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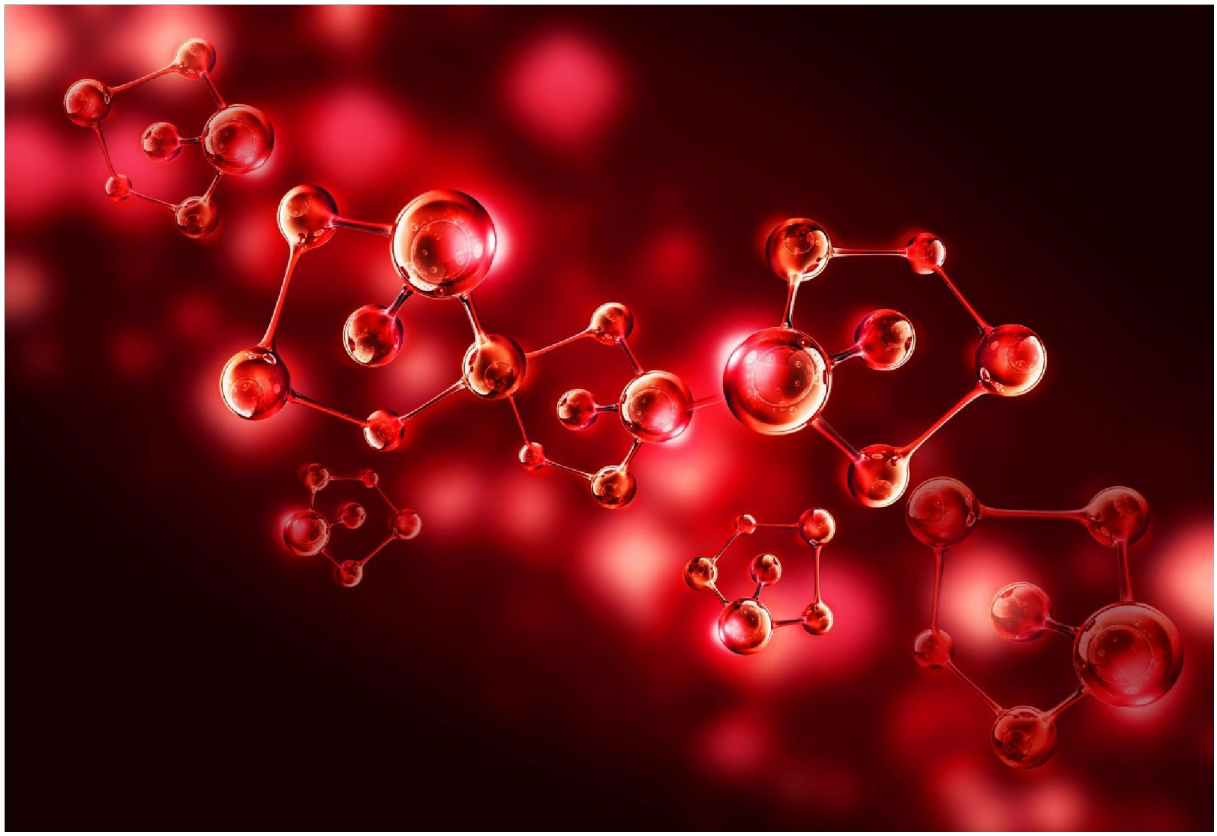

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Comparison of G-banding with Giemsa and Leishman staining methods prior to cytogenetic analysis of hematological malignancies

By: Zahra Ahmadi

Bachelor thesis in Biomedical Laboratory Science performed at the Cytogenetic laboratory at Sahlgrenska academy, University of Gothenburg, 2023

Supervisor: Helene Sjögren, Cytogeneticist, Med.dr

Background: Hematological malignancies are caused by abnormal development of blood-forming stem cells. The defect may occur in the lymphatic or myeloid stem cells and causes different types of leukemia. Both numerical and structural chromosomal abnormalities occur in hematological malignancies. Chromosome analysis, a type of cytogenetic analysis, of bone marrow cells or blood is performed at the time of diagnoses. Correct preparation and staining of slides are essential, affecting chromosome quality and band resolution. Unique and distinguishable band patterns of chromosomes are obtained by staining, making possible to identify and differentiate the chromosomes. The Giemsa staining method is the most commonly used G-banding technique but other staining methods as Leishman can also be used.
The aim of this study is to compare G-banding using the Leishman staining method with the Giemsa staining method and to evaluate if Leishman staining can replace Giemsa staining for the analysis of hematological malignancies.

