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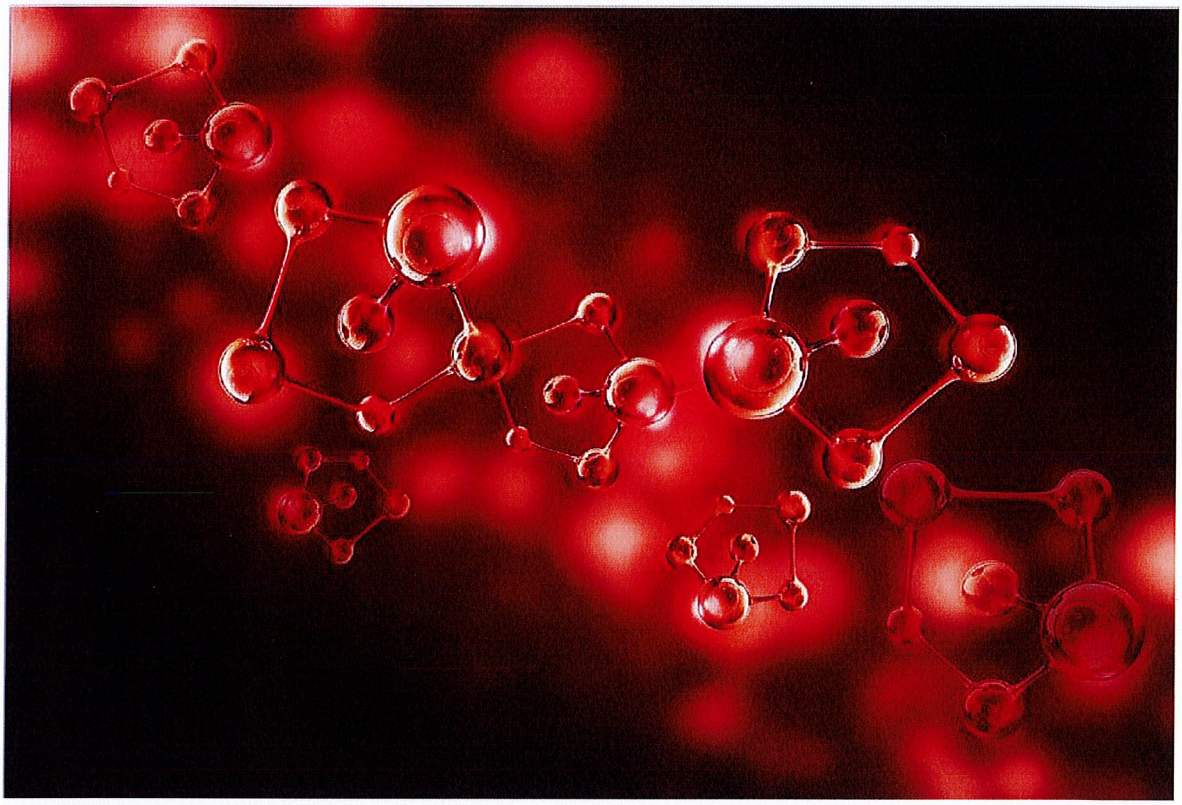

ABSTRACT BOOK 2019

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

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Evaluation of antibody-buffer system combinations for the detection of the neurofilament protein α-internexin in cerebrospinal fluid using single molecular array (Simoa™)

By Nicole Accord

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Psychiatry and Neurochemistry, Sahlgrenska University Hospital, Mölndal, 2019

Supervisors: Kina Höglund, associate professor; Fani Pujol-Calderón, Ph.D student

Introduction: When neurons die, intracellular proteins may leak into the cerebrospinal fluid and circulatory system. Neurofilaments are intermediate filament proteins specific to neurons. These proteins can be measured and used as biomarkers reflecting the current state of the brain. The Type IV neurofilament α-internexin is specific to the central nervous system and is thus a potential biomarker for neuronal damage located there.

Aim: To determine an antibody-buffer system combination with which to continue the method development for detecting α-internexin in cerebrospinal fluid using single molecular array technology. We also aimed to investigate what forms of α-internexin are present in cerebrospinal fluid.

Method: Four different combinations of in-house and commercial antibodies suspended in three various buffer systems were evaluated to determine the combination emitting the strongest signal with the lowest background when analysed with single molecular array technology. To determine the forms of α-internexin present in cerebrospinal fluid, α-internexin was isolated with immunoprecipitation and visualised via western blot.

Results: Using the commercial monoclonal antibody 2E3 as a capture antibody and the in-house monoclonal antibody INA1 as the detector antibody emitted the strongest signals (min=0,111; max=28,510) out of the four combinations across all buffer systems. When suspended in the buffer system 5 mM urea in PBS, 2E3-INA1 had a 35-fold signal-to-noise ratio compared to the antibody combination with the second strongest signal suspended in the same buffer system. In-house antibodies INA1 and INA2 were not able to properly isolate α-internexin with immunoprecipitation using the current protocol.

Conclusion: For further development, the combination 2E3 and the in-house monoclonal antibody INA1 in 5mM urea in PBS emits the highest signal out of the nine evaluated combinations. Urea may have a positive impact on the signal as well as the signal-to-noise ratio, perhaps due to dissolving protein aggregates. Adjustments to the immunoprecipitation protocol are needed to properly evaluate if in-house antibodies isolated α-internexin before different forms of α-internexin in cerebrospinal fluid can be detected. Visualisation with western blot revealed that in-house antibodies INA1 and 16EF bind to other neurofilament proteins in the Type IV family apart from α-internexin.

Deep-learning algoritm möjliggör förkortad insamlingstid vid SPECT/CT bildtagning efter radionuklidbehandling med Lu-177-DOTATATE

By Sahar Akbari

Bachelor thesis in Biomedical Laboratory Science performed at the section for clinical physiology and Radiophysics , Sahlgrenska academy, University of Gothenburg, 2019

Supervisor: Johanna Dalmo, PhD

Background. Today we take a serie of SPECT/CT images after treating cancer with Lu-177. Patients treated with the radioactive isotope Lu-177 suffer either from neuroendocrine tumours (NET) or from hormone-resistant skeletal metastases originating from prostate cancer. Lu-177 is linked to various targeting molecules dependent on disease, Lu-177-DOTATATE to NET patients and Lu-177-PSMA-617 to patients with hormone-resistant skeletal metastases.

Aim. This project aims to investigate how much the scanning time for SPECT/CT imaging can be reduced without affecting precision in the activity determination to various critical organs that has been affected by the tumours such as bone marrow and kidneys.

Method. In total, 14 SPECT/CT images, collected 24 hours after administration, was used. These images were collected with 120 projections. The number of projections were reduced in these original images so that only 30 projections was left. The 30 projection images were then compensated by a new deep-learning (DL) algorithm which interpolated 30 projections back to 120 projections. Reconstruction of all images were made by using either iterative reconstruction OSEM or Monte Carlo based reconstruction SARec. Manually VOIs on kidneys and bone marrow L4 in SPECT/CT images were drawn.

Results. Correlation between OSEM 120 and SARec 120 and all modified reconstructions for 30 projections were found for right and left kidney and bone marrow. Linear regression analysis, normalization and CV between the modified images and the original images performed on right and left kidney and bone marrow, shows that OSEM 30 120 and SARec 30 120 are more accurate than OSEM 30 and SARec 30 since the image noise becomes to high.

Conclusion. Interpolation using the deep-learning algorithm of the angles between the reconstructed 30 projections images reconstructed either with OSEM or SARec gives similar results as standard reconstruction (when all 120 projections were used). In this way, an equivalent result with the OSEM/SARec reconstruction with four factors such as the original reconstruction. This enable a reduction in the total collection time of the SPECT/CT imaging from 33 minutes using images taken with 120 projection (30 s/angle) to 9 minutes using images taken with 30 productions (30 s/ angle).

Optimisation of cell sensitivity assays to detect normal tissue sensitivity to 177-Lu-DOATATE radiotherapy

By Jelena Aleksejeva

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Chemistry laboratory, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisor: Pegah Johansson, PhD, Principal biologist

Cancer is the most common cause of death after cardiovascular diseases. Ionising radiation (IR) is a frequently used cancer treatment. Radiotherapies killing cancer cells by causing damage in their DNA. Normal tissue is, however, also frequently affected by the radiation and is the dose limiting factor. There is a variation in this normal tissues sensitivity to IR among individuals.

The aim of this study was to optimize two previously reported cell sensitivity assays, the cell division assay (CDA) and γ-H2AX assay, to measure individual normal tissue sensitivity to different modes of radiation therapy.

In this project, peripheral blood mononuclear cells (PBMCs) were treated with X-Ray radiation and 177-Lu-DOTATATE *in vitro*. PBMC sensitivity was evaluated using CDA. This was done through the incubating cells with thymidine analogue (EdU), labelled with a fluorescent molecule, that is incorporated into DNA during its active synthesis and analysing cells using flow cytometry. The γ-H2AX fluorescence signal was used as a measure of DNA damage through detection of the nucleosomal histone protein H2AX that is specifically phosphorylated at the site of DNA double strand break. This could be done through incubating cells with anti-γ-H2AX FITC conjugated antibody and flow cytometry. Here, the irradiation doses using X-Ray method and lead containers as a dose regulating tool, were identified. The ability of CDA to measure cell sensitivity to 177-Lu-DOTATATE was demonstrated while the γ-H2AX assay was not sensitive enough to detect the damage induced by 177-Lu-DOTATATE. Further, a biological variation in normal tissue sensitivity to 177-LuDOTATATE was detected using CDA.

The results of this study present a tool for the further research aiming to develop simple and clinically applicable assay to evaluate normal tissue sensitivity in cancer patients *prior* to 177-Lu-DOTATATE treatment.

COMPARISSON BETWEEN MICROWAVEREGULATED TISSUE PROCESSING AND CONVENTIONAL TISSUE PROCESSING

By Albin Aronsson

Bachelor thesis in Biomedical Laboratory Science performed at the department of Pathology, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisor: Katarina Junevik, PhD

Background: Tissue processing is a blanket term and consists of three different processes, dehydration, clearing and infiltration. In the first step named dehydration water is removed from the tissue using increasing concentrations of alcohol. The alcohol is then removed from the tissue during the clearing phase with a reagent that is solvable in both the dehydrationreagent and the infiltrationmedium. The final step is called infiltration and here an infiltrationmedium replaces the clearingreagent and gives the tissue support. If tissue processing is done incorrectly it could damage the tissue and severely lower the quality of tissueslides. Conventional programs for tissue processing can take up to 10 hours. With newer and smarter systems that utilizes microwave heating processing time can be severely shortened. The aim of this study if to see if a new and faster processing program gives the same or better results than conventional programs.

Material and methods: Tissue samples from 40 patients was used in the validation process of the program. Each tissue sample was cut into 2 sections and one was processed with conventional processing and one was processed with a shorter experimental program. The produced slides from the different programs was graded on the number of holes and tears that appeared in the tissue from a scale of 1 to 5 were 1 represented no holes and 5 represented a lot of holes. A pathologist evaluated the quality of all the slides and gave it a passing or failing grade. A total of 3 experimental programs were tested in this study.

Results: The results show that experimental program 2 and 3 gave significantly similar results compared to their control programs. When compared, program 3 gave significantly better results than program 2. Program 3 received a 70 % approval rate.

Discussion: The results have shown that the shorter program gives the same results when it comes to overall tissue quality. The sample sizes are however very small and additional tests need to be done to verify these results. The controls only received a 50% approval which means that factors other than tissue processing may have affected the results.

Increased expression of cyclin D1 in MELF myometrial invasive pattern areas compared with conventional tumor glands of endometrial cancer

By Gabriella Bengtsson
Bachelor Thesis in Biomedical Laboratory Science
Department of Clinical Pathology and Cytology, Sahlgrenska University Hospital
Supervisors: Claudia Mateoiu, MD, PhD,
Levent Akyürek MD, PhD, and Karin Sundfelt, MD, PhD

Background: Endometrial cancer is the sixth most common cancer in females. There are two major types of endometrial cancer: type I endometrioid cancer and type II non-endometrioid adenocarcinomas. Microcystic, elongated and fragmented glands (MELF) are specific pattern of myometrial invasion in endometrioid cancer. It is commonly ignored to identify MELF pattern which is associated with a fibromyxoid stromal reaction and lymphatic and blood vessel invasion, leading to a higher risk of recurrence and a poorer clinical outcome.

AIM: The main goal of this study was to characterize the immune phenotype of the MELF pattern and compare it with conventional tumor glands to improve routine diagnostics at the department of Clinical Pathology and Cytology.

Method: Endometrial tumor tissues showing MELF pattern (n =11) were sectioned and immunohistochemically stained using a panel of antibodies detecting progesterone, estrogen, cyclin D1, E-cadherin, β-catenin and galectin-3. Markers CD34 and D2-40 were used to identify possible lymphatic or blood vessel invasion. Level of expression was blindly graded from 0 to 1, where 0 indicates lack of expression, 0,5 partial expression and 1 positive expression.

Results: Expression of cyclin D1 was significantly increased in MELF compared to conventional tumor areas. Progesterone and estrogen showed a reduced expression but did nog reach a statistical difference. No significant differences in E-cadherin, β-catenin and galectin-3 expression were seen between conventional tumors and MELF pattern areas. Lymphatic and blood vessel invasions were detected by CD34 and D2-40 antibodies.

Conclusion: MELF areas can be easily identified by immunohistochemical expression of cyclin D1. Hopefully, this new panel of immunohistochemical characterization will be considered as a useful tool to identify MELF invasion pattern in routine pathological diagnostics.

Sex differences in microcirculation and forearm blood flow during hyperinsulinemic euglycemic glucose clamp: A study on people with type 2 diabetes mellitus

By Kajsa Brattgård

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg laboratory, Sahlgrenska Academy, University of Gothenburg, 2019.
Supervisor: Per-Anders Jansson, Professor.

