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Abstract

Av: Amani Abuwarda

Giardia lamblia, Cryptosporidium spp och *Entamöba histolytika* är anmälningspliktiga och smittsspårspliktiga parasiter. Därför är det mycket viktigt att kunna identifiera dem med en specifik och sensitiv metod som ger svar inom kort tid. Entamöba histolytika**,** Giardia lamblia och Cryptosporidium spp har diagnostiserats i många år genom mikroskopering. Mikroskopering är en gold standard metod för parasitidentifiering i faecesprover i den aktuella laboratorieverksamheten. Metoden kräver erfaren labbpersonal med stor kompetens och ger inte detaljerade resultat. Därför har vi valt att optimera en real-tids PCR metod som är mer sensitiv och ger mer specifik identifiering än den nuvarande detektionsmetoden, mikroskopering. Detta gjorde vi genom att använda humana faecesprover fixerade i formalin vilka tidigare har analyserats via mikroskopering och färska faecesprover. Faecesprover optimerades för DNA/RNA extraktion på en automatiserad extraktionsrobot och analyserades med real-tids PCR med specifika primrar och prober för Giardia lambila, Entamöba histolytika och Cryptosporidium spp. Totalt 79 faecesprover fixerade i formalin var positiva för *Giardia lambila* vid PCR, av dessa har 76 st påvisat *Giardia lamblia* vid mikroskopering. *Cryptosporidium spp* och *Entamöba histolytika* kunde inte detekteras i formalinprover men påvisades i färska faecesprover. Vid typning av *Cryptosporidium spp* påvisades fler *Cryptosporidium parvum* och *Cryptosporidium hominis* i färsk faeces, dock kunde inte alla *Cryptosporidium spp* prover typas med det nuvarande systemet. Dessa data tyder på att formalin-relaterad degradering skapar problem med vissa real-tids PCR system och färska prover är nödvändiga för säker detektion av parasiter i faeces med real-tids PCR. *Giardia lambila* är möjligt att detektera även i formalinfixerade faecesprover och här detekterar vi *Giardia* i prover där mikroskopering hittat en annan parasit. De *Cryptosporidium spp* och *Entamöba histolytika* systemen vi har utvecklad kräver färska faecesprover. Real-tids PCR är en alternativ metod till mikroskopering, då den detekterar med stor känslighet och specificitet och differentierar mellan olika subtyper.

Abstract

By: Maria Ahlqvist Mannesson

A septic infection with bacteremia is a severe condition that requires a fast and correct antibiotic treatment. *Enterobacteriaceae* is a family of gram-negative rod-shaped bacteria that are the most common pathogens that cause these infections. *Enterobacteriaceae* have in increased extent acquired extended spectrum beta-lactamases (ESBL) enzymes that makes them resistant towards cephalosporins, penicillins and in some cases carbapenemes. *Enterobacteriaceae* with ESBL creates great problems with raised mortality, extended length of stay and increased economic costs. The aim of this study was to compare two methods of susceptibility testing, a rapid direct disc diffusion test and the standard disc diffusion test and see how well the methods correspond. A special goal was even to examine how reliable the rapid direct disc diffusion test is to detect ESBL in *Enterobacteriaceae.* The material consisted of data from all positive blood cultures with *Enterobacteriaceae* from October in 2010 to February in 2015 from the department of clinical microbiology at the hospital in Skövde. The total data consisted of 10 302 unique antibiotic bacteria combinations. The result showed that the total agreement between the two methods was 94 %. Major discrepancies were seen in only 2%. Although the result shows good agreement between the methods the results fluctuates for the various antibiotics. The conclusion for this study is that there is a good agreement between the methods and that the rapid direct disc diffusion test is able to detect cefalosporin resistance in *Enterobacteriaceae* in positive blood cultures.

Variation of blood volume in blood culture and colonization of coagulase-negative staphylococci in the cubital fossa.

By: Sahar Ahmad

Bachelor thesis in Biomedical Laboratory Science performed at the department of microbiology, Södra Älvsborgs sjukhus 2015.
Supervisor: Cleas Henning, Professor.

Bacteremia is a condition that is potentially life threatening and requires antimicrobial treatment. Two common sources of error are incorrect volume of blood obtained for study and contamination of the sample through skin bacteria in cubital fossa. By examining the blood volume interval in cultivation bottles one can determine if the method used is optimal. For each milliliter examined the sensitivity changes by 3 %. Examining the bacteria present and the colonization in the area of cubital fossa one may evaluate the contaminations risk of the sample.

The study shows large variation in the blood culture volumes, many of them outside recommended bloodvolume interval, which seriously affects the sensitivity of detecting microorganisms. The result shows modification of the current blood sampling procedure is needed. It also show how the bacterial species and the bacterial density on the skin differ over time and between healthy people. Such knowledge can contribute to modifications in the disinfection routines and method of sampling for blood culture.

Abstract

By:Amina Arslanagic

Background and aims. Adipose tissue is a complex organ that plays important roles in endocrine, immune and metabolic systems. What has been a target for a lot of researchers is founding out what causes adipose tissue dysfunction. Methylglyoxal (MG) a reactive dicarbonyl produced mainly during the glucose metabolism has been a major target in diabetic complications. Previous studies have shown that MG causes oxidative stress, inflammation, macrophage recruitment but have not been able to impair insulin signaling. The aim of this is study is to investigate if MG-induced glycation can impair insulin signaling in hyperlipidemia. **Methods.** One year old Wistar and Goto-Kakizaki rats (non-obese model of type 2 diabetes) was divided in five different groups and feed with different diets during a period of 18 weeks. At the end of that period, the glucose metabolism was evaluated as well the activation of the insulin receptor pathway. **Results.** Wistar rats fed with MG and a high-fat diet showed decreased activity of insulin receptor pathway. MG-induced glycation in hyperlipidemia lead to fibrosis of the adipose tissue, accumulation of PAS-positive components, increased levels of free fatty acids, insulin, CEL and decreased levels of adiponectin. Most of this features were not observed in rats treated with MG feeding a normal diet or in rats feeding a high-fat diet without MG supplementation. **Conclusion.** MG impaired insulin signaling and causes insulin resistance in high-fat diet-fed Wistar rats, contributing to the metabolic dysfunction in obesity and to the development and progression of type 2 diabetes.

Effects of doxycycline induced-mitochondrial stress and UPR^{MT} on 3T3-L1 adipocyte differentiation and functionality

By Christopher Ayres Cea

Bachelor Thesis in Biomedical Laboratory Science
Institute of Neuroscience and Physiology
The Sahlgrenska Academy, University of Gothenburg
Supervisor: Ingrid Wernstedt Asterholm, PhD, Assistant Professor

Abstract

Background. Adiponectin is a hormone that is secreted from adipocytes and is involved in several metabolic processes including the regulation of glucose and fatty acid oxidation. The production of adiponectin is highly correlated to the mitochondrial density and function of the adipocytes. Furthermore, high levels of plasma adiponectin are associated with longevity and reduced risk of both type 2 diabetes mellitus (T2DM) and cardiovascular disease. A recent study from 2013 shows that imbalance between nuclear and mitochondrial encoded electron transport chain proteins through selective inhibition of mitochondrial transcription/translation leads to an extended life span in *C. elegans*. The mechanism for this surprising finding appears to involve induction of the so-called mitochondrial unfolded protein response (UPR^{MT}). It is a cellular stress response that functions to reestablish mitochondrial protein homeostasis that ultimately may lead to long-term favorable biological effects. Gene expression studies on mouse models suggest that UPR^{MT} might function in a similar manner in mammals as in *C. elegans*. The aim of this study was to induce a UPR^{MT} in cultivated mouse adipocytes and investigate if it affects the synthesis and release of adiponectin.

Methods. Mouse 3T3-L1 preadipocytes were used in this study and UPR^{MT} was induced by doxycycline treatment during adipocyte differentiation as well as in non-differentiated fibroblasts. Doxycycline is a tetracycline antibiotic that inhibits both bacterial and mitochondrial translation and thereby causes an imbalance between mitochondrial and nuclear derived proteins. RNA was isolated for gene expression analysis by real-time quantitative RT-PCR. Cell supernatants were collected for analysis of secreted adiponectin using sandwich ELISA.

Results. HSP60 is a chaperone protein involved in mitochondrial stress responses including UPR^{MT}, we have shown that doxycycline treatment increases HSP60 mRNA expression in fibroblasts. Doxycycline treated adipocytes showed tendencies towards increased expression of mitochondrial genes in doxycycline treated adipocytes. Doxycycline treatment had no effect on adiponectin secretion.

Conclusions. Doxycycline induces UPR^{MT} in preadipocytes as judged by an increase in HSP60 expression. The trend towards increased expression of mitochondrial genes in mature adipocytes exposed to doxycycline during differentiation suggest that doxycycline treatment can lead to long-term positive effect on mitochondrial density in adipocytes, but additional studies are necessary.

Abstract

Background and Aim: The atherosclerotic disease is a common health problem in the world and the major reason to different cardiovascular events. An early sign of the disease is an increased thickness of the arterial wall, i.e. an increased carotid intima-media thickness, which is used as a marker of atherosclerosis and for predicting future cardiovascular events. Impaired glucose tolerance is a transient metabolic state leading to type-2 diabetes, and is known to predict future risk of cardiovascular events. This study aims to investigate whether 64-year-old women with impaired glucose tolerance increases their intima-media thickness in the common carotid artery and the carotid bulb more than 64-year-old women with normal glucose tolerance during a 7-year follow-up in the DIWA study. A further aim was to determine factors that might influence the intima-media thickness.

Methods and Results: A total of 639 subjects underwent an ultrasound examination and repetitive use of oral glucose tolerance tests, and were then divided into different groups depending on their glucose status: diabetes mellitus, impaired glucose tolerance and normal glucose tolerance. This study only includes women with impaired and normal glucose tolerance, which gives a total of 399 subjects. When comparing the carotid intima-media thickness at baseline and 7 years later in the common carotid artery and bulb, no statistically significant differences could be found ($P = 0.136$ and $P = 0.274$). After further adjustments of the results for waist-hip-ratio, diastolic blood pressure, fasting glucose, triglycerides, HDL cholesterol, HbA1c and glucose tolerance group, fasting glucose was the only variable that showed to be of significant importance for increase in intima-media thickness in the carotid bulb ($P = 0.038$).

Conclusion: The main finding of the present study is that no significant difference in carotid intima-media thickness between women with impaired glucose tolerance and normal glucose tolerance after a 7-year follow-up could be observed. After adjustments have been made, fasting glucose is the only variable forming a significant difference. There is limited information and a lack of studies investigating the relationship between carotid intima-media thickness and glucose tolerance. For this reason, more studies on this subject should be performed.

