpB, Jo1, SmD,ribosomal P, Ro60 and histones. The various antigens are coated on test strips and are incubated in the test with a human serum. Specifically bound autoantibodies are detected by a goat anti-human IgG labeled with alkaline phosphatase. Detection of autoantibodies against Scl-70 in a serum is verified by a second test at the CLB (Amsterdam), which use a in-house developed Western blot method.
Results and discussion: Recently the sensitivity and specificity of currently commercially available tests for detection of Scl-70 autoantibodies was analyzed in a multicenter study. Among the tested methods was also the kit used in our laboratory. It showed a sensitivity of 95.3% and a specificity of 95.0% (1). A retrospective study of patients tested in our laboratory revealed a discrepancy between the results of our laboratory and that of the CLB. Analysis of the clinical picture of these patients indicated a high percentage of false positives, 6 out of 8, with the INNOGENETICS kit. Currently our study is extended with 6 additional patients, the data will be presented at the meeting.
Conclusion: The Innogenetics kit has, in contrast to previously reported, a low specificity for Scl-70 antibodies.

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72. LifeScan SureStep®Pro en Point of Care Testen in het Medisch Centrum Alkmaar

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Inleiding: Het diabetesteam in het Medisch Centrum Alkmaar, waarin het laboratorium participeert, streeft naar een intensievere begeleiding van diabeten in de kliniek, met een wezenlijke rol voor gespecialiseerde verpleegkundigen. Het bedsite monitoren van de bloedglucose wordt daarom onontbeerlijk. Daarnaast bestaat op meerdere afdelingen behoefte aan een eenvoudige en snelle (gevalideerde) glucosemeting, die de verpleegkundige minimaal belast en waarvan de (analytische) kwaliteit te allen tijde door het laboratorium garandeerd wordt.
Methoden: Het laboratorium heeft een studie gestart met het SureStep®Pro systeem van de firma LifeScan (Johnson& Johnson). Naast de bedsite units is de kern het werkstation, dat op het laboratorium gelokaliseerd is. Dit station ‘bewaakt’ de aangesloten afdelingen. Bedsite units maken vanuit de afdeling via een ‘docking station’ contact met het werkstation: eerst wordt (nieuwe) informatie naar de unit ‘geupload’, waarna de meetgegevens worden ‘gedownload’. Het werkstation beschikt over een uitgebreid data-management voor optimaal beheer en is middels een bidirectionele koppeling verbonden met het lis, waarin de patiëntenresultaten worden vastgelegd. Op de deelnemende afdelingen zijn apparaat-sops en werkvoorschriften aanwezig. Na individuele instructie worden verpleegkundigen een ‘gevalideerde operator’ met een eigen user-id. Het systeem logt alle acties van de users. Bovendien kunnen een re-certificatiedatum en de maximale tijd, waarin een operator niet actief is op het systeem, worden vastgesteld. Bij overschrijding wordt de user-id geblokkeerd.
Resultaten en discussie: Inmiddels participeren naast het laboratorium het daghospitaal, de diabetes-polikliniek en de afdelingen longziekten en neonatologie in de pilot. In de pilot-fase worden de metingen zowel op de bedsite unit als in het laboratorium uitgevoerd. De resultaten van correlatie-studies zijn zeer goed, ook in het lage gebied. De bruikbaarheid in de neonatologie wordt op dit moment bestudeerd. De stripmethode is aantoonbaar zeer robuust.
Desondanks worden afwijkingen gesignaleerd, omdat verpleegkundigen ondanks persoonlijke instructies en goed hanteerbare voorschriften onverwacht blijken af te wijken van vastgestelde procedures.
Conclusie: Het SureStep®Pro systeem lijkt zeer geschikt voor point of care testen. Veel aandacht dient besteed te worden aan de coaching van verpleegkundige operators. De mogelijkheden van het systeem ten aanzien van de ‘gevalideerde operator’ zijn daarbij een zinvol hulpmiddel. Bij aanvang geeft een (langere) periode parallel draaien inzicht in de foutenbronnen op afdelingen.

73. Assessment of the glomerular filtration rate in hypertensive patients using cystatin C

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Introduction: Measurement of glomerular filtration rate (GFR) is essential for studying changes in renal function in patients with renal failure. Several methods are available for the determination of GFR in patients: clearance of creatinine, inulin, radio-isotopes (such as ^{125}I-thalamate and ^{51}Cr-EDTA). All these measurements have considerable drawbacks: the clearance of creatinine is affected by e.g. body composition, an incomplete urine sampling and there is a long list of interferences; the other methods are time demanding, costly and straining for the patient. More recently, cystatin C has been suggested to be a new sensitive marker of the GFR. Cystatin C is a small non-glycosylated 13 kDa basic protein, produced by all investigated nucleated cells at a constant rate and is freely filtered by the kidneys. Cystatin C, as a marker for the GFR, is independent from urine collection and only one 50 µl sample is necessary. As compared to the linearity of 1/[creatinine] against the gold standard ^{125}I-iothalamate, better results have been reported in literature recently for [cystatin C].
Methods: In the present study 44 hypertensive patients (22 men, age: 57.9±8.4 y; 22 female, age: 53.0±16.7 y), who used at least two antihypertensives (e.g. ACE inhibitors), were studied. To assess renal function the ^{125}I-iothalamate clearance was determined. Cystatin C concentrations were measured by means of a particle enhanced turbidimetric immunoassay (DAKO Diagnostics).
Results and discussion: The range of the creatinine concentrations, measured with the enzymatic method, was 40-240 µmol/l . The ^{125}I-iothalamate clearance varied from 22 to 122 ml/min. Comparable to literature, an acceptable linearity was achieved of 1/[creatinine] against the gold standard (y=x + 0.003, r=0.77). However, the linearity of 1/[cystatin C] against the gold standard was disappointing (y=0.01x +0.41, r=0.63).
Conclusion: Our first data suggest that the promising cystatin C marker is not suitable to assess the renal function in hypertensive patients (with antihypertensives).

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74. Microparticles from T cells, granulocytes and endothelial cells circulate in a subset of patients with systemic lupus erythematosus

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Introduction: he cellular origin of circulating microparticles in patients with systemic lupus erythematosus (SLE) was determined, and their relationship with inflammation, coagulation, and endothelial dysfunction was investigated.
*Methods: M*icroparticles from peripheral blood of SLE patients (n=12) and controls (n=12) were analyzed by flow cytometry. Plasma markers of inflammation (C-reactive protein, elastase, and interleukin-6), coagulation (thrombin-antithrombin complexes, prothrombin fragment F_{1+2}), and endothelial dysfunction (von Willebrand factor, soluble E-selectin) were determined by ELISAs. The thrombin generating capacity of microparticles was also assessed.
Results and discussion: Compared to controls, patients had on average 2.6-fold decreased numbers of platelet-derived (p= 0.024), and 3.2-fold increased numbers of erythrocyte-derived microparticles (p=0.001). A subset of the patients (n=3) had microparticles originating from T helper cells, granulocytes, and endothelial cells. Patients had elevated levels of C-reactive protein (16.5-fold, p<0.001), elastase (1.9-fold, p=0.007), interleukin-6 (5.7-fold, p=0.006), thrombin-antithrombin complexes (2.4-fold, p=0.003), and von Willebrand factor (2-fold, p=0.008). Prothrombin fragment F_{1+2} and soluble E-selectin concentrations did not differ from controls (p=0.878, p=0.954, respectively). The thrombin generating capacity of patient microparticles was 1.6-fold increased (p=0.021). No correlations were found between microparticle numbers and markers of inflammation, coagulation, or endothelial dysfunction. Also, the subset of patients with the extra set of microparticles did not differ from the others regarding activation of these processes.
Conclusion: Microparticles in patients with SLE could not be linked to inflammation, coagulation, or to endothelial dysfunction. However, the presence of T cell-, granulocyte- and endothelial cell-derived microparticles in a subset of the patients may be connected to enhanced in vivo apoptosis or ineffective clearance of apoptotic particles.

75. Microparticles from synovial fluid support coagulation exclusively via a factor VII-dependent mechanism

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Introduction. In synovial fluid from arthritic joints the coagulation system is highly activated, resulting in pathological fibrin depositions as rice bodies. For coagulation, a procoagulant surface is required and cell-derived microparticles -in blood- can act as such a procoagulant surface. Interestingly, synovial fluid also contains microparticles. In the present study we determined their cellular origin, thrombin generating capacity and relationship with local hypercoagulation.

Methods. Microparticles, isolated by differential centrifugation from synovial fluid and plasma from rheumatoid arthritis patients (n=10), were identified by flow cytometry. Thrombin generating capacity (thrombin generation test) was assessed in normal plasma without or with antibodies that block coagulation factor VII, XI or XII. Concentrations of prothrombin fragment F_{1+2} and thrombin-antithrombin complexes (TAT) were determined by ELISA to assess the coagulation status.

Results and discussion. Microparticles in synovial fluid originated from monocytes, granulocytes and lining cells, but platelet-derived microparticles were absent. Plasma contained only significant numbers of microparticles originating from platelets and erythrocytes. Although synovial microparticles bound annexin V, this binding was severely impaired when compared to microparticles isolated from plasma. Microparticles from synovium strongly supported thrombin generation and exclusively via a factor VII-dependent mechanism. A subpopulation of the microparticles exposed tissue factor. Concentrations of both F_{1+2} and TAT were highly elevated compared to plasma ($p<0.001$ and $p<0.001$, respectively), but did not correlate with microparticle numbers or their thrombin generating capacity.
Conclusion. Thus, synovium contains microparticles, originating almost exclusively from white blood cells and lining cells, that initiate and promote thrombin formation via a factor VII-dependent pathway.

76. Microparticles from patients with multiple organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms

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Introduction. We investigated the occurrence and thrombin generating mechanisms of circulating microparticles (MP) in patients with multiple organ dysfunction syndrome (MODS) and sepsis.
Methods. MP, isolated from blood of patients (n=9) and healthy controls (n=14), were stained with cell-specific monoclonal antibodies (MoAbs) or anti-tissue factor (anti-TF) MoAb and annexin V, and analyzed by flow cytometry. To assess their thrombin-generating capacity, MP were reconstituted in normal plasma. The coagulation activation status in vivo was quantified by plasma prothrombin fragment F_{1+2}- and thrombin-antithrombin (TAT) measurements.
Results and discussion. Annexin V-positive MP in the patients originated predominantly from platelets (PMP), and to a lesser extent from erythrocytes, endothelial cells (EMP) and granulocytes (GMP). Compared to healthy controls, the numbers of PMP and TF-exposing MP were 2 and 3 fold decreased ($p=0.001$ and $p<0.001$,respectively), EMP were decreased (E-selectin, $p=0.003$) or found equal (CD144, $p=0.063$), erythrocyte-derived MP were equal ($p=0.726$), and the number of GMP was 12 fold increased ($p=0.008$). GMP numbers correlated with plasma concentrations of elastase ($r=0.70$, $p=0.036$), but not with C-reactive-protein or interleukin-6 concentrations. Patient samples also contained elevated numbers of annexin V-negative MP, predominantly of platelet, erythrocyte- and granulocyte-origin ($p=0.005$, $p=0.021$ and $p<0.001$, respectively). Patient MP triggered thrombin formation, which was 2 fold reduced compared to controls ($p=0.008$) and strongly inhibited by an anti-factor XII MoAb (two patients), by anti-factor XI MoAb (eight patients) or by anti-TF MoAb (four patients). Concentrations of F_{1+2} and TAT were 2 and 5 fold elevated ($p=0.005$ and $p=0.001$, respectively) and correlated inversely with the number of circulating MP ($r= -0.65$, $p=0.001$, and $r= -0.51$, $p=0.013$, respectively) and their thrombin generation capacity (F_{1+2}: $r= -0.62$, $p=0.013$).
Conclusion. In patients with MODS and sepsis relatively low numbers of MP are present that differ from controls in their cellular origin, numbers and coagulation activation mechanisms.