Background: Endothelial dysfunction is common in patients with type 2 diabetes mellitus (T2D), and post-menopausal women appear to have a greater impairment of vascular function compared to men of similar age. **Objective:** The study evaluated potential differences in endothelial function and microcirculation in men and post-menopausal women with T2D, and various baseline covariates that could affect microcirculation. **Methods:** Data was collected from an ongoing treatment study with 23 subjects. Microcirculation was assessed as forearm blood flow (FBF) with venous occlusion plethysmography during a 180 minute hyperinsulinemic euglycemic glucose clamp (120 mU/m^2/min) at the end of the six-week study period. The end of the clamp was the basis for assessment of insulin sensitivity. Endothelial function was assessed using peripheral arterial tonometry on two occasions during the study period; at baseline and midpoint. In addition, blood samples for HbA1c and CRP were collected at baseline and during clamp. **Results:** Thirteen men and 10 women participated in the study. There was a significant difference between men and women regarding FBF during clamp ($p = 0.021$-0.041), where men had a higher median FBF than women. There was also a significant variance of FBF among the men ($p = 0.038$), but not among the women. There was a significant difference between men and women's insulin sensitivity ($p = 0.009$) where women had a higher degree of sensitivity than men. Apart from these findings, we observed no significant differences between the sexes, and none of the covariates had significant correlation to either microcirculation or endothelial function. **Conclusions:** Due to the nature of the study, it is not impossible that the treatment administered to the participants has affected the results and should therefore be taken into account when interpreting the reliability of the results. Nevertheless, when looking at a population with T2D, post-menopausal women appear to have higher insulin sensitivity, and a lower degree of microcirculation than men, indicating that women should be target of care and treatments specified on protecting vascular function.

Validation of an LC-MS/MS method for the detection and quantification of benzoylecgonine in human urine

By Bianca Calancea

Bachelor thesis in biomedical Laboratory Science performed at the clinical chemistry laboratory at Norra Älvsborgs Länssjukhus, Trollhättan, 2019.
Supervisor: Kamil Slupecki, Biomedical Analyst

Cocaine is included in the category of central stimulant drugs that affect the central nervous system and causes increased excitement and euphoria, mental and physical overactivity but also reduced need of sleep. In the body, cocaine is broken down into benzoylecgonine and ecgonine methyl ester. Cocaine is excreted via the urine mainly in the form of its metabolite benzoylecgonine. Benzoylecgonine has a longer half-life compared to cocaine and remains detectable for a longer time. For this reason, benzoylecgonine is used as an analyte to detect and quantify a potential cocaine intake. In analytical chemistry, immunochemical screening methods are used for detection and liquid chromatography combined with mass spectrometry for verification.

The purpose of this study was to validate and optimize a new method for the detection and quantification of benzoylecgonine in human urine by liquid chromatography- tandem mass spectrometry on the instruments Agilent 6460 and Agilent 6470. The analysis results were later compared with results from the existing method used on the instrument Waters Xevo TQ- MS.

Urine samples containing benzoylecgonine were analyzed on all instruments. In series variation was examined by analysis of 16 replicates. The intermediate series variation was examined by reprocessing and analysis of 11 replicates, 1times/day. For comparison of the analysis results between the instruments, regression plots and differential plots were created in excel.

Results for mean value (mv), standard deviation (sd) and coefficient of variation (cv%) calculated for in series and inter series variations show good reproducibility and robustness of the method. Comparison of the analysis results shows good linearity and correlation between the instruments.

The conclusion is that this method is suitable for use as a routine method for answering clinical questions about intake or not intake of cocaine.

Abstract

Introduction: Direct oral anticoagulants (DOAC or NOAK) are quickly replacing Warfarin as the most common medication against thrombosis, but they complicate diagnosis by affecting many clotting tests. Most notably the medication tends to give false positive results for Lupus anticoagulantia. The aim of this study was to evaluate the effect of DOAC-Stop, a product said to absorb NOAK in plasma.

Methods: Plasma samples from four healthy subjects were treated with apixaban, rivaroxaban, dabigatran and edoxaban in different concentrations, and then half the samples were treated with DOAC-Stop while the other half was left untreated. All plasma samples, both treated and untreated, were tested for Owren type of the prothrombin time PT(INR), activated partial thromboplastin time (APTT), Lupus anticoagulantia (LA), protein C, free protein S and antithrombin.

Results: DOAC-Stop removed a significant part of the medication from plasma samples, and also normalized APTT and PK. The numbers of false positive results for Lupus anticoagulantia drastically lessened after treatment with DOAC-Stop. Antithrombin, protein C and free protein S were very slightly affected by the addition of DOAC-Stop.

Consclusion: This study suggests that DOAC-Stop would be helpful for performing clotting tests on patients treated with NOAK, especially when testing for Lupus anticoagulantia. DOAC-stop does not in itself interfer with clotting tests, yet removes almost all of the medication from plasma samples.

Difficulty in detecting candida albicans in blood culture when concomitant bacteremia

By Josefine Collin

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Microbiology, Södra Älvsborg hospital, Borås, 2019
Supervisor: Claes Henning, Consultant

Background: Sepsis is a life threatening organ dysfunction due to dysregulated host response to an infection. Polymicrobial bloodstream infections is when two or more microorganism are circulating in the blood, which is associated with higher mortality. Blood is collected in culture bottles and incubated and in blood culture system to isolate the microorganisms. Blood from the culture bottles are then cultured on different agar plates for identification and determination of resistance. Equalis performs external quality assurance in laboratory medicine in Sweden. To validate the blood culture technique, they send out simulated samples containing bacteria or fungi to be identified. In 2017 Equalis sent out samples containing two different microorganisms (*Candida albicans (C. albicans)* with *Escherichia coli (E. coli)* and *C. albicans* with *Staphylococcus aureus (S. aureus)*). The majority of the laboratories were only able to identify the bacteria. The result may indicate that the laboratories have serious deficiencies in the detection of polymicrobial blood stream infections.

Aim: The aim was to find out why the laboratories were unable to detect *C. albicans* when it was mixed with *E. coli* or *S. aureus* in the external quality controls.

Materials and methods: Determination of detection times in the blood culture system BacT/ALERT was investigated for the microorganisms at different concentrations separately and when co-cultured. Several attempts were made to investigate whether the bacteria *E. coli* and *S. aureus* had some effect on growth of *C. albicans* when co-cultured. The experiments were performed both in blood culture bottles and in BHI broth.

Results: The detection time for *E. coli* and *S. aureus* were much shorter than the detection time for *C. albicans*. It was obvious that the detection time for the co-cultures were due to the bacteria. We could not see any inhibitory effect on *C. albicans*.

Conclusion: The fact that the laboratories were unable to detect *C. albicans* in the simulated samples from Equalis seems to be due to large differences in growth time and too low content of *C. albicans*.

Defining the role of astrocytes in regulating striatal neurotransmission

By Felicia Danielsson

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Neuroscience and Physiology, department of Psychiatry and Neurochemistry, Gothenburg university, 2019.
Supervisor: Louise Adermark, docent

The striatum is the major input nucleus to the basal ganglia, and striatal pathology is associated with a wide array of disorders, including neurological disorders such as Parkinson's disease and Huntington's disease as well as psychiatric disorders such as drug addiction or obsessive compulsive disorder. The dorsolateral striatum (DLS), have been proven to be especially important for motor-skill learning and habitual actions. The knowledge regarding how the synaptic output from the striatum is regulated and fine-tuned is limited.

This project aims to outline the function of transporter EAAT, autoreceptors mGluR 2/3 and $GABA_A$ receptors in regarding astrocytes ability to control striatal neurotransmission. The glia cells called astrocytes have proven to have much interaction with the synapses and can monitor and alter the synapses transmission and function. Because of this it is sometimes considered as the third part of the synapse along with the axon and dendrite. One important function astrocytes have is to transport the neurotransmitter glutamate to and from the synapse and thus making sure that there is a good signal-to-noise ratio. In this study the function of the astrocytes are studied by deactivating them with Fluorocitrate and also deactivating receptors and transporters theorized to play an intricate role in astrocytic function. Drugs were used to block the autoreceptor metabotropic glutamate receptors, the astrocytes glutamate transporters EAAT 1/2 and the $GABA_A$ receptor were studied in order to understand how they affect astrocytes and neurotransmission. Results showed that none of the receptors studied seemed to prevent a decrease in neurotransmission caused by blocking astrocytes. Blocking the mGluR 2/3 receptors seemed to have some function in enhancing the FC effect and further lowering neurotransmission but why is not known, and will be outlined in further experiments in the laboratory.

Demonstration of superficial vein thrombosis in conjunction with PVK treatment during hospitalization using sonographic examination.

By Rüya El Hattawi

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology in Östra Hospital Sahlgrenska Academy, University of Gothenburg, 2019

Supervisor: Anders Thurin

Abstract

Introduction: PVC stands for peripheral vein catheter and is placed into a vein for injections directly into the bloodstream. It is very often used in health care and with this it is possible to inject both nutrients, medicines and other fluids.
PVC can be associated with a number of complications like bruising, infections and extravasation. One of the more common complication is thrombophlebitis.
Thrombophlebitis is a symptomatic thrombosis in a superficial vein, often combined with inflammation of the vascular wall, and can be caused by traumatic vascular injury, infusions, infections and other coagulation disorders. The symptoms may include tenderness, pain and swelling

Aim: The purpose of this work is to see how frequent venous thrombosis is in patients who have peripheral vein catheter (PVC), and if possible assess how the amount of thrombosis changes with different duration of PVC treatment.

Method: The study included patients passing clinically physiology. Patients were recruited after documented consent with oral and written information. The PVC area was investigated using the ultrasonic machine Philips iU22 MIMO (Philips Healthcare, Bothell, WA, USA) with transducer L17-5. Image data was collected in DICOM format and analyzed in arrears with measurement of compressed vessel diameter as a marker for thrombosis occurrence.

Results: Of the 15 patients included, there were 8 non-compressible vessels. The difference in diameter between the compressed and uncompressed vessels was significant (0.001). Thrombosis levels did not increase due to difference in duration (2 or 3 days), It was not significant (0.864).

Conclusion: The conclusion is that 8 out of 15 patients showed signs of thrombosis in the vein in which PVK was placed. There was no significant difference in the amount of thrombosis between the different duration groups 2 - and 3 days.

Experience and competence in thumb-ECG analysis is required to make the correct diagnosis of atrial fibrillation

By Oskarina Fargo

Bachelor thesis in Clinical Physiology performed at the department of Clinical Physiology at Sahlgrenska University Hospital, Östra, 2019.
Supervisors: Sverker Jern, professor and senior physician in cardiovascular physiology
Christina Claesson, biomedical scientist

Background. Correct diagnosis of atrial fibrillation is important in healthcare. Using reliable diagnostic methods to detect various clinical forms of atrial fibrillation reduces the risk of under- and overdiagnosis. Overdiagnosis causes healthy individuals to be diagnosed with atrial fibrillation, which may increase the risk of bleeding if treated with antithrombotic medication. Underdiagnosis leads to sick patients with atrial fibrillation being misdiagnosed and not receiving proper treatment for their cardiac arrhythmia, which leads to increased risk of stroke. In this study, we investigate how many thumb-ECG registrations gets the correct diagnosis by three assessors with different experience and expertise in thumb-ECG analysis. The registrations are collected from two groups, a selection group with participants who have atrial fibrillation / irregularly blocked atrial flutter and a comparison group containing healthy participants. ***Aim.*** The aim of this study is to investigate whether competence and experience in thumb-ECG analysis of an assessor is required in order to be able to correctly diagnose atrial fibrillation on registrations from thumb ECG. ***Method.*** To the selection group, patients who have anamnestic and confirmed atrial fibrillation and/or irregularly blocked atrial flutter were recruited. The healthy group consisted of healthy participants who were recruited among the staff who work in the department where the study was performed. The main principle was to perform a diagnostic test by collecting simultaneous measurements from thumb-ECG and Holter ECG. The registrations from Holter-EKG served as a ''gold standard'' when assessing measurements from thumb ECG. Three assessors, a biomedical scientist who is an expert (assessor A), a senior physician who has good experience (assessor B) and a resident physician with no experience (assessor C) in thumb ECG analysis, assessed these measurements. The assessors were allowed to analyze 20 randomly selected measurements from each group. Specificity, sensitivity, positive and negative predictive value were calculated on the number of correct diagnoses.
Results. Sensitivity, specificity, positive and negative predictive value resulted in 100% for assessor A. For assessor B, sensitivity was 80%, specificity 95%, positive predictive value 94% and negative predictive value 83%. For assessor C, the sensitivity was 80%, the specificity 90%, the positive predictive value 88% and the negative predictive value 82%. ***Discussion.*** Assessor A correctly diagnosed all measurements from the selection group and comparison group. Assessor B who received a sensitivity of 80%, under-diagnosed the remaining 20% of the measurements. Assessor C also under-diagnosed 20% of the measurements. Specificity was at 95% and this means that remaining 5% of the measurements were over-diagnosed. The specificity of 90% for assessor C represents an overdiagnosis of 10%. The number of measurements that were under- and over-diagnosed are few but are considered significant because correct assessments are crucial for the patient's health. A single incorrect assessment can lead to a patient getting wrong or no diagnosis. ***Conclusion.*** This study shows that the competence and experience of an assessor is required in the analysis of thumb ECG to make the correct diagnosis of atrial fibrillation.

Sign of impaired elevated filling pressure, indicating impaired diastolic function are observed three years after heart transplantation

By Zeleikha Farzalieva

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Physiology, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisor: Bente Grüner Sveälv PhD, Entela Bollano MD, PhD

Objecives. The primary aim of this study is to investigate if there is a correlation between measurements from echocardiography with Doppler and pulmonary capillary wedge pressure (PCWP) from cardiac catheterization in cardiac transplant patients, three years after transplantation. The secondary aim is to investigate the changes in echocardiographic and invasive parameters in the same patient population from one to three years after transplantation.

Background. Diastolic dysfunction is a known risk factor for increased mortality in cardiac transplant patients. Normally, a few weeks after transplantation, the incidence of diastolic dysfunction and elevated filling pressure decreases, but due to number of rejection episodes, hypertension and myocardial ischemia, diastolic dysfunction may remain elevated. Right heart catheterization is the Gold standard for assessment of filling pressure, but since the method is invasive and not inexpensive, continuous studies are ongoing in the search for non-invasive parameters that could be used instead. Some studies indicate that parameters obtained with Doppler tissue imaging are useful, while other studies do not agree.

Methods. The study enrolled the total of 35 patients who underwent their third post-transplantation annual routine follow-up at the Sahlgrenska Hospital, that included both echocardiography and right heart catheterization. Echocardiographic assessment included measurements from mitral inflow (E/A-ratio, deceleration time (DT)), tissue Doppler (E/E', isovolumetric relaxation time (IVRT)). We analyzed the relationship between the invasive and non-invasive parameters of diastolic function, and the changes between one and three years post transplantation.

