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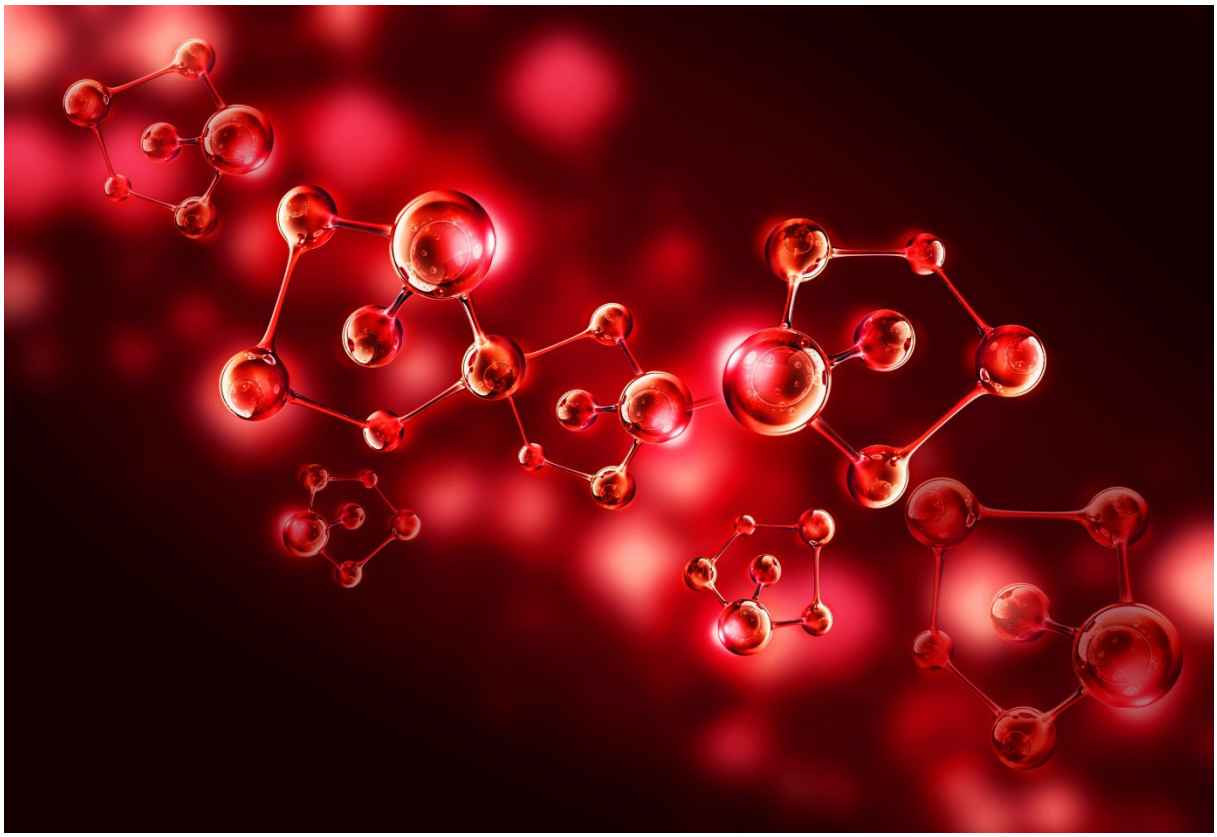

ABSTRACT BOOK 2014

Bachelor's and Master's Theses in Biomedical Laboratory Science

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Differences in automatic diagnosis and localization of myocardial ischemia in two commercially common software-packages -an analysis using modified diagnostic rules

by Tony Adielsson

Bachelor thesis in Biomedical Laboratory Science performed at the section for Nuclear Medicine, Sahlgrenska Academy, University of Gothenburg, 2014.
Supervisor: Milan Lomsky, Med. Dr

Background. Myocardial ischemia is often diagnosed in myocardial perfusion scintigraphy by using a summed difference score, which represents the cardiac muscle cells ability to reverse the oxygen deprevation between provocation and rest. Emory´s Cardiac Toolbox (ECTb) and Quantitative Perfusion SPECT (QPS) are two clinically common software-packages which use similar quantitative methods to detect and evaluate perfusion defects in the left ventricle of the heart. But they process the raw data differently from one another which could have an impact on the results and diagnostic accuracy. **Purpose.** The purpose of this study is to improve the two software-packages capabilities in diagnosing myocardial ischemia. By using three rules in the 17-segment model, this studie aims to modify the automatically summed stress score, summed rest score and summed difference score. This study also aims towards comparing the automated localization and overlapping of ischemic areas between the two software-packages. **Method.** 100 patients with ischemia were chronologically selected from a database of 1052 consecutive patients, which previously had been visually diagnosed without diagnostic tools. 89 of the original 100 were usable in this study. Three rules were implemented in ECTb and QPS; Rule 0, removed negative values from the difference score exclusively in ECTb, similar to existing filters in QPS. Rule 1, removed isolated scores of 1 in segments if the same segment were deemed normal in the corresponding rest/stress images. Rule 2, removed scores in three, adjacent basal segments if surrounding segment were scored with 0. The conformity of scored areas in patients with ischemia were studied and evaluated if the patient were diagnosed as ischemic in both ECTb and QPS. **Results.** Initial diagnostic comparisons showed 68 patients of 89, 38 with ischemi, in consensus between ECTb and QPS (κ=0.53). Rule nr. 0 increased patient compatability to 72, of which 42 patients were ischemic (κ=0.59). Rule nr. 1 lowered the patient compatability to 71, with 41 ischemic patients (κ=0.56). Rule nr. 2 lowered the conformity further to 69 patients in accordance between ECTb and QPS, of which 39 were ischemic (κ=0.52). localization of affected areas in ischemic patients showed overlapping segments in all of the initial 38 patients (mean=54%). **Conclusion.** Rule nr. 0 shows promise in ECTb in providing similar diagnostical results with QPS. Rule 1& 2 provided no increase in diagnostic accuracy. The localization of areas with perfusion defects were similar in ECTb and QPS, with frequent overlapping segments between the two software-packages. These findings suggests that whilst these two software-packages roughly detects myocardial defects in the same areas, the diagnostical capabilities in ECTb can be improved by adapting similar filtering to QPS.

ABSTRACT

The definition of quality controls can be accuracy and reliability of reported test results. It is used for this purpose, and all results must be as accurate as possible, all laboratory operations must be reliable and reporting must be timely in order to be useful in a clinical or public health setting. External quality conrol are control samples sent to different labs as controls for the reliabilty of the analyzes and results of the different labs. Unlike the external quality controls, the internal quality controls are used within a lab before analyzing patients samples.
The aim of this study was to add the external quality control for APTT and PT in the EQAS in Palestine. We wanted to prepare the samples and check the stability of the plasma every week for 4 weeks. From the labs, we wanted to check the inter results and the intra variation and then compare the CV and standarddivision of the labs. For the study 10-12 labs were participating (picked labs that were participating in EQAS).
Material that was used for this study was fresh froozen plasma from the Palestinian National authority (bloodbank) and the method that was used was lyophilization. The plasma was prepared at the Center for quality control in Al-Bireh and then sent out to 12 different labs in Palestine, after checking the stability for 6 weeks.
After receiving the result from the labs, the results were analyzed and we found no outliers for the PT and only 2 for the APTT. That means that these two outliers were excluted from the study. Also the result showed that the samples were stabile.
The conclusion for all this is that we succeeded with our study. Even though we faced some problems with the measurments and values we still managed to keep the samples stabile. This means that the external quality control of the APTT and PT now can be added to EQAS.

By: Lana Ali

The composition of stenotic carotid plaques assessed as percentage white does not correspond to the severity of event in symptomatic patients

By Anna Ander

Bachelor thesis in Biomedical Laboratory Science performed at the
Wallenberg laboratory, Sahlgrenska Academy, University of Gothenburg,
2014. Supervisor: Göran Bergström, MD, PhD

Introduction

Observations of plaque in the carotid artery is said to echo the appearance of plaque in other arteries in the body. Plaque texture may be either heterogeneous or homogeneous, reflecting the distribution of grey-scale tones in the plaque area. Homogeneous is when plaque has uniform consistency regardless of their echogenicity. Heterogeneous, when the plaque is non-uniform consistency with both ecolucent and echogenetic areas. The aim of this study was to investigate if there was a correlation between the type of stroke and the texture of the plaque assessed by Gray-Weale and by Percentage White.

Methods

This cohort comprises of patients who all have had one or more neurological episodes i.e. stroke, transient ischemic attack or Amaurosis fugax in 2010-2013 and when sorted there were 87 patients, with a total of 109 plaques in the cohort. The plaque interpretation program, Semi-Automated Method to Evaluate Echogenicity “SAMEE”, was used to assess plaque echogenicity. SAMEE use the feature “percentage white” to classify the plaques. This feature is derived from the visual subjective Gray-Weale classification.

Results

After assessing the ultrasound images using SAMEE and visually by Gray-Weale, the outcome was that the composition of the carotid plaque assessed by percentage white does not correspond with the severity of neurological event in symptomatic patients. There was no significant variation between the groups in the occurrence of ecolucent and ecogenetic plaques. Neither was there a difference in the number of plaques between the groups.

Conclusion

In conclusion, the present study shows that the composition of stenotic carotid plaques assessed as percentage white and Gray-Weale does not correspond to the severity of event in symptomatic patients.

The gut microbiota affects gene expression by activation of the nuclear farnesoid X receptor (FXR) signaling

By Anna Andersson

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg laboratory, Institute of Medicine.

Sahlgrenska Academy, University of Gothenburg, 2014

Supervisor: Sama Sayin, MD Ph.D Student and Fredrik Bäckhed, Docent, Professor.

In higher animals it is well known that microorganism covers more or less all of the mucosal surfaces and most of them are located in the gastrointestinal tract. Most of microorganisms consist of bacteria but there is also archive, fungi, virus and protozoa. In recent years it has been an increase of research in how the gut microbiota affects their host. The microbiota genom, called microbiom, consist of about 150 times the amount of the human genom. In that way the gut microbiota can be considered as a multifunctional organ. Many studies have shown that in the presents of gut microbiota a nuclear farnesoid X receptor (FXR) becomes more activated and so it affects the gene expression. In doing so it affects the host physiological processes and health. The aim of this studie was to validate this information further and see if the gut microbiota in the presence of the FXR regulates the gene expression. This was achieved by the use of Chromatin Immunoprecipitation (ChIP) and Quantitative Real-Time Polymerase Chain Reaction (QRT-PCR). An experiment test was performed on a FXR protein, which is a transcription factor (TF). When activated, it binds to specific genes of the DNA that determines if transcription starts or stops. Hence FXR regulates the gene expression. The results from this study strengthens what previous studies also argue that FXR upon activation, which increases in the presence of the gut microbiota, affects gene expression and thus a lot of different physiological and metabolic processes for the host. One can use the knowledge gained from this project to find out whether we can change the metabolism of bile acids, glucose and cholesterol by changing the composition of intestinal micro flora and thus the effect on FXR.