77. Procalcitonin and lipopolysaccharide-binding protein. New markers of bacterial infections

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Introduction: Recently, several new fast parameters have been introduced which are more sensitive to bacterial infections than relatively fast but unspecific parameters as C-reactive protein and leucocyte count. These new fast parameters allow a better classification of patients. We have investigated two of these new parameters, lipopolysaccharide-binding protein (LBP) for gram-negative bacterial infections and procalcitonine for bacterial infections in generall
Methods: We performed 67 quantitative immunoluminometric procalcitonin-assays (Brahms Diagnostica GmbH, Berlin, Germany), 24 semiquantitative PCT-tests (PCT-Q, Brahms) and 50 quantitative immunometric sandwich LBP-assays (DPC, Breda, the Netherlands). 11 patients were repeatedly analysed at different occasions while being hospitalized for the same illness
Results and discussion: Procalcitonin-levels > 3.0 ng/ml had a sensitivity of 97.8% and specificity of 94.1% for prediction of the involvement of bacterial endotoxines in general in the disease process in this intensive-care population. One result was false positive based on a negative bacterial bloodculture and one result was false negative. This last result was obtained from a patient whose “tip”-culture revealed Enterococcae and Staphylococcus epidermis. At the same time, this patient had a serum CRP-value of 200 mg/l. LBP-levels > 40 µg/ml had a sensitivity of 98.2% and a specificity of 60.0% in determining the systemic involvement of gram-negative bacteria in patients their disease process. Two results of different patients were false positive based on a negative bacterial bloodculture and one result of a patient was false negative. This last result was obtained from a patient that revealed Escherichia coli and Klebsiella oxytoca from a bloodsample taken at the same occasion and who showed Enterococcus cloacae in his sputum. This patient was diagnosed with a peritonitis. The results of both tests can be obtained within half an hour.
Conclusion: We conclude that in an intensive-care setting these two new markers can help the physician with his differential diagnosis to make a rapid decision about the best possible treatment for his patient.

Gynecologie / Obstetrie

78. Maternal and infant C677T methylene tetrahydrofolate reductase genotypes of Afro-Caribbean women with preeclampsia

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Introduction: The C677T methylene tetrahydrofolate reductase (MTHFR) mutation could be involved in the high plasma homocysteine of preeclampsia. The C677T MTHFR mutation prevalence in preeclampsia was previously investigated in various ethnic populations, revealing both higher and similar prevalence of the maternal C677T MTHFR mutation, compared to pregnant controls. Homocysteine is capable of crossing the placenta and maternal homocysteine levels may be critically dependent on maternal, but possibly also fetal, C677T MTHFR genotype.
Methods: We established, in a retrospective study design, the C677T MTHFR genotypes of 89 preeclamptic Afro-Caribbean women living in Curaçao (Netherlands Antilles), 89 race and parity-matched normotensive controls with uncomplicated pregnancies, 49 children born to the preeclamptic women during their preeclamptic pregnancy, and 49 children born to the controls. C677T MTHFR genotypes were established by PCR-RFLP.
Results and discussion: There were no significant case-control differences in C677T MTHFR genotype frequencies of the mothers, children and the mother/child combinations. However, maternal MTHFR genotype distribution differed among women with mild preeclampsia compared to women with severe preeclampsia (heterozygotes 34.5 vs 9.7%, and homozygotes 3.4 vs 0.0%, respectively; p=0.039). Combined heterozygous and homozygous preeclamptic women had a 5.7 times (odds ratio; 95%CI: 1.5-21.0) higher chance to develop mild preeclampsia, rather than severe preeclampsia. There is growing evidence that preeclampsia is a disorder of heterogeneous causes. Identification of one conclusive genetic disorder is not to be expected, but multiple risk factors (genetic, environmental, combination) are likely to become identified. Different mixtures of underlying etiologies may consequently explain the apparent discrepancies in the association between C677T MTHFR genotype and preeclampsia. This may also explain the presently found MTHFR genotype distribution difference between women with mild preeclampsia and women severe preeclampsia.
Conclusion: C677T MTHFR polymorphism of mother, child and mother/child combination does not constitute a preeclampsia risk factor among Afro-Caribbean women living in Curaçao.

79. Cord vessel arachidonic- and docosahexaenoic acid contents of healthy term infants at birth: negative correlations with anthropometry and positive correlations with gestational age

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Introduction: Arachidonic (AA) and docosahexaenoic (DHA) acids in cord plasma lipid fractions and umbilical artery (UA) phospholipids of preterm infants correlate positively with gestational age at birth (GA), and weight, head circumference and length at birth. Umbilical plasma phospholipid DHA correlates positively with GA of term infants. We correlated in term infants the contents of AA and DHA in UA and umbilical veins (UV) with anthropometry and GA at birth.
Methods: UA and UV of healthy term Caucasian Dutch infants (37-42 weeks; ≥2,500 g; 167 boys and 141 girls) were collected in 1997-1999. Fatty acids were determined with capillary gas chromatography. AA and DHA contents (g%) were correlated [Spearman (S); Partial correlation (PC)] with newborn characteristics at birth (i.e. gender, GA, weight, crown-heel length, and circumferences of the head, arm, abdomen, and upper-leg).
Results and discussion: UA-AA and UA-DHA correlated positively ($p<0.05$). UA-AA, UA-DHA and UV-AA correlated positively with duration of gestation. UA-AA correlated negatively with birth weight, arm and head circumferences of girls and upper-leg-circumference of boys (S), and also with birth weight and circumferences of the head, abdomen, arm and upper-leg (PC controlling for GA, DHA and gender). UA-DHA correlated negatively with birth weight (PC controlling for GA, AA and gender). UV-AA correlated negatively with arm circumference of girls and with birth weight and upper-leg circumference of boys (S), and also with birth weight and circumferences of the abdomen, arm and upper-leg (PC controlling for GA and gender). Analogous to preterms, term infants show positive relations of AA and DHA status parameters with duration of gestation. In contrast to preterm infants, term infants show negative correlations of AA and DHA status parameters with anthropometrics at birth.
Conclusion: AA and DHA do not seem to promote growth in the 37-42 weeks of gestation, when preceding growth has been adequate.

80. Preeclampsia is associated with the Duffy negative phenotype in Curaąao women of West-African descent

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Introduction: We have previously reported higher circulating levels of the chemokine interleukin-8 (IL-8) in preeclampsia. Erythrocytes have chemokine-binding properties. The identified chemokine receptor on erythrocytes proved identical to the Duffy blood group antigen. The Duffy Antigen Receptor Chemokine (DARC) on erythrocytes is considered to act as a sink for circulating chemokines. Individuals of West-African descent do not express erythroid DARC, designated the Duffy

negative phenotype. We determined the frequency of the Duffy negative phenotype in a population of women with preeclampsia in their obstetric history and in controls who had no history of preeclampsia. The study was conducted in the Caribbean Island of Curaçao and the women were predominantly of West-African descent.
Methods: In a retrospective study design we recruited 72 women with preeclampsia in their obstetric history and 55 women who had no history of preeclampsia. The Duffy blood group antigens Fy(a) and Fy(b) are the expression products of the alleles FY*A and FY*B, respectively. Venous EDTA-anticoagulated blood samples were used for the analyses of Fy(a) and Fy(b) by the antiglobulin test. The phenotypes Fy(a+, b-), Fy(a-,b+) and Fy(a+,b+) are the Duffy positive (erythroid) phenotypes; Fy(a-,b-) is the Duffy negative (erythroid) phenotype.
Results and discussion: Preeclamptic women had higher frequencies of the Duffy negative phenotype compared to controls [52.8% vs 27.3%, respectively; OR 2.98; 95% CI (1.40-6.32); p=0.00387]. Preeclampsia proved to be associated with the Duffy negative phenotype. Absence of erythroid DARC results in reduced chemokine binding capacity. It is possible that the Duffy negative phenotype predisposes to high levels of circulating chemokines, notably in conditions with increased chemokine production such as preeclampsia.
Conclusion: Preeclampsia is associated with the Duffy negative phenotype in women of West-African descent. A large population-based trail should determine whether the Duffy negative phenotype constitutes a preeclampsia risk factor.

81. Measurement of MUC-1 mucin in sera of pregnant women with the VIDAS CA 15.3 kit

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Introduction: MUC-1 type glycoproteins are heavily linked oligosaccharides mucins. In normal cells it is expressed at the apical cell surface, but in carcinomas the polarisation of MUC-1 is lost. MUC-1 may be measured by the CA 15.3 assay using two moabs, 115D8 and DF.3 or a competitive format with the B27.29 moab developed later. Pregnant women may have increasing CA 15.3 concentrations.
Methods: To assess possible differences in the MUC-1 composition we measured CA 15.3 concentrations with the sandwich format on VIDAS-automated system (BioMerieux, France), with the BR-MA assay (Immulite DPC, USA) using comparable moabs, and with the BR test (Centaur Bayer, Germany) using a competitive assay with the moab B27.29.
Results and discussion: The mean values for all assays increased with 35% during pregnancy although absolute values differed. The correlation coefficient varied between 0.70 and 0.90 for the healthy population and for the pregnant women at week 12. At week 30 this correlation was lost for the Centaur BR system. This diminished correlation may be due to alterations in glycosylation of the MUC-1 molecule.
Conclusion: Further investigations into the composition of the carbohydrate molecules of MUC-1 are warranted, because the difference in MUC-1 structure may induce an immune response (1). This may be the reason that women who have been pregnant, have lower risk for breast cancer.

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Therapeutische geneesmiddelen monitoring en klinische toxicologie

82. 11-dehydro-thromboxane B2 assay, a suitable screening assay for aspirin use?

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Introduction: The side effects of cyclooxygenase inhibitors like aspirin on the gastrointestinal tract include upper gastrointestinal bleeding and perforation. The frequency of 'over the counter' use of aspirin is probably underestimated because of patient unawareness or denial. We evaluated the usefulness of a simple, rapid and inexpensive enzyme immune assay (EIA) for platelet thromboxane production, which is inhibited by cyclooxygenase inhibitors and might be used as indirect measurement for the use of aspirin.
Methods: Twenty healthy volunteers with no history of bleeding disorders and no NSAID or aspirin use in the last 30 days, ingested one tablet of aspirin of 30 mg, 80 mg, 160 mg or 500 mg (n=5). Urine samples were taken before ingestion and after 1, 2, 4 and 7 days. The urine samples were stored at -20ºC. Thromboxane (Tx) production was monitored by measurement of 11-dehydro-thromboxane B2 in urine, using an EIA kit from Cayman Chemical (Ann Arbor, MI, USA) . Prior to analysis urine samples were purified from interfering substances by solid phase extraction.
Results and discussion: Interassay variation coefficient was less than 10%. Functional assay sensitivity was 15 pg/mmol creatinine and sample recovery was between 74-94%. Before ingestion of aspirin the mean level of 11-dehydro-TxB2 in urine was 108 (range 50-215) pg/mmol creatinine. Twenty-four hours after ingestion of a single dose of 30, 80, 160 or 500 mg aspirin, the 11-dehydro-Tx B2 level decreased respectively with 59% (range 32-49 pg/mmol creatinine), 69% (range 40-70 pg/mmol creatinine), 73% (range 11-29 pg/mmol creatinine) and 76% (15-25 pg/mmol creatinine), all significantly different from the initial level ($p< 0.05$). Seven days after ingestion of a single dose of aspirin the 11-dehydro-TxB2 level was still reduced between 39 and 47%. For high specificity we calculated a cut-off level of 50 pg/mmol creatinine (specificity of 100 % and a sensitivity of 84%). For high sensitivity the cut-off level should be 66 pg/mmol creatinine (specificity 79% and sensitivity 95%
Conclusion: The 11-dehydro-Tx B2 EIA is a simple and quick assay, suitable for indirect screening of aspirin use, and deserving further evaluation in clinical setting.