Trans-thoracic Measured Coronary Flow Reserve in Patients with Stable Coronary Artery Disease is Associated with Left Ventricular Function Assessed by Velocity Vector Imaging

By Tove Brodin

Bachelor thesis in Biomedical Laboratory Science performed at the Dept. of Clinical Physiology.
Sahlgrenska University Hospital, University of Gothenburg, 2015
Supervisor: Sara Svedlund, MD, PhD

Background: Myocardial infarction is a common cause of death in Sweden. The development of new, easily accessible methods for evaluating patients with this disease is therefore important. Using cardiac ultrasound to measure coronary flow reserve (CFR) for evaluation of coronary function is a good option, as the method is inexpensive and risk-free. Velocity Vector Imaging (VVI), which measures tissue velocities in the myocardium, can be used for a more sensitive evaluation of heart function. **Aim:** The primary aim of this study was to apply VVI for assessment of cardiac function in patients with stable coronary artery disease. The secondary aim was to identify the relationship between systolic and diastolic heart function and CFR. **Method:** 54 patients were examined with cardiac ultrasound where CFR was measured and ultrasound images were saved. VVI was applied to the captured ultrasound images to measure tissue velocities and thus enable evaluation of diastolic and systolic function. A correlation analysis was performed on the measured variables and a t-test was used to compare differences between men and women. **Results:** There was a correlation between CFR and diastolic function ($p=0.04$, $r=0.29$), systolic blood pressure ($p=0.002$, $r=0.42$) and tissue movement in systole ($p=0.05$, $r=0.25$) and diastole ($p=0.02$, $r=0.22$). There were no significant differences between men and women in heart function. **Conclusion:** VVI appears to be a useful tool for assessing cardiac function. In this study CFR correlates to diastolic function, systolic blood pressure and tissue velocity.

ABSTRACT

By: Matilda Carlsson

Acute myeloid leukaemia is a haematologic malignancy, which leads to death if left untreated. The disease is located to the bone marrow, where genetically abnormal myeloid cells are produced at a high rate. These cells are immature and have lost their ability to differentiate into mature cells. The production interferes with the normal haematopoiesis, and the cells might infiltrate organs. Mutations in the gene *NPM1* are often found in patients with acute myeloid leukaemia. The three most common mutations are insertions of four base pairs and called A, B and D. It has been shown that *NPM1* mutations can be used as a molecular marker for detection of minimal residual disease after gone-through chemotherapy or allogeneic stem cell transplantation. Through real-time PCR it is possible to quantify the mutations. After the treatment the ratio between expressed copies of mutated *NPM1* and a reference gene has been shown to be connected to the prognosis of the patient.

The aim of this study was to set up a method at the Sahlgrenska University Hospital to quantify the *NPM1* mutations A, B and D through real-time PCR, and use the results to detect minimal residual disease. To do so, different primers and probes were tested, to find the most efficient ones. Samples that previously had been tested positive or negative for *NPM1* mutations with a fragment analysis were analyzed. The study showed that it will be possible to use real-time PCR for minimal residual disease detection in acute myeloid leukaemia. However, further validation is needed before the assay can be applied in clinical routine.

Developing Sample preparation methods for Tandem mass tag (TMT) semi-quantification of proteins and peptides in human CSF with nano-LC-MS/MS

By; Hatice Celik

Supervisor: Jessica Holmén-Larsson, PhD

Bachelor Thesis in Biomedical Laboratory Science performed at Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy University of Gothenburg

To prepare samples for mass spectrometry (MS), different methods can be used for preparation, purification and separation of proteins and peptides. In this study, two methods were compared, the In-solution preparation method and the Filter-aided sample preparation method (FASP) for preparation of cerebrospinal fluid (CSF) samples. In these two workflows, several additional parameters were also tested as different detergents (sodium-deoxycholate, Na-DOC and guanidine hydrochloride, Gu-HCl), the influence of alkylation and Tandem mass tag (TMT) 0-labelling. Also fractionation with high pH (HpH) - reversed phase (RP) - high performance liquid chromatography (HPLC) was tested to decrease complexity in samples. Also enrichment of TMT-labelled peptides as well as clean-up of samples with a single step of strong cation-exchange (SCX) chromatography was tested, and also SCX in combination with immobilized anti-TMT resin. The aim of this study was to find a methodological workflow that maximizes the output of identified and semi-quantified proteins and peptides in CSF.

The results show that the In-solution digestion method with Na-DOC as detergent and in combination with alkylation is the best workflow for identifying and semi-quantifying TMT-labelled proteins and peptides in CSF. However, the FASP method gave higher identification rates without TMT 0-labelling. Further, the alkylation step during sample preparation is contributing to lower technical variation (% CV) between sample replicates. An additional step with SCX chromatography as a single clean-up step for removing unreacted TMT reagent resulted in slightly less identifications, and this did not improve when adding the anti-TMT resin step. By using HpH-RP-HPLC, the identification rates were increased 4-fold (1236 proteins with TMT 0 and 1674 proteins without TMT 0) as compared to the unfractionated samples.

Conclusions from this study were that the In-solution digestion method with Na-DOC is most suitable for semi-quantification with TMT, whereas the FASP method could be more suitable for identification. Even though identification rates were much higher for the HpH-fractionated samples, this can be difficult to perform in larger patient sample studies, since the nano-LC-MS/MS analysis time increases at least 12-fold, and is thus very time consuming.

Abstract

By: Gwendolen Connoly

Streptococcus pneumoniae is a leading aetiological agent for respiratory tract infections and invasive infections, such as bacteraemia and meningitis. The predominant virulence factor is the capsule. Different capsular polysaccharides are used to categorize the species into over 90 serotypes.

The purpose of this study was to characterise 73 *S. pneumoniae* strains from the Culture Collection, University of Gothenburg (CCUG) strain collection and to assess the clonal relatedness between serotypes. The project involved serotype identification using multiplex PCR, biochemical testing with API Rapid ID 32 STREP strips and antimicrobial susceptibility testing using the EUCAST disc diffusion method. Genetic sequencing of the *gro*EL gene was performed on 25 of the strains.

Briefly, the results showed that 39% of the strains were positive for serotype identification and 20% were resistant to at least one of the tested antimicrobials. Analysing biochemical profiles showed that high variability of the strains was conferred to substrates α-GAL, PyrA and β-NAG. When correlating all the test results, we observed highly diverse biochemical and resistance profiles even within a single serotype. Similarly, phylogeny studies showed that only serotypes 1, 3, 4 and 9V clustered with the strain of the respective serotypes. Discrepancies between different identification methodologies were seen during this study.

Overall, the study has shown the high diversity within *S. pneumoniae* strains and improved the CCUG's characterisation of the tested strains. Further testing of pneumococcal strains may provide interesting data on serotype clustering and how that may relate to the phenotypic profile.

ABSTRACT

By: Daniel Cullen

Aim: This study aimed to investigate the activity of canonical Wnt signalling in degenerated human intervertebral disc (IVD) cells, mesenchymal stem cells (MSCs) and co-cultures of degenerated IVD cells and MSCs. The study also aimed to determine if pellet mass area was affected by a low oxygen environment. Furthermore, this study aimed to investigate the presence of ß-catenin in pellet mass culture medium for use as a potential marker of canonical Wnt signalling.

Methods: Human IVDs, MSCs and co-cultures of human IVDS and MSCs were cultured and expanded using a monolayer cell culture system and thereafter cultured using a three-dimensional pellet culture system under normoxic (21% O_2) and hypoxic (10% O_2) conditions. Pellet cultures were collected after 7, 14 and 28 days. Immunofluorescent techniques and computer analysis were used to detect 4 biomarkers of canonical Wnt signalling activity (Frizzled, ß-catenin, Glycogen synthase kinase 3 and Wnt-5a). Computer analysis was also used to compare pellet mass areas. A ß-catenin ELISA was used to detect ß-catenin in pellet mass culture medium.

Results: No significant difference in biomarker expression was found between normoxic and hypoxic culture conditions in either the IVD cell cultures or the co-cultures. A significant difference in GSK3 expression was found however, between normoxic and hypoxic MSC culture conditions on day 7 ($p= 0.0002$). Yet, no other significant difference in biomarker expression was found between normoxic and hypoxic MSC culture conditions. What is more, no significant difference in biomarker expression was found on comparing the three different cell culture types under normoxic or hypoxic conditions and no significant difference in pellet mass area was found between normoxic and hypoxic conditions. Finally, ß-catenin was found to be present in very low concentrations in a number of the pellet mass media samples.

Conclusion: It was evident that canonical Wnt signalling was present in degenerated human IVDs but it cannot be said that the hypoxic environment characteristic of IVDs had a significant effect on this signalling cascade. Moreover, evidence was found to indicate that the signalling pathway was inactive. In addition, it was found that the oxygen level in the cultures did not have an effect on pellet mass area. Finally, ß-catenin was present in some culture media samples but it was not found to be an effective marker of canonical Wnt signalling activity.

Separate Abstract

By: Lily Deland

Neuorblastoma is an embryonal childhood cancer and is the most common extra-cranial solid tumour cancer in children. It originates in the development of the sympathetic nervous system where neural crest cells instead of differentiating into mature neurons, turn into cancer tumours instead. It often manifests in the adrenal medulla, but can develop anywhere along the sympathetic nervous system. Neuroblastoma is a very heterogeneous disease with some of the tumours spontaneously regressing or differentiating and others being very aggressive with 50% chance of survival. One of the genetic markers shown to affect the outcome for these patients is the long non-coding RNA (lncRNA) NBAT-1 on chromosome 6p22. In this thesis work, two lncRNAs (lincTan and lincGen) associated with neuroblastoma, have been amplified, cloned and overexpressed in neuroblastoma cell lines for further analysis of their role in cell viability, as part of an effort to discover new biomarkers and treatment targets for neuroblastoma patients. The overexpression of the transiently transfected cells was successful for both of the lincRNAs investigated.

SEPARATE ABSTRACT

Interaction analysis of CysD domains in the MUC5AC mucin

By Brent De Wijngaert

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Medical Biochemistry, Sahlgrenska Academy, University of Gothenburg, 2015.

Supervisor: Christian V. Recktenwald, PhD

Mucins are large glycoproteins found on or near the surface of non-keratinized cells from the digestive, urogenital and respiratory tract. They form a mucus layer that protects the cells from challenges by changes in the environment. There are two main classes of mucins: secreted and membrane-bound mucins. The intestinal mucus is mainly build by the MUC2 mucin, MUC5AC is found in the respiratory system and the stomach and MUC5B can be found in the lungs and saliva. The cysteine-rich domains (CysD) represent an important but so far not well characterized part of mucins.

In previous studies, it has been shown that the $CysD_2$ domain from MUC2 forms dimers and that they can participate in the network-formation of MUC2 to form a mucus layer. The aim was to expand this analysis to the $CysD_3$ domain from MUC5AC.

The $CysD_2$ domain from MUC2 was purified using affinity chromatography and ion exchange chromatography. The plasmids $CysD_3$-HA and $CysD_3$-MYC encoding the $CysD_3$ domain from MUC5AC were transfected in CHO-K1 cells, in parallel, a co-transfection with different tagged $CysD_3$ was performed. Using far-western blot analysis, no specific signal for the positive control ($CysD_2$ from MUC2) was detected. Therefore, a co-immunoprecipitation approach with HA- or MYC-tagged $CysD_3$ from MUC5AC was applied. Immunoprecipitation with the α-MYC antibody followed by western blot analysis with the same antibody showed that this approach was technically functional. The same results were obtained when an antibody against the HA-tag was used for both immunoprecipitation and western blot. However, when either the antibody against MYC- or the HA-tag was used for immunoprecipitation and the antibody recognizing the other tag was applied for western blot analysis, no signal indicating $CysD_3$ dimerization could be detected.