Stroke volume, measured with Simpson's biplane method and Doppler method, in correlation to a 5 year survival rate

A study in heart failure among the elderly

By Evelina Andersson

Bachelor thesis in Biomedical laboratory science performed at Östra sjukhuset, Sahlgrenska Academy, University of Gothenburg, 2014

Supervisor: Magnus Johansson, M.D and Cecilia Wallentin Gurron, M.D

Background Heart failure, HF, is a common disease amongst the elderly society and is often a sign of an underlying heart disease. It has a high mortality rate that's increasing with the condition of the heart and therefore a correct diagnosis is of great importance. Stroke volume, is one of many measures that can be used for diagnosing the condition, but since HF can have many different causes and comes in many different severities, it's crucial that the method from where it's extracted, is correctly describing the condition of the heart.

Aim The aim with this study is to investigate the stroke volume's importance when it comes to a five year survival rate, but also to examine the differences, and the importance, of the two methods from which stroke volume is measured: Doppler and Simpson's biplane method.

Methods The study was performed in retrospect and the population consisted of 141 elderly patients (>65years) with heart failure. Simpson's biplane method was used to extract Stroke volume and the readings were compared with those measured with Doppler in 2013.

Results Differences in stroke volume as well as the connection between stroke volume and mortality were examined. A significant difference was found between the two methods, $P<0.0001$, and the stroke volume measured with both Simpsons ($P<0.010$) and Doppler($P<0.0001$) was a predictor of mortality within a five year time-span.

Conclusion The study displayed a significant difference between Simpson's biplane method and Doppler. The study also displayed that Stroke volume was a predictor of mortality and therefore that stroke volume is in correlation to a 5 year survival rate.

Effects of ethidium bromide-Induced mitochondrial dysfunction on adipogenesis and adipocyte functionality

By Nanna Andersson Olsson

Bachelor thesis in Biomedical Laboratory Science preformed at the Institute of Neuroscience and Physiology, Sahlgrenska Akademy, University of Gothenburg, 2014
Supervisor: Ingrid Wernstedt Asterholm, Assistant Professor, PhD

Background. High levels of the adipocyte-derived hormone adiponectin are associated with increased insulin sensitivity, a reduced risk of type 2 diabetes mellitus and longevity. The mitochondria have an essential role for adipose tissue function and affects many different processes, including the differentiation and maturation of the adipocytes from precursor cells. A recent study in Caenorhabditis elegans (C. elegance) shows that an imbalance between nuclear and mitochondrial DNA encoded electron transport proteins causes the so-called mitochondrial unfolded protein response (UPR^{Mt}). Interestingly, this is an adaptive stress response associated with an extended life span of the worms.

The aim of this study was to investigate whether the function of mitochondria and induction of the mitochondrial unfolded protein response affect the synthesis and release of adiponectin in adipocytes.

Method. The cell line used in this study was 3T3-L1 preadipocyes. The cells were treated with different concentrations of Ethidium Bromide (EtBr) during and after adipocyte differentiation. EtBr binds to DNA, particularly to circular DNA. An optimal dose of EtBr should thereby selectively inhibit mitochondrial transcription, while leaving the nuclear transcription intact. Gene expression in the mature adipocytes was measured with quantitative real-time PCR and secreted adiponectin was measured with sandwich ELISA.

Results. Low-dose EtBr treatment decreases the mitochondrial transcription of genes involved in the electron transport chain, while transcription of similar genes encoded by the nucleus were unaffected. HSP60, a gene involved in the UPR^{Mt} was however unaffected by EtBr. The EtBr -induced mitochondrial dysfunction was associated with reduced adiponectin expression and secretion. EtBr treatment during adipocyte differentiation inhibited adipogenesis and the resultant immature adipocytes expressed even lower levels of adiponectin than adipocyte treated after differentiation.

Conclusions. EtBr treatment causes an imbalance between nuclear and mitochondrial DNA encoded electron transport proteins without affecting HSP60 expression. Our data support the notion that mitochondrial function, adipocyte functionality and adiponectin synthesis are closely linked. We were however unable to address our initial hypothesis since EtBr did not induce UPR^{Mt}, at least not in a similar manner as reported in C. elegance.

High reproducibility when calculating Bone Scan Index with the quantification program Exini Bone

By Lena Båth

Bachelor thesis in Biomedical Laboratory Science performed at the Section for Nuclear Medicine, Sahlgrenska Academy, University of Gothenburg, 2014
Supervisor: Reza Kaboteh, PhD

Bone scanning is one of the most common methods for detecting bone metastases. They frequently appear in cancers from prostate, breast, kidneys and lungs, and may cause pain and fractures. To detect metastases a radioactive nuclide is injected that seeks to the bone. A gamma-camera detects and processes the radiation into a picture. The most common method for interpretation is visual, and this requires skilled physician, but there is always a risk for misreadings. One disadvantage is that the bone scan doesn't separate metastases from benign conditions. Several attempts has been done in producing computerised programs as a diagnostic tool, as a help for the physicians. One of the programs calculates the percentage of metastases in the skeleton, and is called Bone Scan Index (BSI). Based on this program, a group of scientists and physicians from Lund and Sahlgrenska has constructed a fully-automated computer-program for detection and quantification of bone metastases, Exini Bone. **Purpose:** To verify the reproducibility in the Exini Bone-program, and to examine the quantification in images with a lower image quality. **Material and method:** This was a prospective study; patients who were assigned for a bone scan were asked if they could stay for a second scan. They were divided into two groups, the first 27 patients did extra bone scans with normal speed, and the next 27 did bone scans with double speed. The images were analysed in the Exini Bone-program, regarding its reproducibility. **Results:** 78% of the patients in group 1, and 81% in group 2 were correctly interpreted. The correlation between the two scans was 0,98 in group 1, and 0,97 in group 2. **Conclusion:** The reproducibility is high when scanning with normal speed. It is also high when scanning with double speed, but the results may be affected by an originally low BSI-value in several patients in the second group. The results from this study may be used as a basis for a study with a larger group of patients, to further develop the Exini Bone-program.

Abstract

Validation of LC/MS method for the determination of Pregabalin and GHB in urine.

By: Hassan Chmeis

Pregabalin is a drug prescribed for the treatment of fibromyalgia, and some kinds of nerve pains and seizures. Recently, lot of discussions have risen about the potential drug abuse of Pregabalin. It's even remarkable how much popularity it has gained that it became Pfizer's second-best selling drug 2012. Gamma hydroxybutyrate (GHB) is a narcotic classed drug, which we analyse today as a separate metabolite using Gas Chromatography-Tandem Mass spectrometry. Because of the numerous requests from different customers that suspect the abuse of Pregabalin, we decided to set up a method for the analysis of Pregabalin. This method will include the analysis of both Pregabalin and GHB simultaneously with easy sample preparation and high efficiency that will save time and work. The method is set up on an Ultra Performance Liquid Chromatography combined with a triple quadrupole mass spectrometer. The aim of this work was to implement a new method for the determination of Pregabalin and GHB simultaneously and to be able to use it as a routine analysis. The method showed within-day precision of cv=3,48% and a SD=0,03.

SEPARATE ABSTRACT

The impact of *Meis1* overexpression on proliferation and apoptosis of *HoxA9* murine bone marrow cells.

By Urmi Chouhan

Bachelor Thesis in Biomedical Laboratory Science performed at Sahlgrenska University Hospital, University of Gothenburg, 2014.

Supervisor: Laleh S. Arabanian, PhD, Postdoctoral researcher. Institute of Biomedicine, Department of Clinical Chemistry and Transfusion Medicine, University of Gothenburg, Sweden.

HoxA9 and *Meis1* are two transcription factors which have been introduced as key regulators in acute myeloid leukemia (AML). Their complex causes a rapid development of AML *in vivo* giving the patients a poor prognosis. Furthermore their complex leads to an accelerated proliferation and self-renewal ability in the cells harbouring the dimeric *HoxA9* and *Meis1*.

The aim of this study was to investigate if *HoxA9* and *Meis1* complex has influence on proliferation and apoptosis of cells. Our recent investigation on gene expression of *HoxA9* and *Meis1* revealed a list of genes differentially expressed in these cells. Among them, *Anxa1* an extracellular anti-inflammatory protein was shown to be downregulated in these cells. In addition to the investigation of *HoxA9*+*Meis1,* the impact of *Anxa1* and its effects on apoptosis and proliferation in the presence of *HoxA9* and *Meis1* complex was studied.

To study the oncogenic abilities of the transcription factors a Trypan Blue exclusion assay was executed to test the viability of the cells. Flow cytometry was used to measure the apoptosis with the use of two dyes; APC-Annexin V and 7-AAD.

It was confirmed that the *HoxA9* +*Meis1* complex showed a significantly upregulated proliferation capacity and a downregulated apoptosis. Clarifying that overexpression of *Meis1* together with *HoxA9* promotes proliferation of leukemic cells and counteracts apoptosis. It was observed that the *Anxa1* did not influence the *HoxA9* and *Meis1* complex as expected.

These results imply that the *HoxA9* and *Meis1* complex is promoting an accelerated proliferation rate and a downregulated apoptosis rate. The *Meis1* target *Anxa1* does not counteract the viability or apoptosis rate of the *HoxA9* and *Meis1* complex. Consequently, further studies should be executed in order to fully understand the role of *Anxa1* in leukemia and develop a treatment which prohibits *HoxA9* and *Meis1* to form a complex together or bind into DNA in order to give the patients a better overall survival.

Hämmad produktion av IL-6 och IFN b hos monocyter stimulerade med klinisk isolerade pneumokocker.

Sabina Dhakal

Abstrakt: Pneumokocker (*Streptococcus pneumoniae*) är en grampositiv bakterie, associerad med hög mortalitet och morbiditet främst hos barn under 5 år och vuxna över 65 år men även hos i övrigt friska individer. Den orsakar både invasiva pneumokock sjukdomar (IPD) och icke- invasiva sjukdomar.
Syftet med studien var att undersöka hur olika pneumokock serotyper aktiverar monocyter till cytokinproduktion. Om de olika serotyperna har förmågan att stimulera olika immunsvar.
I den här studien undersökte vi pneumokockserotyper vilka isolerats dels från patienter som var i övrigt friska och dels från patienter i riskgrupp. Monocyter renade från lättcellskoncentrat stimulerades med de olika pneumokocker serotyper.
Vi fann att serotyp 7F gav lägst IL-6 respons både på RNA och protein nivå. Även serotyp 3 och 23F gav ett lägre IL-6 respons på mRNA nivå. Inga av de kliniskt isolerade serotyperna stimulerade ett IFN b respons hos monocyterna.
Resultaten indikerar på att det finns skillnader mellan olika pneumokock serotyper när gäller att aktivera fagocyterande celler till cytokinproduktion men inga skillnader kunde direkt härledas till isolat från en viss patientkategori.

SEPARATE ABSTRACT

Poor evidence for higher sensitivity with T-SPOT.TB compared to QuantiFERON-TB Gold In-tube in the diagnosis of latent tuberculosis infection

By: Jeanette Ekberg

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of Biomedicine, Department of Infectious Diseases at Clinical Microbiology, Sahlgrenska Academy, University of Gothenburg, 2014.

Supervisor: Madeleine Ingelsten, PhD.