83. Genotyping in psychiatric patients: an overview

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Introduction: The cytochrome-P450 (CYP) enzymes CYP2D6 and CYP2C19 are involved in the metabolism of numerous psychopharmaca. Both enzymes are genetically polymorphic. Several mutant alleles are known, associated with enzyme activities ranging from ultrafast to a complete absence. Therefore, metabolic capacity varies, producing intersubject differences in therapeutic response and side-effects at standard recommended doses. By the use of genotyping methods every individual can be classified as either a poor (PM), an intermediate (IM), an extensive (EM) or an ultrarapid metabolizer (UM). Identification of PM and UM subjects is of potential clinical importance for adjustment of doses in drug therapy, to assure therapeutic efficacy.
Methods: In our psychiatric hospital we perform CYP genotyping routinely since 1997. All new patients are screened for the three most common defect gene variants of CYP2D6 (i.e. 2D6*3, *4 , *5), the CYP2D6 gene duplication and the non-functional CYP2C19*2 allele. Most of those patients start psychoactive drug therapy. When their serum drug concentration exceeds the therapeutic index by more than 10%, the patients genotypical state, drug dose and co-medication are considered by the clinical chemist, in consultation with the pharmacist. If the serum drug level is still abnormal after dose adjustment and/or changing medication, while no aberrant genotype was detected, the patient is screened for the less common defect gene variants of CYP2D6 (i.e. 2D6*6, *7, *8, *11, *12, *14) and CYP2C19 (2C19*3, *4, *5).
Results and discussion: About 1000 patients are admitted into our hospital every year, of whom about half are re-admissions whose genotype is already known. PMs and UMs are found with prevalences of 9.7% and 3.0%, respectively. After dose adjustment or drug selection based upon genotypical state, a therapeutic serum drug level is reached in almost every patient.
Conclusion: After 3 years of experience we can conclude that CYP2D6 and CYP2C19 genotyping, performed routinely after admission into a psychiatric hospital, is a good help in individualisation and optimization of psychoactive drug therapy. Compared with other psychiatric hospitals, overall no extra costs are associated with this practice.

84. CYP3A4*1B en de farmacokinetiek van midazolam in pasgeborenenen

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Inleiding: Bij de afbraak van geneesmiddelen speelt het cytochroom P450 3A4 (CYP3A4) een belangrijke rol. Dit enzym komt in de lever en de darm tot expressie. Er bestaat een grote interindividuele variatie in de farmacokinetiek van geneesmiddelen die via CYP3A4 worden gemetaboliseerd. Genetische polymorfismen in het CYP3A4 allel kunnen hier deels verantwoordelijk voor zijn. Het CYP3A4*1B allel heeft een A(-290)G mutatie in de promoterregio. De rol van deze mutatie op de klaring van geneesmiddelen werd onderzocht door middel van het sedativum midazolam. Midazolam wordt door CYP3A4 gemetaboliseerd tot 1-hydroxy-midazolam.
Methoden: Midazolam (0,1 mg/kg) werd oraal of intraveneus toegediend aan 30 vroeggeboren kinderen: 24 Caucasisch, 2 Mediterraan, 1 Negroïde, 1 Spaans en 2 onbekende komaf; zwangerschapsduur 26-34 weken, 3-13 dagen oud. Acht bloedmonsters werden gedurende 24 uur afgenomen voor de bepaling van midazolam en 1-hydroxy-midazolam, waarna farmacokinetiek werd berekend. CYP3A4*1B mutatie analyse werd uitgevoerd met behulp van PCR-RFLP (1). Groepen werden vergeleken met behulp van de Mann-Whitney U test.
Resultaten en discussie: Van de 30 patiënten bleken 24 wild type, 4 heterozygoot en 2 homozygoot voor CYP3A4*1B te zijn; dit geeft een allelfrequentie van 13,3%. De midazolamklaring na intraveneuze toediening was gelijk voor wild typen en CYP3A4*1B dragers (0,1 versus 0,18 L/kg/h; p=0,26). De midazolamklaring na orale toediening bleek significant hoger voor CYP3A4*1B dragers (0,12 versus 0,43 L/kg/h; p=0,048). Na correctie voor co-medicatie was er slechts een trend zichtbaar (p=0,099).
Conclusie: CYP3A4*1B dragerschap is niet gecorreleerd met intraveneuze klaring van midazolam, maar mogelijk wel met orale klaring. Dit zou kunnen wijzen op een verhoogde CYP3A4 activiteit in de darm bij CYP3A4*1B dragers.

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85. Perinatal lead exposure in the Roman empire: evidence from archaeotoxicological studies

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Introduction: Historians have since long tried to find a cause for the rapid decline and fall of the Roman empire in the first centuries of the common era. Since the Romans were avid users of lead in water piping and of lead salts as additives to wine and stewed foods, an obvious explanation for the fall of Rome is lead (Pb) poisoning. The proverbial madness of the emperors Claudius (d. 54) and Nero (d. 68) may well be related to lead encephalopathy. This 'plumbism theory' was substantiated by measurement of Pb in Romano-British bones in the 1970ies. It was, however, unknown at which age Pb exposure started. As infants are known to be particularly vulnerable to Pb, it was our aim to establish Pb exposure in Roman infants.
Methods: The cemetery of a Roman castellum in Valkenburg near Leiden at the Rhine, the northernmost continental frontier of the empire, supplied an ideal population for this study. Although juveniles and adults were cremated, stillborn children and neonates were buried in the alkaline soil, in which the skeletons remained quite intact. Moreover, the Pb exchange between soil and bone is considerably less under alkaline than under acid conditions. We developed a method to

measure Pb in bone samples based on flameless atomic absorption spectrometry. Bone samples (80 mg) from the femoral shaft gave the most reproducible results. Trabecular bone is less suitable because of intrusion of soil particles.
Results and discussion: In the femora of 33 stillborns and infants we found a Pb concentration of 103 ± 87.4 μg/g bone (mean ± SD, range 12 - 388). In surrounding soil, Pb was 18 - 21 μg/g, indicating that diagenesis of Pb from soil to bone was limited. Control femoral bone samples were obtained at autopsy of stillborn and deceased Dutch infants. In all instances, the Pb content was below the detection limit of 2 μg per g bone. Normal blood lead concentrations in contemporary Dutch infants do not exceed 50 μg/l .
Conclusion: It is concluded that 2000 years ago infants in a society with a Roman lifestyle were heavily exposed to lead, likely to result in health effects.

Stoornissen in het intermediair metabolisme

86. Transaldolase deficiëntie: een nieuw aangeboren stofwisselingsdefect in de pentose fosfaat route

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Inleiding: polyolen, suiker alcoholen, zijn verbindingen die in het menselijk lichaam en in lichaamsvloeistoffen voorkomen. De herkomst van deze verbindingen is niet bekend. Ook is het metabolisme van de meeste polyolen nog onopgehelderd. Hier presenteren wij een patient met stapeling van polyolen. Vlak na de geboorte presenteerde dit meisje, dochter van verwante ouders, zich met hepatomegalie. Een leverbiopsie op de leeftijd van 3 jaar toonde een levercirrhose en geen aanwijzingen voor een stapelingsziekte. Ondanks uitgebreid metabool onderzoek werd geen oorzaak voor de levercirrhose gevonden. Wel werden afwijkingen gevonden in het suiker- en polyol profiel van de urine, naar aanleiding waarvan uitgebreid onderzoek werd gedaan.
Methoden: suikers en polyolen in urine, bloed en CSF werden geanalyseerd met gas chromatografie. Naar aanleiding van de afwijkende bevindingen werd een enzym assay opgezet, die het mogelijk maakte transketolase en transaldolase te bepalen in erythrocyten en lymfoblasten. De cellen werden geïncubeerd met ribose-5 fosfaat, waarna vorming van suiker fosfaat intermediairen werd gevolgd met GC-NPD (stikstof fosfor detectie). Moleculaire studies werden verricht op cDNA en genomisch DNA niveau.
Resultaten en discussie: in urine werden verhoogde concentraties van D-arabitol, ribitol en erythritol gevonden. De pentoses xylulose en ribose waren slechts marginaal verhoogd. De concentraties polyolen in plasma waren licht verhoogd; in CSF waren zij vrijwel normaal. Het afwijkende polyol profiel bracht ons tot de hypothese dat er een defect was in de pentose fosfaat route. Naar aanleiding hiervan werd een enzym assay ontwikkeld. Hiermee werd een normale activiteit van transketolase aangetoond in de cellen van de patient. De activiteit van transaldolase was echter onmeetbaar laag, zowel in erytrocyten als in lymfoblasten. Door middel van sequencing van het transaldolase gen werd een deletie van 3 baseparen aangetoond. Deze deletie leidt tot afwezigheid van serine 171 in het transaldolase eiwit. Dit aminozuur bevindt zich in een sterk geconserveerd gebied.
Conclusie: Wij presenteren de eerste patiënt met een aangeboren defect in de reversibele fase van de pentose fosfaat route: transaldolase deficiëntie. Deficiëntie van dit enzym leidt biochemisch tot stapeling van pentitolen en tetritolen en gaat klinisch gepaard met levercirrhose. Wij adviseren om bij levercirrhose van onbekende origine suikers en polyolen in urine te bepalen.

87. A 31 bp VNTR in the cystathionine β-synthase (CBS) gene is associated with reduced CBS activity and elevated post-load homocysteine levels

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Introduction: Molecular defects in genes encoding enzymes involved in homocysteine metabolism may account for mild hyperhomocysteinemia, an independent and graded risk factor for cardiovascular disease (CVD). Although heterozygosity for CBS deficiency is excluded as a main genetic cause of mild hyperhomocysteinemia in vascular disease, mutations in (non)-coding DNA sequences leading to mildly decreased CBS expressions have never been investigated. In the present study, we investigated the possible involvement of the CBS non-coding region in mild hyperhomocysteinemia (mHH) and CVD.
Methods: We analyzed two silent polymorphisms and two repeats in the CBS gene (i.e. 699C->T, 1080T->C, GT-repeat and 31-bp repeat) as markers in linkage disequilibrium with a possible pathogenic mutation, and assessed their association with fasting, post-methionine load, and delta (increase upon methionine loading) homocysteine in 190 patients with arterial occlusive disease, and in 381 controls.
Results and discussion: No differences were observed in genotype and haplotype distribution, nor in allele frequencies for both polymorphisms and repeats between cases and controls. Both the two polymorphisms and the GT-repeat showed no effect on fasting as well as post-methionine load homocysteine concentrations. The 31-bp repeat consist of 17,18, 19 or 21 repeat units and showed a significant increase in plasma homocysteine concentrations after methionine loading with increasing number of repeat units. The 31-bp repeat spans the exon-intron border and thus may affect splicing. To investigate whether the repeat causes alternative splicing, we conducted PCR on cDNA to check for possible multiple cDNA products and evidence was obtained.
Conclusion: The 31-bp repeat is the first common cause of mHH in the CBS gene. Every single repeat contains a possible splice site according to the consensus sequence for splice signals (1), probably causing alternative splicing. PCR on cDNA demonstrated indeed the presence of alternative CBS cDNA leading to abnormal CBS protein

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88. Family Screening in response to Heriditary Hemochromatosis Case-Finding: an efficient and cost-effective approach for early HH recognition

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Introduction: A guideline from the Dutch Health Council upon early recognition of Heriditary Hemochromatosis (HH), one of the most frequent genetic diseases in Caucasians, appeared recently. In this context, the family of an index patient was screened upon the C282Y and H63D HFE gene mutations.
Methods: The C282Y and H63D mutations were determined separately by PCR RFLP as described by Feder et al.; an alternate reverse primer, as described by Jeffrey et al., was used for amplification of exon 4.
Results and discussion: The index patient, a 71 yr old female with asthenia and elevated s-ferritin, was found to be homozygous for the C282Y mutation. Out of eight younger sisters four were proven to be homozygous for C282Y as well, two were compound heterozygous (C282Y/H63D) and two were homozygous for the H63D mutation. Early clinical symptoms (asthenia, arthralgia, aminotransferase increase) and ferritin elevations were recognized in 4 sisters. The index patient had 6 children; all of them were recognized as C282Y homozygotes. Manifest iron overload and symptoms of HH were detected in two children so far (45 yr; 42 yr). Three nieces in the same age were documented to be compound heterozygotes with transferrin saturations of 62%; 43% and 55% but without cellular iron overload.
In this unique family in which the major and minor HFE gene mutations co-appear, eleven C282Y homozygotes and five compound heterozygotes were recognized in two generations in 33 screened family members. In the eldest generation (52-71 yr) the disease was clinically overt in five out of nine sisters. In the younger generation the disease came to expression in two C282Y homozygotes (1 M, 1 F) already at 40 yrs of age.
Conclusion: From this study it becomes obvious that family screening in response to case-finding is a very efficient and cost-effective approach for early recognition of HFE gene mutations in young offspring.