Based on these results we can conclude that the CysD domains need their native conformation to be able to form dimers and no linear sequence stretches are involved in the dimerization. Furthermore, the $CysD_3$ domain from MUC5AC does not form homodimers suggesting a different function for CysD domains in MUC5AC.

Abstract:

By: Greta Domarkaite Tighe

Chronic lymphocytic leukemia (CLL) is a haematological malignancy which mainly affects the elderly. It is a clinically heterogenous disease with staggering differences in disease outcome and survival between the two main prognostic groups. Immunoglobulin heavy-chain variable (IGHV) mutational status is a well known biomarker of prognosis as relating to favourable prognosis (IGHV mutated) and poor prognosis (IGHV unmutated). DNA methylation is one of the main elements of epigenetic modifications. It is a known factor in cancer development and can lead to silencing of genes vital to normal cell function. Differential DNA methylation in CLL, which leads to differential gene expression, can play a role in the development of the disease. However, much still remains unknown about the effects of methylation on genes involved in CLL and the exact pathways to the development of the heterogeneity of the disease.

Our project was designed to validate the methylation status of significantly differentially methylated genes selected from genome-wide methylation studies. We analysed 70 CLL patient samples, composed of 35 IGHV mutated and 35 IGHV unmutated samples. DNA methylation was analysed using a quantitative technique known as pyrosequencing. Significantly differentially methylated genes were further analysed for differential gene expression using quantitative real-time PCR.

Our findings validated significant differential methylation ($p < 0.00001$) of both *SOX3* and *SOX17 genes*. Furthermore, mRNA expression analysis revealed significant differential expression of *SOX3* ($p = 0.00072$) and *SOX17* ($p = 0.003$) genes due to differential methylation. Our results add vital information to the current knowledge of CLL and may lead to further discoveries in the pathogenesis of this devastating haematological malignancy.

The seasons influence on APAP compliance – is there a difference between summer and winter?

By Annie Dyne

Bachelor thesis in Biomedical Laboratory Science performed at the Pneumology department in Hospital de Santa Maria, Institute of Biomedicine, 2015.
Head supervisor: Cristina Canhão and Deputy supervisor: Bente Grüner Sveälv

Background and Aim. The aim of this study was to, by comparing automatic positive airway pressure (APAP)-compliance during a month in summer and a month in winter, determine if there was a significant effect of temperature. To do that, the patients was since earlier diagnosed with obstructive sleep apnea (OSA) and had been treated with APAP for over a year.

Method. The studied population of 14 Portuguese patients had been diagnosed with OSA by polysomnography or a cardiorespiratory polygraphy test and treated with APAP for over a year. The study was a retrospective and comparative analysis. By meeting and interviewing the participants, that information together with the data from the patients hospital files and APAP-memory card, was used to fill the created database. Also the Epworth sleepiness scale was used to assess and measure the sleepiness.

Results. There was seen a significant difference between compliance of APAP during seasons ($p<0.05$). The ESS summer and winter did however not show any significant differences ($p>0.05$). Other findings was that there was a difference when comparing "apnea-hypopnea index (AHI) before treatment" with the "patients best experienced season" ($p<0.05$).

Conclusion. The conclusion is that the winter is the best season for APAP-compliance. However the patients experience about the best season does not match the compliance data.

Separat Abstrakt

Metodjämförelse mellan Cytolyt och PBS med immuncytokemisk färgning

Av: Mari Farhadi Kohan

Kandidatuppsats i biomedicinsk laboratorievetenskap utförd vid institutionen för klinisk patologi och genetik, avdelning cytologi, Sahlgrenska akademin, Göteborgs universitet, 2015.
Handledare: ÖL Christina Kåbjörn Gustafsson, PhD

Dagens metod, att förvara cellmaterial i PBS (Phosphate-buffered saline) är inte optimal för vidare analyser. Syftet med den här studien var därför att utvärdera en ny metod för fixering av cellmaterial, samt jämföra Cytolyt fixering och PBS (Phosphate-buffered saline) transportmedium. I undersökningen förbereddes cytologiskt cellmaterial med cytospin metod. Jämförelsen gjordes med genomförande av immuncytokemiska färgningar. För att uppfylla syftet krävdes även att ett flertal immunfärgningsmetoder (CD3, CD20, CK7, Kappa light chain, Lambda light chain, S100, Ber-EP4 och MNF116) validerades. Det finns många immunologiska metoder som i nuläget används vid diagnostik. Resultaten av färgningarna varierar eftersom överföring av cytologiskt material till objektglas sker på olika sätt.

För metodutvecklande syfte utvärderades metodernas fördelar och nackdelar. Immuncytokemiska analyser gjordes av celler med åtta olika antikroppar, vilket sedan utvärderades. Kvaliteten på det Cytolyt fixerade cellmaterialet utvärderades med ljusmikroskopi. Resultat av färgning och morfologisk kvalitet granskades och bedömdes utifrån en tre-graderad-skala.

Den slutsats som drogs efter granskning, jämförelse och bedömning, är att båda metoderna har sina fördelar och nackdelar. För att komma fram till en mer detaljerad slutsats krävs att fler prov och antikroppar studeras.

Nyckelord: Cytolyt, PBS (Phosphate-buffered saline), Cytospin, May-Grünwald Giemsa och Immuncytokemi.

The test-retest reliability for VO2max, VCO2 and RER in ergospirometry testing in patients with migraine

By Emelie Forsgren

Bachelor thesis in Biomedical Laboratory Science performed at the School of Sport Science, Department of Food and Nutrition and Sport Science, University of Gothenburg, 2015
Supervisor: Bente Grüner Sveälv (Leg. BMA, Med. Dr) Emma Varkey (Leg. sjukgymnast, Med Dr)

Background. Migraine is a common neurological disorder that causes severe headache and often nausea, sensitivity for light and sound and can be triggered by exercise. This fact is a possible explanation to why patients with migraine perform less physical activity compared to healthy controls. Exercise is the corner stone for a high physical fitness, i.e maximal oxygen uptake (VO_{2max}), that can be measured by an ergospirometry test. **Aim.** The aim of this study was to assess the test-retest reliability for VO_{2max}, carbon dioxide production (VCO_2) and the respiratory exchange ratio (RER) and also to investigate how the VO_{2max} should be measured in order to obtain the highest value; the mean value of the 30 last seconds or the highest 15 coherent breaths that gives the highest oxygen uptake (VO_2). **Method.** A bicycle ergospirometry test-retest was performed on 15 patients with migraine. The parameters that was obtained and studied was; VO_{2max}, VCO_2, RER, maximal watt, maximal heart rate(HR), HR before the test, HR 4 min recovery, Borg's rating of perceived exertion (RPE) scale for breath and leg fatigue. Paired sample T-test was used to analyze the correlation and the significance level. A Bland-Altman chart was used to analyze the reliability between test-retest.

Results. The correlation (r) and the significance (p) were studied for each parameter. The coefficient of variation (CV%) was studied for VO_{2max}, VCO_2, RER, maximal watt, maximal HR, HR before the test and HR 4 min recovery. CV%=5.8, r= 0.96 and p=<0.0001 for VO_{2max}. CV%=4.1, r=0.97 and p=<0.0001 for VCO_2. CV%=2.7, r= 0.81 and p=<0.0001 for RER. The highest value of VO_{2max} was measured at the 15 coherent breaths that gave the highest VO_2. **Conclusion.** We can conclude that a single ergospirometry test is sufficient in order to measure maximal VO_{2max}, VCO_2 and RER.

ABSTRACT

Detection of neutralizing autoantibodies against interferon gamma

By Therése Gustafsson

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of Biomedicine, Department of Clinical Immunology and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, 2015.

Supervisors: Pontus Thulin, M.D. PhD and Bengt Andersson, M.D. Associated Professor.

Interferon gamma (IFN-γ) is released by the immune system during infection to stimulate macrophages via STAT1-phosphorylation to phagocytose and kill the invading microorganisms. Neutralizing antibodies against IFN-γ have been noticed in patients with disseminated non-tuberculous mycobacterial infection and in patients with infections caused by other intracellular pathogens. The antibodies against IFN-γ bind to IFN-γ and prevent the stimulation of macrophages and granulocytes, which leads to less effective immune response against intracellular pathogens. To be able to diagnose and treat these patients correctly we developed a multiplex method for detection of antibodies against IFN-γ that can be used for clinical purposes. We have observed that a relatively large number of patients fail to produce IFN-γ in QuantiFERON-TB gold test which is an IFN-γ release assay used to evaluate if patients have been exposed to tuberculosis. In this test immune cells from the patients are expected to have measurable IFN-γ in the test positive control. We analysed samples from 60 patients that could not be evaluated in the QuantiFERON-TB gold test due to a lack of measurable IFN-γ in the positive control. We hypothesized that the lack of measurable IFN-γ could be caused by antibodies against IFN-γ. 40 samples from healthy blood-donors served as a control group. In the multiplex method 10 % of patients and 5 % of healthy blood donors were positive for anti-IFN-γ antibodies respectively, verifying the presence of antibodies against IFN-γ. To examine if the antibodies neutralized IFN-γ mediated signaling we used a flow cytometry method to detect phosphorylation of STAT1. Three out of six patients were found to have neutralizing antibodies to IFN-γ, while none of the two healthy blood donors that were positive for antibodies against IFN-γ in the multiplex method had neutralizing activity. The use of magnetic microspheres coupled to the cytokine of interest makes it possible to rapidly detect antibodies in a small sample volume and to extend the method so that multiple antibodies can be measured at the same time. The flow cytometry method is reliable and with a monoclonal antibody against signaling intermediates it is possible to accurately determine if the antibodies disrupt cytokine signaling, and hence could have pathological relevance.

Prognostic significance of left ventricular isovolumic time in medical care for elderly patients with heart failure

By Ronza Haddad

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology , Sahlgrenska University hospital/ Östra, Sahlgrenska Academy, University of Gothenburg, 2015

Supervisors: MD. Qays Al-modares and MD. Magnus Johansson.

Abstract

Heart failure is defined by a disorder of the heart's function, which in turn gives increase to many other complications in other organ systems. By means of a number of parameters and analytical forecast for the development of heart failure, isovolumic time, mitral filling time and ejection time are the parameters used in this study. **Background:** isovolumic time (isovolumic relaxation and contraction) is the time that is lost in each heart cycle. The longer the isovolumic time becomes the less effective the cardiac cycle. **The aim** of our study was to test the hypothesis that the isovolumic time in the left ventricle can predict survival in senior patients with heart failure.

Method: 163 patients with heart failure, age elderly than 65 years. 95 were deceased and 78 of them were women. Using apical pulsed Doppler of left outflow tract of the aorta and mitral valves, measured ejection time (ET), which is the time from aortic valve opening to closing, and RR interval, filling time for mitral valve(MFT) which is the time from mitral valve opening to closing in milliseconds and RR interval was also measured. The total isovolumic time was calculated by the following equation: The value of ET, and MFT was multiplied by the value of HF to get the times in seconds/ minutes. The values were used according to the following equation: T-IVT = 60- (ET + MFT). **Results:** The total isovolumic average time was 9,28 ± 7,03 sec / min for deceased patients and 8,96 ± 4,98 sec/min for alive patients with P value = 0,77. The mean ejection time was 20,57 ± 5,78 sec / min for deceased and 20,02 ± 2,48 sec/min for alive patients, the P value was= 0,53. The filling time from mitral 30,14± 4,16 sec / min for deceased and 31,02± 4,47 sec/ min for alive patients and the P value was= 0,28.