At present, the Serological Department of Clinical Microbiology at Sahlgrenska University Hospital in Gothenburg is using the QuantiFERON-TB Gold In-tube for detecting latent tuberculosis. This method has sometimes proven insufficient to patients that are immune compromised, as these patients fail to produce a response in the positive control, and therefore are evaluated as indeterminate. This has also been recognized in prior studies and partly explained, as there are correlations between low lymphocyte (CD4) counts and the indeterminate interferon-gamma assay results. In this study we wanted to compare the two different interferon-gamma release assays that are out on the market, the QuantiFERON-TB Gold In-tube and the T-SPOT.TB. And to evaluate if the T-SPOT.TB gives better results for patients with a suppressed immune defense and will work to their advantage in the future, since it also has been suggested prior to this study. During the months of march-may of 2014 a total of 164 blood samples, from 82 patients, were collected (to both assays) from different healthcare facilities through Västra Götalandsregionen. Of these 82 samples, for T-SPOT.TB, 19 (23%) were excluded from the study, due to low cell counts, and not analyzed. This study shows that of all 63 patient samples, that were analyzed, 37% were positive, 63% were negative and 13% were determined as borderline/indeterminate. The two assays had a good concordance, assessed here with Kappa coefficient ($\kappa = 0,74$), similar to what has been shown in prior studies. Three samples were determined as indeterminate in the QuantiFERON-TB Gold In-tube and became negative in the T-SPOT.TB-assay. These are too few results to decide if the T-SPOT.TB really is superior to QuantiFERON-TB Gold In-tube for the immune compromised patients, but this gives an indication that this might be the case.

Anti-bacterial peptides in the intestine in patients with ulcerative colitis before anti-TNF therapy start

By Reem Elias

Bachelor thesis in biomedical Laboratory Science performed at laboratory medicine, Sahlgrenska Academy, University of Gothenburg, 2014

Supervisor: Maria Magnusson PhD, University of Gothenburg, Inst for Biomedicine, Dept for Microbiology and Immunology

Abstract

Background: Ulcerative colitis is an inflammatory bowel disease that causes symptoms such as diarrhea, blood in stool, pain and fatigue. The disease often affects young people and is lifelong. The inflammation occurs in relapses with periods of illness and health. Anti-inflammatory medications are used to treat patients, and in many cases there is a need to have treatment for a long time. When no other treatments work, patients can receive anti-TNF therapy. Anti- TNF is an antibody against the pro-inflammatory cytokine TNF (tumor necrosis factor alpha) and is a biologic therapy, but less than 70 % of patients respond to treatment. **Aim**: The aim of this study was to investigate whether patients with ulcerative colitis, who will respond to anti-TNF α therapy (responders), have higher levels of anti-microbial peptides in the gut than non-responders before therapy initiation.

Methods: Biopsies from 30 patients were collected (18 responders and 12 non-responders) that would initiate anti-TNF therapy. The samples were biopsies from the inflamed area taken at endoscopy examination before treatment started. We analyzed AMPs at the mRNA level by RT-PCR, protein levels by dot blot and bacterial killing using flow cytometry.

Results: There were no differences in disease activity between the patient groups before treatment started. Responders had higher mRNA levels of Defensin 5 than non-responders while there were no differences in mRNA levels of cathelicidin, lysozyme or hBD2 of responders and non-responders. Results showed that the responders and non-responders had similar protein levels of Defensin 5 in the intestine. Results also showed that biopsy lysates from responders and non-responders had similar capacities to kill E. *coli* bacteria.

Conclusion: We found no differences in protein levels of Defensin 5 which may explain why some patients respond to treatment and others do not. However, we detected higher mRNA levels of Defensin 5 for therapy responders and this may be worth following up in future studies.

Left ventricular diameter underestimates ventricular dilation in patients with aortic- and mitralregurgitation - a comparative study of transthoracic ecocardiography och cardiac magnetic resonance

Maria Eliasson

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2014
Supervisor: Sinsia Gao, MD, PhD

Purpose: Severe aortic- or mitralregurgitation causes volume overload and left ventricular dilation. Left ventricular diameter by Transthoracic echocardiography (TTE) is recommended by guidelines in assessment of ventricular enlargement in grading of valvular regurgitation and before decision of valve surgery. The purpose of this study was to examine the precision of left ventricular end diastolic diameter ($LVEDD_{TTE}$) and left ventricular end diastolic volume ($LVEDV_{TTE}$) by echocardiography in grading of left ventricular dilation, using left ventricular end diastolic volume by cardiac magnetic resonance (CMR, $LVEDV_{CMR}$) as gold standard.

Methods: In this prospective study 53 patients with valvular regurgitation before valve surgery (28 patients with mitral regurgitation, 25 patients with aortic regurgitation) were included along with 20 healthy volunteers. All individuals were examined with TTE and CMR on the same day. After measuring $LVEDD_{TTE}$, $LVEDV_{TTE}$ and $LVEDV_{CMR}$, predicted values based on reference values and Z-scores ((observed-predicted)/SD) was calculated for each individual. Agreement in grading of left ventricular enlargement between these three parameters were evaluated using kappa coefficient.

Results: The mean age±SD was 50±16 years and 20 % were women. The indication for surgery was based on symtomatic regurgitation (96 %) or significant left ventricular dilation (4 %). Despite strong correlation between $LVEDD_{TTE}$ and $LVEDV_{TTE}$($r= 0.76$), $LVEDD_{TTE}$ and $LVEDV_{CMR}$($r= 0.80$) as well as $LVEDV_{TTE}$ and $LVEDV_{CMR}$ ($r= 0.95$, $P <0,001$ for all), there was a significant underestimation of left ventricular dilation by $LVEDD_{TTE}$ in comparison with both $LVEDV_{TTE}$ and $LVEDV_{CMR}$ (mean difference±SD Z-score -3.6±3.3 and -3.8±3.6 respectively, $P < 0.001$ for both), while there was no significant difference between $LVEDV_{TTE}$ and $LVEDV_{CMR}$ (mean difference Z-score -0.25±1.6, $P= 0.31$). $LVEDV_{TTE}$ showed significantly better agreement with $LVEDV_{CMR}$ in grading of left ventricular dilation in comparison with $LVEDD_{TTE}$ (weighted kappa (95 % confidence interval) 0.76 (0.49-0.79) and 0.31 (0.16-0.40) respectively).

Conclusions: $LVEDD_{TTE}$ underestimates grade of left ventricular dilation in comparison with $LVEDV_{TTE}$. In clinical practice underestimation of left ventricular volume and severity of valvular regurgitation could mean delay of diagnosis and surgery.

The protein UBTD1 and UBTD2:s subcellular location

By: Louise Eriksson

Bachelor thesis in Biomedical Laboratory Science perfomed at the Department of Medical Biochemistry and cellbiology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2014.
Supvervisor: Jennifer Uhler, PhD.

The protein UBTD2 has previously been localized to the mitochondria indicating that the protein may interact with mitochondrial processes. UBTD1, a homolouge to the protein UBTD2, was discovered when a yeast-2-hybridization was performed with TWINKLE, which indicates that the protein, as UBTD2 is located and have a function in the mitochondria, possibly involved in the mitochondrial DNA replication. The proteins contains an ubiquitin-like domain, but unlike ubiquitin cannot conjugate to other proteins. The aim for this study was to determine the proteins, UBTD1 and UBTD2:s subcellular location.

UBTD1/2-flag and UBTD1/2-GFP was created using PCR, the PCR-products was later transformed into chemically competent *E. coli* to generate a large amount of DNA. DNA was extracted, purified and analysed before T-REx-293 cells was sucessfully transfected, generating four different stable cell lines. The proteins subcellular location was assessed by Western blot, cell fractioning and fluorescence microscopy.

The result from this study cannot be used to determine the subcellular localization for UBTD1 and UBTD2. Cellfractioning and Western blot indicates that UBTD1 are located in the mitochondria, but was not confirmed by fluorescence microscopy . Therefore further studies are required, mainly in regard to the fluorescence microscopy that didn't result in detailed images of the cells, proteins and organells. A new study must therefore involve a better staining of the cells and better technique to receive detailed images.

Potential differencing of Bacillusspecies with gene sequencing of gyrB and Matrix Assisted Laser Desorption/Ionization-Time Of Flight Mass Spectrometry

By Alexandra Gillberg

Bachelor thesis in Biomedical Laboratory Science performed at the Culture Collection University of Gothenborg (CCUG), Sahlgrenska academy, University of Gothenburg, 2014
Supervisor: Liselott Svensson Stadler

Background and aim: Some species within the genus Bacillus is known to be hard to distinguish from each other. It is primarily the *B. cereus* group and the *B. subtilis* group. Early studies have shown that species identification with the *16S rRNA* gene is difficult due to the similarities with other species. Instead the focus has now shifted to the work of finding a housekeeping gene that can help with the identification. At the same time we will look at how Matrix Assisted Laser Desorption/Ionization-Time Of Flight Mass Spectrometry (MALDI TOF MS) identifies the bacteria. The aim of this study is to look at some housekeeping genes, primarily *gyrB*, and to determine the reliability with MALDI TOF MS. **Method**: Strains from the *B. cereus* group, the *B. subtilis* group and some other species were chosen for sequencing with *16S rRNA* and the *gyrB* gene. The results were presented as dendrograms and compared to each other. Parallel with these methods, MALDI TOF MS was used as an identification tool; dendrogram from this method were also compared to those of sequencing. **Results**: When comparing the different methods to each other we discovered that in most cases the dendrograms gave similar results. As expected sequencing with 16S rRNA showed high similarity between species within the groups, the *gyrB* gene showed lower similarity. With MALDI TOF MS we were not able to determine every strain down to species level, but were in most cases able to tell which group the strain belonged to. **Conclusion:** The *gyrB* gene shows potential in being a determinative housekeeping gene for *Bacillus*. But to be able to use it we need to make sure that the differences between species is stable and large enough.

Eosinophils inhibit T cell proliferation without detectable effect of IL-7, IL-13, TGF-β or PD1

By: Evelina Gustafsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Infectious Diseases, Institution of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2014

Supervisor: Jennie Andersson, PhD

The disease Graft-verus-Host-Disease is characterized by an excessive T-cell activation, which in acute cases can cause death. Regulatory T cells can regulate T cell activation using proteins. The aim of this study was to check whether eosinophils could affect the T cells similarly, ie. inhibiting the T-cell proliferation.

Eosinophils and T cells were purified from donor blood and was then put together with anti-CD28, recombinant IL-7, IL-13 and TGF- β , and antibodies PD1 , IL -13 and TGF- β in various combinations as well as isotype IgG1 in anti- CD3 coated wells. Subsequently, plates were incubated and on the second day radioactive thymidine was added. Plates were harvested and then radioactivity was read by using a β - counter.

Eosinophils compared with neutrophils inhibited 80.07 % and 54.65 % respectively of T cell proliferation. No significant effect could be demonstrated by the proteins IL-7, IL -13, TGF- β or antibodies towards IL -13, TGF- β and PD1, on eosinophilic inhibition of T cell activation.

The results suggest that our hypothesis that eosinophils have an inhibitory effect on T cell activation is correct, however it is not known what mechanism is responsible for the inhibition.