89. X-gebonden creatine transporter defect: een nieuw creatine deficientie syndroom

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Inleiding: Wij presenteren een zeven jaar oude jongen met een nieuwe vorm van Creatine (Cr) deficiëntie. De jongen vertoonde op 6 jarige leeftijd ontwikkeling- en taal achterstand. Zijn motoriek was normaal en hij was matig hypotoon. De Magnetische Resonantie Spectroscopie (MRS) van de hersenen vertoonde een bijna volledige afwezigheid van het Cr-signaal. De familieleden van de index patiënt hebben klachten die overeenkomen met het erfelijkheidspatroon van een X-gebonden aandoening. De MRS en de klinische verschijnselen van de index patiënt suggereerde oorspronkelijk de diagnose guanidinoacetaat methyltransferase (GAMT) deficiëntie. Het GAMT-enzym methyleert guanidinoacetaat (GUAC) waardoor (Cr) wordt gevormd in de lever, nier en pancreas. De Cr-transporter (SLC6A8), welke gelocaliseerd is op Xq28 is belangrijk voor opname van Cr in verschillende weefsels, met name spier en hersenen.
Methoden: GUAC- en Cr-niveaus zijn met behulp van stabiele isotoop dilutie gas chromatografie/ massa spectrometrie bepaald. DNA en RNA is geïsoleerd uit fibroblasten van de index patiënt en drie familieleden. Het creatinetransportergen is vervolgens geamplificeerd en gesequenced.
Resultaten en discussie: In tegenstelling tot GAMT-deficiënte patiënten was GUAC normaal en Cr verhoogd in urine en plasma van onze index patiënt. Orale Cr-suppletie resulteerde niet in herstel van de Cr-piek bij MRS, noch in klinische verbetering. GAMT-deficiëntie werd daardoor uitgesloten. Het feit dat Cr afwezig is in de MRS van de hersenen, de verhoogde Cr-niveaus in plasma en urine en het geslachtgebonden erfelijkheidspatroon van de familie suggereerde dat de patiënt lijdt aan een defect in de Cr-transporter. DNA-sequentieanalyse van de Cr-transporter toonde een hemizyogote nonsense mutatie en bevestigde dat dit een nieuw X-gebonden Cr-deficiëntiesyndroom betreft. Drie vrouwelijke familieleden zijn heterozygoot voor de mutatie.
Conclusie: We hebben met behulp van MRS en moleculaire analyse een nieuwe X-gebonden ziekte geïdentificeerd, die veroorzaakt wordt door een disfunctionele creatinetransporter.

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90. The molecular basis of Dutch infantile nephropathic cystinosis

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Introduction: Infantile nephropathic cystinosis, an inborn error of metabolism with an autosomal recessive inheritance pattern, is characterized by lysosomal storage of the amino acid cystine due to an impaired transport of cystine out of the lysosomes. Initial diagnosis consist of the renal Fanconi syndrome and crystals in the cornea. Oral therapy with cysteamine lowers the intracellular cystine content. Recently, the gene coding for the integral membrane protein cystinosin, which is responsible for membrane transport of cystine (CTNS) was cloned. Mutation analysis of the CTNS gene of Caucasian patients revealed a common 57-Kb deletion, and several other mutations spread throughout the entire gene.
Methods: In the present study, we developed an improved screening method for the detection of the common 57-Kb deletion. Eleven Dutch cystinosis patients were screened by use of this method. The remaining alleles were screened for other mutations by genomic sequencing of the different exons.
Results and discussion: By use of our improved method we detected the 57-Kb deletion in 59% of the Dutch alleles. Genomic sequencing of the remaining alleles revealed three

previously described mutations. Furthermore, we studied a possible genotype-phenotype relation of the homozygous deleted patients, which could not be demonstrated in our study group.

Conclusion: In addition to biochemical determination of cystine in leucocytes and fibroblasts, molecular genetic analysis enables prenatal diagnosis and facilitates identification of carriers.

91. Betaine-homocysteine methyltransferase (BHMT): Genomic sequencing and relevance to hyperhomocysteinemia and cardiovascular disease in humans

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Introduction: Elevated homocysteine levels have been associated with artheriosclerosis and thrombosis. Hyperhomocysteinemia is caused by altered functioning of enzymes of its metabolism due to either inherited or acquired factors. Betaine-homocysteine methyltransferase (BHMT) serves, along with methionine synthase, as a facilitator of methyl group donation for remethylation of homocysteine into methionine, and reduced functioning of BHMT could theoretically result in elevated homocysteine levels. Recently, the genomic sequence of the BHMT gene was published. Mutation analysis may reveal mutations of the BHMT gene that could lead to hyperhomocysteinemia.
Methods: In the present study, we performed genomic sequencing of the BHMT gene of 16 vascular patients with hyperhomocysteinemia.
Results and discussion: Three mutations were detected in the coding region of the BHMT gene. The first one was an amino acid substitution of glycine to serine (G199S), which was found only in the heterozygous state. The second mutation was a substitution of glutamine to arginine (Q239R), and the last mutation was an amino acid substitution of glutamine to histidine (Q406H). The latter was also found only in heterozygous state. The relevance of these mutations was tested in a study group, which consists of 190 cases with cardiovascular disease and 601 controls. The influence of these mutations on homocysteine levels was investigated.
Conclusion: None of the three mutations led to significantly changed homocysteine levels. In addition, no differences in genotype distribution between cases and controls were found. So far, our results provide no evidence for a role of defective BHMT functioning in hyperhomocysteinemia or subsequently in cardiovascular disease.

92. Dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil associated toxicity

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Introduction: Dihydropyrimidine dehydrogenase (DPD) is responsible for the breakdown of the thymine analogue 5-fluorouracil (5FU), thereby limiting the efficacy of the therapy. Inherited (partial) DPD deficiency is increasingly recognized as an important pharmacogenetic syndrome. To evaluate the importance of this specific type of inborn error of pyrimidine metabolism in the etiology of 5FU toxicity we have determined the DPD activity in peripheral blood mononuclear cells (PBM cells) of 36 cancer patients suffering from severe toxicity after the administration of 5FU.
Methods: The activity of DPD was determined by a radiochemical assay. PCR amplification and sequencing of the genomic regions containing the 23 DPD exons was carried out using intron-specific primers for each exon.
Results and discussion: In approximately 50% of the patients a decreased activity of DPD was detected in the PBM cells which was comparable to that observed in obligate heterozygotes. Analysis of the gene encoding DPD for the presence of mutations already identified a number of patients who where heterozygous for the common splice site mutation IVS14(G+1)>A. Thus, our results clearly show that a partial DPD deficiency can cause severe and even lethal toxicity in case these patients are treated with 5FU.
Conclusion: Since according to the literature the prevalence of a partial DPD deficiency has been estimated to be around 3% it is very important to screen tumor patients for the presence of this particular type of inborn error of pyrimidine metabolism prior to the treatment with 5FU.

93. Dihydropyrimidine dehydrogenase (DPD) deficiency: novel mutations in the DPD gene

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Introduction: iDPD is the initial and rate-limiting enzyme in the catabolism of the pyrimidine bases thymine and uracil. DPD is also responsible for the breakdown of 5-fluorouracil (5FU), thereby limiting the efficacy of the therapy. In children, the deficiency of DPD is often accompanied by a neurological disorder but a considerable variation in the clinical presentation among these patients has been reported. Identification of disease-causing mutations in the DPD gene will allow rapid pre-screening of patients at risk.
Methods: PCR amplification and sequencing of the genomic regions containing the 23 DPD exons was carried out using intron-specific primers for each exon.
Results and discussion: In a group of 6 patients suspected of having a complete DPD deficiency we could identify 7 novel mutations including 6 missense mutations D949V (exon 22), I370V (exon 10), P86L (exon 4), S201R (exon 6), H978R (exon 23) and I560S (exon 13) and 1 deletion of two nucleotides (1039-1042delTG). The latter mutation causes a frameshift leading to a premature stop codon shortly thereafter.
Conclusion: So far, 14 different mutations have been identified in 22 families presenting 26 patients with the IVS14+1G>A splice-site mutation being the most common one. The identification of these mutations will facilitate the detection of tumor patients heterozygous for a DPD deficiency prior to therapy with 5FU.

94. Isoforms of human CTP synthetase

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Introduction: CTP synthetase (EC 6.3.4.2) catalyses the formation of CTP from UTP with the concomitant deamination of glutamine to glutamate. CTP synthetase is generally regarded as the rate-limiting enzyme in the synthesis of cytosine nucleotides from both de novo and uridine-salvage pathways. An increased activity of CTP synthetase exists in tumour cells compared to their normal counterparts. The increased activity of CTP synthetase proved not only to be linked to the proliferation rate of the tumour cells but also to the process of malignant transformation. In yeast, two isoforms of CTP synthetase exist encoded by two different genes. So far, it is not known whether isoforms of human CTP synthetase exist.
Methods: A cDNA clone homologous to human CTP synthetase was identified in an EST library. Amplification of the lacking 5' and 3'-cDNA region was performed with the Marathon Ready cDNA kit. Expression of wild-type and the isoform of human CTP synthetase was performed in the E. coli strain JF618.
Results and discussion: A cDNA clone was obtained containing the full coding sequence of an isoform of human CTP synthetase. The cDNA contained an open reading frame encoding a protein of 586 amino acids. The nucleotide sequences of the open reading frames of both isoforms of CTP synthetase were 67% homologous. The predicted protein sequence of both isoforms showed 74% identity. Complementation of the E.coli cytidine requiring mutant lacking CTP synthetase with either the wild-type or isoform of CTP synthetase completely restored the growth in the absence of added cytidine.
Conclusion: At least two functional isoforms of CTP synthetase exist in man. The differential expression of both isoforms in tumour and normal tissues as well as the characterization of the gene is underway.

95. Analysis of serum pristanic and phytanic acid stereo-isomers in Zellweger syndrome, Refsum disease, D-bifunctional protein deficiency and alpha-methylacyl-coa racemase deficiency

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Introduction: Naturally occuring phytanic acid is a mixture of (3R,7R,11R)- and (3S,7R,11R)-phytanic acid. After peroxisomal alpha-oxidation, which is not stereoselective, both (2R, 6R,10R)- and (2S,6R,10R)-pristanic acid are formed, of which only the latter is a substrate for peroxisomal beta-oxidation.
Methods: Phytanic and pristanic acid and their stereoisomers were analyzed by GC/MS as their S-1-phenylethylamine derivatives in serum samples of patients with Refsum's disease, Zellweger syndrome and D-bifunctional protein deficiency.
Results and discussion: GC/MS analysis of phytanic and pristanic acid stereoisomers as their S-1-phenylethylamine derivatives in serum samples of patients with Refsum's disease (n=7) showed that the ratio 3R/3S-phytanic acid was 7/3, similarly to what was observed in Zellweger syndrome (n=5) and D-BP deficiency (n=6), most likely reflecting the ratio of these isomers in a normal diet. The ratio 2R/2S- pristanic acid ratio was 4/6 in Zellweger patients and 6.5/3.5 in D-BP deficiency. In patients with alpha-methylacyl-CoA racemase deficiency (n=3) the 2R/2S- pristanic acid ratio was 7.5/3.5.
Conclusion: Our data show that in contrast to the exclusive accumulation of the bile acid biosynthesis intermediates 25R-THCA and 25R-DHCA, no exclusive pristanic acid diastereomer accumulates in racemase deficiency, probably due to the dietary origin of racemic pristanic and phytanic acid, whereas THCA is produced endogeneously only.