Conclusions: Total isovolumic time couldn't predict survival in elderly patients with heart failure.

Abstract

By: Elinor Hansson Wilcken

Background and aim: Methylglyoxal (MG) is a highly reactive compound derived mainly from glucose and fructose metabolism. The mechanisms related to adipose tissue microvascular dysfunction are unknown, however studies showed that MG-induced glycation may contribute to vascular dysfunction in adipose tissue. It causes alterations in the tissue such as macrophage recruitment, impaired angiogenesis and decreased irrigation independent of obesity. Obesity itself is associated with low-grade inflammation due to tissue expansion which will lead to hypoxic areas in the tissue. The tissue will compensate the hypoxia with increased angiogenic factors to expand the vascular system. The aim of this study was to see how MG-induced glycation would affect tissue irrigation and adaption when MG administration was combined with a high-fat diet. **Methods:** Groups of rats were divided with different diets; control with normal diet, normal diet with MG administration, high-fat diet (HFD), high-fat diet combined with MG administration (HFDMG), and non-obese type 2 diabetic Goto-Kakizaki (GK) rats with a normal diet. We did Western blot analysis to determine levels of proteins involving adipose tissue metabolism as well as histological and quantifying analysis. **Results:** The results indicated that HFD combined with MG administration impairs the adipocytes ability to adapt to the high-fat diet. Adipocytes did not grow properly, and inflammation and fibrotic factors were elevated in rats with MG administration. Other proteins essential for lipid storage and lipolysis were decreased in the combined diet. **Conclusions:** The result from this study combined with other studies from the same group suggest that MG administration causes adipose tissue dysfunction and impairs the adaptation to a high-fat diet and the hypoxia that follows. The abnormalities in lipid storage and lipolysis might lead to elevated levels of free fatty acids in the blood which might be a contributing factor to the development of diabetes type-2, but future research has to be done to address this matter.

Detection of *vanA* and *vanB* genes in Vancomycin - Resistant Enterococci (VRE) using Multiplex TaqMan Real- time PCR

By Rajaa Hassan

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Microbiology, Norra Älvsborgs Länssjukhus, NÄL. Trollhättan, 2015.

Supervisor: Ingvar Eliasson (Dr Med Sci), Darinka Andersson (Leg. Biomedical scientist)

Background: Vancomycin Resistant Enterococci (VRE) have become an important pathogen in recent years. VRE have been recognized as a major cause of hospital- acquired infections, nosocomial infections. Emergence of VRE has become a challenging problem in health care institutions worldwide. Therefore, a rapid detection of these bacteria is very important for limitation and prevention spread of nosocomial infections caused by these bacteria. Conventional methods for VRE- screening which are based on culturing take 3-5 days to provide results. A multiplex TaqMan real- time PCR used in this study for rapid detection of *vanA* and *vanB* in *Enterococcus faecium* and *Enterococcus faecalis*, the most important species of VRE that responsible for majority of human infections. **Subjects and Methods:** A total of two hundred clinical specimens (faecal and rectal swabs, secret from unidentified places, secret from anus and urine) sent to the laboratory for VRE- screening under Mars-May 2015 were included in this study. Clinical specimens were already analyzed by routine culturing using *VRESelect* and they showed negative results. Five known *vanA* positive strains, one known *vanB* positive strain and a panel of reference strains other than enterococci were tested in the study also. Specimens and bacterial strains were enriched in Bile Esculin Azide broth 24- 48 hours. DNA was extracted and purified using QIAsymphony DSP Virus/Patogen-mini-kit. Sample preparation and assay setup was carried out by Qiasymphony SP/ AS instrument. Multiplex TaqMan real- time PCR was carried out by RotorGene 6000 and Q RotorGen. **Result:** The sensitivity and specificity of PCR assay in this study were found to be 100 %. PCR assay showed to be more rapid compare with culturing and the assay showed to be capable to detect both *vanA* and *vanB* at the same time. **Conclusion:** Multiplex real- time PCR is a rapid and accurate technique for detection of *vanA* and *vanB* in VRE. This method offered results at least one day earlier than culture. The use of multiplex real- time PCR would have important implications for an effective control and limitation of nosocomial VRE infections.

SEPARAT ABSTRACT

Yeast mitochondrialDNA-replication using a human DNA-polymerase

By Sandra Hedlund

Bachelor thesis in Laboratory Science performed at the department of medical chemistry and cellbiology, Sahlgrenska Academy, University of Gothenburg, 2015.
Supervisor: Zsolt Szilagyi, Researcher

Mitochondria are unique organelles in eukaryotic cells. They are responsible for producing energy in a form that can be used by the cells. The mitochondria also has its own genome, the mtDNA, that encodes for a few proteins necessary for energy production although all proteins necessary for mtDNA maintainance, replication och transcription are encoded in the nucleus and imported into the mitochondria from the cytoplasm. MtDNA is replicated by the mtDNA-polymerase gamma, PolGA. Alot of mutations found in PolGA has been identified as causative of disease. Exacly how these mutations affect the mitochondria is not understood which is why a *in vivo* system to study the functions of PolGA could be important. A yeast system using the fission yest *S.pombe* could be a good system using the yeast similarities with higher eukaryotic organisms. *S.pombe* has been used widely as a model for cell cycle control and DNA-repair and works perfectly as a model for studies about the mitochondria because of its similarities with eukaryotic cells. And so the aim of the study was to explore the possibility of using a human mtDNA-polymerase to replicate mtDNA in fission yeast. As material for the reactions we used human cDNA, cloning plasmids and yeast DNA. With PCR we amplified the mitochondria polymerase gene, POLGA, its accessory factor, POLB, tags, HA och MTS from the pog1 and pfh1 genes. Using fusion PCR we then constructed fragments containing the gene, tag and MTS in a determined order and with ligation and heat shock transformation we introduced the fragments into expressionplasmids 41x, 81x and 42x, 82x containing leucin and uracil markers respectively. As the fusion PCR for POLGA didn´t work as planned we had to change tactic and used primer that cut the gene into two smaller parts which were fused together in two seperate PCR reactions, solving our problem. The plasmids were cleaved using different restrictionenzymes to determine the orientation of the fusion PCR products in the plasmids. When the cleavages didn´t turn out as expected, new plasmids were introduced, 3X and 4X, and the experiments were reproduced. Because of lack of time the question if a human mtDNA polymerase can be used to replicate yeast mtDNA is left unanswered. The material used also needs to be looked over to find out what went wrong before the experiment can continue.

SEPARATE ABSTRACT

Method Evaluation and study of Mg effect in the healing of bone fracture in the tibial bone in rats

By: Sara Hedlund

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Medical Biochemistry and Cell Biology, Sahlgrenska Academy, University of Gothenburg, 2015.
Supervisor: Håkan Nygren, Professor

Background: Osteoporosis has long caused problems in areas such as orthopedics and innovation required new research. Magnesium (Mg) has attracted attention due to its good osteoconductivity
and osteoinductivity. Properties that may affect the natural fracture repair in bone fractures. Various methods are used to analyze and study bone tissue such as ESEM, CTscan and Light microscopy.

Methods and materials: Two imaging techniques, ESEM and MicroCTscan were evaluated to examine which method is most suitable to measure size differences by comparing measurements on periosteum, endosteum and the cortical thickness of untreated samples, 3 and 6 weeks. Effects of Mg on fracture repair and the normal bone healing was studied under the light microscope and size measurements on the periosteum, endosteum and the cortical thickness was performed on 1, 2 and 3 week samples to investigate whether Mg contributes to increased bone growth.

Results: Measurements from the X-ray technology (CTscan) resulted in no significance. Measurements from ESEM measured significant growth of periosteum1 ($p = 0.003<0.05$), endosteum1 ($p = 0.026<0.05$) and the cortical thickness1 ($p = 0.05$). The periosteum is significantly larger with MgO treatment at 1 week ($p = 0.006<0.05$) and 2 weeks ($p = 0.006 <0.05$). Also the cortical thickness was significantly thicker treatment at 1 week ($p = 0.0002<0.05$) and 2 weeks ($p = 0.01<0.05$).

Discussion: The method evaluation suggests that ESEM is an more sensitive method to detect differences with limited numbers of samples. The difference between the methods may be due to the fact that X-ray technique is more automatic in the selection of areas in the sample to produce a final image. With ESEM the imaging is done more manually. Mg has a good anabolic effect on bone formation in fractures and contributes to increased growth of bone with a bone inducing effect but this study does not tell if the treatment contributes to increased strength in the bone.

Conclusion: ESEM achieved significant differences when compared with the X-ray technique. However repeated experiments are necessary to adequately ensure that the method is more suitable. Treatment with MgO contributes to rapid bone formation and has bone inducing effects. Further experiments with Mg may show additional anabolic effects of the metal and how this can be applied in experiments concerning osteoporosis (OT).

ABSTRACT

The vast majority of *Escherchia coli* are harmless and even necessary for our digestion. However, there are some strains that acquired the characteristics that cause diseases. One of such pathogenic type of E.coli is *Enterohaemorrhagic Escherchia* (EHEC). EHEC is a pathogenesis bacterium responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) worldwide.

The aim of this study was to subtype EHEC with MLVA (Multiple- Locus Variable number tandem repeats Analysis) and to correlate results with PFGE (Pulsed Field Gel electrophoresis). A total of 91 pieces of frozen EHEC isolates was analyzed by the loci of ten Variable Number Tandem Repeats (VNTR). The loci were amplified by three multiplex PCR and one single PCR, resulting in amplicons of different sizes, which were assigned in an allele number. The combined results for all VNTR loci were adapted as a Multiple- Locus Variable number tandem repeats Analysis and each isolate was assigned with a MLVA-type code number, which were used for strain comparison. The results indicated that PFGE had higher discrimination ability than MLVA. The 91 EHEC isolates could be characterized according to 82 different sub-typed with PFGE and 72 different sub-typed with MLVA.

We conclude that the PFGE was higher discrimination ability than MLVA, but with adjustment and optimized MLVA protocols, the MLVA can be replaced by PFGE in future.

Abstract

By: Chris Häggqvist

Background: Obesity leads to unhealthy adipose tissue and there is risk of obesity associated disease and metabolic syndrome. Adipose tissue is an organ that promotes health by producing hormones and eliminating harmful metabolites. One of the ways its effectiveness can be increased is by increasing the mitochondrial biosynthesis; this is referred to as browning. The increased activity of the adipose tissue will improve metabolite elimination and might facilitate weight loss. There is a protocol for differentiation of 3T3-L1 cells that will induce a browner phenotype. This protocol would make 3T3-L1 cells more relevant for our experiments concerning the effect of oxidative stress on cell browning and differentiation.