Average artifacts can be eliminated in a safe way in EEG

By: Lina Gustafsson

Bachelor thesis in Biomedical Laboratory Science, performed at Clinical Neurophysiology, Sahlgrenska Academy, University of Gothenburg, 2014.
Supervisor: Anders Hedström, MD

Background: Electroencephalography (EEG) is a medical research method where the cerebral cortex electrical activity is registered. EEG is often used to diagnose epilepsy. With 19 measure electrodes and one reference electrode pathological activity can be located. The reference electrode contributes to the EEG-signal as much as the other electrodes. To eliminate this contribution different montages are used. The average reference montage is known to produce artifacts if the sample vector with potential values contains outliers, which drive the mean away from zero. The purpose with this study is to test if three algorithms can reduce the average artifacts in average montage in EEG.

Materials & Methods: From unidentified EEG-registrations sample vectors was chosen, 62 from routine EEG-registrations, 70 from an EEG-registration with seizure activity and 70 from an EEG-registration with epileptiform activity. Momentum based algorithms was used to delete the sample vectors that makes the mean differ much from zero. The reference artifact could then be eliminated. To see the effect of the algorithms range, standard deviation and skewness normalized before and after processing of the algorithms.

Results: Range, standard deviation and skewness in routine EEG, seiuzure activity and epileptiform activity was unaltered or reduced after processing of the three algorithms.

Discussion: Two outcomes were seen. In the first case the spread of the sample vectors had skewness near zero, no sample vectors was then deleted. In the other there was outliers. After these were deleted the skewness were closer to zero. The three algorithms therefore had an effect and the mean artifact was unaltered or decreased. This procedure was safe and didn't produce any new artifacts.

ABSTRACT

By: Tove Hellqvist

BACKGROUND: Alzheimer's disease is the most common form of dementia and is diagnosed with cognitive loss tests and supplementary biomarker tests of Aβ and tau in cerebrospinal fluid. Measurements of the biomarkers today are based on immunoassays which have been proven to be difficult to reproduce. To be able to have a more reproducible method for $A\beta_{38, 40 \& 42}$ a liquid chromatography tandem mass spectrometry method was developed. The aim of the study was to validate the method so it could be used in pharmaceutical studies and eventually to produce reference material.
METHOD: A solid phase extraction and liquid chromatography tandem mass spectrometry method was used to validate the $A\beta_{38, 40 \& 42}$ measurement. The used method have been developed earlier for quantification of $A\beta_{42}$. The method is based on a variety of processing steps including spiking of internal standards, extraction and separation of analytes and calibration in human and artificial cerebrospinal fluid. After sample workup and solid phase extraction, the eluted analytes were put in a vacuum centrifuge to concentrate and dry, before it was stored in a freezer at -80oC. When it was time to analyse the samples it was resuspended in a small volume and placed in the liquid chromatograph and mass spectrometer for detection and quantification. The validation had several analytical steps which needed to pass for the validity of the method; carry over, matrix dependent ion suppression, measuring range, limit of quantification, precision, trueness, recovery and stability of the analyte.
RESULTS: The limit of quantification was set to150 pg/mL for $A\beta_{38 \& 42}$ and 1000 pg/mL for $A\beta_{40}$ and the measuring range was linear between 150 pg/mL-4000 pg/mL for $A\beta_{38 \& 42}$ and 1500 pg/mL- 40000 pg/mL for $A\beta_{40}$. The results from the precision study showed a day to day variation and reproducible factor of CV <11% for high and low levels of $A\beta_{38, 40 \& 42}$ in cerebrospinal fluids. No carry over could be detected between samples.
DISCUSSION: The validation of $A\beta_{38, 4o \& 42}$-method could not be completed due to instrument failure. The completed analytical tests shows that the method is well developed and reproducible, thus the validation probably would pass after all tests have been analysed.

Presence of FBS and high insulin concentration may enhance differentiation of human preadipocytes into adipocytes.

By Erika Hidebring

Bachelor thesis in Biomedical Laboratory Science performed at the department of Neuroscience and Physiology the Sahlgrenska Academy, University of Gothenburg, 2014.
Supervisors: Mickaël El Hachmane, PhD and Charlotta Olofsson, PhD

Introduction: Obesity is becoming a worldwide epidemic causing diabetes, heart disease, hypertension, and cancer. The weight gain and fat accumulation seen in obese people occur when the food intake exceeds the body's energy requirement. White adipose tissue is the major part of an adult body's total fat mass and serves as a protector of internal organs as well as an energy reservoir. Adipose tissue also acts as an endocrine organ and secretes molecules called adipokines. They affect the immune system and regulate the metabolism. A low secretion of a specific adipokine called adiponectin, is seen in obese patients and is thought to be associated with insulin resistance. Adipogenesis is an essential process that differentiate mesenchymal stem cells or preadipocytes into mature adipocytes. To provide the preadipocytes cultured in vitro with an appropriate molecular environment for the differentiation process, the cultivation medium needs to be chosen carefully.

Aim: The aim of this project was to evaluate two protocols used for *in vitro* differentiation of human preadipocytes into mature adipocytes. The differentiation quality was assessed based on the lipid storage and adiponectin production and secretion.

Materials and methods: Subcutaneous preadipocytes were cultivated and differentiated for up to 14 days following two protocols, provided by AstraZeneca and Professor Martin Wabitsch. The lipid storage of the cells was visualised by Oil Red O staining and the basal and stimulated release of adiponectin were measured using ELISA.

Results: The lipid storage was significantly higher in the cells treated with the Astra protocol compared to the Wabitsch protocol. Adiponectin production was also about 10 to almost 100 times higher than with Wabitsch protocol. Basal adiponectin release ranged from 3 to 7% of total production in Astra cells. Measuring of stimulated adiponectin release was not detectable for the Wabitsch cells and seemed to be highest for the Astra cells at day 10 and 12.

Conclusion: The Astra protocol might give a higher rate of differentiation of human preadipocytes into mature adipocytes, than the Wabitsch protocol does. This needs to be further investigated due to the cell death and/or lack of differentiation in the Wabitsch cells. The results may relate to the differences in composition of the two protocols. The Astra cells were treated with 3% FBS and a high insulin concentration, whereas no FBS and a low insulin concentration was used in the Wabitsch protocol.

A History of Mild Food Restriction Elicits Divergent Adaptive Responses in Male and Female Rats

By: Sarah Holmström

Bachelor Thesis in Biomedical Laboratory Science performed at the Section for Metabolic Physiology, Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, Göteborgs University 2014. Supervisor: Karolina Skibicka, Assistant Professor (Ph.D)

The rates of obesity are increasing at an alarming rate throughout the world. The repercussions of this pandemic are accumulating socio-- economic burdens on an already weakened healthcare and economic infrastructure. Obesity sufferers seek to alleviate their condition by attempting to reduce their excess weight. Dieting by means of caloric restriction is a very common and effective weight-- loss method. However, at a 2 or 5-- year follow-- up many of the patients return to their pre-- diet body weight or worse they even gain more body weight. Preclinical data suggest that a history of dieting may change the balance of major neurotransmitters controlling feeding behavior and emotionality. The mechanisms that regulate food intake are situated in the brain and comprise of two distinct pathways: the homeostatic pathway and hedonic pathway. They control feeding behavior such as food reward, motivation and craving palatable food. In this project our aim was to determine whether the food reward behavior is impacted by a history of caloric restriction. 40 Sprauge-- Dawley rats, 20 males and 20 females were the experimental models used in this study. The hypothesis was tested in two groups of rats, one group (control, n=20 of which 10 were females and 10 were males) was allowed to eat as much as they want (ad libitum), the other group of rats (n=20 of which 10 were females and 10 were males) was exposed to 3 cycles of caloric restriction (to 66% of calories eaten at baseline) to model the human pattern of repeated dieting. The rats were then allowed to gain weight to the level of body weight measured in *ad libitum*-- fed control rats in between each dieting cycle. After the first 2 cycles of 4-- day restriction the rats started their training in the operant boxes, with the final goal of performing progressive ratio operant test, which is a method of testing that is well established for assessing changes in Motivated behavior. The current study uncovered an interesting relationship between a mild dieting history and gender. The females adapted to dieting by reducing their physical activity yet also reduced motivation. The males did not change their physical activity yet increased food motivation, which is more in line with our hypothesis. It is possible that a history of restriction changed the hedonic processing of the rats in general. This is consistent with a history of food restriction inducting emotional disturbance some even progressive to depression in humans.

Abstract

Impact of a type II diabetes treatment, Exendin-4 on the serotonin system.

By Camilla Höglund

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, 2014. Supervisor: Karolina Skibicka, Assistant Professor

Background: Exendin-4 (Ex-4) is a GLP-1receptor agonist and a clinically approved treatment for type II diabetes. Ex-4 has an extended period of action, prolonged pharmacokinetics and high apparent *in vivo* potency when compared to endogenous GLP-1, these are only some of the reasons that Ex-4 has been utilized as a therapeutic agent in treatments of type II diabetes. Furthermore Ex-4 has been associated with a progressive dose-dependent weight-loss and is now evaluated as a potential weight loss therapeutic in clinical trials. Weight loss after can be achieved after peripheral application of Ex-4(clinical route), the drug, however has to reach the brain in order to reduce food intake. To date little is known about the central mechanism mediating the food intake suppression effect of Ex-4. **Aim:** The aim of this project was to increase understanding of the neuroanatomical and neurochemical targets engaged by the drug EX-4. **Method and results:** In order to determine the neurochemical changes after GLP-1 receptor activation male Spargue-Dawley rats were centrally (into the lateral ventricle) injected with EX-4 or vehicle (control condition). Two brain areas, the dorsal raphe and the hypothalamus, important for regulation of food intake were microdissected and tissue was the flash frozen. Subsequently the mRNA was extracted, cDNA synthesized and the expression of genes associated with the central serotonin system was determined via real time PCR. Ex-4 significantly decreased the expression of a gene associated with GABA signaling, Gad-1 ($p<0,031$) in the non-food deprived group in the dorsal raphe. In the dorsal raphe Ex-4 increased the expression of two serotonin receptor genes 5-HT_{2c} ($p<0,036$) in the non-food deprived group and 5-HT_{1a} ($p<0,031$) in the food deprived group. There were no significant changes in any genes measured in the hypothalamus. **Conclusions:** This study determined that brain activation of GLP-1 receptors by Ex-4 leads to changes in serotonin associated genes. This is an important finding since the serotonin system is a key target of obesity drugs currently available on the market. Thus, results presented here link Ex-4 with the hypothalamic serotonin signaling, which further supports a potential role of Ex-4 as an anti-obesity drug and indicated a probable mechanism of action of this drug.