96. Plasma analysis of di- and trihydroxycholestanoic acid (DHCA and THCA) stereoisomers using HPLC tandem mass spectrometry and its application in peroxisomal alpha-methylacyl-CoA racemase deficiency

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Introduction: It has been demonstrated that the peroxisomal beta-oxidation system is stereospecific, because the first enzyme, branched-chain acyl-CoA oxidase, can only handle (2S)-substrates. For this reason, another enzyme called alpha-methylacyl-CoA racemase is also involved in the beta-oxidation of branched-chain fatty acids. This enzyme is able to convert (2R)-pristanoyl-CoA, (25R)-DHC-CoA and (25R)-THC-CoA into their (S)-stereoisomers. This conversion is essential for degradation of these substrates, because in case of DHCA and THCA only the (25R)-stereoisomers are produced from cholesterol.
Methods: HPLC/tandem mass spectrometry was used to analyze the C27-bile acid intermediates in plasma from patients suffering from various diseases.
Results and discussion: We analyzed the C27-bile acid intermediates accumulating in plasma from patients with racemase deficiency (n=3), Zellweger syndrome (n=4), and adults with cholestatic liver disease (n=6). In racemase deficiency we detected an exclusive accumulation of free and taurine conjugated (25R)-THCA and free (25R)-DHCA, whereas in Zellweger syndrome and cholestatic liver disease the S/R-ratio was between 0.3-0.5 for free THCA, taurine conjugated THCA and free DHCA.
Conclusion: We have developed an easy and reliable method to diagnose alpha-methylacyl-CoA racemase deficient patients by plasma analysis.

97. Abnormal cardiolipin and phosphatidylglycerol remodeling in Barth syndrome

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Introduction: Barth syndrome (MIM 302060) is an X-linked disorder characterized by a cardioskeletal myopathy, intermittent neutropenia, 3-methylglutaconic aciduria, mophologically abnormal mitochondia and variable respiratory chain dysfunctions. The gene responsible for Barth syndrome has been cloned (G4.5) and shares homology with a family of acyltransferases, suggesting a role in phospholipid metabolism.
Methods: Skin fibroblasts from control subjects, patients affected by Barth syndrome and patients with other genetic diseases were cultured according to standard conditions followed by addition of radiolabelled linoleic acid and analysis of the incorporation of radioactivity into PG and PL using thin-layer chromatography.
Results and discussion: Using cultured skin fibroblasts from six patients with five different mutations in the G4.5 gene, we show that the incorporation of linoleic acid into phosphatidylglycerol (PG) and cardiolipin (CL) is dramatically reduced, whereas the incorporation of saturated and monounsaturated fatty acids in these phospholipids is normal. The deficient incorporation of linoleic acid into PG was not observed in normal controls and in patients with documented respiratory chain defects or cardiomyopathies of unknown origin, implicating that this abnormality is specific for Barth syndrome and not secondary to mitochondrial dysfunction. Linoleic acid incorporation into PG and CL is the first specific biochemical test which discriminates Barth syndrome from clinically or biochemically similar defects and suggests a role for the G4.5 geneproduct in PG and CL remodeling.
Conclusion: We conclude that the abnormal acyl composition of PG and CL might explain the mitochondrial dysfunction observed in Barth syndrome.

98. Detection of a severe case of tyrosine deficiency by a diagnostic urinary neurotransmitter metabolite pattern

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Inleiding: Tyrosine hydroxylase (TH) deficiency is an inherited defect in catecholamine synthesis. The enzyme is expressed in brain and adrenal medulla. Treatment with L-DOPA usually leads to spectacular clinical improvement. Diagnosis relies on a characteristic pattern of biogenic amine metabolites in the CSF, while urinary metabolite patterns are usually normal or at least not typically abnormal. Here we describe a boy of 18 months who presented with oculogyric crises, severe motor retardation, truncal hypotonia and peripheral hypertonia.
Methods: HPLC analysis of biogenic amines and metabolites with fluorometric and electrochemical detection.
Results and discussion: Urinary excretion of catecholamines and metabolites was severely decreased, while serotonin and 5-HIAA were excreted in normal amounts. This was highly indicative for TH deficiency and the diagnosis was confirmed by CSF analysis and establishment of a new homozygous mutation in the TH gene. L-dopa treatment however was unsuccessful. The severity of the clinical presentation and unresponsiveness to L-DOPA treatment suggests an atypical form of TH deficiency and raises serious questions about the pathophysiology of this disorder. The finding of urinary in addition to CSF metabolic abnormalities might be related to this variant.
Conclusion: This case shows the importance of metabolite analyses in urine of TH deficient patients, not only as a diagnostic tool, but also to provide further insight in the pathophysiology of this still poorly understood disorder.

99. Unusual biochemical and clinical presentation of severe biotinidase deficiency

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Introduction: Biotinidase deficiency interrupts the biotin cycle, which leads to biotin depletion and Multiple Carboxylase Deficiency. This results in accumulation of many carboxylase substrates in the body fluids and a serious clinical picture characterized by neurological symptoms, skin rashes and alopecia. Early diagnosis and biotin treatment normally leads to spectacular recovery and good prognosis. Therefore biotinidase deficiency is included in some neonatal screening programs.
Here we present a 5-year-old girl of non-consanguinous parents who was admitted for metabolic investigations because of severe autistiform psychomotor retardation, convulsive disorder and choreo-athetosis, spasms and hemiparesis. The child had developed normally until the age of 3.5 years.
Methods: GC-MS for organic acids; ESI-tandem MS for acylcarnitines; spectrophotometric technique for biotinidase measurement.
Results and discussion: Metabolite profiles were unremarkable, except for a slightly elevated urinary 3-OH-isovaleric acid excretion and a high-normal plasma 3-OH-isovalerylcarnitine. Because of the possibility of partial biotinidase deficiency, mainly based on the unexplained convulsive disorder, plasma biotinidase activity was measured and, surprisingly, revealed a deficiency (1.6 % of mean normal activity). The absence of the characteristic biochemical abnormalities in this severe and atypical case is as yet unexplained, but raises doubts about the reliability of metabolite-based neonatal screening programs for biotinidase deficiency.
Conclusion: biotinidase activity measurements should be included in general metabolic work-up of patients with neurological disorders and in neonatal screening programs.

100. Human complex I defects can be resolved by monoclonal antibody analysis into distinct subunit assembly patterns

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Introduction: Isolated enzymatic deficiency of the first oxidative phosphorylation complex, complex I, is most frequent entcountered among oxidative phosphorylation disorders. The total outcome of an extensive mutational analysis study in 20 complex I-deficient patients revealed in about 35 % mutations in complex I structural genes. In addition to genetic analysis, it is important to have protein-based approaches for detecting and further characterizing of complex I deficiencies. We present Western blot data to aid in diagnosis and understanding of Complex I deficiencies.
Methods: Mitochondrial enriched fibroblast fractions of a total of 11 different patients were examined. Four patients had undefined Complex I defects, while the other patients had defects in individual structural complex I genes (NDUFV1, NDUFS2 (2 patients), NDUFS4 (2 patients), NDUFS7 and NDUFS8). Antibodies were generated by immunizing mice with purified bovine Complex I or recombinant human proteins. Western blotting using different antibodies against the individual respiratory chain complexes and a set of monoclonal antibodies which react with 39, 30, 20, 18, 15, and 8 kD subunits of Complex I were used. Sucrose gradient centrifugation were used to distinguish catalytic versus assembly defects.
Results and discussion: Quantified Western blot data, displayed that in most of the patients cell lines the levels of the 39 kD subunit of Complex I, but not that of any of the other OXPHOS subunits probed, were reduced. A significant reduction in the levels of one or more components of the complex was seen in all patient samples; except in the patient with a mutation in NDUFV1. Some patients, which have unidentified mutations, show remarkable similarity in the pattern of subunit loss, suggesting that one unidentified gene is involved in the underlying defect. Sucrose gradient centrifugation showed that the 39 kD and 20 kD subunits of most patients elute at a similar position to that of control Complex I (900 kD), indicative of complete or near complete assembly. However, in one patient, those subunits migrate in subcomplexes of around 200 kD and 500 kD respectively, indicating that assembly of Complex I is poor.
Conclusion: Western blot experiments to characterize respiratory chain defects by complex, or complexes involved, is relatively rapid, easily performed and requires much less sample than enzymatic assays. The comparison of subunit profiles as shown here allows patients to be sorted as in genetic complementation studies so that with wider screening of patients, a group of possible Complex I assembly factor mutants can be collected for chromosomal analysis and gene identification.

Literature

1. Triepels RH, Hanson BJ, Heuvel LPWJ van den, Sundell L, Marusich MF, Smeitink JAM, Capaldi RA. Human complex I defects can be resolved by monoclonal antibody analysis into distinct subunit assembly patterns. J Biol Chem; in press.

101. Carnitine biosynthesis in man

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Introduction: Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is a vital compound, which plays an indispensable role in the transport of activated fatty acids across the inner mitochondrial membrane into the matrix, where beta-oxidation takes place. Furthermore, carnitine is involved in the transfer of the products of peroxisomal beta-oxidation, including acetyl-CoA, to the mitochondria for oxidation to CO2 and H2O in the Krebs cycle. Many organisms, ranging from bacteria to mammals, are able to synthesize carnitine. In man, carnitine is synthesized in kidney, liver and presumably in brain, from the essential amino acids lysine and methionine.
Protein-bound lysine is trimethylated on its gamma-amino-group by a protein-dependent methyl transferase using S-adenosylmethionine as the methyl donor. Upon degradation of these proteins, ypsilon-N-trimethyllysine is made available for carnitine biosynthesis. The released gamma-N-trimethyllysine is hydroxylated at the 3-position by ypsilon-N-trimethyllysine hydroxylase, the first enzyme in the pathway. Beta-Hydroxy-ypsilon-N-trimethyllysine aldolase catalyses the cleavage of beta-hydroxy-ypsilon-N-trimethyllysine into gamma-trimethylaminobutyraldehyde and glycine. Subsequently, the aldehyde is oxidized by gamma-trimethylaminobutyraldehyde dehydrogenase yielding gamma-butyrobetaine. Finally, gamma-butyrobetaine is hydroxylated at the 3-position by gamma-butyrobetaine hydroxylase to form carnitine.
The aim of our studies is to identify all four enzymes of the carnitine biosynthesis at the molecular level to investigate whether a defect in carnitine biosynthesis can be disease-causing.
Methods: Using a variety of methods we have set up assays for each individual enzyme with the aim to purify the proteins and sequence/identify the purified proteins either by Edman degradation or by using MALDI-TOF/Q-TOF Mass Spectrometry. The peptides sequences are then used to screen the online databases to identify the corresponding cDNA/gene.
Results and discussion: With this strategy we so far have identified two of the four enzymes, gamma-trimethylaminobutyraldehyde dehydrogenase and gamma-butyrobetaine hydroxylase, catalyzing the penultimate and ultimate step, respectively. We recently also identified the first enzyme in the pathway: ypsilon-N-trimethyllysine hydroxylase. The ypsilon-N-trimethyllysine hydroxylase gene is localized on chromosome X and we currently are investigating whether defects in this gene may be responsible for disorders mapped to this chromosome region.
Conclusion: We have made substantial progress with respect to the full enzymatic and molecular characterization of the enzymes involved in carnitine biosynthesis and will use this information to identify patients with a defect in one of these enzymes.

102. Biochemical and molecular diagnosis of isoprenoid biosynthesis defects: mevalonic aciduria and hyper-IgD and periodic fever syndrome

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Introduction: Mevalonic aciduria (MA) and hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS) are two autosomal recessive inherited disorders both caused by a deficient activity of the enzyme mevalonate kinase (MK) resulting from mutations in the encoding MVK gene. MA is a severe multisystemic disorder, characterized by psychomotor retardation, failure to thrive, hepatosplenomegaly, anaemia and recurrent febrile crises. HIDS is a relative benign condition, in which patients suffer, as in MA, from recurrent fever episodes associated with lymphadenopathy, arthralgia, gastrointestinal problems and skin rash. Thus far, disease-causing mutations could only be detected by analysis of MVK cDNA, however, we recently resolved the genomic structure of the MVK gene. This enables mutation analysis at the genome level.
Methods: Using standard molecular techniques we have set up methods to allow amplification of each individual exon making us of specific primers devised on the basis of the gene sequence.
Results and discussion: We here report the biochemical and molecular characterization of 27 patients with an MK deficiency. In total, we identified 15 different mutations of which 9 were not reported before. Furthermore, the sequence analysis confirmed all previously reported genotypes based on cDNA analysis. Comparison of the genotypes with the corresponding MK enzyme activities provides new insights in the genotype/phenotype relation between HIDS and MA.
Conclusion: We have resolved the genomic structure of the mevalonate kinase gene which will be of great help in deciphering the molecular basis of mevalonic aciduria and hyper-IgD and periodic fever syndrome.