Method: Cell suspensions from nine bone marrow samples, with different hematological malignancies, were dropped on slides after which G-banding was performed using Giemsa staining method as well as the Leishman staining method. The cells, which were in metaphase, were studied. The methods were compared regarding the total number of analyzable metaphases and their banding quality.

Result: There is a significant difference (p=0,008) regarding the analyzable metaphases between Leishman and Giemsa staining methods. There are significantly more analyzable metaphases with the Leishman staining method. The median was 22% metaphases using Leishman compared with 13% with Giemsa. Sign test did not show any significant difference between the banding quality of the metaphases. However, with the Leishman staining method, better chromosome spreading of the metaphases was seen visually with more distinct band patterns and contrast between light and dark bands on the chromosomes.

Conclusion: This study has shown that the Leishman staining method can replace the Giemsa staining method for the G-banding, in order to perform a cytogenetic analysis of hematological malignancies. Leishman staining method resulted in significantly more analyzable metaphases as well as more distinct contrast of the bands.

EVALUATION OF THE ANTIVIRAL EFFECT OF BIOCIDAL DISINFECTANT WIPES UTILIZING BACTERIOPHAGES AS A MODEL SYSTEM

By: Dina Al Robai

Bachelor thesis in Biomedical Laboratory Science performed at the RISE Research Institutes of Sweden AB, 2023

Supervisor: Lucia Gonzales Strömberg, PhD

The last Covid-pandemic has lifted the need to study the effect of disinfectants in the inactivation of viruses. In contrast to eukaryotic virus, like SARS-CoV-2 which is a BSL-3 organism, bacteriophages are used as surrogates and are used as model systems for inactivation studies given its similar structure and genetic material composition. Biocides are in general being studied for inactivation of bacteria but the effect on different types of virus is not well known. The aim of this study is to measure antiviral activity of biocides using enveloped bacteriophage Phi6 and non-enveloped bacteriophage MS2 as model systems. Bacteriophages were propagated according to ISO 16604:2004 using double layer agar plates with the top agar containing the host bacteria. The virucidal activity was initially tested according to ISO 18184:2019, which is a standard to determine the virucide activity of textiles. Two different wipes, a baby wipe containing cocamidopropyl, PG-dimonium chloride phosphate), as well as Disinfect wipes, containing didecyldimethylammonium chloride, alkyl (C12-16) dimethylbenzylammonium chloride, alkyl (C12-16) dimethyl(ethylbenzyl) ammonium chloride, were tested as well as the biocides benzalkonium chloride (BAC16) 16 0.1% , Polyoxyethylene (10) tridecyl ether (Brij10) 0.1% and 2,2-Dibromo-3-nitrilopropionami (Dcoit) 0.1%. An optimized protocol for propagation of bacteriophage Phi6 was developed where the temperature for the topagar was modified from the initially indicated of 45°C to 40°C given that the host bacteria showed to die at this temperature since it propagates at 25°C. The volume of bacteria per ml in the top agar was also modified from 5 µl/ml to 2 µl/ml, showing a better display of the plaques. It was not possible to determine the virucidal activity following the ISO 18184:2019 since the time for incubation did not show results. A follow up with 1 minute exposure time gave results showing effect to Phi6 but not for MS2. The results show that enveloped virus, Phi6, is inactivated by Brij10 0.1%, BAC16 0.1%, Baby wipes and Disinfect Wipes at the 1 min incubation and also at a low concentration. However, it is not inactivated by Dcoit 0.1%. Results for the non-enveloped virus, MS2, showed no change in virus reduction, indicating that the biocides had no effect on this non-enveloped virus. Experiments showed that ISO 18184:2019, which describes the antiviral activity of textiles, is not suitable for wipes, and ISO 16604:2004 is not suitable when propagating MS2 regarding the temperature of top agar when the host bacteria is killed. The conclusion is that the optimal temperature for topagar together with *P. aeruginosa* was 40°C. The optimal bacterial concentration when propagating Phi6 is 2 µl/ml. The enveloped virus Phi6 is inactivated by the following biocides at short exposure times and low concentrations, except for non-enveloped MS2, which is not inactivated. ISO 18184:2019 is not optimal when studying wet wipes since an incubation of 0 and 3 hours are not representative reference points.