The search for suitable blood donors to build a three-cell-antibody screen with heterozygous and homozygous set using different phenotypes determination methods

By Dhara Indaria

Bachelor thesis in Biomedical Laboratory Science performed at Transfusion medicine NÄL, Trollhättan, 2014
Supervisor: Kristina Gamnis, Certified biomedical scientist, Anne-Marie Olsson, Head of department of Transfusion medicine, NU health care, Jan Konar, Unit surgeon general of Clinical Immunology and Transfusion medicine, SU/Sahlgrenska

Humans have different types of erythrocyte antibodies. Some of them occur in the body naturally such as immune antibodies and autoantibodies. Human also have alloantibodies. When a blood transfusion is needed these must be considered cause otherwise there is a risk of hemolytic transfusion reaction. An antibody screen is used to find irregular erythrocyte antibodies. An irregular erythrocyte antibody is an antibody that the body doesn't have naturally when a specific antigen is missing. The purpose of the study was to find three or more possible blood donors to create a new three-cell-antibody screen. Ninety samples of whole blood were taken from donors with the blood group 0+ and 0- and collected in EDTA-tubes. The blood donors were tested for specific erythrocyte antigens such as M, N, Lea, Leb, S, P1, Cw, Kpa, Lua, s, k, Fyb, Jka and/or Jkb. Phenotype determination NaCl (to find IgM-erythrocyte antibodies), IAT tube technique (to find IgG-erythrocyte antibodies) and phenotype determination with enzyme gel technique are the techniques that were used to find the erythrocyte antigens. Of all the tested blood donors there were three that were a match and that could be used to put together a new three-cell-antibody screen. The first blood donor had the following phenotypes: D +, C -, c +, E +, e -, Cw -, M +, N -, S -, s +, P1 +, K -, k +, Lea -, Leb +, Fya +, Fyb +, Jka +, Jkb -, Kpa -. The second one had: D +, C+, c -, E -, e +, Cw -, M +, N +, S +, s -, P1 +, K +, k +, Lea -, Leb +, Fya +, Fyb -, Jka +, Jkb +, Kpa -. The third one had these phenotypes: D -, C -, c +, E -, e +, Cw -, M +, N +, S -, s +, P1 +, K -, k +, Lea -, Leb +, Fya -, Fyb +, Jka -, Jkb +, Kpa -. In conclusion the study fulfilled its purpose. A three-cell-antibody screen with homozygote and heterozygote pairs were put together using these three blood donors. The result of the antibody screen was good because all the important erythrocyte antigens were included except Cw. Although the screen could have been even better if there had been more time to find suitable blood donors and if even more blood donors had been tested. Conclusion: the more time there is to do a study and the more blood donors that are tested, the better chance of getting an antibody screen of higher quality.

Measurement of maximum venous flow with plethysmography and sonography after deep vein thrombosis

By: Nina Isaksson

Bachelor thesis in biomedical Laboratory Science performed at Clinical Physiology,
Sahlgrenska Academy, University of Gothenburg, 2014
Supervisor: Anders Thurin, PhD.

Background. Deep vein thrombosis (DVT) is a quite common disease of the lower limbs. The course of disease after DVT is often straightforward with no major complications. In the acute phase, DVT is complicated by pulmonary embolism which is life threatening and in the long run it usually occurs some degree of post-thrombotic syndrome (PTS). PTS contributes both continuing outflow obstruction and deep, chronic venous insufficiency due to damaged venous valves. However, people who suffer from occlusion of the deep venous system are experiencing their condition very individual. Some patients function as normal without any obstacles while others may have big problems. But for analyzing DVT and PTS plethysmography is the only applied method. Ultrasound has never regularly been used for the measurement of maximum venous flow.
Aim: The aim of this study was to compare the strain-gauge plethysmography with ultrasound and examine the maximum venous flow in patients with post- DVT. By examining the maximum venous flow in patients with post- DVT, we can study the technology that provides the most accurate readings. The secondary aim is to evaluate the patients' quality of life after being diagnosed with deep vein thrombosis.
Method and result. We studied 10 subjects with post- DVT or with newly diagnosed in the popliteal vein and the external iliac vein. Methods that were used were sonography and strain-gauge plethysmography. Mean difference was compared between the sick and the healthy leg. For the values obtained with doppler technique between sick and healthy leg p-value was 0.0012 (< 0,05) and all measurement with plethysmography p-value was 0.00024 (< 0,05). To observe patients' quality of life a questionnaire was used.
Conclusion. It is concluded that there are significant differences between the maximum venous flow in the diseased and healthy leg regardless of the method. The methods identify low flows in patients with deep vein thrombosis and high flow rates in patients without DVT. Therefore, both methods can be used for mapping of maximum venous flow. The quality of life in patients with deep vein thrombosis seems very varied. Some didn't feel anything while others noticed some symptoms. The sociability between family and friends seemed to be positive.

Utvärdering av molekylära och immunohistokemiska metoder för att detektera EML4-ALK- förändringar i icke-småcellig lungcancer

Oskar Jevås

Abstract

In non-small cell lung cancer a new target for molecular based therapies has been identified. The standard method for detecting the EML4-ALK fusion gene is at present fluorescence in-situ hybridization. This method is slow, costly and sometimes difficult to evaluate. In this study several commercial PCR-kits and combinations thereof for detecting the fusion gene EML4-ALK were evaluated, where upon both new patient material and previously analyzed material were tested with both the evaluated PCR-kits and immunohistochemistry. The material was analyzed for EML4-ALK and ALK over expression respectively.

It was found that none of the PCR methods chosen after evaluation could detect all of the reported EML4-ALK variants. It was also found that immunohistochemistry could probably be useful as a screening method and possibly also for therapeutic guidance.

Separate Abstract

Comparative study of three cytological methods: Microfiltration, Thinprep and Cytospin in combination with EZ Megafunnel, for diagnosis of atypical cells in urine and bladder wash fluid.

By: Marie Karlström Janhagen

Bachelor thesis in Biomedical Laboratory Science performed at the laboratory of clinical pathology and cytology, Unilabs, Skaraborg hospital Skövde, 2014
Supervisor: Gunila Chebil, M.D.

Every year more than 2000 people in Sweden get diagnosed with cancer in the urinary bladder. This type of tumor has a big propensity for relapse and in some cases progress, it is therefore important to have simple and cost efficient diagnostical methods. One of these methods is cytology, with which you look for malignant cells in urine and bladder wash fluid. The purpose of this study was to compare two cytological methods for preparing urinary cytological material, against the routine method that is used today, and to decide which of these methods is the best one to use as a routine method at the cytological laboratory. The methods compared in this study were; Thermo Scientific Cytospin® in combination with Thermo Scientific Collection Fluids and EZ megafunnel™, Hologic Thinprep and microfiltration with Millipor Microfilter, the current routine method at the laboratory. This study consisted of 45 samples that were all separated and prepared according to the three methods. The slides were evaluated according to a protocol regarding the variables morphology, background, cellularity and coloration. The result of this limited study indicates that the Thinprep method has qualitative advantages; it was the method that most of the diagnostics considered to have the best morphology, coloration and background. The cheapest and most time efficient method was the microfiltration method. The size of the specimen does not allow any clear evaluation of possible differences in specificity and sensitivity. The conclusion is that even though Thinprep is both more expensive and not as time efficient it is the better choice when it comes to diagnostics.

Faktorer som påverkar HRV mätningar med eMotion

By Zahra Khorshidi
Bachelor thesis in biomedical Laboratory Science performed at Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2014
Supervisor: Professor Peter Friberg, Docent Yun Chen

Abstrakt

Bakgrunds: Autonomic nervous system (ANS) can be divided into the sympathetic system that prepares the body for fight or flight and the parasympathetic system that is activated at rest. When stressed, the sympathetic system is activated, resulting in increased heartbeats. This alternation can be measured with a time series assessment consisting of the time-intervals between consecutive heartbeats, heart rate variability (HRV). HRV can be used as a stress marker. Our **aim** was to investigate how acute mental stress, by performing Stroop test (color test), effects HRV. **Method and Results:** We examined fourteen healthy subjects (5 males and 9 females, mean age 21 $\pm$ 4,2 years and mean BMI was 22 $\pm 4,74$ kg/ m^2) in two distinct days. We registered RR intervals using eMotion sensor, during three time points (5 minutes rest, 3 minutes stress and 5 minutes recovery). We studied whether reactivity index and recovery index were influenced by different factors (BMI, smoking, sleep, exercise). We compared blood pressures, heart rate (HR), HRV parameters such as RR intervals and root mean square successive difference (RMSSD) from the three time points using two-way repeated measurement ANOVA. The result showed that smoking and obesity reduced RR-interval and raised HR. The results show an increase in heart rate during stress and a decrease in heart rate during recovery. Furthermore they show that those who are smokers have high frequency at rest than non-smoking individuals. **Conclusion:** Stress affects HRV and one can see decrease in HRV in obese individuals and smokers.

SEPARAT ABSTRACT

Proinflammatory cytokines and neurogenic inflammation in peritoneal dialysis

By Alexandra Larina

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2014
Supervisor: Magnus Braide, Associate Professor

Background

Previous studies show that peritoneal dialysis (PD) triggers a local inflammation and releases a variety of inflammatory cytokines, which gradually decreases normal function of the peritoneum. However the release mechanisms remain unknown. Recent studies show that PD not only triggers the release of those inflammatory cytokines but also triggers neurogenic inflammation through activation of Transient Receptor Potential Vanilloid 1 (TRPV1) with the release of the neuropeptides such as substance P (SP). The hypothesis is that the neurogenic inflammation with its neuropeptide release triggers increased production of proinflammatory cytokines in PD. Therefore, pharmacological treatment of neurogenic inflammation with TRPV1- and SP-antagonists will prevent gene expression of proinflammatory cytokines, and thereby prevent inflammation of the peritoneum.

Methods

In this study, 21 rats were assigned to different treatment groups. All rats that were not included in the control group underwent peritoneal dialysis for 4 hours, after which a biopsy sample of the diaphragm muscle was taken from each rat. The expression of inflammatory cytokines were then measured and studied by quantitative real time PCR.

Conclusions

The results were not significant, but it showed some trends. Groups that received treatments with the TRPV1-antagonist and SP-antagonist had the lowest average expression of TGF-β. This means that the pharmacological treatment with TRPV1- and SP-antagonists still might have inhibited cytokine release induced by PD, and thereby reduced inflammation.

Phosphatase and tensin homolog (PTEN) protein in human endometriosis

By Jakob Linde

Thesis in biomedical laboratory science performed at the institution of neuroscience and physiology, Sahlgrenska Academy, University of Gothenburg, 2014 Supervisor: Ruijin Shao, Ph.D. M.D.

The endometrium is a complex hormone regulated mucous membrane of the uterus, its tissue type is the location of a very common and yet not completely understood gynecological disease named endometriosis, which is defined as the presence of ectopic endometrial tissue in the form of endometriotic lesions outside the uterine cavity, is one of the most frequent gynecological diseases. Due to its pain symptoms, chronic progression and high recurrence rate, this estrogen dependent disease is often associated with a severely altered quality of the patients' private and professional life. A tumor suppressor gene named PTEN has been shown to be absent or mutated in many types of cancer including endometrial cancer, but has also been associated with endometriosis, yet current studies have not taken the menstruation cycle into consideration with its expression in mind. Here we show though immunological methods that the expression of PTEN differs between different types of endometriosis implantations compared to normal endometrium, and that it is highly expressed in the cell nucleus.