Results. With Spearman's rank correlation coefficient, we observed a statistically significant relation between the invasive PCWP and non-invasive E/A-ratio, $p=0.014$. Other diastolic parameters as IVRT, DT and E/E' did not show any relation with invasive data ($p > 0.05$). Further, we found a significant elevation in the diastolic parameter E/E' from one to three year after transplantation (7.2 vs 8.3, $p=0.003$), indicating an elevated filling pressure.
Conclusion. In this study, we can conclude that there is no relationship between invasive and non-invasive parameters that can be used for assessing PCWP in heart transplant patients three years after transplantation. However, we observed with echocardiographic assessment, a sign of impaired elevated filling pressure, indicating impaired diastolic function three years after transplantation.

No Association between Low Ankle-Brachial Index and Cardiovascular Events Among Middle-aged Men

By Rut Forsberg Bernevång

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, 2019
Supervisor: Caroline Schmidt, Associate Professor

Objective: To examine whether there is an association between low ankle-brachial index and cardiovascular events in a group of middle-aged, Swedish men.

Background: Atherosclerosis is often the cause for cardiovascular diseases, such as myocardial infarction, stroke and peripheral artery disease. The ankle-brachial index (ABI) is a method used to identify peripheral artery disease in the legs, where a low ABI (≤0.9) indicates peripheral artery disease.

Material and Method: The study was conducted on a group of 388 subjects, all men aged 58 and with Swedish ancestry. ABI was calculated for a. dorsalis pedis and a. tibialis posterior bilaterally, and the subjects were divided into 2 groups; those with low ABI (≤0.9) in at least one of the 4 examined blood vessels and those with ABI within the reference interval (0.91-1.39) in all of the examined blood vessels. Cardiovascular events were specified to be myocardial infarction, angina pectoris, stroke, claudication, and revascularization. During a mean follow-up period of 5.0 years after the ABI-measurements, data whether the patients had a cardiovascular event or not was collected from the national patient register, and the subjects were then divided into those that had an event and those without any events.
Subjects with high ABI (≥1.14) in at least one blood vessel, or that had a cardiovascular event before the study, or with insufficient data were excluded from the study(n=127).

Results: Out of 261 subjects, 44 had a low ABI, and out of them 3 had had a cardiovascular event. For those with ABI within the reference interval, 20 of them had had a cardiovascular event. Fisher's exact test showed a p-value of 0.775 when examining a potential association between low ankle-brachial index and cardiovascular events.

Conclusion: There was no significant association between low ankle-brachial index and cardiovascular events in a group of middle-aged, Swedish men.

FREE CIRCULATING NUCLEIC ACIDS IN CEREBROSPINAL FLUID AS A DIAGNOSTIC TOOL FOR INFECTIONS IN THE CENTRAL NERVOUS SYSTEM

By Elin Frank

Bachelor thesis in biomedical laboratory science, 2019
Institute of Biomedicine at Sahlgrenska academy, University of Gothenburg.
Supervisor: Hedvig Engström Jakobsson PhD, Josefin Olausson PhD

Introduction: Early diagnosis of central nervous system infection is essential to prevent mortality or neurological sequelae. The most common cause is virus, followed by bacteria, fungi and protozoa. There is over a hundred known viruses that has potential to cause infection in the central nervous system. Nevertheless, in a substantial number of cases the cause of infection remains unidentified. Today diagnostic methodology uses targeted analysis, where the test is built on clinical expectations, anamnesis and experience. For some pathogens these methods have a limited sensitivity. Therefore, improved methods are needed to identify causative pathogens of infection in the central nervous system. Free circulating nucleic acids are small fragments of DNA that circulate freely in body fluids outside the cells. Free circulating nucleic acids in plasma is used in cancer diagnostics very favourably where tumour-DNA is a part of the cell-free DNA. When diagnosing a brain tumour, studies have indicated that cell-free DNA in cerebrospinal fluid provides improved results compared to plasma due to lesser cellular contamination. Thus, we propose that the sensitivity of microbial detection will increase if cell-free DNA is used instead of total DNA in cerebrospinal fluid.
Aim: The aim of this study is to investigate whether cell-free DNA in cerebrospinal fluid can serve as a diagnostic tool in clinical microbiological diagnostics.
Method and materials: Using a variety of commercial kits, cell-free DNA is isolated from cerebrospinal fluid. Kits are then evaluated by total double stranded DNA quantification and fragment length analysis that will determine size distribution of DNA. Furthermore, cell-free DNA integrity is investigated using a quantitative PCR, where the ratio of a long and a short gene is calculated to determine the amount of genomic contamination. Cerebrospinal fluid positive for viral infection, virus-negative controls, as well as dilution series of virus-positive samples are performed in order to investigate sensitivity and specificity of cell-free DNA to detect infection. Therefore, a virus-specific real time PCR is utilized to detect and quantify the virus.
Result: This study proposes that cell-free DNA in cerebrospinal fluid might have a unique size profile. Two fragment lengths cell-free DNA were in majority, ranging from approximately 160-200 and 340-400 base pairs long. The study also found an indication that the size of cell-free DNA depends on the origin of infectious agent and response from the host.
Conclusion: The study concludes that cell-free DNA marketed extraction kits were preferable to automatized extraction methods. Furthermore, the study contributed with information about the size profile of cell-free DNA in cerebrospinal fluid, which will lead to adjustments in future analysis. This study also supplied suggestions of improvements for downstream research. Thus, the results of this study indicate a great potential for the use of cell-free DNA for microbial detection in cerebrospinal fluid.

SEX, CHESTPAIN AND KNOWN CARDIOVASCULAR DISEASE – REFERRAL INFORMATION ASSOCIATED WITH PATHOLOGICAL OUTCOME WITH MYOCARDIAL PERFUSION IMAGING

By Emilia Gianello

Bachelor thesis in Biomedical Laboratory Science preformed at the department of clinical physiology at Östra hospital, Sahlgrenska Academy, University of Gothenburg, 2019.
Supervisor: Gert Hermansson, MD, PhD.

Background and aim: To identify, quickly diagnose and prevent complications for coronary artery diseases is one of the healthcare's primary tasks. Myocardial perfusion imaging is a method to identify and to see the spread of possible perfusion defects in the myocardium. The aim of this study was to retrospectively evaluate the outcome with myocardial perfusion imaging and to investigate whether certain symptoms or risk factors described in the referral can signal that we will find something pathological in the study result. We will also specifically examine how the outcome looks for patients with left bundle branch block (LBBB) in rest-electrocardiogram (ECG). **Method**: The study population consisted of 255 patients that under February and March 2018 had done a myocardial perfusion imaging examination at Östra hospital, Gothenburg. All patients' referrals were analysed and information about sex, LBBB, symptoms, risk factors and other referral information was obtained and included in the collection protocol. Furthermore the outcome of the examination was acquired and whether the patient was hospitalized or not. To compare the different variables with the outcome the Fishers exact test was used. **Results**: Three patients were excluded from the study due to patient-specific reasons. Out of 252 patients there was 194 (77%) with normal perfusion in the myocardium, 25 patients had a reversible perfusion defect, 13 patients had a constant perfusion defect and 20 patients had both a reversible and constant perfusion defect. 17 patients had LBBB on rest-ECG, and 5 of them had perfusion defects. Among all pathological patients 9 % had LBBB. A significant difference were shown on sex ($p = 0{,}0001$), chest pain ($p = 0{,}045$) and known cardiovascular disease ($p = 0{,}0001$). **Conclusion**: The majority of patients examined had a normal outcome on myocardial perfusion imaging, only 23% had some type of perfusion defect in the myocardium. Among the patients with pathological outcome (58 patients) do 9% have LBBB on rest-ECG, the presence of LBBB showed no increased risk for ischemia and/or myocardial infarction in our study population. The referral information that showed a significant difference are sex, chest pain and known cardiovascular disease.

Knockout of the Estrogen receptor alpha in CCL19 positive stromal cells do not cause an increased number of osteoclasts

By: Madeleine Haraldsson

Bachelor thesis in Biomedical Laboratory Science preformed at Department of Rheumatology and Inflammations Research, Institute of Medicine, Sahlgrenska Academy at University of Gothenburg, 2019.

Supervisors: Ulrika Islander (Associate professor), Julia Scheffler (PhD)

Background: Osteoporosis is often a consequence of age and declining sex hormones, especially estrogen, in both males and females. Studies have shown that estrogen mediates its effects on bone remodeling through estrogen receptor alpha (ERα), inducing for example apoptosis in osteoclasts and a decrease in osteoclastogensis. A previous study made at the Department for Rheumatology and Inflammation research at the University of Gothenburg, showed that a conditional knockout of ERα in chemokine ligand 19 positive stromal cells in mice, *CCL19*-Cre*ERα*$^{flox/flox}$ mice (*CCL19ERα*KO), resulted in decreased bone mineral density compared to control mice (*ERα*$^{flox/flox}$). The aim of this study was to elucidate if the decreased bone mineral density in the knockout mice is due to increased production and differentiation of osteoclasts in the bone marrow compared to control mice.
Material and Method: To elucidate the potential of the bone marrow to produce and differentiate osteoclast in the mice, a primary osteoclast culture was preformed using bone marrow from *CCL19ERα*KO and *ERα*$^{flox/flox}$ mice. The osteoclasts were counted and compared between the mice. Immunofluorescence, haematoxylin-eosin staining, and flow cytometry was also done to examine the morphology of the stomal cell compartment and the subtypes of stromal cells in the lymphnodes, to see if there were any difference between the mice. The flowcytometry was done with *CCL19ERα*KO, *ERα*$^{flox/flox}$ and *CCL19*-Cre mice, while the immunofluorescence and haematoxylin and eosin staining were done with *CCL19ERα*KO and *ERα*$^{flox/flox}$ mice.
Results: The flowcytometry results showed significantly decreased levels of fibroblastic reticular cells in the *CCL19*-Cre mice, compared with both *ERα*$^{flox/flox}$ and *CCL19ERα*KO mice. Furthermore, *CCL19*-Cre mice displayed a significantly higher expression of CD40 and a significant lower expression of MHC I on these cells compared with the two other genotypes. The immunofluorescense, haematoxylin-eosin staining, and the osteoclast culture showed no significant differences between the *CCL19ERα*KO and the *ERα*$^{flox/flox}$ mice.
Discussion: The difference seen in *CCL19*-Cre mice is most likely due to the fact that these mice came from a different breeding setup compared to the other two genotypes. Our results from the osteoclast culture showed no significant difference between the *CCL19ERα*KO and *ERα*$^{flox/flox}$, suggesting that the difference in bone mineral density seen previously in these two genotypes most likely are not due to the osteoclastogenisis in the bone marrow.

Examining echocardiography answers and measurements of the right ventricle for collecting data for training a software

By Sumaya Hassan

Bachelor thesis in Biomedical Laboratory Science performed at the institution for medicine, University of Gothenburg, 2019

Supervisors: Ola Hjelmgren (MD Clinical physiology) and Eva Hagberg (MD Clinical Physiology)

Background: To measure and diagnose the size and systolic function of the right ventricle (RV) is of outmost importance. With the help of 2D-echocardiography different views of the RV can be analysed and measured, such as the right ventricular outflow tract (RVOT) and 4-chamberview. By training a software built on convolutional neural network (CNN), the diagnosis of the RV will be much more effective and faster.

Aim: Can the answers from the physicians that already exists be categorised into different groups? Also, can a correlation between objective measurements and the groups be seen? And if so, is it possible to train a software based on the results to recognize a normal/abnormal size and function of the RV?

Method: 5 different categories were made for the RV size and 6 groups were made for the systolic function, all from the answers the physicians had given from 2015. However, the groups for the size became 2 groups when the statistics were being made just so the study population could be big enough in each group. Firstly 250 patients were divided into the 5 groups and then 61 of these patients were randomly selected. Secondly, objective measurements were made on the 61 these patients in RVOT and 4-chamberview. Finally, a Mann-Whitney U test was performed to see the significance level between the groups, and a comparison was made between the objective measurements and the reference values that already existed.

Results: There was a significant difference between all the groups when measuring the RVOT and 4-chamber view. Between group 1-2 and 3-5 a significance level was seen with a p-value of 0.003 when measured in RVOT. A significance level was also seen between group 1-2 and group 3-5 with a p-value of 0.0000008 when measured in 4-chamberview. Also, it could be seen that group 3-5 had a dilatation both in RVOT and 4-chamberview when measured objectively and compared to the reference value.

Conclusion: There is a correlation between the categories that were made up based on the answers from the physicians and the objective measurements. This study can be a foundation for training a software that will be able to measure the RV size and function to ease the clinical work.

Abstract

Seronine/threonine kinase BRAF is mutated in around 66% of malignant melanomas, and in 15% of all human cancers (80% *BRAF*^{V600E}). BRAF^{V600E} drastically upregulates the MAP kinase pathway, leading to cell survival and proliferation. Selective BRAF^{V600E} inhibitors exist and treatment is highly effective, however patients quickly develop immunity to the drug. Preliminary results show a supposed upregulation of TGF-β2 in malignant melanoma cell treated with PLX4720.

TGF-β2 regulates cell proliferation, survival and homeostasis and plays an important role in keeping cancer cells from growing. However cancer cells are able to avoid the effects of TGF-β2 and turn it's effects to their advantage. To study the effects of PLX4720 induced expression of TGF-β2 in cancer cells, two different malignant melanoma cell lines, both PLX4720-sensitive and -resistant cells, were cultured and treated with PLX4720. qPCR results revealed an increased TGF-β2 gene expression in sensitive malignant melanoma cells treated with PLX4720, but no increase in resistant malignant melanoma cells treated with PLX4720. Western blot results revealed that the PLX4720-sensitive cells were actually sensitive, and the PLX4720 resistant cells were actually resistant, to PLX4720 treatment.

In conclusion this thesis revealed that PLX4720 resistance reverses the upregulation of PLX4720 induced TGF-β2 expression.