103. Molecular basis of the peroxisome biogenesis disorders: Identification of the mutant PEX-gene by means of complementation analysis in 207 patients followed by mutation analysis mains

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Introduction: The disorders of peroxisome biogenesis (PBDs) include Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), infantile Refsum disease (IRD) and a number of phenotypic variants in between ZS, NALD and IRD. Earlier studies have shown that there is profound genetic heterogeneity within the PBD-group. We have now extended these studies and have performed complementation analysis in 207 PBD-patients. In all patient' cell lines detailed studies were done including global assays like de novo plasmalogen biosynthesis, C26:0 and pristanic acid Beta-oxidation and phytanic acid alpha-oxidation followed by individual enzyme activity measurements, and immunoblot analysis plus immunofluorescence microscopy using antibodies against catalase and the ALD-protein.
Methods: Skin fibroblasts were cultured and used for complementation analysis which involves fusion of the cells by means of polyethylene glycol. After 3 days in culture the heterokaryons were inspected for the presence or absence of peroxisomes by means of catalase immunofluorescence microscopy.
Results and discussion: Our results further show strong overrepresentation of one particular complementation group representing PEX1-deficiency. We show that 136 of the 207 (66%) patients we analyzed belong to this group. Thanks to the successful use of yeast mutants disturbed in peroxisome biogenesis enormous progress has been made with respect to the identity of the genes underlying each of these complementation groups with the result that 11 of the 12 so-called PEX-genes are known now.
Conclusion: Our results show that the group of peroxisome biogenesis disorders is extremely heterogeneous with at least 12 distinct genetic groups with one group being most frequent.We have used this increased knowledge to resolve the molecular defect in patients belonging to the various complementation groups.

104. Mutations in the gene encoding peroxisomal alpha-methylacyl-coa racemase cause adult-onset sensory motor neuropathy

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Introduction: We present a new peroxisomal disorder due to alpha-methylacyl-CoA racemase deficiency in three patients, two of whom suffered from adult-onset sensory motor neuropathy. In addition, one (male) patient had pigmentary retinopathy suggesting Refsum disease whereas the other (female) patient had upper motor neuron signs in the legs suggesting X-ALD. Full analysis of peroxisomal metabolites in plasma revealed a similar pattern including elevated pristanic acid and di- and trihydroxycholestanoic levels but normal very-long-chain fatty acids pointing to a defect in the peroxisomal beta-oxidation of 2-methyl branched-chain fatty acids. Studies in fibroblasts revealed reduced pristanic acid beta-oxidation but normal activities for branched-chain acyl-CoA oxidase, D-bifunctional enzyme and branched-chain thiolase. These puzzling results prompted us to focus on the enzyme alpha-methylacyl-CoA racemase which converts (2R)-pristanoyl-CoA, (2R)-DHC-CoA and (2R) THC-CoA into their (2S)-isomers which are the true substrates for beta-oxidation.
Methods: 2-Methylacyl-CoA racemase activity was measured using a newly devised method based on the ability of the enzyme to convert (2R)-THC-CoA into (2S)-THC-CoA and their resolution by HPLC. Standard molecular techniques were used to clone the human cDNA and perform mutation analysis.
Results and discussion: We first set up a reliable assay for racemase in fibroblasts and found a full deficiency in all patients. We subsequently cloned the human racemase cDNA

and identified clear-cut mutations. Expression studies in E.coli showed that these mutations completely inactivate the enzyme (Ferdinandusse et al. (2000), Nat. Genet. 24, 188-191).
Conclusion: The identification of 2-methylacyl-CoA racemase deficiency in patients with adult-onset sensory motor neuropathy has major implications for the diagnosis and subsequent treatment of adult-onset neuropathies of unknown origin.

105. Biochemical and molecular diagnosis of cholesterol synthesis defects: Smith-Lemli-Opitz syndrome

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Introduction: Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive developmental disorder characterized by facial dysmorphism, mental retardation and multiple congenital anomalies. The disorder is caused by a deficient activity of 7-dehydrocholesterol reductase (7-DHCR), the enzyme catalyzing the final step in cholesterol synthesis, which is the conversion of 7-dehydrocholesterol (7-DHC) into cholesterol. As a consequence, most patients have low serum cholesterol and elevated 7-DHC (and 8-DHC) levels.
Methods: Our laboratory has developed a complete package for post- and prenatal diagnosis of SLOS both at the biochemical and the molecular level, including serum analysis of sterol and bile acids by GC-MS, analysis of de novo cholesterol synthesis in fibroblasts of patients by 14C-mevalonate incorporation, measurement of specific 7-DHCR enzyme activity in cell lysates by ergosterol conversion and mutation analysis of the DHCR7 gene to detect the disease-causing mutations. Finally, we have generated 7-DHCR-specific antibodies to study the effect of the various mutations on the protein by immunoblot analysis.
Results and discussion: Currently, we have performed postnatal analysis in 32 patients clinically diagnosed with SLOS and performed 4 prenatal analyses. So far, 27 different mutations have been identified which when compared with the corresponding 7-DHCR enzyme activities provide insight into the genotype-phenotype relationship of SLOS.
Conclusion: We have made substantial progress in the biochemical and molecular diagnosis of SLOS which is of importance for the correct pre- and postnatal diagnosis of this syndrome.

106. Biochemical and molecular diagnosis of cholesterol synthesis defects; X-linked dominant chondrodysplasia punctata (CDPX2)

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Introduction: X-linked dominant chondrodysplasia punctata (CDPX2), also known as Conradi-Hunermann-Happle syndrome, is a male-lethal disorder characterized by asymmetric rhizomesomelia (shortening of limbs), hyperkeratosis and ichthyosis. Recently, it was demonstrated that CDPX2 is caused by a deficient activity of sterol Delta8-Delta7 isomerase, one of the enzymes involved in cholesterol biosynthesis. As a consequence, abnormal amounts of the sterol intermediates cholest-8(9)-en-3bèta-ol and cholesta-5,8-dien-3bèta-ol (8-DHC) can be found in cultured fibroblasts of patients.
Methods: A complete package has been developed for the postnatal diagnosis of CDPX2 both at the biochemical and the molecular level, including serum sterol analysis by GC-MS, analysis of sterol synthesis in patient's skin fibroblasts grown in lipoprotein-depleted medium and mutation analysis of the EBP gene to detect disease-causing mutations.
Results and discussion: Currently, our laboratory has confirmed the clinical diagnosis of CDPX2 in 10 suspected patients both at the biochemical and molecular level. Biochemically, serum of most patients contained readily detectable elevated levels of cholest-8(9)-en-3bèta-ol. Both sterols were also clearly detected in fibroblast cultures of patients grown in sterol-free medium. Mutation analysis of the EBP gene, which codes for the sterol Delta8-Delta7 isomerase, revealed different mutations. In a few patients the mutations were of maternal origin but most mutations were de novo in agreement to the sporadic nature of CDPX2.
Conclusion: We have made great progress with respect to the biochemical and molecular diagnosis of X-linked dominant chondrodysplasia punctata (CDPX2) which will be of great help in the actual diagnosis of patients.

107. Cholesterol biosynthesis in human PEX5 fibroblasts and PEX5 mouse livers

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Introduction: The cerebro-hepato-renal syndrome of Zellweger is a fatal inherited disease caused by deficient import of peroxisomal matrix proteins. The pathogenic mechanisms leading to extreme hypotonia, severe mental retardation and early death are unknown but these clinical abnormalities include ones also frequently observed among patients with inborn errors of cholesterol biosynthesis. In addition Zellweger patients show hypocholesterolemia. Cholesterol has long been recognized as an essential component of mammalian cell membranes and myelin, and as a precursor for steroid hormones and bile acids. In addition, a novel crucial role for cholesterol in mammalian embryonic development has been discovered recently. For a long time, it has been assumed that the enzyme reactions of the isoprenoid pathway, which produces cholesterol, take place in the cytosol or the endoplasmic reticulum, but evidence is now accumulating that at least some steps of the pathway occur in peroxisomes. Additional support for a peroxisomal localization comes from the observation that both mevalonate kinase and phosphomevalonate kinase contain consensus peroxisomal targeting sequences. Proteins containing such sequences can be imported into peroxisomes via specific import receptor proteins.
Zellweger patients of complementation group 2 have mutations in their pex5 gene that encodes the import receptor for

the majority of peroxisomal matrix enzymes. As a consequence most enzymes become mislocalized to the cytosol, often resulting in degradation and/or inactivation.
Methods: In search for additional evidence that cholesterol biosynthesis partly occurs in peroxisomes, we have measured the activities of enzymes of the isoprenoid pathway in fibroblasts of pex5 patients and in livers of pex5 Zellweger mouse model.
Results and discussion: In human fibroblasts no significant differences were observed in enzyme activities. In livers of pex5 mice, enzyme activities were slightly elevated when compared to controls.
Conclusion: No indication of lower cholesterol biosynthesis in fibroblasts of pex5 patients or in livers of pex5 Zellweger mice has been observed. If cholesterol biosynthesis (partly) takes place in peroxisomes, mislocalization of the enzymes involved does not lead to a decrease in enzyme activities

108. Moderate citrullinemia without hyperammonemia in a child with mutated and deficient argininosuccinate synthetase

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Introduction: Most patients with a defective urea cycle show hyperammonemia, at least after protein intake. We present a young patient with an argininosuccinate synthetase (ASS) deficiency without obvious ammonia elevations.
Methods: Clincal examination of the 4-months-old girl from non-consanquineous Turkish parents revealed feeding problems, restlessness, microcephaly and left-sided hypertonia. Right-sided hemiatrophy and temporoparietal brain wasting were seen with brain MRI. EEG showed hypofunctional and moderate irritative activities.
Metabolic screening was performed using standardized methods. Methodology of in vitro NMR, enzyme studies, and mutation analysis has been described previously.
Results and discussion: A moderate increased blood level of citrulline was observed (200-350 microM) and an elevated urinary excretion of citrulline (600-900 micromol/mmol creat). No other abnormalities in amino or organic acids or purines/pyrimidines were seen. Blood ammonia content was normal even after a meal and protein loading. Allopurinol administration did not result in generation of orotic acid. In vitro NMR revealed the presence of a substantial amount of N-acetylcitrulline in urine.
The ASS activity was below detection limit in patient's fibroblasts.Sequencing of cDNA demonstrated the hitherto unknown G1,085T point mutation in exon 14 of the ASS gene, which could be confirmed in genomic DNA.
Conclusion: The findings in this patient clearly underline that moderate citrulline increase in blood and urine can form an important indication for ASS deficiency. Rather characteristic features for urea cycle defects like hyperammonemia and hyperglutaminemia can be absent even under stressed conditions. Also the phenotype can be little characteristic in ASS deficiency.

109. Confirmation of the enzyme defect in the first case of ureidopropionase deficiency

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Introduction: In man, the pyrimidine bases uracil and thymine are degraded via a three-step pathway leading to the synthesis of ß-alanine and ß-aminoisobutyric acid, respectively. A number of patients have been described with defects of the first two enzymes of the pyrimidine degradation pathway presenting with a considerable variable clinical phenotype including neurological problems. Very recently, the first patient with the putative defect of ß-ureidopropionase, the third enzyme of the pathway has been reported. Ureidopropionase (EC 3.5.1.6) catalyses the conversion of N-carbamyl-ß-alanine or N-carbamyl-ß-aminoisobutyric acid into ß-alanine or ß-aminoisobutyric acid, ammonia and CO2. This 17 month old girl presented with muscular hypotonia, dystonic movements and severe developmental delay.
Methods: Differential analysis of the dihydropyrimidine and N-carbamyl-Beta-amino acids in urine was performed by isolation of the compounds by cation and anion chromatography in different fractions followed by amino acid analysis before and after acid hydrolysis. The enzyme activity was measured with a radiochemical assay using radiolabeled N-carbamyl-ß-alanine. The reaction product 14CO2 was subsequently measured by liquid scintillation counting.
Results and discussion: Analysis of a urine sample revealed strongly elevated levels of N-carbamyl-ß-alanine and N-carbamyl-ß-aminoisobutyric acid. We had the opportunity to confirm the presumptive ureidopropionase deficiency by measuring the enzyme directly in liver tissue. In the patient's liver the ureidopropionase activity was undetectable low (< 0.05 nmol/mg/h). In 10 control liver samples the ureidopropionase activities ranged form from 36 to 165 nmol/mg/h with a mean activity of 69 ± 37 nmol/mg/h.
Conclusion: Our findings confirm for the first time that the affected patient suffers from a complete ureidopropionase deficiency.