ABSTRACT

Determination of the epitope of monoclonal antibody 1G12 to the C-terminus of Glypican-3 with phage display

By Tamara Ljubičić

Bachelor thesis in Biomedical Laboratory Science performed at the Science and Development Department at Fujirebio Diagnostics AB, 2014
Supervisor: Eva Röijer, PhD

Hepatocellular carcinoma is the third reason for death caused by cancer in the world and a big problem is to diagnose in an early stage due to no symptoms. Several scientific studies have showed specific overexpression of Glypican-3 in early as well as later stages of hepatocellular carcinoma. Fujirebio Diagnostics AB evaluated different anti-Glypican-3 antibodies, among them monoclonal antibody 4A5 and 1G12. The aim of this study is to epitope map these anti-Glypican-3 antibodies, thus gaining useful information for development of an immunoassay.

Phage display technique using 12- and 7-mer randomized peptide libraries with four rounds of panning was utilized to affinity select peptide sequences specific for the epitope. Every round of panning was followed by an amplification step of the selected phage (eluate), which was used in upcoming panning. After the fourth round of panning phage DNA isolated from individual clones binding with high affinity to the antibodies were sequenced.

Epitope mapping was performed by pairwise sequence alignment. No sequences specific for Glypican-3 were recognized using antibody 4A5. A possible explanation for that unsuccessful epitope mapping can be that the antibody binds to a conformational dependent epitope and that the 7-mer and 12-mer peptides are too short to take such a structure. Alternative methods are suggested for future successful epitope mapping of monoclonal antibody 4A5. Consensus sequences for antibody 1G12 resulted in a mimotope discovery of four amino acids critical for binding in the C-terminal of Glypican-3. The location of the corresponding epitope to the C-terminal part of GPC3 agrees well with the protein fragment used as immunogen for establishment of mAb 1G12.

Preferred velocity – a stable individual preference of passive velocity is not connected to active velocity

By Amanda Luong

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of Neuroscience, Sahlgrenska Academic of Gothenburg University, 2014

Supervisor: Ilona Croy, Post-Doctor

Introduction: Slow stroking is perceived most pleasant if happening at a velocity range between 1-10 cm/s and CT fiber codes the pleasant touch sensation, however it is unknown, if a person have an individual preferred pleasant touch velocity. The purpose of the study was to execute the stability of the preferred passive velocity. We also aim to study the velocity in relation to the passive touch.

Methodology: 30 participants [22.17 ± 2.574 years (mean, SD; standard deviation), 13 men and 17 women] were included for the study of passive and active touch. Passive velocity was measured using a robot device with a soft brush to perform the strokes on the arm. Velocities run pairwise of each high and slow velocity in random order and after every pair the subject responded to which of the speed they prefer. Three sequences were run through to complete the test with an average speed calculated as a final outcome. This was tested twice on two different occasions. Right after the passive touch, the active touch was implemented. The task was performed on an artificial arm with a velocity tracker to record the speed of the movement along the arm. Two questionnaire concerning the sexuality, relationship and social touch was included for exploratory analysis.

Results: Our data confirms that there is an individual preference in passive velocity and that this is stable ($r= 0.869$, p-value <0.001). However no correlation was found between active and passive velocity ($r= 0.045$, $p=0.813$).

Conclusion: The study confirms that there is an individual preference on passive velocity and results shows that it is stable. However the passive velocity is not connected to active velocity. This opens the discussion, of how we learn to stroke someone.

Abstract

By: Jennifer Mattson

This thesis consists of an evaluation of a new in vitro diagnostic point-of-care system for the quantification of acute phase protein haptoglobin. LifeAssays®Feline Haptoglobin). Acute phase proteins are bio-markers of systemic inflammation and can be used for diagnosis and monitoring of inflammation [1]. The evaluation was done by 79 pieces of exisiting serum samples from both healthy and sick cats analyzed with point-of-care system. The samples came from cats of different age, race and gender. The samples were divided into three categories: healthy; disease with systemic inflammation and other disease. The results show a very good agreement between the haptoglobinkoncentration as measured by the point-of-care system and the patient's clinical condition. The clinical sensitivity was set to 91%, and clinical specificity of 100%.
Keywords; cat, diagnostics, acute phase protein, haptoglobin.

Separat Abstract

Survey of investigating methods at Sahlgrenska nuclear units regarding V/P SPECT shows differences which may impact on the image quality

By Axel Mellqvist

Bachelor thesis in Biomedical Laboratory Science performed at the nuclear departments of
Sahlgrenska University Hospital and Östra Hospital,
Sahlgrenska Academy, University of Gothenburg, 2014
Supervisor: Martijn van Essen, MD, PhD

Background: Pulmonary embolism is a serious disease that can lead to several complications and even death. Image Techniques is primarily used for diagnostics where Multiple Detector Computed Tomography and Ventilation/Perfusion SPECT (V/P SPECT) are the leading diagnostic tools. V/P SPECT is a nuclear medical examination, which means that radioactive isotopes are used and thus eliminating problems such as allergy to contrast media and investigating in patients with renal insufficiency. Examination with V/P SPECT is divided into two parts with the first part examining lung ventilation by inhalation of a radioactive gas and the second part examining the lung perfusion, i.e. blood flow, through an intravenous injection of a radioactive isotope. The diagnosis is based on the image material and the patient's clinical symptoms. The purpose of this study was to survey research methods at Sahlgrenska University Hospital and Östra Hospital to detect any differences, which might be relevant to the image quality.

Method and materials: Information about study procedures was provided by biomedical scientists responsible for method of examination and medical physicists at both hospitals. Observations were also made by active participation in examinations at each hospital. Patient data consisted of 20 patients from the SU/S and 20 patients from the SU/Ö with suspected pulmonary embolism examined with V/P SPECT. In both departments, questionnaires with variables that could affect the image quality were filled in during each V/P SPECT scan. Data for total count, countrate and quota between perfusion and ventilation countrates were collected. Statistical calculation with Mann-Whitney U test was then performed for quotas.

Results: The differences in examination procedures were small. The technical equipment was different. Gamma cameras of another brand with other types of collimators were used at Östra. There were statistical differences in the count values appealing to higher sensitivity in the examinations at Östra.

Conclusion: This study shows that there is a significant difference between the quota values (perfusion / ventilation) at SU/S and SU/Ö. The study identified some differences between devices that could affect: the length of the analysis time and the type of collimators. In the light of previous studies, the choice of collimators is probably the most important difference and this should be taken into account in the future development/quality-work for V / P SPECT.

ABSTRACT

By: Stella Nakate

Background: Clinical laboratories require an accurate and precise 1,25 dihydroxy vitamin D quantification assay to assist in the diagnosis and management of disease associated with vitamin D deficiency. 1,25 is the active form of vitamin D formed in the kidney after the hydroxylation of the normally measured hepatic 25 hydroxy vitamin D form. In this study the partially automated IDS-iSYS 1,25 dihdroxy vitamin D assay´s performance was validated against IDS-1,25 dihydroxy vitamin D enzyme immunoassay.

Methods: Twenty de-identified left over patient serum samples were used to compare IDS-1,25 dihydroxy enzyme immunoassay with IDS-iSYS 1,25 dihdroxy vitamin D assay. Twelve replicates of the house control serum were assayed to assess within-run precision and three replicates in house control were assayed as separate aliquots on three different days to assess between-run precision. Dilution of linearity and lower limit of detection were assessed by diluting the in-house controls using 0.9% sodium chloride respectively.

Results: The IDS-iSYS assay showed good performance in reproducibility, sensitivity and specificity thereby acceptable precision. The IDS- ISYS assay was less time consuming compared to the IDS- enzyme immunoassay that includes a laborious manual ELISA assay part that takes more than 20 hours to complete.

Conclusion: The ISDS-iSYS automated assay was the better assay to use in clinical laboratory settings. With good performance results, it was less time consuming and reduced the amount of manual handling which improved reproducibility and quality assurance. However both methods required extraction with multiple manual pipetting steps which if reduced or entirely automated would further improve performance and hopefully enable standardization necessary for standardizing the reference intervals.

Abstract

By: Sharon Ngéno

Background/aim of the study: Automated ejection fraction (Auto EF) is an innovative method that automatically determines ejection fraction. It uses a database of more than 10,000 endocardial tracings to identify and track the endocardium and rapidly calculate left ventricular volumes and ejection fraction. The manual biplane Simpson's method is the standard method used to calculate ejection fraction in two dimensional echocardiography, it is however time consuming. The aim of this study is to compare Auto EF method with manual biplane Simpson's method to determine whether Auto EF is a reliable and effective method of determining ejection fraction.

Materials and methods: The study group consisted of 150 patients whom had undergone a standard two-dimensional transthoracic echocardiography at the Sahlgrenska university hospital and ejection fraction (EF) determined by manual biplane Simpson's method. Ejection fraction (EF) was later on calculated using Auto EF method on the same images of the 150 patients. The results by Auto EF and manual biplane Simpson's method were then compared.

Results: Ejection fraction determined using Auto EF method correlated well with Biplane Simpson's method, r= 0.852 with minimal intraobserver and interobserver variability.

Conclusion: Auto EF is a fast and reliable method of determining ejection fraction. It has clinical potential and should be used in clinical practice.

Abstract

By: Manh Nguyen

Introduction: Oropharyngeal cancer afflicts the posterior region of the oral cavity, such as tonsils, tongue base and soft palate. Afflicted regions can be further categorized into two groups based on the involvement of human papilloma virus. As a defense against viral infections, the innate immunity has evolved viral restriction factors, one being a group of mRNA editing enzymes referred to as *Apolipoprotein mRNA editing, catalytic polypeptide like 3*. A commonly shared characteristic of the mentioned group of proteins is the zinc-dependent binding domain, which deaminates cytidine of viral and cellular single stranded nucleic acids, turning it into uracil.

In recent cancer research articles, elevated levels of *Apolipoprotein mRNA editing, catalytic polypeptide like 3* were detected in several cancer types, amongst them cervical and oropharyngeal cancer, which have been associated with human papilloma virus infection. Furthermore, these studies reported a novel mutational pattern in a wide array of cancer types – referred to as *kataegis*. A correlation was made between the frequency of mutational patterns and the transcribed levels of *Apolipoprotein mRNA editing, catalytic polypeptide like 3*. These findings indicate that these proteins play a major role in *kataegis*. It has therefor been proposed that abnormally high levels of those enzymes not only engage in innate immunity against viral infections but could also inadvertently act on cellular DNA and potentially be a part of a formerly unknown mechanism of oncogenesis.