Validation of antibody staining for bcl-2, CD5, CD19 and CD30 on a new automated platform (Dako Omnis)

By Meiying Jaritram

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Pathology/Cytologi, Halland Hospital, Halmstad, 2019.
Supervisors: Tomas Seidal, MD, PhD and Frida Kronqvist, specialist Biomedical laboratory scientist

Background: Immunohistochemistry is a method which antibodies are used for identifying antigens in a tissue section or smear, by visualizing antigen-antibody binding with chromogen-substrate system. Antibody validation is important for understanding the cell function in both normal and pathological tissues, and as for diagnostic. Detection of bcl-2 is important for diagnosis of lymphoid malignancies. CD5 is a T-cell marker used for classification of T-cell and B-cell malignancies, lymphoma and leukemias. CD19 is often used as an additional marker in B-cell lymphoma after-treatment. CD30 can be detected in such as classical Hodgkin Lymphoma. Nowadays, automated staining platforms have become more common in most laboratories. In order to increase productivity and quality assurance, we have changed to a new automated platform Dako Omnis. The antibody validation was therefore necessary to be able to ensure sensitivity and specificity of antibodies, when changing the instrument. The aim of this study was to validate antibody staining for bcl-2, CD5, CD19 and CD30 on the new staining platform Dako Omnis.
Method: Antibody staining for bcl-2, CD5 and CD19 used a multiblock containing 2 cases of DLBCL, 2 cases of follicular lymphoma grade II, and control tissues (tonsil and appendix). Antibody staining for CD30 used a tonsil tissue, 2 cases of Hodgkin Lymphoma and 2 cases of embryonal carcinom in testis. Different antibody dilutions were tested in staining for CD19 and CD30 while ready-to-use antibody dilutions were used in staining for CD5 and bcl-2. All tests were performed with HIER in low and high pH, with mouse linker and without linker. Stainings were then compared with the reference staining on Autostainer Link 48.
Results: Staining of bcl-2 showed an optimal result with high pH and linker. CD5 showed an optimal staining in high pH without linker. Staining of CD19 was optimal by using dilution at 1:50 with high pH and linker. For CD30 the optimal staining resulted in dilution at 1:100, high pH and linker.
Conclusion: Validation of antibodies on new platform Dako Omnis produced comparable results with staining on the older platform Autostainer Link 48. In this study, we have found that most antibodies were stained better by using linker, while CD5 was stained better without linker. An antibody concentration used on an instrument can give a result with good or poor staining, depends on staining protocols, appropriate pH and materials used for that instrument.

Low prevalence of colistin resistance among dogs and *E. coli* isolated from urine samples in Halland

By: Louise Johansson

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Microbiology, Hallands hospital, Halmstad, 2019.
Supervisor: Ingegerd Sjögren, microbiologist.

Introduction: Colistin is an old antibiotic effective against gram-negative bacteria. It was abandoned in the 1970s because of its toxicity, but nowadays it has gained a new role as a last-resort antibiotic in the fight against multidrug-resistant gram-negative bacteria, especially carbapenem-resistant strains. Until recently, colistin resistance was thought to occur entirely because of chromosomal mutations and the overall resistance has been low. The discovery of plasmid-mediated resistance in 2015 then led to an increased worry of a greater spread worldwide. Since 2015, the phenomena of plasmid-mediated colistin resistance has been investigated all over the world and the mobile colistin resistance genes *mcr-1* to *mcr-8* have been identified. Colistin resistance and the presence of *mcr*-genes must be followed both globally and locally to make us better prepared for upcoming resistance problems and the formation of pandrug-resistant bacteria. Knowledge about colistin resistance in different environments may also serve as a predictor, as reservoirs of colistin resistant bacteria and *mcr*-genes in the environment increase the risk of the spread to humans.

Aim: The aim of this study was to determine the prevalence of colistin resistance in clinical isolates of *E. coli* from human urine samples in Halland, Sweden and among *Enterobacteriaceae* isolated from dogs.

Method: In this study, 278 human consecutive isolates of *E. coli* and 53 samples of dog faeces were screened for colistin resistance. The samples were cultured on selective agar and antimicrobial susceptibility testing was performed using broth microdilution for colistin and disk diffusion for other antibiotics. Colistin resistant isolates were sent to the reference laboratory for detection of *mcr*-genes using whole genome sequencing.

Results: Out of 278 *E. coli*-isolates, one was clearly colistin resistant (MIC 8 mg/L), leading to a prevalence of 0,36%. The colistin resistant isolate showed no existence of *mcr*-genes. Among 53 samples from dogs, no colistin resistant *Enterobacteriaceae spp* were identified.

Conclusion: The prevalence of colistin resistance in Halland is very low in clinical *E. coli*-isolates. Companion animals do not serve as reservoirs for colistin resistant bacteria and *mcr*-genes and since resistance is rare in our local environment the situation seems to be stable enough to not be a threat within the near future in Halland.

No Differences in Echocardiographic Parameters in Patients with Pulmonary Arterial Hypertension - Before and After Specific PAH-targeted Therapy

By: Josefin Johnsson

Bachelor thesis in Biomedical Laboratory Science performed at Institution of Medicine, Apartment of Clinical Physiology, Sahlgrenska University Hospital, University of Gothenburg

Supervisor: Clara Hjalmarsson MD PhD, Bente Grüner Sveälv PhD

Introduction: Pulmonary arterial hypertension (PAH) is a rare and serious cardiopulmonary disease, which mainly affects the small pulmonary arteries and arterioles. The PAH subgroups includes idiopathic/hereditary pulmonary arterial hypertension (IPAH/HPAH) and associated pulmonary arterial hypertension (APAH) that can be associated to several diseases, e.g. connective tissue disease (CTD). Among CTDs, systemic sclerosis (SSc) is most often accompanied by PAH. The PAH-specific treatment options have progressively evolved during the last decade, resulting in better quality of life and reduced hospitalization and mortality. Transthoracic echocardiography (ECHO) has an important role in screening and treatment monitoring of patients with PAH, due to the high accuracy, availability, low cost, non-ionizing and non-invasive technology. **Aim:** The primary objective of this study is to assess if the standard ECHO parameters recommended by the European Society of Cardiology (ESC)/European Respiratory Society (ERS) Guidelines are improved within one year after the given treatment; a secondary aim is to compare these parameters at baseline and follow-up in patients with IPAH/HPAH versus patients with APAH. The null hypothesis (H0) of the study would be that there are no differences in echocardiographic parameters between the time of diagnosis and one-year follow up. However, due to a low number of patients, the study does not have the statistical power to detect such differences; therefor the study should be regarded as mainly descriptive. **Method:** It is a retrospective study which is based on data recorded in N=50 patients who meets the criteria for inclusion. The patients were diagnosed with PAH (IPAH/HPAH or APAH) at Sahlgrenska University Hospital, from January 23, 2008 and forwards. The baseline was set by the day of diagnosis, confirmed by RHC. Mann-Whitney U test (continuous variables) and Chi-square test (categorical variables) and Wilcoxon signed-rank test (continuous variables) were used for the statistical comparisons. **Result:** The stroke volume was significantly increased at follow-up compared to baseline ($p=0.041$, $p=0.025$, $p=0.026$); also, the right ventricular systolic pressure (RVSP) and the presence of pericardial effusion were significantly different between the IPAH/HPAH group and the APAH group at baseline. **Conclusion:** The only echocardiographic parameter that was improved at follow-up was the stroke volume. However, due to the low number of patients included in the study, the null hypothesis that claims that there are no differences between baseline and follow-up cannot be rejected.

Abstract

Inter- and intravariability within echocardiographic 2D-parameters

By: Alexander Jönsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2019.

Supervisors: Anita Persson, PhD, Angela Poller, MSc, Leg BMA

Background: Echocardiography is a fast and relatively cheap examination that allows the observer inspect the patient's heart structures in real time. It is a non invasive method that does not emit ionizing radiation. Echocardiography is performed with an ultrasound machine that produces high frequencies with the compression of a piezoelectric crystal. There will always be some variability between and within sonographers. The question is how significant that variation is and what effect it has in a clinical setting. This study focuses on the measurements of 2D-parameters acquired from several previously performed echocardiographies. The aim of the study was to determine if there exist a variability within and between sonographers.

Method: Five patients that had good image quality from an echocardiographic examination were selected for the study. All echocardiographic personnel at the Sahlgren´s University hospital were asked to participate in the study. In the end 15 echocardiographers participated in the study. The software that was used to measure the different parameters was Echopac, GE Healthcare. To calculate the variability of the different parameters the coefficient of variation was calculated for each parameter.

Result: A variability of >10% was deemed to be a significant variation. The intervariability was as high as 26.35% (ESVI) and as low as 2.68% (VKd). Meaning there were some significant variation in a few parameters. For intravariability the highest variation was 8.93% (ESVI) and the lowest 0.861% (VKd) meaning there was no significant intravariability.

Conclusion: There is less variability within the same observer than between different observers when measuring 2D-parameter from an echocardiographic examination.

Automatic classification of ^{18}FDG-PET/CT uptake pattern in bone and bone marrow in patients with lymphoma as a first step of an automated software.

By Armin Krupic

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2019.

Supervisor: May Sadik (Biomedical scientist, PhD)

Background: Lymphoma is a type of cancer that occurs in the lymph nodes and other lymphoid tissue. Lymphoma can be divided into two groups: Hodgkin's lymphoma and non-Hodgkin's. ^{18}FDG PET/CT is a commonly used and effective examination method for investigating the spread of the disease and for staging according to Ann Arbour classification. A biopsy is frequently done in connection to the PET/CT for bone marrow examination as the lymphoma can occur extra-nodal in the bone marrow but can be refrain if there is a focally increased uptake in the bone marrow on PET/CT that indicates for bone marrow involvement. In earlier studies artificial intelligence has been applied for automatically segmenting skeletal bones that is fast, reproducible and highly precise. The long-term goal is to develop a software that automatically evaluates ^{18}FDG uptake pattern in bone/bone marrow in lymphoma patients that can be used to an aid when reviewing PET/CT-material so that no pathologic uptake is missed. The specific goal whit this study is to evaluate if an artificial intelligence (AI) software can be trained to quantify and separate pathologic uptake patterns from normal uptakes in newly diagnosed lymphoma patients. **Method:** All patients with biopsy verified Hodgkin's or B-cell lymphoma that had undergone a PET/CT-examination at Clinical physiology, Gothenburg, Sahlgrenska University Hospital between 2011-2016 were retrospectively included in the study (151 patients). The axial skeleton including 49 bones were segmented automatically in the CT-images and ^{18}FDG uptake vas recognized and quantified in the corresponding PET-images. the standardized uptake value (SUV) from the uptake patterns was calculated to a total lesion uptake (TLU), $TLU = (averageSUV - threshold) \times skeletal\ volume$ resulting in a specific threshold for each patient. All data that was classified as positive or negative by the AI software was compared to a gold standard that consisted of patient data about medical history, type of lymphoma, blood values, bone marrow biopsy and Ann Arbour stage. Statistic tests were used to find out how well the AI software distinguish pathologic from normal bone/bone marrow ^{18}FDG uptake pattern. Sensitivity, Specificity, Positive (PPV) and Negative (NPV) predictive values and ROC-curve with the area under the curve (AUC) was calculated. **Results:** Out of 151 patients, 20 had a focal bone/bone marrow involvement. The AI software identified 14 patients with pathologic uptakes and 127 with normal uptakes. The sensitivity and specificity were 70% and 97% respectively. The PPV and NPV were calculated to 78% and 95% respectively. The best cut-off value was with ROC-curve determined to 0,8 which included 70% (14 patients) true positive cases and 3% (4 patients) false positive cases. The AUC was calculated to 0,86. **Conclusion:** An automated software based on AI was trained to identify pathologic uptake patterns in bone and bone marrow. The result indicates that the test is a good basis for further testing in the development of an automated software.

Inter- and intra-observer variability in Doppler-echocardiography

By Ajla Kulovac

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisors: Anita Persson (PhD), Angela Poller (Biomedical analyst)

Background. Doppler-echocardiography is a well-established method for evaluation of cardiac function and hemodynamic parameters. The most frequently used Doppler-techniques for assessment of the cardiac function, are colour Doppler, continuous-wave Doppler (CW), pulsed-wave Doppler (PW) and tissue velocity imaging (TVI). Due to the growing popularity of method, the need for good reproducibility of the Doppler-parameters also increases. Previous studies show that a large inter- and intra-observer variability in measurements of Doppler-parameters exists and may potentially affect diagnosis and treatment of a patient. Therefore, it is of outmost importance for the variability to be as minimal as possible. **Aim.** The aim of the study was to evaluate the inter- and intra-observer variability in measurements of the most commonly used parameters obtained by Doppler-echocardiography. **Method:** 5 patients that underwent echocardiography year 2018-2019 were randomly selected. 16 experienced echocardiographic personnel at the Department of Clinical Physiology, Sahlgrens's University Hospital, performed the measurements on all patients. Each observer first measured the Doppler-parameters for every patient on one occasion, before repeating the measurements on a different occasion. In order to evaluate the inter- and intra-observer variability, coefficient of variation (CV %) was used. **Result.** Large inter- and intra-observer variability (CV >10%) was observed for many parameters, but not for all patients. Deceleration time had the largest inter-observer variability (CV 11.7-32.1%) and also a large intra-observer variability (CV 0.0-27.7%), whereas the lowest variability (CV <10%) was seen for measurements of heart rate. **Conclusion.** The reproducibility for the Doppler-parameters is interpreted as moderate to very good. The large variability observed for deceleration time indicates a highly affected reproducibility.

Development of a fusion primer system for next generation sequencing of Norovirus Genotype II.4

By Virpi Kytönen

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Virology, Sahlgrenska Academy, University of Gothenburg, 2019
Supervisor: Maria Andersson, PhD

Project aim: The aim was to develop a fusion-PCR method for sequencing of Norovirus (NoV) GII.4 with Next Generation Sequencing (NGS). This was done by studying the functionality and sensitivity of a fusion primer system that binds to the most variable regions of the NoV GII.4 genome. The method development is included in a study that examines the spread of the virus in a NoV outbreak at the hospital of Kungälv in 2013.

Introduction: NoV is a pathogen that causes the winter vomiting disease in Sweden and the genotype NoV GII.4 is the type that usually is associated with acute gastroenteritis in hospitals. In clinical diagnostics the virus is detected by qPCR and in sequencing with NGS the NoV GII.4 is amplified with a conventional PCR method. The designing of primers is constantly under development and fusionprimers can be used to incorporate adaptors to the amplified fragments that are later used to catch the fragments in the NGS. When a sequence is obtained, the genotype can be determined and information of the spreading of the virus can be concluded.

Methods: The project studied the sensitivity of the fusion-PCR method and the effect of the barcodes on the amplification process. Quantification of all the patient samples was done with qPCR. Samples with $Ct \leq 25$ was amplified with direct fusion-PCR and the remaining samples were amplified with nested PCR. Verification with gel electrophoresis was made and the amplified samples were joined into four different pooles. The pooles were purified and control of the purification was done with the TapeStation method before sequencing with NGS.

Results: The evaluation of the method sensitivity indicated that the fusion-PCR method is able to amplify NoV-samples with $Ct \leq 25$. The dilution series for nested PCR showed that NoV-samples of $Ct > 33$ are not possible to amplify. The amplification worked for all the samples amplified with direct fusion-PCR, but for the nested PCR reaction, three samples were not amplified. Two of these samples had a Ct-value over 33 and are outside of the method amplification range.