Method: Browning of the 3T3-L1 cells was achieved by a prolonged treatment with Rosiglitazone, insulin, T_3 and IBMX. The effect of the intracellular oxidative state on differentiation was investigated by long-term treatment with low dosages of NAC.

Cells where harvested at day 10 of treatment, and mRNA expression was analyzed with quantitative real time-PCR. Adiponectin levels in the supernatants were measured with ELISA.

Some of the genes that were investigated were UCP1, ATP-6 and adiponectin and pref-1 to assess respectively, how “brown” and differentiated the cells were, as well as their activity.

Results: The results show a 3-4-fold increase in UCP1 compared to 3T3-L1 cells treated with the standard white adipose tissue differentiation protocol, confirming that the cells are pushed to become more brown with the new protocol. The long term NAC treatment also shows that increased ROS levels play a role in the browning process as judged by lower ATP6 levels. The expression of pref-1 and resistin were however similar to controls indicating that the differentiation rate was unaltered.

Conclusion: Our results provide some pieces of evidence that 3T3-L1 cells can be treated to express a browner phenotype and that increased ROS levels may be instrumental for the cells to differentiate into beige adipocytes.

SEPARATE ABSTRACT

Cloning and purification of Oxidoreductase-like domain containing protein 1

By: Louise Jenninger

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Medical Biochemistry and Cell biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2015.

Supervisors: Maria Falkenberg (Professor), Viktor Posse (PhD student) and Emily Hoberg (Biomedical scientist)

The knowledge of the regulatory mechanisms of mitochondrial replication and transcription is inadequate. According to BioGRID's database "Protein and Genetic Interactions" the protein oxidoreductase-like domain containing protein 1 (OXLD1) physically interacts with mitochodrial DNA polymeras γ and mitochondrial genome maintenance exonuclease 1. OXLD1 may therefore be a possible factor involved in the regulation of mitochondrial replication and transcription. The aim for this study is to clone and develop a purification strategy for OXLD1. This enables further studies of the protein which may lead to a greater understanding of how mitochondrial replication and transcription is regulated.

PCR was performed to amplify an OXLD1 gene sequence that included restriction sites for *Nde1* and *HindIII*, and a 6xHistidine tag. The gene sequence was cloned into pET-17b and the recombinant vector was transformed into chemically competent *E. coli* cells. Plasmid DNA was extracted, purified and sequenced and later transformed into competent Rosetta cells designed for gene expression. Gene expression was induced by isopropyl β-D-1-thiogalactopyranoside, cells were harvested and protein was extracted. OXLD1 was successfully purified with immobilized metal ion affinity chromatography with Ni^{2+}-sepharose followed by anion exchange chromatography and gel filtration chromatography.

Studies like replication and transcription assays *in vitro* and knockdown of OXLD *in vivo* are suggested for further examination of OXLD1 functions.

Abstract

Direct Identification of Bacterial and Fungal Pathogens in Positive Blood Culture Broth with Matrix Assisted Laser Desorption/Ionization Time-of-Flight Yields Promising Results

By Ida Johansson

Bachelor Thesis in Biomedical Laboratory Science at the Department of Clinical Microbiology, Sahlgrenska Academy, University of Gothenburg, 2015.

Supervisor: Nahid Kondori, PhD

Early diagnosis and accurate treatment of patients with sepsis are crucial to prevent complications that could become life threatening. In the last decade, Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has been used frequently for identification of microorganisms. Blood culture is still the gold standard for diagnosis of sepsis in microbiological laboratories. Conventional identification of pathogens in positive blood bottles is based on subculturing of the bottles on agar medium and isolation of microorganism before starting the identification process. The aim of this study was to evaluate the performance of MALDI-TOF MS for direct identification of microorganisms in culture-positive blood bottles in order to shorten turnaround time.

A number of 178 blood bottles were analyzed. The positive blood bottles were identified traditionally by subculturing on agar plates and by direct identification by MALDI-TOF MS. Results obtained by these methods were compared. It was shown that MALDI-TOF MS was able to find 1 CFU/ml of bacteria or yeast in spiked blood bottles. Negative association between concentration of microorganisms in blood bottles and time required for detection of microorganisms in bottles was found. The direct identification method identified 42 (84%) of the gram-negative bacteria and 76 (62.3%) of the gram-positive bacteria correctly. It identified 1 (20%) out of 5 of the bottles containing fungi. The results obtained from the study suggest that MALDI-TOF MS could be a promising method for direct identification of bacteria in positive blood samples.

Abstract

By: Kevin Karlsson

As fungal infections are becoming more common and morbid in clinical contexts, so does the need for rapid identification and treatment of these infections. Earlier methods of identification, such as staining and microscopy, has proven to be both time consuming and less reliable than other, more modern methods. Currently, the better method seems to be the *Matrix Assisted Laser Desorption/Ionization – Time of Flight* method. This method uses a compound called matrix, in order to erode samples and ionize the intracellular protein material of these. By using a magnet and a high speed detector, the instrument then creates a spectrum, which is entirely dependent on the mass of the proteins of the fungi. This spectrum is then compared to a database of other spectrums, in order to identify the sample.

The purpose of this study was to evaluate the use of this method at the Microbiological department of Sahlgrenska University Hospital, for easier and faster identification of clinically relevant fungi.

By cultivating a total of 88 previously identified mold patient samples, they were later prepared and analyzed with the instrument and given a confidence value, based on the comparisons with the database. These results were then compared to the results of the conventional method, of staining and microscopy, and were used to evaluate the method.

The most commonly identified species was *Aspergillus fumigatus*, which the instrument correctly identifying these 100% of the time (31 out of 31), while *Aspergillus* as an overall species was correct 75% (45 out of 60) of the time. Less common species were correct at 28.6% (8 out of 28), while both methods overall were consistent in 60.2% (53 out of 88) of the cases.

The conclusion that could be drawn from the study was that the method could be used as screening, specifically for *Aspergillus fumigatus,* while less common species would need to be further studied and added to the database. The method was also considered to be much faster than earlier methods; it is merely the certainty of the results that needs to be improved.

ABSTRACT

Background. Sometimes treated cancer patients suffer from a condition in which their B cells functions work less well after the treatment has ended. These patients often suffer from recurrent infections and respond poorly to vaccine because they can't produce antibodies as they should. Often you want to purify a particular cell type to examine how it works and reacts by exposing them to different subjects. One can use antibodies to purify B cells. CD45 is a structure that is expressed on all human leukocytes while CD19 is expresses at all stage of B cells. By using these antibodies one can identify leukocytes and B cells in whole blood using flow cytometry. **Aim.** The aim of the study was to compare three different methods kit of different manufactures in their ability to purify blood cells and to determine which method is most suitable for purifying B cells from frozen samples. **Method and result.** The methods we used was PluriBead anti-CD19 from pluriSelect, Easysep Direct Human B cell isolation kit from StemCell Technologies and MACSxpress Pan B cell isolation kit from Milteny. The methods was performed on fresh and frozen whole blood from 4 different persons. By performing the methods on person 1, we observed that all the methods worked fine with whole blood but pluriSelect didn't work with frozen whole blood. We decided not to continue with pluriSelect, instead we continued with the other two methods with 3 other persons. The result showed us that EasySep were the best method. **Conclusion.** We observed that it was difficult to purify B cells from frozen whole blood than from fresh whole blood , but we also showed that despite this it was possible to purify B cells from frozen whole blood.

SEPARAT ABSTRACT

Proteasspecificitet vid inflammatorisk nedbrytning av broskproteiner

Av. Sara Kazemi

Examensarbete i Biomedicinska analytikerprogrammet utfört på Klinisk kemi (klinisk molekylär forskning), Sahlgrenska Akademin, Göteborgsuniversitet, våren 2015

Handledare: Ulla Rüetschi, Docent, 1:e Kemist

Artros är en progressiv ledsjukdom där de extracellulära matrisproteinerna i ledbrosket gradvis bryts ner. Nedbrytning av extracellulära matrisproteiner och proteoglykaner leder till irreversibla förändringar i egenskaperna hos det kollagena nätverket. Dessutom resulterar obalans i omsättningen av matrixproteiner ofta i ökad proteolys av molekyler bundna till och exponerade vid ytan av kollagenfibrer, såsom fibromodulin, dekorin, och cartilage oligomeric matrix protein (COMP). Det är viktig och avgörande att kunna identifiera specifika enzymer i den proteolytiska processen som sker vid brosknedbrytningen och orsakar ledsjukdomar, för att kunna förebygga nedbrytning av extracellulära matrisproteiner i tidigt förstadium av sjukdomsutveckling. I det här arbetet studeras den proteolytiska specificiteten hos MMP-1 och MMP-3 för klyvning av extracellulär matrix proteiner. Renframställt rekombinant proteas inkuberas med kända peptidsubstrat för att säkerställa att proteaset har den förväntade enzymatiska aktiviteten. Substraten är inmärkta med acceptor/donormolekyler i N- respektive C-terminalen. Enzymatisk aktivitet mäts genom att registrera hur mycket flourescens som avges vid exitation av peptidsubstratet. Syntetiska peptider från cartilage oligomeric protein (COMP), biglykan och fibromodulin märks in med acceptor/donor (MCA/DNP) och dessa peptider inkuberas med aktivt proteas. Specificiteten bedöms utifrån fluorescensmätningar genom att använda FRET metoden. Slutligen har vi kommit framtill att Fibromodulin peptiden är MMP-3s substrat.

ABSTRACT

Sign of Rheumatic Heart Disease was observed in 3.0% of pregnant women in Kigali, Rwanda. Time for echocardiographic screening in high prevalence areas?

By Anna Kerola

Bachelor thesis in Biomedical Laboratory Science performed at the University Teaching Hospital of Kigali and at Muhima District Hospital in Kigali, Rwanda, 2015
Supervisor: Bente Grüner Sveälv, PhD

Background: An untreated infection of Group A streptococcus might lead to Rheumatic Heart Disease (RHD). In developing countries, RHD is the most common heart disease among pregnant women and causes an increased risk of mortality and morbidity of mother and child. Access to echocardiographic screening is therefore a central issue especially in pregnant women as the pregnancy itself is a challenge for the heart.

Aim: To estimate the prevalence of pathological mitral and aortic regurgitation that could be associated with RHD among pregnant women attending antenatal care in Kigali, Rwanda.

Method: Echocardiographic screening was performed following the World Heart Federation criteria (WHF) for diagnosis of RHD. A portable echocardiography (Vivid-I, General Electric, Fairfield, CT, United States of America) was used to examine pregnant women attending antenatal care in two different health facilities in Kigali. A total of 200 patients underwent screening for detection of pathological aortic and mitral regurgitation associated with RHD.

Result: Mitral regurgitation was found in 77 of the 200 women (39%) and aortic regurgitation in eight of the participants (4%). Applying the WHF-criteria for echocardiographic diagnosing of RHD five of these was considered as borderline RHD (25 cases per 1000) and one definite case (5 cases per 1000) of RHD was detected. In total, a prevalence of 30 cases per 1000 was found including both borderline and definite RHD. Thus, a prevalence of rheumatic heart disease among pregnant women in Kigali was found to be between 0.5-3.0%.

Conclusion: The result of this study indicates the need of performing an extended study with a larger population to determine the accurate number of pregnant women in Kigali with rheumatic heart disease.