Aim: In this thesis we wanted to elucidate whether HPV positive tonsillar cancer biopsies, previously characterized for p16 positivity, correlated with increased APOBEC3B transcript levels. **Materials and Methods**: RNA extracted from thirty tonsil cancer biopsies embedded in paraffin was used in this study, with the purpose of quantifying Apolipoprotein *mRNA editing, catalytic polypeptide like 3* expression levels. The samples were divided into two groups according to occurrence of the HPV tumor suppressor protein p16. Prior immunohistochemical analysis of the samples was utilized to reveal their p16 status whereof fifteen p16 positive and fifteen p16 negative samples were chosen. In the actual experiment, eleven p16 positive and twelve p16 negative biopsies were selected per group, since some samples had to be disregarded due to extremely low RNA concentrations and/or too small sample volumes. The RNA was extracted by staff at the department of pathology, using a commercial RNA extraction kit. We proceeded by performing reverse transcription and quantitative PCR of the RNA samples. **Results**: Our statistical analysis of normalized samples resulted in a non-significant difference. However comparison of the non-normalized samples (Ct values only) showed a significant difference ($p<0,05$). Unexpectedly the results deriving from non-normalized samples indicated a lower *Apolipoprotein mRNA editing, catalytic polypeptide like 3* expression in p16 positive samples compared to the p16 negative group. **Discussion**: The unanticipated results of non-normalized and normalized samples are doubtful as we suspected a varied degradation of RNA samples. Due to the RNA degradation in archival formalin-fixed, paraffin embedded tissue, our results may not accurately reflect the cellular gene expression levels of the protein in tonsillar tumors.

Left ventricular ejection fraction does not predict 5 year survival rates in patients over the age of 65 with heart failure

By Ann Olofsson

Bachelor thesis in biomedical Laboratory Science performed at Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2014
Supervisor: Magnus Johansson (PhD, MD), Cecilia Wallentin Guron (PhD, MD)

Background. Heart failure (HF) has a prevalence of 2-3% in the population. Incidence and prevalence both increase with age rising to around 10% in the very elderly. In order to diagnose and manage patients with HF an accurate assessment of left ventricular ejection fraction (LVEF) is of central importance. Visual assessment of LVEF is often used in clinical routine despite Simpson biplane being the recommended method. Several previous studies have shown a connection between LVEF and mortality in patients with HF, other studies have found no evidence to support that.
Aim. The primary purpose of this retrospective study was to analyze survival in the patient population with regards to preserved and reduced LVEF. The secondary aim was to compare visual estimation of LVEF with the Simpson biplane method.
Method. The study sample included 358 patients over the age of 65 with HF, hospitalized sometime between april 2007-april 2008. The Simpson biplane method was used to measure LVEF in each patient. The results were compared to the previously performed visual assessment. Data on survival was collected from the Swedish death register.
Results. Results showed no significant difference between visual estimation and Simpson biplane (mean±SD 43.1±14.5 vs 41.6±13.0%). In the five year follow-up no significant difference in LVEF was found between the group of patients that were deceased and the group that were still alive.
Conclusion. LVEF could not predict survival in the population. No systematic differences were found between the two methods, however a relatively large interobserver variability was registered.

CAROTID ARTERY INTIMA-MEDIA THICKNESS IS PREDICTIVE OF MAJOR CARDIOVASCULAR EVENTS IRRESPECTIVE OF GLUCOSE TOLERANCE IN 64-YEAR OLD WOMEN

By: Catherine Pachón

Bachelor thesis in Biomedical Laboratory Science, performed at the Wallenberg Laboratory, Sahlgrenska Academy, University of Gothenburg, 2014.
Supervisor: Caroline Schmidt, Associate Professor

Background: Atherosclerosis is a global health problem and is the cause of stroke and myocardial infarction which kills over 20 million people a year. The carotid intima-media thickness (IMT) is used all over the world as a marker of atherosclerosis. The aim of this study was to examine if the carotid IMT was predictive of cardiovascular (CV) events regardless of glucose tolerance in 64-year old women during 7 years of follow-up.

Methods: From a total cohort of 4856 64-year old women, 639, were recruited. Out of these, 36.5% had diabetes mellitus (DM), 32.6% had impaired glucose tolerance (IGT) and 29.7% normal glucose tolerance (NGT).

The IMT in the carotid arteries was examined by B-mode ultrasound. All the participants underwent anthropometric measurements, an oral glucose tolerance test (OGTT) and risk factors and serum concentrations of apolipoproteins were analyzed. **Results**: During 7 years of follow-up, almost 60% of the participants with DM, 22.6% of the participants with IGT and 17.8% with NGT had experienced a CV event. Common carotid IMT showed to be significantly associated with a 1.6 increased risk of CV events during follow-up (HR 1.6, 95% CI 1.2 to 2.0, $p<0.001$). The risk of events was similar after adjustment for co-variates (HR 1.4, 95% CI 1.1 to 1.9, $p=0.005$). **Conclusion:** The present study showed that IMT measured in the common carotid artery (CCA) is predictive for major CV events in 64-year old women irrespective of glucose tolerance.

Abstract

By: Cindy Pho

The mitochondrion is an organelle present in eukaryotic cells and is responsible for the cells energy production by producing adenosine triphosphate (ATP). The mitochondrion has its own genome, which is about 16 kbp in size. The mitochondrial genome encodes 13 essential proteins for the electron transport chain, and two ribosomal RNA and 22 transfer RNA. (1, 2)

The mitochondrial transcription factor A (TFAM) is a DNA-binding protein that is involved in transcription and packing of the mitochondrial DNA. The protein contains two High Mobility Group (HMG) – box, A and B, connected by a linker and a 25-residue C-terminal tail. (3)

TFAM can bind to two promotor sequences of the mitochondrial DNA (mtDNA), the light strand promotor (LSP) and the heavy strand promotor 1 (HSP1), and activates transcription of mtDNA together with the RNA polymerase (POLRMT) and transcription factor B2 (TFB2M). (1, 2, 4)

TFAM has the ability to bend the mtDNA into a U-turn. This U-turn is necessary to active the LSP, but not HSP1. This has been shown by a TFAM-mutant, L6, where the linker of TFAM has been mutated. The mutant lost its ability to bend DNA and showed defects in activation of transcription from the LSP, while the activation ability of HSP1 was as effective as wild type (WT)-TFAM. (3, 4)

A recent study found that Helix 3 of HMG -box A can interact with each other in an anti-parallel pattern and form a TFAM dimer. When interacting amino acids in helix 3 were mutated (dimer mutant), it reduced TFAMs ability to pack the mtDNA. (4)

The aims of this study were to produce four different mutants of TFAM by cloning, protein expression and purification. By manipulating both the WT-TFAM and ΔC25-TFAM with the same mutation sets; L6 respectively dimer. ΔC25 - TFAM is a WT-TFAM which is shortened by 25 amino acids of the C-terminal tail.

To generate the mutations, PCR-based mutagenesis was used. In order to confirm whether the correct mutations has been made, a few colonies was selected from each mutant and sent for sequencing.

A Baculovirus expression system was used for protein expression and protein production in insect cells. To confirm that the clones had the ability to produce TFAM-mutants, a Western blot assay was performed.

When production of the protein was completed, the protein was purified by various purification steps including affinity chromatography and ion exchange chromatography to produce a pure product of TFAM mutants. The analysis of the purification was made, by running SDS-PAGE after each purification step.

The four TFAM-mutants has been cloned, expressed and purified into pure products.

ABSTRAKT

Amplifiering av helgenom från singelceller med Sigma-Aldrich GenomPlex® Single Cell Whole Genome Amplification Kit resp. Qiagen REPLI-g® Midi Kit

Av Anna Rignell

Biomedicinska analytikerprogrammet 180 hp.
Examensarbete 15 hp, våren 2014.
Handledare: Malin Berggren, med.dr.
Camilla Friberg, med.dr.
Klinisk genetik, Sahlgrenska Universitetssjukhuset.

BAKGRUND OCH SYFTE: Preimplantatorisk genetisk diagnostik (PGD) är en metod som används för att upptäcka genetiska defekter hos embryon som skapats via in vitro fertilisering (IVF). Det laborativa arbetet vid PGD involverar avlägsnandet av en cell, en blastomer, från de växande embryona och genetisk analys av dess innehåll. Det krävs då att analyserna utförs på mycket små mängder genetiskt material. Denna begränsade startmängd DNA medför begränsningar i antalet analysmöjligheter. En enda cell innehåller ca 6-7 pikogram (10^{-12} gram) av DNA. Molekylärgenetiska analyser kräver ofta nanogram (10^{-9} gram) av DNA för att kunna genomföras. Helgenomsamplifiering (eng. *whole-genome amplification* - WGA) är en metod som har utvecklats för att lösa detta problem. Vid helgenomsamplifiering kan mycket små mängder genetiskt material amplifieras och ge en större mängd material att använda för vidare analyser. Syftet med detta projekt var att utvärdera de kommersiellt tillgängliga kitten för helgenomsamplifiering av singelceller från företagen Sigma-Aldrich resp. Qiagen. **METOD:** Helgenom från singelceller amplifierades med hjälp av de två företagens olika kit. För att kontrollera resultatet av amplifieringen gjordes gelelektrofores och spektrofotometrisk koncentrationsbestämning. För att kontrollera amplifieringprodukternas användbarhet utfördes multiplex-PCR och minisekvensering. **RESULTAT:** Resultat från koncentrationsmätningar och gelelektroforeser tydde på att en viss amplifiering hade skett i försöken med de två företagens olika WGA-kit. Dock gav ingen av WGA-produkterna fullständiga och tillfredsställande resultat då de användes för genetisk analys. **SLUTSATS:** I dagsläget anses de två utvärderade WGA-kitten ge alltför instabila och ofullständiga resultat för att kunna användas vid PGD i den kliniska vardagen.

Succesful occluded varicose veins with Radiofrequency Ablation checked by Duplex ultrasound

By Melika Robati

Bachelor thesis in Biomedical Laboratory Science performed at Surgical Clinic, Sodra Alvsborgs Hospital, University of Gothenburg, 2014
Supervisors: Christer Drott (PhD) and Sheida Nourbakhsh (Biomedical scientist)

Background. Varicose veins is a common problem both symptomatically and also cosmetically. Venous insufficiency usually affects the lower limbs caused by an increased pressure in the venous system. Varicose veins can be caused by venous insufficiency and cause the vessel wall to weaken and broaden. The valves lose its function, conducting the blood in the right direction and as a result you get a reverse blood flow in the leg. The great saphenous vein (GSV) and the small saphenous vein (SSV) are veins in the lower limbs that suffers from varicose veins. Patients with varicose veins can be treated with Radiofrequency Ablation (RFA). RFA is a technique that involves burning the endothelium causing occlusion of the veins. It is a minor procedure that only requires a local anesthetic. The treatment can result in various complications such as deep vein thrombosis (DVT) and thrombophlebitis. It is therefore necessary for patients undergoing RFA to come back for a postoperative control ultrasound to ensure success of the treatment. **Aim.** The aim of the study was to independently perform Duplex ultrasound on patients after RFA.**Method.**We studied 16 subjects, 9 (56%) women and 7 (44%) men. The mean age was 58±12 (SD) years old. Every subject was examined with a complete examination of the lower extremity venous system.All subjects had undergone RFA treatment.**Result.**We found thrombophlebitis in 2 subjects and all subjects had occluded target vessels.**Conclusion.** Our study showed occlusion of all target vessels and thrombophlebitis in 2 subjects. The evaluations of the patient's view of treatment outcome shows that RFA is a good method for treatment of varicose veins.