Discussion: The sensitivity was determined and the project results proved that the fusion primer system is functional for amplification of NoV GII.4. The results obtained from this project will be used in a further study of the spreading of the NoV GII.4 at the NoV-outbreak at the hospital of Kungälv in 2013.

Introduction and validation of immunohistochemical dual staining Melan-A/ Ki67.

By: Mattias Kåår Johansson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of pathology Sahlgrenska Academy, University of Gothenburg, 2019
Supervisor: Iva Johansson, Senior Consultant Department of Clinical Pathology.

Hematoxylin & eosin is the most used routine staining in histopathology today and is used as one of the first overview staining. Immunohistochemical staining is an technique where one uses antibodies that binds to specific antigens to visualize specific cells in tissue. Melan-A is an transmembranprotein that is used to illustrate the melanocytes. Ki67 is an protein that is expressed in the nucleus during the aktive part of the cell cycle and the antibody is used to stain proliferating cells. HE is used together with Ki67 and Melan-A for diagnosis of malignant melanoma, both as separate stains and as dual stains. Dual staining involves the use of two different antibodies simultaneously and enables the examination of two proteins in one and the same section. Dual staining with Ki67 / Melan-A is an effective visualization method for melanoma by clearly visualizing proliferating melanocytes.

The main purpose of the work is the introduction and validation of immunohistochemical dual staining Melan-A /Ki67. In addition, a comparison of this dual staining with the current local standard which is separate immunostaining of Melan-A and Ki67.The method was tested for validation by staining 8 paraffin-embedded skin specimen with tumors of various kinds, for each case staining consecutive sections with HE, Melan-A, Ki67 and Melan-A / Ki67. The staining was done in Dako Autostainer. The dyes were compared in the Olympus BX46 microscope at 100-400x magnification. New controls for dual staining were made up of cases of metastatic malignant melanoma as positive control and normal skin as negative control.

Dual staining Melan A / Ki67 gave a clear visualization of the proliferation activity of melanocytes in malignant melanoma specimens (Ki67 core positivity and cytoplasmic positivity in Melan-A)

The use of dual staining in routine diagnostics in the pathologist department will improve the work of the pathologists and reduce the amount of histological glass that needs staining.

Sensititre plate DKMGN an alternative method for MIC-determination of ESBL-producing bacteria

By Annelie Larsson

Bachelor's Degree in Biomedical Laboratory Science Exercised at the Laboratory for Microbiology, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisor: Dr. Erika Lindberg, senior lecturer at the University of Gothenburg

Abstract

Background: Around the world, antibiotic resistance is rapidly increasing. Extended spectrum beta-lactamases (ESBL) producing bacteria such as *Enterobacteriaecae* and *Pseudomonas aeruginosa* are one of the worst problems. Antibiotic susceptibility testing (AST) is done with disc diffusion and determination of the minimum inhibitory concentration (MIC) in order to give the patient, the right antimicrobial treatment and with the right concentrations.

Aim: The purpose of this study was to validate a broth dilution method; Sensititre with the DKMGN plate for MIC-determination of resistant *Enterobacteriaceae* and *Pseudomonas.*

Material and methods: The material used in the study are 47 bacterial strains with validated MIC-values from the reference laboratory in Växjö as well as two control strains, *Escherichia coli* (CCUG 17620) and *Pseudomonas aeruginosa* (CCUG 17619) from Culture Collection University of Gothenburg (CCUG). AST with MIC was also done on eighteen clinical isolates collected at Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden. The DKMGN plate contains seventeen different antibiotics available in different concentrations: meropenem, gentamicin, ciprofloxacin, amoxicillin / clavulanic acid, colistin, tigecycline, ceftazidime, imipenem, aztreonam, ceftolozane / tazobactam, piperacillin / tazobactam, cefotaxime, ceftazidime / avibactam, ertapenem, amikacin, tobramycin and trimethoprim / sulfamethoxazole. The method was validated with a focus on reproducibility, incubation time and correct MIC values in relation to the control values.

Results: The results showed a categorical agreement of 94% and the essential agreement of 92%. The method showed good reproducibility with little dependence on incubation time within the recommended time interval. The DKMGN plate showed good results for all analysed antibiotics and with low differences between different bacterial species. The largest differences were seen at 18 hours of incubation.

Discussion: The broth dilution method with the DKMGN plate is an alternative method to be used for AST at clinical microbiology at the Sahlgrenska University Hospital and is also a good economic alternative since the cost is lower for a larger number of MIC values than for current diagnostic methods.

Neutrophil activation is increased in pregnant patients with systemic lupus erythematosus compared to healthy controls

By Gunilla Larsson

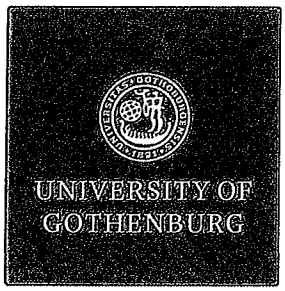
Bachelor thesis in Biomedical Laboratory Science performed at Dept. of Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, 2019.
Supervisor: Anna-Carin Lundell, PhD

Systemic lupus erythematosus (SLE) is an autoimmune chronic inflammatory disease that predominantly affects women. Women with SLE have a higher risk for pregnancy complications compared to healthy women, but the immunological mechanisms behind this remains to be identified. Many non-pregnant SLE-patients display increased neutrophil activation and they often have a subset of activated neutrophils in blood with a low density, called low-density neutrophils (LDN). LDN are not present in healthy individuals. These activated neutrophils may damage the blood vessel endothelial cells. The aim of this project was to investigate if the number of neutrophils differs during pregnancy in pregnant women with SLE compared to healthy pregnant controls, and if neutrophils are more activated in pregnant women with SLE than in healthy controls. Another aim was to analyze the presence of and the degree of activation in LDN in these two groups.

This project is part of the multicenter SLE-Placenta study, in which first-time pregnant women with SLE and healthy first-time pregnant controls are recruited and donate blood samples in the first, second and third trimester and at delivery. Whole blood was taken from the blood samples to be analyzed and the rest of the whole blood was gradient centrifuged to separate LDN from normal-density neutrophils. The number of granulocytes in whole blood, where the vast majority where neutrophils, was measured with flow cytometry. Activation of whole blood neutrophils, as well as normal- and low-density neutrophils was examined by flow cytometry. The degree of activation of the neutrophils was examined based on the cell surface expression of CD11b and CD62L. The amount of LDN was also analyzed with flow cytometry by measuring the proportion of granulocytes of all the CD45-positive low-density leukocytes.

There was no significant difference in the number of granulocytes between pregnant women with SLE and controls during pregnancy. However, the normal-density neutrophils were more activated in the SLE-patients than in the controls. LDN were activated both in SLE-patients and controls, but SLE-patients had a significantly higher amount of LDN. There was a large variation in the amount of LDN in pregnant women with SLE, ranging between 1,85% - 68,4%.

These results suggest that there is an increased activation of neutrophils in pregnant women with SLE compared to healthy pregnant women, both because the normal-density neutrophils have a higher degree of activation and because many pregnant women with SLE have a large amount of activated LDN. It remains to be examined if this increased neutrophil activation is associated with a higher risk of pregnancy complications among women with SLE compared to healthy women.



SAHLGRENSKA ACADEMY

OCCURRENCE OF ANTIBIOTIC RESISTANCE AGAINST 7 OUT OF 9 TESTED ANTIBIOTICS IN IRISH WATER ENVIRONMENTS.

By: Rebecka Larsson

Institute of Biomedicine, Sahlgrenska Academy, Gothenburg University and
Department of Biological and Health Sciences, Technological University of Dublin
Supervisor: Shane C. Dillon Ph.D. Assistant lecturer at TU Dublin

Abstract

The development of antibiotic resistant bacteria is a worldwide problem and our excessive use of antibiotics has accelerated the process. The production of novel antibiotics has almost stopped, and physicians have had to turn to outdated ideas in the treatment of their patients. Furthermore, research has shown that the transfer of antibiotic resistance genes (ARGs) occurs between environmental bacteria and human pathogens, making it more important than ever to fully understand the environmental resistome. Water and waste water treatment plants have been established as reservoirs for the transfer of ARGs between bacteria and as facilitators for the transportation of ARGs to humans through drinking water, irrigation of crops and recreational activities. The aim of this study was to investigate the occurrence of common ARGs in Irish water bodies exposed to agricultural, industrial and human waste water run-offs. The study was performed on 6 water samples collected across Ireland and the methods used to investigate the occurrence of ARGs were cultivation on antibiotic containing plates and amplification of certain ARGs using PCR. The results showed occurrence of ARGs against 7 out of the 9 tested antibiotics in the cultivation study: Ampicillin, Methicillin, Penicillin G, Nalidixic acid, Streptomycin, Trimethoprim and Vancomycin, and the ARGs *blaTEM* and *cmr*, conferring resistance to β-lactams and Chloramphenicol respectively, were detected in all samples with PCR. The study failed to further identify any of the cultivated bacteria. These results show the occurrence of ARGs in Irish water bodies and this study can act as an initial survey into the environmental resistome in Irish water bodies. Nevertheless, more research is needed to explore the full extent of the resistome.

Elucidating the impact of PI3K mutations on arachidonic acid metabolism

By Amanda Lidevi

Bachelor thesis in Biomedical Laboratory Science performed at Sahlgrenska Academy, University of Gothenburg, 2019 Supervisor: Victoria Rotter Sopasakis, phD

The protein PI3-kinase is a protein that is involved in the insulin signalling cascade and cell growth and proliferation in human cells. In the past, studies have been made to evaluate if mutations in the p110 subunit of the protein, specifically in the isoform called p110alpha, might be associated with different types of cancers. The indication for this is that a high prevalence of mutations in the gene PIK3CA, which is the gene coding for p110alpha, has been found in patients who suffers from various cancers.

Previously to this study, another study was conducted where mice were created with mutations in the PIK3CA gene in two hotspot locations; E545K and H1047R. The mice in this study quickly developed a condition of fatty liver, where the mechanism behind this effect is unknown. A whole genome sequencing was performed, where a lot of genes seemed to be upregulated that are involved in arachidonic acid metabolism.

To investigate this, we used the same mice from the previous study to further compare the expression of the genes that showed a high fold change in the mutants. RNA from the liver was extracted from five groups of the mice: two groups with mice expressing either of the two mutations and three reference groups (n=5 per group). The RNA was converted to cDNA, to be analysed with qPCR technique and comparative statistics. Contrary to our expectations, the gene expression was low in most genes, and the differences between the groups with mutations and the controls were generally small. However, three genes, called Hao2, Acot5 and Cyp2a22, were significantly increased in the mice expressing H1047R compared to mice expressing the wildtype gene. The functions of the related proteins to the genes involved are 2-hydroxyacid oxidase activity (Hao2), hydrolysis of AcylCoAs to coenzyme A and free fatty acids (Acot5) and arachidonic acid epoxygenase activity (Cyp2a22). Although the effect of H1047R on the expression of these genes adds clues to the underlying mechanism of this particular mutation, the overall conclusion of this study is that the effect of E545K and H1047R mutations of PI3K on the massive lipid accumulation in the liver is not mediated through arachidonic acid metabolism. Further studies are encouraged to unravel the mechanisms behind this phenomenon.

Div. of Hematology, Dept. of Internal Medicine
Medical University Graz, Austria

By Cassandra Lindahl
Supervised by Alexander Deutsch, PhD

Molecular etiopathogenesis of aggressive lymphomas: A gene expression analysis of chemokines, interleukins and eukaryotic initiation factors

Background: The diffuse large B-cell lymphoma (DLBCL) is an aggressive non-Hodgkin lymphoma (NHL). Its prognosis varies with the subtypes and relapses are frequent. The nuclear orphan receptor Nr4a1 has previously been found to have immunoregulatory functions, and to act as a tumour suppressor. In NHLs, a changed gene expression profile concerning interleukins (ILs) and chemokines have been observed while dysregulations of eukaryotic initiation factors (eIFs) in general are related to oncogenic development.

Aims: The first aim of this bachelor's thesis was to define the dissemination behaviour of primary testicular lymphoma by comparing its chemokine receptor (CR) profile to central nervous system lymphoma. The second aim was to identify the interleukin genes regulated by Nr4a1 by comparing expression profiles in murine *Eμ-Myc* derived aggressive lymphomas with and without *Nr4a1* loss. The last aim was to investigate which eukaryotic initiation factors were related to the prognosis and outcome of aggressive lymphomas such as primary central nervous system lymphoma (PCNSL).

Methods: Semi-quantitative Real-time PCR (RQ-PCR) was used for gene expression analysis for CRs, ILs and eIFs in patient and mouse cohorts. Statistical analyses were performed for comparing the different genotypes in the IL-related and eIF-related studies of the project. Survival analysis was performed for eIFs to relate expression to clinical outcome.

Results: The chemokine profile could not be investigated because of non-detectable RQ-PCR results. *Il7* and *Il12b* were higher expressed in lymphoma with *Nr4a1* loss in immunodeficient mouse models. For eIFs, a higher expression of *EIF1A*, *EIF2B3* and *EIF3D* and a lower expression of *EIF2A*, *EIF4EBP1* and *EIF4G3* was found in PCNSL as compared to non-neoplastic germinal B-cells. When compared to clinical data, 7 out of 16 eIFs were associated with poor cancer-specific survival when expressed at higher levels: *EIF1*, *EIF2B4*, *EIF2B5*, *EIF2S1*, *EIF3L*, *EIF4A2* and *EIF5*.

Conclusions: No results could be generated for the chemokine receptor expression profile in primary testicular lymphoma potentially because of presence of inhibitors in the tissue. The interleukin results indicate that NR4A1 is implicated in the regulation of *IL7* in aggressive lymphoma cells, and that *IL12b* might be expressed by immune-cells in the tumour microenvironment. Furthermore, the data from the eIF project suggest that eIFs might be vital in the pathogenesis of PCNSL and can hopefully be used as clinical prognostic markers for risk stratification or eventually as targets for PCNSL treatment.

Bachelor thesis in Biomedical Laboratory Science
University of Gothenburg

Spring semester 2019

No correlation for diastolic function and left ventricular filling pressure between global longitudinal strain and the pulmonary capillary wedge pressure in heart transplant recipients, 3 year follow up

By Mehrdad Mokhtari

Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg 2019
Supervisor: Entela Bollano MD, PhD, Bente Grüner Sveälv PhD

Background: Amongst heart transplant recipient diastolic dysfunction is a rather common complication that occurs for a multitude of reasons. Presence of diastolic dysfunction is linked to increased morbidity and mortality.