ABSTRACT

By: Anna Komarovska

Background: Enlarged and reduced compliant atrium is a common finding in patients with heart failure, caused by elevated filling pressure in the left ventricle. Enlarged chambers on the left side of the heart will also over time lead to a pressure increase in the pulmonary circulation and pressure loading on the right side of the heart. This implies a poor prognosis and often death as a result. An important tool in both diagnosis and prognosis of these patients is transthoracic echocardiography. Systolic deceleration time of pulmonary venous flow and estimation of atrial size are one of the parameters which can enhance the role of echocardiography to detect high- risk patients. **The aim** of the study was to observe the predictive value of 5-year mortality of the systolic deceleration time of the pulmonary vein and visual assessment of the bilateral difference in the atrial size in older patients with heart failure. **Methods:** The study population included 289 patient hospitalized for heart failure whom had a previous transthoracic echocardiography examination. Pulmonary venous systolic deceleration time and visual comparison of atrial size were analyzed from an echocardiographic 4-chamber view. Mortality data were collected after 5 years. **Results:** The mean age of our population was 79 ± 7. 17 years, of whom 43% were females and 195 died. The deceleration time in the dead group was (160. 35 ± 44.95) while it was (170.07 ± 41.77) in the living group (p=0.17). Patients with largest right atrium showed lower survival rate (15,9 %) than patients with equal sized atrium or largest left atrium (29.5%, 36.6%, respectively) (logrank= 8.80, p= 0.012) **Conclusion:** Our study shows that visual comparison of atrial size can be used to predict 5-years mortality in elderly patients with heart failure.

ADP-induced aggregation in platelet concentrates deteriorate sharply during the first storage day.

By: Ely Koyun

Bachelor thesis in Biomedical Science performed at the Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, 2015

Supervisor: Camilla Hesse PhD

Platelets goes through changes in the structure and function when blood is removed from the donor until the platelet concentrate is produced and distributed to patients. Previous studies has shown that Adenine di-phosphate (ADP) -induced platelet aggregation in platelet concentrates deteriorate during storage. The agonist ADP activates platelets by 3 purinergic receptors P2Y1, P2Y12 and P2X1.Vasodilator stimulated phosphoprotein (VASP) is an intracellular actin-regulatory protein that can be used to study if the P2Y12 receptor is blocked by endogenous ADP from platelets. P-selectin is a protein found in the inner wall of the α-granules. When platelet activation by agonists (in this study ADP) take place platelets release α-granules and the inner walls of the granules is exposed on the outside of the cell. P-selectin is expressed on the platelet surface and used as a marker to ensure that the platelets are activated. The aim of this study was to examine if ADP-induced aggregation is impaired in platelet concentrates and what may be the cause of the deterioration. Flow cytometry was used for VASP fosfosforylation and P-selectin expression where measurements conducted on platelet concentrates from apheresis platelets. Multiplate analyzer, a whole blood aggregation instrument was used to assess platelet function with impedance technique. Platelet concentrate was also evaluated with the instrument. We studied 26 healthy apheresis donors. 14 unirradiated samples was analyzed directly after the donation, 6 unirradiated and 6 irradiated samples was analyzed after one storage. The results indicated that unirradiated platelet concentrates analyzed one day after the donation was deteriorated. Irradiated samples seems to raise the average value of the ADP-induced aggregation. The material is however small, and therefore further evaluation in a larger population could be of additional value. Further studies might also include the study of how the ADP receptor is affected.

Scintigraphic additional value of combined planar imaging and SPECT/CT at suspected parathyroid adenoma

By Lisa Lagervall

Bachelor thesis in Biomedical Laboratory Science performed at Nuclear Medicine/Clinical Physiology, Sahlgrenska University Hospital, University of Gothenburg, 2015 Supervisors: Martijn van Essen (MD, PhD) and Johan Svalbacke (MD)

ABSTRACT

Background. Various approaches for Tc-99m-sestamibi parathyroid scintigraphy are in clinical use. Planar imaging can be performed with Tc-99m-sestamibi only or in combination with Tc-99m-pertechnetate for background subtraction, i.e. thyroid gland activity. A tomographic scan (SPECT) and a combined computed tomography (CT) is often performed as an addition to planar imaging, to further improve the anatomical localization of hyperfunctional parathyroid tissue. There are several studies that suggest that combined SPECT/CT is superior to planar imaging and SPECT solitary. However, the addition of SPECT/CT as a routine procedure during parathyroid scintigraphy is currently being investigated. **Aim.** The aim of this study was to perceive the value of added SPECT/CT during parathyroid scintigraphy, and furthermore study some clinical factors that may influence the outcome of the scintigraphy. **Methods.** The study population consisted of 20 patients that had undergone parathyroid scintigraphy at Sahlgrenska University Hospital, Gothenburg, Sweden, during January – April 2015. Each result from the examinations was retrospectively rated by one physician in regard to whether the addition of SPECT/CT could detect more lesions and whether it could localize the findings from the examinations, and finally an overall additional value was stated. Clinical factors of the patients that were studied were preoperative serum levels of parathyroid hormone (S-PTH) and whether they had undergone surgery in the neck area earlier. **Results.** Added SPECT/CT was considered valuable in 30 % of the patients and possibly valuable in 25 % of the patients. In 55 % of the examinations SPECT/CT seemed to better localize radionuclide uptake. Clinical factors of the patients, i.e. preoperative S-PTH and whether they had undergone surgery in the neck area earlier had no significant effect on the results. **Conclusion.** SPECT/CT was considered valuable in 30 % of the examinations. Both planar imaging and SPECT/CT could beneficially be performed as a routine procedure. Continued studies should include a larger study population and postoperative histopathology results as gold standard.

ABSTRACT

By: Katja Lind

Retinopathy of prematurity (ROP) is a serious disease that can cause vision impairment in extremely premature infants. The disease is characterized by a pathogenic vascularization of the retina. At the more serious form of ROP, these abnormally developed blood vessels can lead to retinal hemorrhage and scar formation in the retina which in extreme cases can lead to retinal detachment and blindness. There are many risk factors for the development of ROP. Today radioimmunoassay (RIA) technology is the most common assay for IGF-1 analyses. For some analytes, assays based on multiplex immunoassay (Luminex) technology have demonstrated advantages over enzyme–linked immunosorbent assay (ELISA) and (RIA). However, comparative studies between the RIA or ELISA and multiplex luminex technology if IFG–1are missing. The purpose of this study is to evaluate and compare multiplex luminex technique with the well-established RIA method, for the analysis of IGF-1. Seventy blood samples from preterm infants with the gestational age below 32 weeks were analyzed. For statistical data analysis intra-assay, inter-assay and correlation was measured. The agreement between methods was studied using Bland–Altman plot.The Spearmans correlation coefficient between the assays was 0,226, p =0,066. The Bland-Altman plot showed non-significant correlation between multiplex and RIA method. The intra assay variation showed IGF–1 values slightly higher in the multiplex method than with RIA–method. Inter–assay variation showed large and irregular variability of internal serum controls with multiplex in comparison with RIA–method. I conclusion the study showed poor agreement of luminex and RIA methods, particularly in the low measuring range. Further studies are needed to answer the question of what causes this, and if the methods correlates better in a higher measuring range.

Comparison of transportation system for feces pathogens for initiating ESwab in the Clinical practice

By: Anette Lundqvist

Bachelor thesis in Biomedical Laboratory Science performed at Department of Clinical Microbiology, Hallands Hospital, Halmstad, 2015
Supervisor: Ingegerd Sjögren, PhD Clinical Bacteriology

When a new system for diagnostics of feces pathogens was introduced a couple of years ago at the Department of Clinical Microbiology in Halmstad, a new transport system seem to be required. With the new diagnostic system only the positive patient samples needs to be cultured. This leads to a prolonged storage time for the transport tube in the refrigerator for up to four days. The aim of this study is to evaluate if the feces pathogens survives better in the new transportation system containing liquid Amies medium compared to the transportation tubes the laboratory uses today. To compare these two transport systems three different bacteria where used, *Shigella sonnei, Salmonella typhimurium* and *Campylobacter jejuni.* With the method based on viable count, equal amount of bacteria in suspension was added to the different transport systems and they were then stored in the refrigerator for four days. Every day including the day the bacteria were added to the tubes, samples were taken and cultured in different dilutions on to different agar plates. When the results were analyzed there was no significant difference between the tubes for *Shigella sonnei* and *Salmonella typhimurium*. But *Campylobacter jejuni* had grown nearly nothing at all on the selected agar plates. Another try gave almost the same result but a second agar plate was added and some colonies were formed. The conclusions from this study is that the new transport system containing liquid Amies medium with its positive benefits is sufficiently good to be inserted in the daily routine.

SEPARATE ABSTRACT

The development and validation of an LC-MS/MS method for quantification of cortisol in urine

By: Vala Dögg Marinósdóttir

Bachelor thesis in Biomedical Laboratory Science performed at the Laboratoriemedicine, Clinical Chemistry, Sahlgrenska Academy, University of Gothenburg, 2015.

Supervisors: Ulla Rüetschi, Docent, Chemistry
Thorleif Jonsson, Chemist

One of the recommended analysis for investigating patients with symptoms of hypercortisolism is by measuring 24 h urinary free cortisol. Many different methods have been published during the last years for measurement of cortisol with some variations in reference values because of interfering substances especially concerning immunoassays. Many laboratories today have gone from immunoassays to quantification of cortisol with the LC-MS/MS method which have shown to be both more selective for cortisol and suitable for routine analysis. A validation of a new method involves establishing numerous tests to identify any changes or influences that could affect characteristics of the method and to which extent. The aim of this study was to test parameters to see if the method was suitable for routine analysis in this laboratory which previously have measured urinary free cortisol with the chemiluminiscence immunoassay (CLIA) method, a non-accredited method for cortisol. The parameters tested in this study were precision, linearity, accuracy, carryover, interferences and stability. The accuracy was tested by measuring 40 patient samples previously tested with LC-MS/MS in Lund and 11 external controls previously tested with an in-house CLIA method. The interferences tested were 20- α-dihydrocortisol, 20-β-dihydrocortisol, 11-α-hydrocortison and prednisolone. The method showed precision with intra- and inter-assay <5% and linearity from 5-1500 nmol/L with $R_2 = 0.999$ and no carryover was detected. The material used was stable and showed linear regression with corresponding method in Lund. However this study did not show great advantages for the LC-MS/MS over the CLIA method which in this study can be related to a narrow testing range in the external controls. The study indicates good results for the method to be established into routine analysis shortly with only a few parameters left to be tested.

Abstract:

Erythrocyte transketolase is a thiamine dependent enzyme diagnostically used for assessing the thiamine status. Alcoholism and malnutrition is the most common cause for thiamine deficiency throughout the world. Thiamine deficiency may lead to beriberi, a disease that affects multiple organ systems, including the central and peripheral nervous systems.

The transketolase activity is routinely assayed using a colorimetric detection method. However, this method has caused several problems for the laboratory. Our purpose of the study was to test if a NADH-dependent transketolase assay would solve these problems.