Diversen

110. Evaluatie van heparine-gelbuizen

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Inleiding: De receptie van het LAKC is verantwoordelijk voor de pre-analytische bewerking van patiëntenmonsters die door het LAKC geanalyseerd worden als ook monsters die door diverse laboratoria binnen en buiten het AMC geanalyseerd worden. De belangrijkste bestemmingen van heparine-plasma zijn de routine klinische chemie en de speciële klinische chemie (beiden LAKC) en het Specieel Laboratorium voor Endocrinologie (SLVE). In het kader van vergaande automatisering van de pre-analytische bewerking van patiëntenmonsters is het LAKC genoodzaakt om van glazen heparine-bloedafnamebuizen over te gaan op kunststoffen heparine-bloedafnamebuizen met een scheidingslaag van gel (de zogenaamde heparine-gelbuizen). Er is in dit project onderzocht of de wijziging van buistype invloed heeft op de verkregen analyseresultaten van ondermeer ACE, CRP, haptoglobine, lipase, Mg (Hitachi 912); CKMB, digoxine, PSA (Bayer ImmunoI); TYBC, transferrine, vitamine A/B12/E, foliumzuur, Cu, Zn (diverse methodieken); ferritine, beta-HCG, FSH, LH, cortisol (DPC Immulite); en andere endocrinologische parameters bepaald met diverse methodieken (schildklierpakket, progesteron, testosteron, oestradiol, DHEA-sulfaat, SHBG, groeihormoon, prolactine, insuline).
Methoden: Per bepaling is er een patiëntenvergelijking (Passing & Pablok) uitgevoerd waarbij de resultaten verkregen met bloedplasma uit heparine-gelbuizen (y) vergeleken zijn met de resultaten verkregen met bloedplasma uit de traditionele glazen heparine-buizen (x). Bij het merendeel van de bepalingen was het mogelijk om de evaluatie uit te voeren zonder extra materiaal bij de patienten af te nemen.
Resultaten en discussie: Per bepaling zullen naast de analysemethode en analytische variatiecoëfficient de resultaten van de patiëntenvergelijking getoond worden. Enkele voorbeelden van de resultaten van patiëntenvergelijkingen zijn hieronder weergegeven:
CRP: y=0,988x+0,012, N=19, r=0,999, range=0-220 mg/l .
PSA: y=0,981x+0,014, N=13, r=1,000, range=0-40 µg/l .
TSH: y=x, N=29, r=0,998, range=0-5 mE/l .
Testosteron: y=0,958x+0,081, N=16, r=0,988, range=0-25 nmol/l.
LH: y=1,019x-0,022, N=16, r=0,998, range=0-45 E/l .
Conclusie: Voorlopig lijkt de overgang van glazen heparine-buizen naar plastic heparine-gelbuizen binnen het LAKC en SLVE voor wat de analyseresultaten betreft geen problemen op te leveren.

111. Evaluatie van de "point-of care" TAS analyser voor de bepaling van hirudine in plasma: vergelijking met een conventionele laboratoriummethode

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Inleiding: Hirudine is een eiwit uit het speeksel van de bloedzuiger, dat zeer krachtige anti-trombine werking bezit. Hirudine wordt momenteel in klinische studies onderzocht en hierbij wordt de concentratie van hirudine gecontroleerd, meestal met de ecarinetijd (ECT). Deze stollingstest is gebaseerd op het slangengif ecarine. De ECT kan gemeten worden met de TAS analyser, een point-of-care apparaat dat in volbloed diverse stollingsbepalingen kan uitvoeren. Het detecteert stolling met magnetiseerbare ijzerpartikels (1). Wij vergeleken de TAS-ECT met een conventionele ECT (2).
Methoden: De TAS-ECT (Cardiovascular Diagnostics Inc.; Raleigh, NC, USA) werd gemeten in citraat volbloed met Thrombin Inhibitor Management reagenskaartjes. De conventionele ECT werd in plasma gemeten volgens Nowak (2). Voor calibratie gebruikten wij PEG-hirudine (LU-87981; Knoll). De correlatie werd onderzocht met 145 monsters van patiënten met onstabiele angina pectoris die deelnamen aan een klinische studie.
Resultaten en discussie: De TAS-ECT was volkomen lineair met de hirudine concentratie tussen 0 en 3,0 µg/mL (r=0,9997). De intra-assay imprecisie (VC) van de TAS-ECT in bloed met hirudine (4,3%) was groter dan de conventionele ECT (0,8%). De inter-assay imprecisie (7,1%) was ook groter (2,4%). De variatie werd in beide methoden groter na conversie naar hirudine concentratie. De correlatie was evenwel uitstekend (r=0,956; 95% BI 0,939-0,968).
Conclusie: De precisie van de TAS-ECT is minder goed dan de conventionele ECT, maar de TAS analyser lijkt wel acceptabel. Naar onze mening verdient de conventionele ECT, ook al om financiële redenen, toch de voorkeur.

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112. Overzicht van de kwaliteitscontrole voor de meting van delta aminolevulinezuur, porfobilinogeen en porfyrines in urine door een twintigtal Nederlandse ziekenhuizen

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Inleiding: Voorlopers van de heemsynthese worden met de urine uitgescheiden. De meting hiervan wordt gebruikt om porfyrie aan te tonen. De externe kwaliteitscontrole van de SKZL in een twintigtal ziekenhuizen, kan een indruk geven van de nauwkeurigheid van die metingen.
Methoden: De gegevens van de SKZL enquetes over 1996 t/m begin 2000 voor ALA, PBG, totaal porfyrines, coproporfyrine en uroporfyrine zijn verzameld. De variatiecoëfficiënten (VC) per enquete over de deelnemende labs zijn berekend en worden grafisch weergegeven in relatie tot de concentratie en in verloop van de tijd.
Resultaten en discussie: In de concentratierange binnen het referentiegebied varieren de VC's voor ALA: 20% - 45%; PBG: 45% - 55%; totaal porfyrines: 20% - 45%; uroporfyrines: 20%

- 60% en coproporfyrines: 40% - 200%. In de concentratierange boven de referentiewaarde was dit voor ALA: 15% - 40%; PBG: 15% - 40%; totaalporfyrines: 20% - 60%; uroporfyrines 20% - 60%; coproporfyrines: 30% - 50%. Deze resultaten zijn teleurstellend. De VC's worden gedurende de periode dat dit onderzoek loopt niet lager. De verhouding van uro- en coproporfyrines per urine analyse tussen de labs is ook erg variabel. VC's varieren van 26% - 67%. Dit wijst op grote variatie in uitvoering van de HPLC techniek en/of ontbreken van juiste calibratie van de betreffende standaarden.
Conclusie: Het is noodzakelijk om bij voorkeur via de SKZL, commuteerbare calibratoren te verstrekken om lagere VC's te bereiken en daardoor de kwaliteit van de porfyrine diagnostiek te verbeteren.

113. Commutability Assessment of Candidate Calibrators using a split-patient-sample between-field laboratory design; a modification on the NCCLS EP14-P protocol

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Introduction: The growing importance of accuracy and harmonization of results in clinical chemistry has led to the launching of the project "Calibration 2000". This harmonization can be achieved by using commutable calibrators.The official NCCLS EP14-P protocol for studying commutability of test sera requires the analysis of fresh patient samples together with the test sera both by a field method and a comparative reference method. This is cumbersome and costly, especially when several field methods have to be studied. A practical modification is presented: the so-called Twin-study, to be illustrated with the study of calibrators for HDL-cholesterol.
Methods: Laboratories were selected on the basis of dissimilar methods and analysed selected patient sera together with the candidate calibrators by their own field method. 84 laboratories were included. The protocol consisted of the exchange of 12 fresh patient sera between each two laboratories. After splitting these samples, one half of the samples was sent to the other laboratory that, in turn, proceeded likewise for their patient specimens. All patient samples were then analysed again, together with the candidate calibrators.
Results and discussion: 42 datasets from all lab couples were analysed by plotting the results of lab 1 on the X-axis and lab 2 on the Y-axis. The distance of the location of the calibrator to the regression line through the patient data divided by the scatter of the patient data around the line is called the normalized residual. A test serum was considered commutable when its normalized residual is less than 3.
Based on the cumulated results of all participating laboratories a scatterplot with the normalized residuals was constructed. This permits an objective choice for a future commutable calibrator material.
Conclusion: The Twin-study design is a practical modification of the more stringent NCCLS protocol to study matrix effects and commutability of sera to be used as possible calibrators.

114. Ethanol on the Vitros-700 analyzer

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Introduction: Alcohol is a possible cause of coma of unknown etiology and alcohol intoxication may mimic diabetic coma, cerebral trauma and drug overdose. Since an intracranial trauma as a cause of unconsciousness requires immediate surgical intervention, it is important to assess the role of alcohol without delay.
Methods: We evaluated the determination of ethanol on the Vitros-700 analyzer (Ortho Clinical Diagnostics). This determination uses a dry, multilayered, analyticial slide. Ethanol in the sample is oxidized to acetaldehyde by alcohol dehydrogenase. The coenzyme NAD+ is reduced to NADH. The acetaldehyde is trapped in the TRIS buffer in the reaction layer to drive the reaction to completion. The concentration of ethanol is determined by the increase of the NADH concentration at 340 nm after a 5 minute incubation at 37°C. The analysis was compared with a gas-chromatographic method (Hewlett Packard 5890 Series II). 40 patient serum samples were used to compare the two methods using Passing and Bablok regression analysis. Reproducibility was assessed by measuring two control samples 20 times.
Results and discussion: A good correlation was found between the Vitros and gas chromatography: correlation coefficient 0.99, intercept -0.021 (µg/l) (95%CI -0.104 - 0.057), slope 0.97 (95%CI 0.93-1.00), Syx was 0.082, (n=105). No statistically significant difference was found among ethanol-results obtained with the two methods. The bias between Vitros and gas chromatography was –0.17 µg/l (agreement limits –0.80 to 0.46 µg/l). The mean within run CV was 1.2%. The analytical recovery was 93-105% for the Vitros method and 99-112% for gas chromatography.
Conclusion: In conclusion, the Vitros offers a fast determination of the ethanol concentration with acceptable intra-assay variation and good correlation with gas chromatography.

115. Can the oral methionine-loading test be shortened?

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Introduction: Hyperhomocysteinemia is defined as an elevated fasting plasma homocysteine and/or elevated plasma homocysteine 6 hours after oral methionine loading. Logistic problems limit the use of the methionine-loading test. We validated whether a shorter methionine-loading test is as accurate as the original 6-h test in identifying hyperhomocysteinemic patients.
Methods: Plasma homocysteine was determined in EDTA-blood from women with a history of pre-eclampsia (n=39, of which 39% were homozygous for the thermolabile MTHFR C677T variant) after 12 hours fasting and 3 and 6 hours after an oral methionine load (0.1 g/kg body weight).
Results and discussion: Correlation between 3-h and 6-h plasma concentration of postload and delta (postload minus

the fasting value) homocysteine was good: for both parameters, 3-h plasma homocysteine levels accounted for 85% of the variability in 6-h plasma homocysteine levels. Linear regression line equations were y = 0.7x + 6.1 for postload homocysteine and y = 0.6x + 3.6 for delta homocysteine.
Conclusion: This study shows that the 3-h methionine-loading test can accurately identify hyperhomocysteinemic patients, in line with a previous study (1). A shorter methionine-loading test (3 h in stead of 6 h) may facilitate its use in clinical diagnosis and epidemiological studies.