Right-sided heart catheterization is the gold standard for measuring the left ventricular filling pressures (LVFP) but this method is an invasive procedure that exposes the patient for risks. Therefore, a noninvasive marker that could measure LVFP would be of utmost importance for the patients as this would be much safer and simpler to monitor them.

Aim: The goal of the study is evaluate if there is an agreement for left ventricular filling pressure and diastolic function in heart transplant recipients between the methods of cardiac catheterization and speckle tracking echocardiography (STE).

Method: Thirty patients were examined by heart catheterization and 2D speckle-tracking Echocardiography (STE) three years after heart transplantation. We investigate the association between echo parameters of LVFP and invasively measured pulmonary wedge pressure (PCWP).

Results: No significant relations between global longitudinal strain from echocardiogtaphy and PCWP from heart catheterization could be observed. Coefficient of variation for intravariability was measured: 14 % for ejection fraction (EF) and 6 % for GLS 3P. Regarding the Intervariability, the coefficient of variation between the observers were: EF 11.5% and for GLS 3P 14.9 %.

Conclusion: We could not observe any agreement between the two methods right-sided heart catheterization and speckle tracking echocardiography for ventricular filling pressure and diastolic dysfunction in heart transplant recipients.

A pilot study of multiplex PCR with BD-MAX system for detection of atypical airway pathogens gave promising results for further validation

By Frida Molin

Bachelor thesis in Biomedical Laboratory Science performed at Klinisk mikrobiologi, Norra Älvsborgs Länssjukhus, Trollhättan

Supervisors: Erika Lindberg, University Associate Professor, Leg. BMA, Ph.D. and Rajaa Hassan, Leg. BMA

Community-acquired pneumonia caused by atypical pathogens is today analyzed by multiplex real-time PCR. This is an effective method for detection of the pathogens which are hard and takes time to culture for detection. Today samples for analyzing off atypical pathogens like Chamydophila pneumoniae, Chlamydophila psittaci, Legionella pneumophila and Mycoplasma pneumoniae are sent to SU for detection since NÄL does not have a method for this. The aim of this study is to see if a multiplex real-time PCR for atypical respiratory pathogens is in interest for NÄL.

Respiratory tract secretions were collected in double samples where one was sent to SU and the other to NÄLs study. The method that was tested was BD-MAX™- system (BD) and b-CAP Primers and probes (BioLegio). The results were then compared between SU and NÄL. Out of 25 samples and 9 positive controls, did 3 results differ. The sensitivity and specificity were calculated with the obtained results. The sensitivity for Chamydophila pneumoniae and Legionella pneumophila was 100% and 75% for Mycoplasma pneumoniae. The specificity for Chamydophila pneumoniae, Legionella pneumophila and Mycoplasma pneumoniae was 100% and 96% for Chlamydophila psittaci.

The samples that differ were low positive, which may be an explanation of the difference. Other possible reasons can be that the double samples wasn't similar. Despite this does the method have good presumptions to become a sensitive and specific analysis and the execution is simple and fast. This study concludes that a validation of the method will be done at NÄL later this year.

Evaluation of the existing reference range for children between 0-2 years when assessing bone marrow

By: Sofia Nadi
Bachelor thesis in Biomedical Laboratory Science performed at the Section of Bonemarrow laboratory at the Department of Clinical Chemistry, Sahlgrenska Academy, University of Gothenburg, 2018
Supervisor: Ruth Wickelgren, Sofia Grund and Maud Andersson

Introduction: At the bone marrow laboratory in clinical chemistry (the Sahlgrenska University Hospital) the existing reference intervals between children 0-2 years have been questioned as it does not seem to be consistent with morphological assessments. Information regarding age-specific cell distribution in the bone marrow in children is limited in comparison to the blood. There are not many studies that calculated cells separately but instead put together the different cell types in categories based on their poesis. This leads to the result that these values cannot be not used to create a reference interval. There are also few studies examining if there are differences in cell distribution in bone marrow between gender and ethnicities.

Aim: The aim of the study was to investigate whether the existing reference range is a accurate representation of the cell distribution for healthy children between 0-2 years. Furthermore, it is also studied if a reference interval can otherwise be obtained based on the study's material that may be used in practice. Due to known limitations of the study, the existing reference range for adults who can otherwise be used in the activity is also tested. The study also focuses on finding out if there is any difference in cell distribution between the sexes and between ethnicities.

Method: Previous results of cell distribution in bone marrow from differential counted bone marrow smears by the bone marrow laboratory of patients between age 0-2 years during a ten-year period between 2008-2018 was collected from the database. Through assessment from 552 patients only 29 patient was identified as being healthy. By plotting the values of the healthy population (n=29) with the existing reference range the percentage within the existing reference range could be examined. Same method was used to examine the existing reference interval for adults. Reference intervals from the healthy population was obtained by percentiles and the difference between gender (male =14 n, female= 15 n) was calculates using Mann-Whitney U-test.

Results: The percentage of values within the existing reference range for children was less than 95% in all cell types. The percentage of values within the existing reference range for adults was less than 95% in all cell types, except for plasmacells. Reference interval, median and interquartile was obtained for each celltype. No significant difference of cell distribution between genders was found ($p>0,05$).

Conclusion: With this study, we have found that the existing reference range for children between 0-2 years used in the Bone marrow laboratory in Clinical chemistry (Sahlgrenska University Hospital) does not correspond to the distribution of the respective cell type in the bone marrow of healthy child between 0-2 years. Neither the existing reference interval for adult nor the obtained reference range can be utilized in practice. No ascertain can be made regarding difference between in cell distribution between genders and ethnicities.

Validation of performance for diagnostic methods in different real-time PCR instruments

By: Katja Nyberg
Bachelor thesis in Biomedical Laboratory Science performed at the Virology department, Clinical Microbiology, Sahlgrenska University Hospital 2019.
Supervisor: Maria Andersson, PhD.

Background: Validation of instruments is of great importance in the process of developing a method or in replacing it to a new, better one. The primary reason for doing this is because obtaining correct results is vital. It is especially crucial with correct results when analyzing human herpesviruses, because they can manifest with vague symptoms. There is often specific therapy available, and laboratory readings must be correct so that the patient can get a correct diagnosis and eventual treatment. Real-time PCR has been described as the method of choice for analyzing human herpesviruses, thanks to its high sensitivity and specificity.
Aim: The aim of the study was to validate and find out if equivalent results could be obtained from four real-time PCR instruments, ABI 7300, QuantStudio 5, QuantStudio 6 and QuantStudio 7. Should one instrument be under maintenance or broken it would be good to know if the other instruments were reliable enough to be used as backup.
Methods: Real-time PCR with TaqMan probe was run in two different modes, standard or fast. Standard was run for all four instruments and fast for only two of them. The agents used in this study were positive cytomegalovirus and Epstein Barr virus samples with concentrations between 3-5 (log10), and negative cerebrospinal fluid samples spiked with positive bladder secretion samples (positive for herpes simplex virus type 1 and 2, and varicella zoster virus) with a cycle threshold value of around 22 which were also diluted. These agents were used in validation for standard and fast mode. Feces samples positive for different agents were also used, but only for the standard mode and were run in a multiplex PCR format.
Results: The instruments gave equivalent readings for all agents. The results were presented in log10 of the quantity, for each sample with average and the standard deviation for all instruments. The average of the standard deviations for each human herpesvirus agent run in standard mode were for cytomegalovirus 0,05, Epstein barr virus 0,08, herpes simplex virus type 1 0,06, herpes simplex virus type 2 0,03 and varicella zoster virus 0,02.
Conclusion: All four instruments have similar performance and give equivalent readings for the tested agents run in the standard mode. The same goes for fast mode and the agents that were run there. The instruments give reliable results and can be used as backup.

Potential stem cell niche in the right atrioventricular area of the human heart

By Charlotta Orback

Bachelor thesis in Biomedical Laboratory Science performed at Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, 2019

Supervisor: Kristina Vukusic, BMA, PhD

Traditionally, the heart has been looked upon as an organ that cannot regenerate or renew itself. But research, using the C^{14} method, has shown that cardiac muscle cells are renewed by about 1% per year. That indicates that stem cells exist in the heart, but their location is unknown today. Stem cells in other tissues, reside in a stem cell niche, which is a microenvironment where stem cells are protected in a passive state until activated. In a previous study on rats, researchers observed a region in the atrioventricular junction with feature of a stem cell niche. Is it possible that a similar stem cell niche exists in the human heart? One study, show that specific cardiac stem cell biomarkers and a possible stem cell niche, were located in the left atrioventricular junction in the human heart, in the same area as in the rat model. In this study, we examined the presence of stem cells and a possible stem cell niche in the right atrioventricular junction and right ventricles. Tissue from 8 organ donors were stained with an immunofluorescence method. At the right atrioventricular junction, in the boundary between right ventricle and valve, we detected cells expressing both the stem cell markers; WT1, Nkx2.5, SSEA4, MDR1 and the hypoxia marker Hif1α. We also noticed that the expression of the tested stem cell biomarkers was much higher in the right atrioventricular junction than in the right ventricles with the exception of donors who have had a cardiac arrest that showed a lower expression in the atrioventricular junction. Taken together we suggest that the atrioventricular region of the Tricuspid valves could be a potential stem cell niche and it is important to further investigate the functionality of these detected cells, in future studies, to understand if they could give rise to new heart tissue. Since cardiovascular diseases are the most common causes of death in Sweden today, the finding of a stem cell niche in the heart would be of great importance to better understand how regeneration of heart tissue occurs. It would open up to the possibility of using regenerative medicine to repair damaged heart tissue after, for example, myocardial infarction. New future therapies based on repair of damaged heart tissue, could lead to longer and better life for people suffering from heart disease.

Platelets and angiogenesis – induction of tube formation in vitro and high protein expression of anti-angiogenic factors in platelet releseate

By Areen Qunaibi

Abstract

Retinopathy of Prematurity (ROP), as a short gestation related disease, is a disease causing problem in the vision and might lead to blindness in severe cases among numerous preterm infants worldwide. In addition to low gestational age, low birthweight and exposure to hyperoxia after birth, several other risk factors for development of ROP have been reported. Low count of platelets, which was observed with high rate among infants especially preterm ones, also has been reported in association with development of ROP in preterm infants. We aimed in this study to establish and evaluate possible methods to study the angiogenic effect of platelets on endothelial cells and investigate the differences in expression of angiogenic factors of platelets in adults and cord blood, respectively. For that purpose, activation of platelet by Thrombin Receptor Activator Peptide 6 (TRAP-6) was performed. Then the activated platelets were used in tube formation assay to investigate angiogenic effect of platelets on Human Umbilical Veins Endothelial cells (HUVEC). Proteome Profiler Array was used to study protein expression in platelets' releasate prepared from adult and cord blood samples.

It has been shown that tube formation in HUVECs were induced both by releseate from activated and resting platelets. The proteome screening membrane revealed high expression of anti-angiogenic factors, and the expression was even higher in platelet releseate from cord blood compared to that from adult blood. From these experiments, it can be concluded that the protocol used for activation of platelets needs optimization for induction of specific release of α-granule content. The high expression of anti-angiogenic factors in the releseate suggests that platelets are important providers of these factors at the period when the maturing retina signals for more vessels.

Potassium concentration in packed red blood cells is affected by time of storage and irradiation

By: Natalie Roslund

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisors: Camilla Hesse, senior lecturer and Lisa Kylbring, leg. BMA

Introduction: Anemia is one of the reasons why a patient could need a transfusion of red blood cells. Erythrocytes for transfusion can be stored for up to 42 days in 2–6 °C, which affects the quality of the red blood cells and increases the level of potassium due to a failure in the sodium potassium pump at this temperature. The method of analysis used in the study was ion-selective electrodes.

Aim: The aim of the study was to observe the changes of the potassium concentration when the red blood cells were stored in different temperatures or were irradiated.

Method: One unit of erythrocytes, stored in 2–6 °C, was observed for 42 days to observe a raise in potassium concentration during a longer period. Samples of the unit was taken every other day. Another unit was irradiated, and samples were taken continuously for a total of 16 days and was later compared with a non-irradiated unit. All samples were analyzed on Cobas 6000.

Results: The concentration of potassium in the erythrocytes increased during 42 days from <1,5 mmol/L to 55,1 mmol/L. Irradiation did also affect the concentration in the packed red blood cells and after 16 days the concentration of the irradiated unit had increased to almost twice the concentration of the reference unit.

Conclusion: Potassium concentrations increases in erythrocytes while stored in 2–6°C and after irradiation. This depends on mechanisms, like the sodium potassium pump, have shown to be inactive at 2–6°C. This means that the potassium ions diffuse out of the cells and cannot be transported back, this causes a potassium raise in the extracellular space. The higher levels of potassium in irradiated units also depend on a damaged cell membrane.

Evaluating sampling techniques of carbapenem-resistant *Enterobacteriaceae* in hospital environment.

By Sara Runestam

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Microbiology, Hallands hospital, Halmstad. 2019

Supervisor: Ingegerd Sjögren, microbiologist

Introduction: In the health care a current problem is the spread of nosocomial infections, for example caused by carbapenem-resistant Extended spectrum Beta-Lactamase producing bacteria (ESBLcarba). Despite this problem there are no standardized method for environmental sampling in the health care settings. The antibiotic group carbapenems are a broad-spectrum antibiotic that are usually used as a last resort treatment of infections caused by gram negative bacteria. Carbapenem-resistant *Enterobacteriaeae* produce an enzyme called carbapenemas that break down the antibiotic group carbapenem. For this trial E-swab are evaluated, it is a nylon flocked swabs with a thin and open layer of nylon fibers with very good absorbent and eluting ability of sample material.

Aim: The aim of the study was to develop a new technique to sample carbapenem-resistant *Enterobacteriaeae* in hospital environment after treatment of a patient. In the trial the E-swab and foam swab was evaluated towards the rayon-tipped swab used in the old method. The new swabs were enrichment in Tryptone Soya broth with hope for even better exchange of found bacteria.

Method: Four bacterial strains, three *K. pneumoniae* and one *E. coli* were diluted into different concentrations and inoculations was applied on glass slides. The old sampling method with rayon swabs was evaluated against sampling with E-swab and foam swabs. When sampling, a "swab-rinse" technique was used. For this "swab-rinse" technique one moist swab was used to sample the test area, thereafter a dry swab to soak up the remaining fluids on the surface.