Although we optimized the NADH-dependent assay we couldn't discriminate a deficient sample from a sample with normal thiamine levels that were identified with the colorimetric assay. In summary to use the NADH-dependent assay as a diagnostic tool for identifying thiamine deficiency the described method needs to be further evaluated.

ABSTRACT

Tissue preparation for immunohistochemical studies of bone growth related to magnesium implants – a new method

By Sofia Neergaard-Möller

Bachelor thesis in Biomedical Laboratory Science performed at Department med Biochemistry and Cellbiology, Sahlgrenska Academy, University of Gothenburg, 2015
Supervisor: Håkan Nygren, Professor

The most commonly used method for histologic preparation of bone tissue utilize fixation with formalin and decalcification, which both have withdrawals when it comes to immunohistochemical staining. The aim of this study was to develop a method for tissue preparation and immunohistochemical staining of bone, not comprising formalin fixation and decalcification, which can be used for studying osteocalcin in bone from rats treated with magnesium implants. Tibiae from rats were fixed by freeze substitution and sectioned with a diamond saw. Different ways to mount the sections on glass slides, perform the immunohistochemical staining for osteocalcin and mount the cover glass were evaluated and the protocol was modified based on the results. Secondary antibodies with fluorescein isothiocyanate and fluorescence microscopy were used for visualization. Sections prepared with the final protocol were compared with paraffin sections of formalin fixed, decalcified bone. Furthermore a pilot study on magnesium implants in rat tibia was performed. There were differences in the appearance of the fluorescence between the two methods, but the localization of osteocalcin in the sections were essentially the same. In some parts the fluorescence was a little too strong, partly due to the thickness of the sawed sections, so some adjustments according the secondary antibody are recommended for further studies. The pilot study indicated that 2 and 3 weeks after surgery animals with magnesium implants had larger areas positive for osteocalcin than sham operated animals. The developed method for tissue preparation of bone for immunohistochemical studies of osteocalcin is time saving and also requires less resources than formalin fixation and decalcification, making it suitable for research purposes.

EEG suitable as a prognostic marker for thrombectomy

By Jessica Nilsson

Bachelor thesis in biomedical laboratory science performed at the Department of Clinical Neurophysiology, Sahlgrenska Academy, University of Gothenburg, 2015 Supervisors: Mikael Elam (MD, PhD) and Linda Lundblad (PhD)

Background. Stroke is a common disease which causes reduced blood flow in the brain. Brain functions controlled from affected areas deteriorate and urgent care is vital in this state. A stroke can be caused by a cerebral hemorrhage (approx. 15 %) or a thrombus (approx. 85 %). An emerging treatment for stroke caused by thrombus is thrombectomy. Studies have shown its usefulness, and in some cases its superiority, to the alternative treatment thrombolysis.
To evaluate the effects of acute stroke treatments, a modified Rankin 90 days scale (mRS90) is used, ranging from 0 (no symptoms) to 6 (death). Electroencephalography (EEG) has been suggested as a possible tool to improve the clinical evaluation of early treatment.

The aim of this study was to evaluate EEG as a prognostic marker for the efficacy of thrombectomy.

Method and result. We studied 20 stroke subjects after completed thrombectomy. Subjects were registered with EEG at three occasions; the morning after thrombectomy, 24 h after the first registration and after a follow up period of 3-9 months. EEG findings were related to the corresponding mRS90 values. In addition, we compared clinical routine visual EEG interpretation with a quantitative spectral analysis of EEG. Our main findings were that 1) the early EEG findings predicted the clinical outcome evaluated with mRS90, and that 2) traditional visual analysis was more reliable than spectral analysis.

Conclusion. Visually interpreted EEG can be considered of clinical value as a prognostic marker for outcome after thrombectomy following ischemic stroke.

SEPARATE ABSTRACT

Set up and validation of high-sensitivity flow cytometric analysis of leukemic cells in chronic lymphocytic leukemia

By: Monica Nilsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska Academy, University of Gothenburg, 2015.
Supervisors: Linda Fogelstrand, MD, PhD
Marianne Johnsson, Biomedical scientist

Introduction. Analysis of minimal residual disease for patients with chronic lymphocytic leukemia is requested from hematology clinics in Västra Götalandregionen. The analysis can be used for monitoring drug therapy, follow-up after stem cell transplantation and for future research in the field. The method could also be used for early detection of relapses of the disease. **Aim.** The aim of this thesis was to develop and validate an antibody protocol for the analysis of minimal residual disease in patients with chronic lymphocytic leukemia for use at the section of flow cytometry. **Method and Results.** A total of 17 blood samples were analyzed; 10 from healthy subjects and 7 from patients previously diagnosed with chronic lymphocytic leukemia. Every sample was evaluated with the 8-colur flow cytometry analysis and antibody protocol. The results from the dilution study showed a sensitivity of 0,008 %. Because of the background of normal CD5+ B lymphocytes were the limit of detection 0,018 %. The variability of the method was CV 4.93 %. **Conclusion.** The antibody protocol for minimal residual disease had a higher sensitivity than the previous diagnostic method that was in routine at the laboratory, with a higher number of cells analyzed and with a distinct separation of normal CD5+ B lymphocytes and leukemic cells. The analysis will soon be in operation in clinical routine in the laboratory.

Volumetric capnography provides information about ventilation-/perfusion-mismatch in adults with asthma

By Alexandra Norén Eilertsen

Bachelor thesis in Biomedical Laboratory Science performed at the Laboratory of pulmonary research, Skaraborgs hospital Skövde, 2015
Supervisor: Dr. Per Gustafsson, MD, PhD

BACKGROUND The involvement of the peripheral airways is an established component of asthma and their involvement have been difficult to assess with conventional lung function tests. Volumetric capnography has shown promise in detecting ventilation inhomogeneity and changes in airway size but it's correlation to indices derived from multiple breath washouts, which is a better method than spirometry to detect peripheral airway involvement, has not been described in adults with asthma. **AIM** The aim of this study was to investigate if indices derived from volumetric capnography could show involvement of the peripheral airways that correlated either to indices from spirometry or multiple breath washouts and if volumetric capnography could be used as an effort-independent alternative to spirometry in the diagnosis of asthma. **METHOD** Lung function data for one hundred and one adult asthmatics where collected from a previous study. All of the asthmatics had underwent spirometry and nitrogen multiple breath washouts from which the volumetric capnographies where obtained. Statistical tests used where one-way ANOVA with Tukey's post hoc test and Pearson's and Spearman's regression analysis. $P<0,05$ was considered to be a statistically significant difference. **RESULTS** When divided into groups, based on the severity of obstruction assessed by nitrogen multiple breath washout, the normalized phase III and alveolar deadspace derived from volumetric capnography differed between all three groups and correlated moderately with corresponding N_2-MBW derived indices. **CONCLUSION** In conclusion, this study shows that different indices derived from volumetric capnography roughly correlates with corresponding indices derived from nitrogen multiple breath washout. The flaws in the consistency most likely represents different degrees of ventilation/perfusion mismatch. Volumetric capnography therefore provides additional information to inert gas washouts, but cannot replace it. Volumetric capnography provides more information about the peripheral airways than spirometry and could most likely, with further developing, be used as

a non-effort alternative to spirometry. Further studies are needed to determine the informational value that volumetric capnography seems to contribute with.

Sammafattning

Av: Fahimeh Norozi

Urinvägsinfektion (UVI) är den mest vanligaste infektion sjukdom. UVI är vanligare hos äldre patienter, både män och kvinnor.

Den vanligaste bakterien vid urinvägsinfektion (cirka 80 %) är *Escherichia coli* som kommer från tarmfloran.

Syfte är att jämföra reproducerbarheten och bedöma arbetsinsatsen vid urinodling med blodagar/Krom-agar mot nuvarande teknik blodagar och CLED-agar.

Nuvarande teknik: avläsning skedde oberoende av två olika avläsare och dokumenterades oberoende av varandra.

Ny teknik: avläsning skedde oberoende av två olika avläsare och dokumenterades oberoende av varandra.

Nuvarande teknik jämfördes med ny teknik: avläsning skedde oberoende av två olika avläsare och dokumenterades oberoende av varandra.

Vid kontroll av bakteriemängd användes *E. coli* (CCUG17620) och *Enterokocks faecalis* (CCUG9997).

Vid studiens slut kunde blodagar/Krom-agar ersätta blodagar och CLED-agar. Eftersom Krom-agar är snabbare och effektiverare än CLED-agar. Igenkännande vid identifiering av bakterier sker snabbare med Krom-agar på grund av att bakterie producerar olika kolonipigment.

NFkB-reglering av herpes simplex virus typ-1-inducerade fukosyltransferaser i humana fibroblaster

Av Jens Nygren

Biomedicinska analytikerprogrammet 180 hp
Examensarbete 15 hp, våren 2015

Handledare: Kristina Nyström, PhD
Avdelningen för infektionssjukdomar, Institutionen för biomedicin
Sahlgrenska Akademin, Göteborg

Virus nedreglerar generellt uttrycket av sin värdcells egna gener för att kunna ställa om produktionen till virusens gener, samt de gener ur värdcellens genom som gynnar virusets spridning. Herpesvirus har tidigare visats öka uttrycket av sialyl Lewis X, ett glykoprotein som används av T-celler för att binda till selektin på insidan av kärlväggen och till slut lämna blodbanan och ta sig till inflammationshärdar.

Mycket är okänt om mekanismen med vilken herpesvirus, i det här fallet HSV-1, ökar uttrycket av sialyl Lewis X. Hittills har man i odlade lungfibroblaster visat att viralt dsRNA aktiverar proteinkinas R, och att HSV-1-infektion ökar transkriptionen av FUT3, FUT5 och FUT6, som kodar för fukosyltransferaser som syntetiserar sLeX. Vi försöker här genom att i samma celltyp blockera olika protein med droger och mäta uttrycket av generna med RTqPCR att ta reda på vilken signalväg som resulterar i uppregleringen av dessa gener, som aldrig annars uttrycks tillsammans.

Våra resultat visar en dosberoende inhiberande effekt av panepoxydone i HSV-1- infekterade fibroblaster, vilket tyder på att proteinkomplexet NFkB är involverat i den virala aktiveringen av dessa gener, samtidigt som den dosberoende stimulerande effekten av samma drog i oinfekterade celler vittnar om att processen styrs av komplexa signalvägar.

Separate Abstract

Method development of Dimethylmethylene Blue (DMB) in Spectrophotometric Determination of Glycosaminoglycans (GAG) in Urine.

By: Rosanna Nyqvist

Bachelor thesis in Biomedical Laboratory Science performed at the Department of clinical Chemistry, Sahlgrenska Academy, University of Gothenburg, 2015.
Supervisors: Maria Blomqvist PhD, Carlos Cabrera Rodriguez M. Sci.