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116. INK-zelfevaluatie op basis van het eigen CCKL-kwaliteitshandboek

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Inleiding: Binnen de Nederlandse medische laboratoria werken CCKL (Coördinatie Commissie ter bevordering van de Kwaliteitsbeheersing van het Laboratoriumonderzoek op het gebied van de Gezondheidszorg), INK (Instituut Nederlandse Kwaliteit) en tot voor kort NIAZ (Nederlands Instituut voor de Accreditatie van Ziekenhuizen) naast elkaar. De CCKL-accreditatie wordt nu door het NIAZ overgenomen en beide systemen zijn derhalve geharmoniseerd. Het CCKL-kwaliteitssysteem is verankerd in de 15 hoofdstukken van de accreditatiegids, gerangschikt volgens de inzichten van de beroepsgroepen. Het INK-managementmodel, gebaseerd op het EFQM-model (European Foundation for Quality Management), beschrijft middels een negental items de laboratoriumprocesgang en de interactie met de interne en externe omgeving. Deze benadering is toepasbaar op ieder soort organisatie of onderdeel daarvan.
Methoden: De CCKL-accreditatierichtlijn werd nauwgezet vertaald naar het INK-scoringssysteem, dit leverde 115 items op. Vervolgens evalueerden een drietal laboratoria zichzelf o.b.v. het kwaliteitshandboek en de nodige aanvullende informatie.
Resultaten en discussie: Naast het CCKL-kwaliteitshandboek was er aanvullende informatie nodig voor de INK-zelfevaluatie. Het CCKL-model behandelt voornamelijk de kwaliteit van analytische en daarmee rechtstreeks verbonden processen. Andere aspecten zoals beleid, financiën, personeel, klanten, maatschappij e.d. komen minder aan bod. Het INK-model daarentegen beschrijft het hele laboratorium en de omgeving waarmee het interageert. Bovendien wordt het proces van continue verbetering op alle aspecten van het laboratorium sterk benadrukt in het INK-model.
Conclusie: De INK-zelfevaluatie en nadien eventueel de INK-accreditatie is het logische vervolg op de CCKL-accreditatie. Het natuurlijk aanzetten tot verbetercycli, objectivering van het kwaliteitsniveau en benchmarking zijn zaken die als vanzelf worden bereikt door het uitvoeren van de INK-zelfevaluatie. Deze gestandaardiseerde vergelijking levert een transparantie op die voor de beroepsgroep kwaliteitsverbeterend kan werken. Vergelijking op basis van dezelfde aspecten, maar dan samengevat als indicatoren is een logische vervolgstap.

117. Eerste ervaringen met Point Of Care Testing op de NICU in Zwolle (tevens voordracht)

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Inleiding: De toenemende intensivering van zorg op de intensive care vraagt om een steeds snellere beschikbaarheid van resultaten van laboratorium diagnostiek. Op de neonatale intensive care (NICU) van de Isala klinieken in Zwolle wordt sinds 1 november 2000 ervaring opgedaan met een decentraal geplaatste analyser voor de analyse van bloedgassen, glucoses en electrolieten (ABLTM735, Radiometer). De afnames en analyse van bloedmonsters worden verricht door daartoe opgeleide en bevoegd verklaarde verpleegkundigen. De resultaten zijn direkt beschikbaar voor de NICU. Doordat de analyser op de NICU identiek is aan de analyser op het centrale laboratorium zijn de analisten van het laboratorium blijvend in staat om de noodzakelijke ondersteuning te bieden bij problemen. Dit wordt nog vereenvoudigd doordat beide analysers zijn verbonden met een centraal draaiend softwarepakket (Radiance-TM) voor besturing en troubleshouting op afstand en door een volautomatische module voor de regelmatige kwaliteitscontroles op analyser op de NICU.
Methoden: In de index maand februari 2001 (drie maanden na de introductie van point of care testing op de NICU) vindt systematische analyse plaats van het aantal analyses (bloedgassen, glucose, electrolieten) per patiënt. Deze gegevens worden vergeleken met identieke produktiecijfers uit februari 2000.
Resultaten en discussie: De hypothese wordt getoetst dat decentraal testen op de neonatale intensive care een afname van het aantal analyses op een per patiënt basis tot gevolg heeft en secundair hieraan een vermindering van het aantal bloedtransfusies.
Conclusie:. De subjectieve ervaringen van artsen en verplegend personeel op de NICU zijn overwegend positief. De nieuwe werkwijze laat meer ruimte om laboratoriumdiagnostiek te verrichten op klinische indicatie waardoor het aantal routinematige analyses kan worden beperkt.

118. Patiëntenstromen bij de poliklinische bloedafname in het AMC

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Inleiding: Het patiëntenaanbod bij de poliklinische bloedafname in het AMC fluctueert sterk. Daardoor variëren de wachttijden voor bloedafname voor patiënten aanzienlijk. Om deze variatie te verkleinen kan enerzijds de personele bezetting worden geoptimaliseerd naar de patiëntenstroom. Anderszijds kan de patiëntenstroom zelf beïnvloed worden. Geruime tijd geleden is de personele bezetting al afgestemd op het patiëntenaanbod. In de huidige studie wordt de patiëntenstroom in kaart gebracht en gekeken of het mogelijk is ook deze te beïnvloeden.

Methoden: 375 patiënten voor bloedafname zijn gedurende 6 dagdelen geënquêteerd. Met de antwoorden zijn gegevens verzameld over: tijd van bloedafname, aanvragend arts, aanvragende afdeling, de aard van de aanvraag (cito of niet), het al dan niet nuchter zijn van de patiënt, tijdstip van het poliklinisch consult.
Resultaten en discussie: De meeste patiënten zijn afkomstig van poliklinieken uit het AMC (90,3%), de resterende patiënten van huisartsen. Van alle patiënten komt 67,8% vlak voor of na een consult en zal niet genegen zijn op een ander moment te komen. In de ochtend komt 18,7 % van de patiënten nuchter voor bloedafname; deze patiënten zijn gebonden aan de ochtenduren. Het percentage cito's is afhankelijk van het dagdeel, en dus afhankelijk van het type spreekuur en betreft 2 tot 12 % van de bezoekende patiënten.
Deze gegevens wijzen uit dat het potentieel beïnvloedbare deel van het patiëntenaanbod bestaat uit patiënten die niet gerelateerd aan een consult en/of niet nuchter komen (26%). Aan hen kan gevraagd worden op een ander moment te komen ten einde de variatie in wachttijd te verkleinen.
Conclusie: In deze studie is voor het eerst de patiëntenstroom bij de poliklinische bloedafname in het AMC in kaart gebracht. Het blijkt dat slechts een kwart van de patiëntenstroom potentieel beïnvloedbaar is. Momenteel wordt overleg gevoerd met poliklinieken hoe deze patiëntengroep bereikt kan worden.

119. Are systematic reviews systematic in laboratory medicine? (tevens voordracht)

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Introduction: In evidence based laboratory medicine, application and interpretation of diagnostic tests is based on current best evidence from scientific research. Systematic reviews are gradually replacing single studies as the highest level of evidence of effectiveness of diagnostic and therapeutic interventions. Unsystematic reviews mix opinions and evidence. A systematic review applies scientific methods for the search of the available scientific research and to minimise bias in retrieving, interpreting and summarising these results. As with any new method, there are concerns that the methodology is inappropriately applied. Diagnostic studies with methodological shortcomings may overestimate the accuracy of a diagnostic test. The Methods Working Group on Screening and Diagnostic Tests of the Cochrane Collaboration is updating existing guidelines on how to perform a systematic review.
Methods: All the systematic reviews in the field of clinical chemistry and laboratory haematology that could be identified in Medline, EMBASE and other literature databases up to december 1998, were evaluated.
Results and discussion: We studied 23 systematic reviews of diagnostic trials. These reviews shared the same basic methodology, however there was much variation in the methods applied. We observed differences in quality criteria for inclusion of primary studies and differences in the analysis of the factors that cause heterogeneity of the results, and in the summary statistics used to pool the data from the primary studies. The results of the primary studies were often heterogeneous which was partly due to design flaws in the primary studies, but was also inherent to the varying study designs in the diagnostic trials.
Conclusion: The evaluated 23 systematic reviews identify areas in the methods of systematic reviewing where consensus is lacking, such as quality rating of primary studies, analysis of heterogeneity between primary studies and pooling of data. A sound systematic review will do justice to the evidence in the primary studies. New guidelines both for reporting studies of diagnostic accuracy of medical tests and for performing systematic reviews will contribute to the quality of these studies.

120. Kalibratie 2000: een tweelingstudie van kandidaat kalibratoren voor bepaling van fibrinogeen, factor VIII, en antitrombine

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Inleiding: De voornaamste voorwaarde waaraan een kalibrator moet voldoen is het vertonen van hetzelfde gedrag als patientenmateriaal bij gebruik van verschillende meetmethoden (commuteerbaarheid). Het vaststellen van commuteerbaarheid wordt gedaan met tweelingstudies. De Stichting Subcommissie Stolling van de CCKL vormt een task force "stolling" in het project Kalibratie 2000.
Methoden: Door de task force werden verschillende kandidaat kalibratoren geselecteerd voor fibrinogeen (3 gevriesdroogde materialen), voor factor VIII (1 diepgevroren en 2 gevriesdroogde materialen), en voor antitrombine (1 diepgevroren en 2 gevriesdroogde materialen). Deze materialen werden gezonden aan 36, 20, en 30 laboratoria voor bepaling van fibrinogeen, factor VIII, en antitrombine. Deze laboratoria werden in paren aan elkaar gekoppeld, waarbij niet de bepalingstechnieken maar de geografische ligging bepalend waren. Ieder paar werd verzocht minstens 30 citraatplasma's van patienten te verzamelen, verdeeld over de bepalingsrange. Ieder plasma werd in twee porties ingevroren. Na uitwisseling van de ingevroren plasma's tussen de partnerlaboratoria werden alle plasma's in drie runs geanalyseerd. In iedere run werden tevens de kandidaat kalibratoren geanalyseerd.
Resultaten en discussie: Voor ieder paar werden regressielijnen van de uitslagen van de patientenplasma's berekend. In het algemeen was er aanzienlijke spreiding van de individuele uitslagen rond de regressielijnen. De positie van de kandidaat kalibratoren ten opzichte van de regressielijnen was bevredigend: de absolute afwijking gedeeld door de spreiding was in alle gevallen kleiner dan 2. Dit houdt in dat de kandidaat kalibratoren commuteerbaar zijn. De tussen-lab variatiecoefficienten van de uitslagen van de kandidaat materialen waren: 7,4, 7,2, en 10,9 % (fibrinogeen); 10,0, 12,3, en 19,4 % (factor VIII); 7,2, 6,6, en 14,0 % (antitrombine).
Conclusie: De onderzochte kandidaat kalibratoren zijn commuteerbaar.

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121. The use of prostate-specific antigen (PsA) and other kallikreins in the early diagnosis of prostate cancer (tevens voordracht)

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Introduction: Prostate specific antigen (PsA) is considered to be the best and most widely used tumor marker in urology today. There are, however, still several questions to be resolved. Among other items, these are related to the tissue specificity, the predictive value after radical surgery, the development of assays for other members of the human kallikrein family, the use of PsA and in discriminating between benign and malignant disease, its value in population screening.
Methods: An overview of recent literature was prepared from which answers to some of the questions raised are extracted.
Results and discussion: PsA expression is not restricted to the prostate and the number of tissues found to express PsA now includes breast, endometrium, salivary glands, ovary, lung, renal carcinomas, adrenal adenoma and thyroid. The relative contribution of non-prostatic tissues to the plasma levels remains to be determined. For practical purposes, this is of potential relevance only when monitoring patients who succesfully underwent radical prostatectomy. The relevance of other members of the human kallikrein family which at a recent count consists of 14 members (1) is under investigation and some authors claim that assay of some of these, i.e. human glandular kallikrein 2 (hK2) or prostase (KLK-L1 protein, human kallkrein 4 or hK4) might contribute significantly to the discrimination between patients with benign and malignant prostatic tumours (2). The debate on screening for prostate cancer has not been finished. Arguments pro and con are easily found and a rational and generally accepted conclusion might never be reached. One author claims that introduction of wild screening with PsA has even eradicted “asymptomatic prostate cancer” leaving men who would otherwise live happy lives worrying about their PsA level (3).
Conclusion: The role of PsA in disease monitoring remains undisputed. For screening and/or case finding the answer to other questions, like whether PsA might enable discrimimation between dormant and actively developing prostate cancer, needs to be answered first. It is submitted that serial determinations, to determine the PsA velocity rather than single-point screening might be more effcient.

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