The E-swab and foam swab was incubated in Tryptone Soya Broth with over night before culturing. The swabs were incubated in broth containing both the antibiotic ertapenem and meropenem. After sampling the different bacterial strains in different concentrations, it was cultured on ESBLcarba agar from CHROMagar (Paris) for 12 to 24 hours.

Results: The obtained results showed a detectable dilution of the test bacteria of $10^4 - 10^6$ cfu/ml. The best exchange of bacteria was obtained with E-swab and foam swab sampling and enrichment in TSB-broth with ertapenem, the detectable level of the bacterial strains *K. pneumoniae* were 10^4 cfu/ml.

Conclusion: The best exchange of found bacteria was obtained when sampling with the foam swab and E-swab. We can conclude that the old method when using rayon-tipped swabs did not give good exchange and should not be used for environmental sampling. It was also seen that an important part of culturing the samples were enrichment in broth containing the antibiotic ertapenem.

Diagnostics of lower respiratory tract infections with Breath Explorer

By Afrah Saleh

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Virology laboratory, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisor: Rickard Nordén, PhD

Respiratory tract infections are categorized into two groups - upper and lower respiratory tract infections. The upper respiratory tract infections consist of common cold, epiglottitis, pharyngitis, laryngotracheitis and sinusitis. These infections are usually harmless, but can be dangerous for, for example, infants. Through good hand washing and avoiding infecting other individuals, these infections can be prevented.

Lower respiratory tract infections consist of pneumonia, bronchitis and bronchiolitis. These infections can be much more serious, and in some cases lead to death. Bacteria are usually the cause of the greater proportion of lower respiratory tract infections.

Influenza A virus is a common pathogen that occurs during the winter and can lead to pneumonia. This condition can be dangerous for some risk groups such as elderly people, people with chronic cancer or immune deficiency.

For some viruses, there is vaccine for the affected, while for bacteria, it is mostly antibiotics that are prescribed for patients.

A new method is called Breath Explorer (BE), and consists of a small instrument that measures exhalation particles when exhaled into the instrument. In theory, with this method, it may be possible to determine whether a patient has lower respiratory tract infection, while with the traditional methods in which samples are taken in the throat or nose, it is not possible to determine whether the infection is localized to the lower respiratory tract or only in the upper. The aim of the project was to be able to develop a new method for lower respiratory tract diagnosis.

Samples were collected on patients who were admitted to the infection clinic at Östra Sjukhuset for serious respiratory tract infection.

A nucleic acid extraction, cDNA synthesis, preamplification and RT-qPCR were done to evaluate which method which gave the optimum result. Various master mixes were tested to see which one works the best.

RNA extraction, using the Pure Link Viral Mini kit, and RT-qPCR gave the best result.

AI-based detection of lung cancer in PET/CT

By Saba Salehian

Bachelor thesis in Biomedical Laboratory Science performed at the nuclear medicine section, Sahlgrenska Academy, University of Gothenburg, 2019
Supervisor: Reza Kaboteh, Supervisor

Background: Lung cancer is one of the indications to perform FDG-PET/CT. Benign and malign tumors can, diagnostically, be separated by FDG-PET/CT. Artificial intelligence (AI) is a software that can be developed for some different purposes, such as ease the clinical work, in this case find pathological uptakes in lungs. The Aim of this study was to develop a fully automated tool that is based on artificial intelligence (AI), to find pathological uptake in FDG-PET/CT examinations in patients with known lung cancer or with suspected lung cancer.

Method: A total of 115 patients underwent FGD-PET/CT due to suspected lung cancer or for known lung cancer. The study population was divided into two groups; a training group (80 %) and a validation group (20 %). A nuclear medicine expert manually marked the pathological uptakes that were visible in FDG-PET/CT examines. Later a Convulotional Neural Network (CNN) was trained to classify each image voxel as either normal lung or lung lesion. The PET/CT images were the input for the software.

Results: 26 abnormal lesions were, totally, detected in 16 of the 17 patients of the validation group. The sensitivity was 91 % and the positive predictive value (PPV) was 85 %. One of 17 patients did not have any abnormal lesions and the AI-method correctly marked no lesions in this case.

Conclusion: The outcome values of the study showed that this could be developed into a method for diagnostics of lung cancer.

ABSTRACT

Comparative studies of colonization factors and enterotoxins expressed by Enterotoxigenic *Escherichia coli* (ETEC) isolates infecting humans and animals

By: Alba Sallova

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Microbiology and Immunology, Institute of Biomedicine, Sahlgrenska Academy, University of Gotherburg

Background: Enterotoxigenic Escherichia coli (ETEC) is the most common cause of diarrhea in children in developing countries as well as travelers to these areas. Today there is not treatment for infection caused by ETEC. The bacteria adhere to the small intestine with surface structures called colonization factors (CF). Once established ETEC produces enterotoxins which leads to the diarrheagenic symptoms. ETEC can produce two types of toxins, heat-labile (LT) and heat-stable (ST). These virulence profiles differ between strains who infect animals and strains who infect humans. Although, recent studies have shown that there are similarities between the human CF, CS30, and the porcine CF, F6. This suggests that there might be an ETEC strains that can infect both humans and animals. **Aim:** The aim of the study was to study the virulence profile on animal and human ETEC to get a greater understanding of their adhesive abilities. To understand more about the colonization factor can lead to a development of a vaccine which can prevent the bacteria from establishing in the intestine. In addition to this we wanted to investigate if there is a strain that can infect both humans and animals. **Methods:** DNA was extracted from 500 human-associated strains for whole genome sequencing. PCR was performed to determine toxins and CF on 40 human-associated strains that had earlier been tested positive for CS30, 38 animal-associated ETEC and 20 CS6 strains. In addition to this phenotypic analysis was performed on the CS6 strains. **Results:** For the CS30 positive isolates our results differed from earlier tests performed by our collaborators. Only 23 strains were tested positive for CS30. The toxin profiles and CFs were determined for the 38 ETEC, although, 18 strains remained with an unidentified CF. **Conclusion**: In conclusion, further studies need to be made to understand the relation between human and animal ETEC and their adhesive abilities in order to develop a working vaccine. Although, the findings in this project gives a guide for future analysis that are of great importance in understanding more about Enterotoxigenic *Escherichia coli*.

Characterization of lymphocyte populations in children with ulcerative colitis and Crohn's disease

By Emili Spasovska

Bachelor thesis in Biomedical Laboratory Science performed at the department of infectious diseases, institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2019
Supervisor: Hardis Rabe, Researcher.

Background. Inflammatory bowel disease (IBD) consists of two major subcategories: ulcerative colitis and Crohn's disease. In our previous study, BIT, we have found that newly diagnosed and untreated children with Crohn's disease had a low percentage of memory B cells and a high percentage of naive B cells compared to children with ulcerative colitis and the control group. Our hypothesis is that Crohns's disease is associated with deficient B cell maturation or activation. Follicular T helper cells (T_{FH}) and follicular regulatory T cells (T_{FR}) are involved in the differentiation of naive B cells into memory B cells or plasma cells in the germinal center, but how they contribute to the low proportion of memory B cells in children with Crohn's disease is still unknown.
Aim. The aim of the study was to develop a flowcytometry panel to distinguish T_{FH} and T_{FR} cells in the blood, and to develop a cell culture technique to induce cytokine production from T_{FH} and T_{FR} cells in healthy adults. Further on, we wanted to investigate how treatment affects the proportion of naive, activated, and memory T and B cells in the circulation of children with ulcerative colitis and Crohn's disease.
Method. Blood from 6 healthy adults was obtained to identify T_{FH} and T_{FR} cells, as well as for cytokine simulation. The cytokine stimulation experiments were performed in three different ways: with ready to use cell stimulation cocktail and with anti-CD3 and anti-CD28 with or without cell stimulation cocktail, before further analysis with flow cytometry. In an ongoing study including either children (n=18) with newly diagnosed and untreated ulcerative colitis and Crohn's disease or treated children, blood samples were collected for analyzing T and B cell phenotypes in the circulation. The control group consisted of patients (n=5) with suspected IBD but in which the IBD diagnosis was refuted after diagnostic work-up.
Results. With the developed flow cytometry panel, we could distinguish T_{FH} and T_{FR} cells in the circulation. Stimulation for cytokine production was optimized after 6 hours of stimulation with cell stimulation cocktail only. The results of the IBD study showed a lower percentage of naive T cells in treated patients with Crohn's disease compared to untreated patients. In contrast, the percentage of circulating effector memory T cells was higher in treated patients with Crohn's disease compared to untreated patients with Crohn's disease. Regarding the percentage of memory B cells, we found a lower percentage in untreated patients with Crohn's disease compared to the control group.
Conclusion. Stimulation of PBMC for cytokine production from T_{FH} and T_{FR} cells showed best results with only cell stimulation cocktail for 6 hours of stimulation to study the production of IFN-γ and IL-21. In order to optimize induction of IL-10, the cell stimulation technique needs to be further developed. Treatment of IBD appears to affect the B and T cell phenotype in the circulation to a more effector T cell phenotype after treatment. The results of the IBD study are preliminary as more patients will be included in the study.

A comparison between two DNA extraction kits for the implementation of *RHD* screening on cell free fetal DNA

A bachelor thesis in biomedical laboratory science performed at the department of clinical immunology and transfusion medicine, Sahlgrenska academy, University of Gothenburg, 2019.

By: Alva Strand

Supervisor: Pauline Isakson PhD.

Background: Cell free fetal DNA are short DNA fragments in pregnant women's bloodstream originating from fetal cells in the placenta. These DNA fragments have previously been utilized for genomic determination of the *RHD* blood group of the fetus. The *RHD* gene, consisting of 10 exons, determines the expression of the RhD antigen on the red blood cells. Fetal hemolytic disease can occur if an RhD negative pregnant woman forms antibodies against the RhD antigen that the fetus possesses. Anti-D prophylaxis can be administered to prevent the formation of these antibodies. The determination of the fetal blood group therefore serves the purpose of determining which pregnant individuals are at risk of immunization and therefore should receive anti-D prophylaxis.

Aim: The aim of the study was to determine which of the two DNA extraction kits *QIAsymphony DSP Virus/Pathogen Midi Kit* and *QIAamp Circulating Nucleic Acid Kit* were best suited to use in a routine *RHD* screening setting.

Methods: DNA was automatically extracted from 24 plasma sample duplicates, stored at -80°C from 0 to 501 days, with the *QIAsymphony DSP Virus/Pathogen Midi Kit* and *QIAamp Circulating Nucleic Acid Kit.* The *RHD* gene was detected in the isolated DNA using real-time polymerase chain reaction to amplify exon 4 of the *RHD* gene.

Results: The *QIAsymphony DSP Virus/Pathogen Midi kit* extracted more DNA from the plasma than the *QIAamp Circulating Nucleic Acid kit.* Out of 24 samples in total, 21 samples yielded results which were deemed as reliable, by the used internal control, after extraction with the *QIAsymphony DSP Virus/Pathogen Midi Kit* whereas 13 reliable results were obtained with the *QIAamp Circulating Nucleic Acid Kit.*

Conclusion: The *QIAsymphony DSP Virus/Pathogen Midi kit* was deemed more appropriate to use for purposes of screening the *RHD* gene of fetuses. The conclusion was reached mainly because the *QIAsymphony DSP Virus/Pathogen Midi kit* was shown to determine the *RHD* status of the samples whether they were stored in -80°C for a short or for an extended period of time. This is likely due to *QIAsymphony DSP Virus/Pathogen Midi kit* extracting a higher concentration of DNA as compared to the *QIAamp Circulating Nucleic Acid kit.*

Platelets obtained by apheresis technique differ from pooled platelets from whole blood donations regarding several quality markers

By Maja Torstensson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Transfusion Medicine, Sahlgrenska University Hospital, 2019
Supervisor: Camilla Hesse, Senior lecturer

Platelets play a vital role in hemostasis, meaning the blood's ability to coagulate and prevent thrombosis. Platelet transfusions can be given to patients who suffer from thrombocytopenia to prevent or treat bleeding. They can also be given to patients with platelet defects. Platelet concentrates can be stored for up to seven days in room temperature but during storage morphological and biochemical changes occur which cause a decline in quality, what is typically referred to as platelet storage lesions. The quality of the platelet concentrates can be assessed with a number of in vitro analyzes. The aim of this study was to compare platelet concentrates created using an apheresis procedure with pooled platelet concentrates derived from whole blood by measuring several markers on day 1, 3 and 7 of storage time. The parameters being measured were platelet concentration, pH and metabolism (glucose and lactate). The platelets ability to aggregate upon stimulus with the agonists ADP, ASPI and TRAP were also measured. Finally, the three markers CD62p, CD63 and phosphatidylserine for platelet activation were studied. The main findings of the study were that the apheresis concentrates had a higher platelet concentration, a lower degree of activation and a better ability to aggregate. Because of some limitations of the study, more research is needed on the subject.

Difference between vendors measuring global longitudinal strain in patients receiving Herceptin treatment

By Eric Täll

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2019

Supervisors: Anita Persson, PhD and Angela Poller

Background. Patients with breast cancer have a 15-30% chance of developing a more aggressive disease caused by overexpression of a growth hormone gene. Treatment with chemotherapy may include the drug Herceptin which has cardiotoxic effects. Speckle tracking echocardiography (STE) evaluates the global longitudinal strain (GLS) as a measure of the systolic function of the left chamber and is recommended to assess regularly in patients receiving Herceptin. However, studies have shown that GLS values differs between vendors which may have clinical implications.

Aims. The aim of this study was to determine if there are differences between the two vendors (EchoPAC and TOMTEC) used for measuring GLS at Sahlgren's University Hospital in patients receiving Herceptin treatment.

Method. Patients with optimal image quality allowing GLS analysis by speckle tracking were included from the Image and Function Register. After exclusion of 14 patients due to sub-optimal image quality, GLS analysis could be performed twice on 30 patients. The average GLS and GLS from each of the individual apical views were compared between the two vendors.

Results. Mean GLS absolute values (± 1SD) for EchoPAC were 20.6% (± 2.9%) for average GLS, 20.3% (± 3.0%) in the four-chamber view, 20.6% (± 3.1%) in the three-chamber view and 20.9% (± 3.4%) in the two-chamber view. Mean GLS absolute values (± 1SD) for TOMTEC were 21.2% (± 2.9%) for average GLS, 21.8% (± 3.2%) in the four-chamber view, 21.7% (± 3.3%) in the three-chamber view and 21.6% (± 3.1%) in the two-chamber view. Comparison showed a statistically significant bias for the average GLS (-0.6), in the four-chamber view (-1.5) and in the three-chamber view (-1.2). The difference in the two-chamber view was not proven to be significant at the $P<0.05$ level. ($P=0.65$)

Conclusion. This study shows a significant difference between EchoPAC and TOMTEC. Since this difference might lead to clinical implications the same vendor should be used for follow up examinations.