Mucopolysaccharidosis (MPS) is a metabolic disorder that is devided into seven groups depending on which enzyme deficiency. These conditions lead to glycosaminoglycan (GAG) storage in lysosomes. MPS has very severe symptoms like retardation and bone abnormatilities, many patients do not survive childhood. A common initial analyse for this condition is detection of GAG in urine with Alcian blue. The reagent will bind to GAG and the complex concentration will be measured with a spectrophotometer. This method is time consuming, relatively unstable and can give varied results.
Therfore we investigated the possibility of chaning to dimethylmethylen blue, which is a more rapid and accurate method that might increase the patient security and decrease response time. In this method we simply mix centrifugated urin with DMB-reagent. After five minutes at room temperature the absorbances are measured by spectrophotometer at two wavelengths, 530 nm to measure aborbance maximum and 595 nm for the absorbance minimum of the DMB-GAG-complex. Using the difference between minimum and maximum enhance the linearity of the calibration curve.
Different tests were preformed to optimize the protocol of the DMB method.
Buffers, DMB-concentration, cuvettes and other aspects were examined.

Our evaluations prove great linearity and a stable method, although the control ERNDIM did not show the expected value of 46,6 mg GAG/L. Therefore further development needs to be done to gain an accurate method.

Abstract

By: Tomoko Okamoto

Studies using brain imaging and postmortem brain tissues have revealed reduced brain volumes and cell density in the prefrontal cortex and limbic regions in patients with bipolar disorder (BD). This suggests presence of disturbed neuronal circuits and impairment of neuroplasticity in these regions (1). This may be partly examined in vitro by the migratory ability of patient-derived neural stem cells in comparison with those of healthy controls.

For this, we used neuroprogenitors (NP) differentiated from the induced pluripotent stem (iPS) cell lines that were previously created from subcutaneous adipocyte samples, which derived from BD patients and healthy controls (2). Neurospheres cultured from NP were used for migration assays in a culture medium that was made to direct cortical neuron differentiation (3).

After NP migration out of the neurospheres, the length of radial neurite outgrowths were measured and quantified by using image processing and analysis software.

Logistic regression was used to predict diagnosis (bipolar|control) from the predictors square-root transformed migration ratio, the two types of cultivation plates used, and their interaction. Thus, controlling for plate and the plate x migration ratio interaction, the migration term was clearly significant in an Effect Likelihood Ratio test ($p=0.0023$). The overall nominal logistic fit was significant ($R2=0.079$; $n=125$; $p=0.0040$). Higher migration ratios were correlated with the bipolar group.

Abstract

Animal trials in addiction biology research are an important tool, in the regards of understanding how different brain regions communicate during drug stimulation. Dependingon the effects to be studied, the alcohol can be administered in various ways. A standard dose of alcohol intraperitoneally (i.p.) is 2.5 g ethanol/kg, due to the high, yet physiologically relevant gained concentration. Basedon the dopamine elevations formed by said i.p. concentration, the Addiction Biology Unit at the University of Gothenburg has determined the standard concentration of alcohol administered through local perfusion to be 300 mM, due to the similar dopamine responses (~30% elevation). The aim of this study is to set up a method for analyzing ethanol in samples from *in vivo*microdialysis studies. Then compare the actual concentrations of ethanol in the brain at standard doses of i.p. and local perfusion, to investigate whether the dose by local perfusion correspond to the same, high but physiologically relevant dose given i.p.

*In vivo*microdialysis were used to determine the ethanol concentration in the rat brain, after systemically and locally administered standard doses of ethanol (2,5 g/kg and 300 mM resp.). For ethanol analysis, a spectrophotometric method with enzymatic reaction process was used. The results of the *in vivo*microdialysis is however difficult to interpret, because of malfunctions in the ethanol analysis method resulting inloss of data. Also, many of the probes were believedto have a greater active space than acceptable, resulting in falsely high ethanol concentrations, especially in the locally administrated animals. Based on these results, it is not possible to draw any conclusions regarding the relevance of the ethanol concentration in the brain, gained from 300 mM local perfusion. In future ethanol studies in this field, another method should be selected.

Quantification of volume flow in venous reflux using ultrasonography shows reproducibility and a correlation between volume flow and vessel diameter

By Alex Siman

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Physiology at Östra Hospital, Sahlgrenska Academy, University of Gothenburg, 2015
Supervisor: Anders Thurin MD

Introduction: Venous insufficiency is a disease that affects a large part of the Western world, and is characterized by loss of function in the veins, usually in the legs. The primary method for quantification of venous reflux in individual veins is venous ultrasonography. The aim of this study was to investigate if an ultrasonography examination of the veins could provide reproducible measurements to quantify venous reflux in the great saphenous vein. In addition, we also wanted to observe the relationship between proven reflux and the correlation to the symptoms/quality of life among the patients'.

Methods: In the present study, a total of 35 patients (48 legs) underwent venous ultrasonography. Examined parameters were the diameter of the vein, time average velocity, peak reflux velocity and average flow. A modify version of the AVVQ (Aberdeen Varicose Vein Questionnaire) was used to correlate the symptoms/quality of life with the reflux.

Result: A correlation between the diameter of the vein and the average flow could be discovered (R-value 0,52). Overall, a poor relationship between the average flow and the symptoms/quality of life could be demonstrated.

Conclusion: The present study indicates a certain relationship between the average flow and the diameter of the vein, but in this current study no correlation could be established among the patients' symptoms/quality of life and the average flow. A further study in larger group of patients including, more patients with liposclerosis and/or venous ulcerations should be conducted to obtain better data.

SEPARATE ABSTRACT

Prevalence of Hepatitis E virus (HEV) in Swedish pigs in different types of farms

By: Leyla Temiz

Bachelor thesis in Biomedical Laboratory Science performed at the Department of clinical microbiology-virology, Sahlgrenska Academy, University of Gothenburg, 2015

Supervisors: Heléne Norder, Professor and Marie Karlsson, Biomedical scientist

Hepatitis E is caused by hepatitis E virus (HEV), which a non-enveloped virus of 27-35 nm in size. The genome is single-stranded RNA. HEV is zoonotic; it can infect both humans and animals. HEV infection is mainly fecal-oral and can occur via contaminated food and water, and also by blood transmission. HEV that infect humans are classified in four genotypes. Genotypes 1 and 2 are found exclusively from humans and occur mainly in Asia and Africa. Genotypes 3 and 4 occur worldwide and infect animals and humans. HEV genotype 3 has been detected in pigs in different parts of the world also in Sweden. Since pigs excrete large quantities of HEV in their feces, they are considered to be primary host for the infection. The virus has also been found infecting many other animals, including deer, moose and rodent, which can also be a source for human infection. The purpose of this study was to investigate the prevalence of hepatitis E in Swedish piglets and sows; if it differs between different types of farms and at different years of sampling. For detecting HEV RNA in fecal samples from pigs, PCR was used, which is a sensitive and specific method. The study material consisted of 128 feces samples from 13 pig farms in Sweden. From each farm 10 samples from piglets and 3-4 samples from sows were collected. HEV RNA was detected in 9 of the 128 feces samples (7%), regardless of type of farm. This result was compared with result from a year earlier, which showed that there is no difference in prevalence of HEV infection between the farms and year of collection. There were only piglets excreting the virus, while all samples from the sows were negative for HEV RNA. This study demonstrates that HEV is common in Swedish pigs and that the frequency does not vary between years. The pig is considered to be a source for the HEV dissemination as mentioned, which was confirmed by this study, although furthermore a sequencing of the virus strains needed to investigate if these viruses remain in the farms or are reintroduced with each pig herd and if these strains are found infecting humans.

Abstract

By: Therese Vadman

Platelets are an important factor in the hemostasis. Patients with thrombocytopenia or defects in platelet function is given platelet transfusion as treatment. Due to the limited storage of platelets, incompatible ABO-matched platelets is given, which has shown reduced effect on platelet recovery and higher mortality in comparison when ABO-matched platelets is used.

The aim of this study was to analyze how platelets were affected after transfusion *in vitro* between patients with blood group A and platelet apheresis of blood group O with high titers of anti-A. Impedance aggregometry was used to study platelets before and after the transfusion. For detection of antibodies bound to the platelets after transfusion a flow cytometry method was tested.

Aggregation was measured before and after transfusion in eleven cases, as well in the seven apheresis used in the transfusion. Titers of apheresis with associated blood typing tube was performed with indirect antiglobulin test and direct agglutinations test. Flow cytometry was performed with 7 different methods.

The results showed an average increase in aggregation with three out of the four reagents that were used in the impedance aggregometry. In 45 % of the transfusions the direct antiglobulin test were positive. Only 3 of 7 cases showed high titer in apheresis. The method for flow cytometry did not function.

Due to the vital role of platelet transfusions in modern healthcare, stricter rules for the use of platelet concentrates are justified. Further studies on how platelets are affected in transfusions is needed. For the results in this study to be significant, inclusion of a larger number of subjects are required.

Abstract

By: Oscar Wiberg

Introduction: Atherosclerotic plaques in the carotid arteries are one of the main sources of origin for cerebral thromboembolism. There are several factors which may determine the formation of atherosclerosis. It has been stated long ago that the occurrence of plaque between the left and right carotid artery is not equally distributed; the left side being more commonly exposed.

Aim: The aim of this study is to investigate if there is a difference in occurrence of atherosclerotic plaques between left and right carotid artery among men and women in Gothenburg assessed by B-mode ultrasonography.

Methods: 1097 subjects in the age 50 to 65 underwent carotid sonography examination, investigating plaque occurrence at the near and far wall of the common carotid artery, the internal carotid artery and the bulb on both sides. Plaques were defined according to the Mannheim consensus.

Results: Counts of plaque occurrence in the left vessel were 503 and 492 in the right, total of 995 plaque occurrences, there was no statistical significant difference P=0.637. A paired sample t-test showed no significant difference in the number of plaques in the left carotid artery and number of plaques in the right carotid artery P=0.087.

Conclusion: There is no difference in atherosclerotic plaque occurrence or number of plaques between the left and right carotid arteries among the general population of the Gothenburg area, aged 50 to 65.

ABSTRACT

Av: Martin Wietzke

Syfte

Att studera reglerad exocytos av adiponektin från vita adipocyter.

Metod

Preadipocyter från cellinjen 3T3-L1 differentierades till mogna adipocyter med insulin/dexametason/IBMX. Exocytos av adiponektin stimulerades vid 32° C med insulin, β3-agonisten CL-316,243 samt den cAMP-höjande kombinationen forskolin/IBMX. Frisatt adiponektin mättes vid tidpunkter mellan 0 och 90 minuter och analyserades med sandwich-ELISA och relaterades till den totala proteinkoncentrationen, mätt med Bradford Protein Assay. Differenserna i koncentration jämfört med den negativa kontrollen (5mM glukos) testades med students t-test.

Resultat

Kombinationen forskolin/IBMX gav en signifikant ökad adiponektinutsöndring från 15 minuter. Inkubering med CL-316,243 gav liknande resultat som forskolin/IBMX medan stimulering med insulin endast visade signifikant ökad adiponektinfrisättning vid senare tidpunkt (60 min).

Slutsats

Resultaten från denna studie är i enlighet med egna publicerade resultat och bekräftar att en ökning av cAMP stimulerar adiponektinfrisättning via en direkt effekt på det exocytotiska maskineriet. I kontrast till detta indikerar våra mätningar med insulin att detta hormon ökar frisättningen av adiponektin via mekansimser som ligger mer uppströms i signallerinsvägarna (effekt vid senare tidpunkter). Denna studie visar att adiponektinfrisättning stimulerad av cAMP och insulin sker via olika mekanismer.