Strain-Gauge-plethysmography and Airplethysmography: Comparable methods in quantitative assessment of total venous function

By Tara Shams

Bachelor thesis in Biomedical Laboratory Science, performed at Clinical Physiology,

Sahlgrenska Academy, University of Gothenburg, 2014

Supervisors: Lena Karlsson (PhD, MD), Mikael Ekman (Ph Lic, MSSC)

Background. Veins are the blood vessels in the circulatory system that transports blood towards the heart. These vessels contain valves and their task is to prevent the blood from flowing backwards. Venous insufficiency is a condition that affects the lower extremities and is mainly caused by poorly functioning venous valves. If left untreated the condition can cause pain, swelling and eventually wounds. The most commonly used method for diagnosing venous insufficiency is duplex ultrasound, although sometimes complemented by methods such as plethysmography. **Aim.** The aim of this study was to evaluate if the simpler plethysmographic method, Airplethysmography, was comparable with the more established Strain-Gauge-plethysmography in terms of ability to estimate total venous function. **Method.** We studied 27 subjects, who all had previously undergone a duplex ultrasound examination to determine the venous function in either one or both legs. All subjects were examined once with our reference method, Strain-Gauge-plethysmography, and four times with Airplethysmography, where all four measurements differed in terms of cuff pressure. Two parameters were used to quantify the venous insufficiency, T50 and T90. **Results**. We found a good accuracy between the two methods for the parameter T50, regardless of cuff pressure. **Conclusion.** We conclude that Airplethysmography may replace the Strain-Gauge-plethysmography in quantitative assessment of total venous function.

Milrinone induces Takotsubo-like cardiac dysfunction in rats

By Anna Sundström

Bachelor thesis in Biomedical Laboratory Science, Spring semester 2014
Wallenberg laboratory, Sahlgrenska Academy, University of Gothenburg
Supervisor: Björn Redfors, PhD-student and Elmir Omerovic, Associate Professor

Background: Takotsubo cardiomyopathy, or TC, is a rare and reversible form of heart failure characterized by an apical and mid ventricular dyskinesia, hypokinesia or akinesia, sometimes combined with apical ballooning, that cannot be explained by coronary occlusion.

While the pathophysiology of TC remains unknown, several studies have shown that overexposure of catecholamines can cause overstimulation of cardiac β-receptors, which in turn can cause TC.

When a β-receptor is activated by extracellular catecholamines, intracellular adenylyl cyklase starts to convert ATP to cAMP. cAMP functions as a second messenger that increases the hearts contractility, heart rate and conduction velocity. cAMP is eventually broken down by an enzyme called phosphodiesterase 3. Milrinone is a phosphodiesterase 3 inhibitor that functions by inhibiting the breakdown of cAMP. This enhances the effect of cAMP, which in turn enhances the effect of β-receptor activation.

Objective: The aim of this study is to determine if the phosphodiesterase 3 inhibitor Milrinone can induce TC-like cardiac dysfunction in rats.

Method: This study included 12 rats that were randomized into two groups. The rats were anesthetized and an intra-arterial catheter connected to a pressure monitor was inserted in the right carotid artery of each rat. Baseline hemodynamic data such as blood pressure and heart rate, as well as body temperature was continuously recorded during 5 minutes.

After the baseline registration, one group (n=6) was intraperitoneally injected with 25 mg/kg Milrinone and the other group (n=6) was injected with saline. Hemodynamic data was continuously recorded once again, this time during 90 minutes. The rats were then examined with echocardiography to determine whether TC-like cardiac dysfunction had developed or not. The rats were then sacrificed.

Results: All rats injected with Milrinone expressed decreased arterial blood pressure followed by an elevated heart rate. Four out of the six rats injected with Milrinone developed apical akinesia (between 3,9 and 8,5%).

Discussion: The most important conclusion that can be made from this study is that Milrinone can induce TC-like cardiac dysfunction in the form of apical akinesia in rats.

Abstract

By: Sabrina Syed

Background: Aortic stiffness is an indicator for future cardiovascular diseases. A measurement used to predict this is pulse wave velocity (PWV). The Vicorder is a fairly new oscillometric device which is used to measure the PWV, and therefore a validation of the method is needed. ***Method:*** Fourteen individuals (5 men and 9 women) were recruited to undergo studies of reproducibility of the device and observation if measurements done in different positions affect the PWV. The mean age was 20.1 years and BMI was 22.5 kg/m^2. We measured PWV on two distinct days. ***Result:*** The intra- and inter-assay coefficient of variation of PWV was 3.41 % and 8.28 % respectively. All individuals underwent measurement of PWV in different body positions. PWV changed significantly from laying down to sitting, and from laying down to standing. Though there is no difference between the two distinct days. ***Conclusion:*** Measurement of PWV is reproducible. The results show that there is no difference from day to day measurements, or difference in measurement from different examiners. Though there is significant difference between different postures.

SEPARATE ABSTRACT

AB0-antibodies in platelet concentrates effects aggregation in in-vitro transfusion, measured with impedance aggregometry.

By: Karolina Torstensson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of clinical chemistry and transfusion medicine, Institute of biomedicine, Sahlgrenska Academy, University of Gothenburg, 2014.
Supervisor: Camilla Hesse, Senior Lecture

Therapeutic platelet transfusion is clinically given to patients with active bleeding, thrombocytopenia or patients with a defective haemostatic function. Platelet concentrates has a short lifespan and are expensive to produce. The guidelines are to give patients AB0-identical platelets, but this is not mandatory. Because of the concentrates short lifespan and their expensive manufacture, many hospitals supply an inventory blood group 0 concentrates. Even though adverse effects of transfusion when given platelet concentrates are quite rare, research has proven that patients benefit when given AB0-identical platelets. Most of the research in the field has a focus on the platelet survival rate.
This study focus on the AB0-antibodies effect on the platelets receptor response measured with impedance aggregometry.

An in-vitro platelet transfusion of platelet apheresis was performed on total 12 different subjects. Patient samples of whole blood collected in Hirudin test tubes was diluted 20 % with PBS and 89x109/L platelets was added. Impedance aggregometry was measured before and after in-vitro transfusion as well as 60 ± 10 minutes after, with MultiPlate® analyzer. The single donor apheresis was titrated for IgG and IgM AB0-antibodies. And the whole blood added with in-vitro transfusion was examined with DAT-test and checked for haemolytic levels.

The study shows an average decrease in receptor activity in bloodgroup A-subjects transfused with single donor apheresis from bloodgroup 0 both directly and after 60 ± 10 minutes. For bloodgroup AB it shows an average decline directly after in-vitro transfusion, but not after 60 ± 10 minutes. Bloodgroup 0 and B had an average increase directly and after 60 ± 10 minutes in three of four parameters measured with impedance aggregometry. Furthermore four of the seven subjects in bloodgroup A and AB tested DAT-positive. The lowest IgG-titre with a positive DAT outcome had titre 1:16.

The study need to be extended with more subjects for a more conclusive result. But the study shows that it is possible that very low titres of AB0-antibodies effect platelet receptors.

ABSTRACT

Correlation between CT targeted stimulation and sexual experience.

By Vicktoria Wagnbeck

Bachelor Thesis in Biomedical Laboratory Science performed at Institute of Neuroscience and Physiology, 2014.
Sahlgrenska University Hospital.
Sahlgrenska Academy, University of Gothenburg.

Supervisor: Ilona Croy (Dr. rer medic. Dipl. Psych., Scientist). Emma Jönsson (M.Sc).

Background and Aim. Touch is an important part of our well-being, both for humans and animals. It is part of our communication, a way to explore the surroundings but also to provide care and show affection. Touch can also convey and evoke different kinds of emotions and can also lead to arousal. Areas of our body that has no direct connection to the genitalia can still lead to erotic arousal. However, many people suffer in today's society of lack of tactile stimulation, a phenomenon called touch hunger.

The aim of the study was to determine if there is a correlation between CT-specific stimula-tion and sexual experience. Furthermore coherence between the personal experience of touch, social touch in daily life and CT-specific stimulation was examined.

Material and Method. We recruited 47 participants (20 men and 27 women) who rated eroticism, pleasantness and intensity on a visual analogue scale after being stroked by a soft brush with CT-targeted and non-targeted velocities from 0.3 to 30 cm/s. They were also given two questionnaires to fill in. These focused on their partnership, sexuality and social contact. Participants also responded to a computer based questionnaire about erogenous zones.

Result. The analysis revealed a significant main effect of velocities in erotic, pleasing and intense experience of CT stimulation. It also showed a relationship between the erotic and pleasurable experience of the touch. Furthermore, there was a significant interaction be-tween the CT-targeted pleasantness ratings and the reported sexual activity.

Analysis of CT stimulation and Hotness survey showed significance for all categories; erotic, pleasing and intense experience. Furthermore, there was a significant coherence between relationship length and the mean erotic experience of CT stimulation. A main effect of gender was also found in the analysis.

Conclusion. CT stimulation was experienced as erotic and the perception of CT targeted stimulation was further more related to the sexual behavior and relationship status of the participants. I therefore conclude that the CT system is involved in the processing of eroticism.

The ITPA (inosine triphosphate pyrophosphatase) gene has a ribavirin-like effect on HCV treatment, but does not affect self-healing in hepatitis C genotype 2/3 infection

By Hanna Wirdelius

Bachelor thesis in Biomedical Laboratory Science, performed at Dept. of Infectious Diseases/Virology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Sweden, 2014, Supervisor: Kristina Nyström, PhD.

A recent study from our group analyzed the frequency of two polymorphisms, rs7270101 and rs1127354, in a group of patients infected with hepatitis C genotype 2 and 3. These polymorphisms are situated in the gene coding for ITPase. When present, they result in a decrease in ITPase activity. This was proven to improve the outcome of treatment, usually ribavirin and interferon, for hepatitis C genotype 2 and 3 (NORDynamIC).This is suggested to correlate due to a ribavirin like effect of low ITPase levels. Ribavirin treatment and decreased levels of ITPas, leads to low levels of GTP respectively high levels of ITP. These nucleotides are then incorporated in the viral genome and causes mutations.

The aim of this study was to study the effect of ITPase in the establishment of chronic hepatitis C virus infection. Therefore, the correlation between a lack of ITPase and the chances of self-healing from the virus, were investigated. Another part of the aim was to explain how the activity of ITPase can affect the infection, when treated with ribavirin and interferon. To do so, the connection between ribavirin treatment and a decreased ITPase activity was studied.

The frequency of polymorphisms, rs7270101 and rs1127354, which are correlated to the level of ITPase activity, where studied in 403 healthy Swedish blood donors. The polymorphisms were analyzed by real-time PCR, using the allelic discrimination plot. Each individual's level of ITPase activity was determined using data from another study together with the polymorphisms. The predicted ITPase activity was then compared to the NordynamIC study. There was no difference in the results of the two groups. Since there was no difference, it could be determined that the groups were similar (two tailed p-value: 0,983). Further, we compared the treatment response and ITPase activity in a group of patients treated only with ribavirin. A trend could be seen among the patients with low levels of ITPase. They were more likely to achieve SVR (sustained virological response). Due to a small sample size, this was not significant (p-value 0, 06).

These results indicate that a decrease in ITPas activity provides a ribavirin-like effect, by protecting against a relapse of hepatitis C virus infection after finished treatment. This discovery could possibly lead to better treatments that are tailored on an individual basis.