REFERENCE VALUES OF MEAN OVERNIGHT SATURATION

Using the European Sleep Apnoea Database

By Louise Valfridsson

Bachelor thesis in Biomedical Laboratory Science performed at the Centre for Sleep and Vigilance Disorders, Sahlgrenska Academy, University of Gothenburg, 2019.

Supervisors: Ludger Grote MD, PhD, Ding Zou MD, PhD

Background. More than 20 000 sleep diagnostic tests are performed in Sweden annually. However, reference intervals for mean overnight oxygen saturation during sleep for different age and gender categories are lacking. We aim to establish reference intervals for overnight mean saturation in a large population of individuals investigated during sleep. **Method**. Oxygen saturation data was analysed in individuals (n=3,225 adults) from the European Sleep Apnoea Database (ESADA) which collects data from 29 sleep centres in 18 European countries. Patients with comorbid sleep apnoea (Apnoea-Hypopnea Index ≥5 events/hour), pulmonary disease or heart failure were excluded from the analysis. Reference intervals for mean overnight oxygen saturation were established and stratified for gender, Body Mass Index (BMI) and for age classes. A Generalised Linear Model (GLM) was applied to determine the independent contribution of these factors on mean saturation. **Results**. Mean overnight saturation was 95.0% for the entire cohort (95% confidence interval (CI) 94.9 to 95.1%), 94.8% for males (n=1798) (CI 94.7-94.9) and 95.3 for females (n=1427) (CI 95.2-95.3), $p<0.001$). Age had a significant influence on mean nocturnal saturation (95.8%, 95.4%, 95.1%, 94.6%, 94.2% and 93.9% for those aged 18-29, 30-39, 40-49, 50-59, 60-69, and ≥70 years, $p<0.0001$). Each increase of BMI unit reduced the saturation mean by 0.1% (CI 0.09-0.11), $p<0.001$. Current smoking was associated with a reduction of mean saturation by 0.6% (CI 0.4 - 0.7%), $p<0.001$. **Conclusion**. Our study of a large European population defined reference intervals for mean overnight saturation stratified for important confounders like age, gender and BMI. Our data may be applied in daily sleep medicine practice.

Key words: Oxygen saturation – sleep – age – Body Mass Index - gender

Prevalence of heart failure with preserved ejection fraction and clinical correlation in patients with rheumatoid arthritis

By Evelina Wadheden

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2019.

Supervisior: Sara Svedlund (MD, PhD)

Background: Heart failure with preserved ejection fraction (HFpEF) is a globally increasing health issue with a high prevalence around the globe. According to today's research, there are no available treatments for this condition. The underlying mechanisms of HFpEF are still unknown, but recent studies have shown that comorbidities can cause microvascular dysfunction in the coronary endothelium. However, there's a lack of knowledge regarding a possible correlation between microvascular dysfunction and different patient groups, such as rheumatoid arthritis (RA).

Aim: To investigate whether elderly patients with RA have an increased incidence of coronary microvascular dysfunction and HFpEF.

Method: A cross-sectional study was performed on RA patients with and without HFpEF. Parameters were measured with an echocardiographic examination, carotid intima-media thickness and laboratory tests. Values were then compared between the two cohorts using a chi-square-test and independent t-test.

Results: 13 RA patients with HFpEF (32%) and 28 RA patients without HFpEF participated in this study. In both cohorts, the majority of the participants were women, 85% and 82% respectively. The mean age of the patients in the HFpEF cohort were 72.9 years, while the mean age of the non-HFpEF cohort were 71.5 years. Furthermore, the HFpEF cohort had a significantly higher prevalence of hypertension ($p=0.001$), elevated NT-proBNP levels ($p=0.001$), decreased mitral valve deceleration time ($p=0.029$), increased E/e' ($p=0.019$), lower mean and maximal LAD coronary flow velocities ($p=0.004$ and $p=0.005$) than the non-HFpEF cohort. However, no differences in carotid intima-media thickness were observed.

Conclusion: There is a high prevalence of HFpEF in patients with RA and they are very likely underdiagnosed. Decreased coronary flow velocities in HFpEF cohort suggest a more microvascular origin rather than a macrovascular one which may play an important role in the development of HFpEF in patients with RA.

Importance of dietary fibre for the preservation of mucosal crypts in a model of radiation therapy to the pelvic area

By Malin Warholm
Bachelor thesis in Biomedical Laboratory Science performed at the Department of Oncology
Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University
Supervisior: Cecilia Bull (PhD)

Background: One of the most common treatments used in pelvic area cancer is radiotherapy. The radiation usually eliminates the cancer effectively but also damages the tissue of the intestine. This injury affects patients all over the world and gives them a lower quality of life with problems such as excessive gas discharge, excessive mucus discharge, blood discharge, fecal leakage syndrome and fecal-urgency syndrome.
Patients are often advised to eat a low fiber diet during treatment to avoid flatulence and bowel dysfunctions despite a lack of scientific basis. However, there are a few studies that indicate that a high fiber intake can have beneficial effects on the health of the intestine.
Aim: The purpose of this study was to investigate whether dietary fiber in the diet protects the gut against radiation-induced loss of crypt.
Method: Eight groups of 10 mice in each, assigned 4 different diets were started 2 weeks before the radiation to 1-18 weeks after radiation. The diets consisted of a group of 0% fiber, a group of 15% fermentable oat bran, a group of 5% oat bran and 10% non-fermentable microcrystalline cellulose, and a group which received the usual diet for laboratory mice, consisting of approximately 15% of an unknown mixture of fiber. Each diet group had a control group that was anesthetized under the linear accelerators without being irradiated. Mice were irradiated with 4 MV nominal photon energy, with a linear accelerator twice daily, the fractions were given at a dose rate of 3.2 Gy / min, with 12 hours inbetween. After week 1 and week 18, the animals were sacrificed and histologically prepared for microscopic examination.
Result: Quantification of the crypts showed that one week after radiation, the number of remaining healthy crypts was similar between the dietary groups. Nevertheless, intake of dietary fibre resulted in a larger increase of crypt numbers over time, when compared to fiber-free diet. The composition of fermentable versus non-fermentable fiber did not appear to have a major impact on the outcome, but normal chow resulted in fewer crypts regardless of whether the animals were irradiated or not. In addition, a fiber-free diet resulted in less crypts over time in sham-irradiated animals.
Conclusion: Our findings suggest that the consumption of fiber does not play a role in the survival of crypts directly after irradiation, but in the long run, dietary fiber consumption appears to be protective. In addition, the amount of fiber increases the number of crypts considerably in the healthy intestine after a long period of consumption. Furthermore, we suggest that the dietary fiber source in the standard chow given to laboratory mice may not be optimal with regards to their intestinal health.

A retrospective analysis of the Method used in Vein Sonography performed at Sahlgrenska Academy 2004-2018 including Descriptive Accounting of various outcomes

RETROSPEKTIV METODANALYS AV VENSONOGRAFIER UTFÖRDA PÅ SAHLGRENSKA UNIVERSITETSSJUKHUSET 2004-2018 INNEFATTANDE EN DESKRIPTIV REDOVISNING AV UTVALDA FYND

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Physiology, Sahlgrenska Academy, University of Gothenburg 2019.
Supervisor: Anders Thurin. By Matina Wodstrup.

Introduction: At the department of physiology in Sahlgrenska Academy vein sonographies are achieved by mostly biomedical analyst. Between the years 2004 and 2018, about 15 499 investigations of the lower extremities were accomplished at this section. Data from this time interval was gathered in a data system called ÅderDok that has been received, including information about insufficiencies, diameters and patient-data.

Aim: The aim with this study is to evaluate the used method in these patient-investigations but also analyse some data from the results documented in received data with descriptive statistics considering gender, age and amount of examinations.

Method: Data that has been used is retrieved as an Excel-file with information from the data system ÅderDok. The statistical program practised is Statistical Package for the Social Sciences. First all data was elucidated thereafter some comparison between the patients' gender, examinations per year and the patients age were made.

Results: Data could not be properly organized since further information about the abbreviations was not released from the department. Beyond this mishap the results from earlier research corresponds with the results concluded in this study scilicet women are more often affected due venous diseases and age is a risk factor for ailment in the veins.

Conclusion: The most important missing part is uniformity. More structure is needed when it comes to documenting the results from the forthcoming patients' examinations. Otherwise the group of exanimated patients quadrate with earlier results in similar analyses.

Optimization of Storage of Peripheral Whole Blood for use in the Cell Division Assay

By Carolina Wäppling

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, 2019

Supervisor: Pegah Johansson, PhD

Introduction: The cell division assay is a new way of calculating the optimal dosage of cancer treatment in individuals. It uses flow cytometry and 5-ethynyl-2'-deoxyuridine (EdU) to analyse cell proliferation in the presence of cancer treatments such as radiotherapy and cytostatics. In order for the assay to be fully optimized for routine clinical use it must be evaluated whether the samples can be stored in a way that doesn't influence results, namely by cryopreservation.

Aim: The aim of this study was to find the method of cryopreservation that is best suited for the CD-assay samples, and that can be carried out by the sampling laboratories.

Methods: All samples used are excess routine samples diagnosed as healthy by the haematology department at Sahlgrenska university hospital. These samples were then either separated into PBMCs using lymphoprep or cryopreserved as whole blood. Cryopreservation of whole blood was done using either a 1:1 ratio cocktail 1 (10%DMSO, 30% RPMI, 60%FBS) or cocktail 2 (non-diluted DMSO) at a final concentration of 5%. After thawing of the whole blood, PBMCs were separated using lymphoprep. Both the fresh samples and the cryopreserved samples were then incubated for 72h in different mitomycin C concentrations, after which EdU was added. After 16h the samples were stained according to a click-it protocol, and analysed using flow cytometry. The number of EdU positive cells were then used to calculate cell sensitivity by comparing the non-treated samples to the treated ones.

Results: It was found that both cryopreserved PBMCs and PBMCs separated from cryopreserved whole blood can be used in the CD-assay. Using non-diluted DMSO with a final concentration of 5% leads to more cells surviving cryopreservation. Blood could be cryopreserved in vacuette blood tubes as well as cryotubes, but cryopreservation in vacuette blood tubes led to more cell loss. There was no significant difference between whole blood that had been cryopreserves for 9 days versus 16 days, in either cell sensitivity results or cell number.

Conclusions: Cryopreserving samples for use in the CD-assay is possible, and it is possible to do it in a way that can be done in sampling laboratories without access to specialised materials. Thus, the aim of the study has been achieved.

Detection of PVK-associated superficial vein thrombosis with sonographic examination in conjunction with myocardial scintigraphy

By Sara Zaben

Bachelor thesis in biomedical Laboratory Science performed at Clinical Physiology Östra, Sahlgrenska Academy, University of Gothenburg, 2019
Supervisor: Anders Thurin

Abstract

Background: Peripheral venous catheters (PVC) are widely used in medical care for infusion of drugs, fluid, nutrition and blood transfusion. In connection with PVC, complications can arise. A common complication is thrombophlebitis, which involves thrombosis in a superficial vein, usually combined with inflammation. Symptoms that may occur with thrombophlebitis are edema, pain and erythema. Anti-inflammatory drugs and anticoagulants are an alternative to more severe symptoms.

Aim: The aim of this study is to investigate the presence of superficial blood clots (thrombosis) in patients who have peripheral venous catheters in the arm for one day for a myocardial scintigraphy examination, with repeated ultrasound examinations.

Methods: The study included a total of 11 patients undergoing a 2-day myocardial scintigraphy. The patient was examined at three different times using ultrasonography. The transducer was applied in cross-section and the PVC was visualized with repeated compressions of the vein. Short clips were saved, and compressed vein diameters were noted at each examination.

Results: The result was presented for a total of 11 patients between 58 and 82 years. Already on the first examination day, day 1, the vein diameter for 9 patients was calculated over 1 mm. After 24 hours, all patients showed a vein diameter over 1 mm. At the end after removal of PVC, 8 patients had a vein diameter below 1 mm. The remaining 3 patients had residual vein diameter over 1 mm (1.1 - 2.7 mm).

Conclusion: Most patients had signs of superficial vein thrombosis as early as the first examination with PVC treatment. All patients developed superficial vein thrombosis after 24 hours, which was resolved in 8 of 11 patients after removal of PVC.

The Mutagenic Effect of Bisphenol-A on Mitochondrial DNA; A molecular based study on Mus Musculus C2C12 Cells.

By Pauline Zoughbi

Bachelor thesis in biomedical laboratory science performed at the section of Molecular Biology, Sahlgrenska academy, University of Gothenburg, 2019

Background: Bisphenol-A (BPA) is a compound used in the production of plastics and many other different products including food cans and water bottles. It is a chemical to be considered. It's widespread, it's close proximity to food and beverages, makes us humans more susceptible to contact it on everyday basis. BPA has become a public health concern since it is one of the world's highest produced and consumed chemical. Many adverse health effects were reported, yet on molecular basis a little is known, the link between BPA and mutagenicity therefore must be investigated. And that brings us to the aim of this project, which is to seek and further investigate the mutagenic effect of BPA on mitochondrial DNA (mtDNA) using Mus musculus C2C12 cells.

Methods and Materials: The experiment started with cell culturing where the cells were grown under controlled conditions and treated with different doses of reagents in a period of eight days per each dose. This was then followed by DNA extraction and a long template PCR of mtDNA was performed followed by gel electrophoresis to confirm the amplification process. Subsequently the PCR products were purified and sequenced with different primers and finally analyzed with Basic Local Alignment Search Tool (BLAST).

Results: The results obtained are referred to cells treated with 12.5 μg/ml only. the results of the sequenced fragments of the mtDNA were compared to the reference wild type of Mus musculus mtDNA, the base pairs were identical meaning there was no mutations observed. Except for the C6247T mutation that altered the cytosine base to a thymine base at position 6247. However, this mutation was observed on the controls as well.

Conclusion: Due to the limitation encountered this study could not give a definite answer regarding the link between BPA and its mutagenic effect on mtDNA. Stating the need for further investigations regarding the impact of BPA on mtDNA, this could be done by determining the effect of the higher concentrations of since this will give a better indication regarding BPA as a mutagen.