Study of THP-1 monocytes differentiation and polarization induced by cancer cells adapted to breast cancer patient- derived scaffolds.

By Ella Alhällen
Bachelor thesis in Biomedical Laboratory Science performed at Göran Landberg group,
Sahlgrenska center for cancer research, University of Gothenburg, 2023.
Supervisor: Elena Garre, PhD

Background: A important factor in tumor progression and development of breast cancer is the tumor microenvironment (TME). There are different types of components included in TME, for example cancer cells, extracellular matrix and fibroblasts. Through different signaling molecules and other interactions the different cells in TME can interact with each other. In our innate immune system, we have monocytes that can differentiate into macrophages. Macrophages are the most abundant immune cell in tumors and have high plasticity which means that they can polarize into different types in response to signals from TME. To simplify, macrophages can be divided into classically activated M1 macrophages and alternatively activated M2 macrophages. M1 macrophages release pro- inflammatory cytokines meanwhile M2 macrophages anti- inflammatory cytokines. The cytokines M2 macrophages release promotes tumor progression and are therefore often associated with bad cancer prognosis. A good model to further understand how the TME affects THP-1 monocytes is patient- derived scaffolds (PDS). PDS is tumor material that is decellularized and therefore gives a good representation of a tumor since the PDS have an intact extracellular matrix and associated proteins. Since the PDS is decellularized it is also a useful model to repopulate cancer cell lines on.

Aim: The aim with this project is to study how the aggressive features and secreting factors cancer cells gain from growing in PDS affects THP-1 monocytes, but also to study THP-1 monocytes ability to infiltrate PDS repopulated with cancer cells.

Method: Two PDSs from two different breast cancers were repopulated with two different cancer cell lines., MDA-MB-231 and MCF7 for 21 days. The condition media from the PDSs cultivated with cancer cells were then added to THP-1 monocytes. THP-1 monocytes were also added directly to cancer growing in PDS. Infiltration was analysed with Immunohistochemistry with antibody marker CD86 and Mayer's hematoxylin staining. To study THP-1 monocytes differentiation and polarization RT-qPCR was used to analyse changes in gene expression of M1/M2 markers.

Results: When the samples gene expression were analysed, an induction of M2 markers could be seen for the samples that had been cultivated with condition media from cancer cell line MDA-MB-231 growing in PDS. This indicates that cancer cells growing in PDS affects THP-1 monocytes. For the immunohistochemistry staining the negative control became positive and therefore the results from the other staining's cannot be used since they are not reliable.

Conclusion: In conclusion, PDSs repopulated with cancer cell lines do affect THP-1 monocytes differentiation into M2 macrophages. Since it is known that M2 macrophages releases anti- inflammatory cytokines that promote tumor progression, it could possibly be a good target for cancer therapy.

The role of the alpha 7 nicotinic acetylcholine receptor (α7nAChR) in myocardial infarction

By Zeinab Mohamed Ali

Bachelor thesis in Biomedical Laboratory Science performed at the Circulation physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, 2023.

Supervisor: Maria Johansson

ABSTRACT

Cardiovascular disease is a global health challenge and the leading cause of death. Atherosclerosis, an inflammatory disease of the arteries, is a major cause of cardiovascular disease. A heart attack occurs when a lack of oxygen in the heart muscle is caused by blockages in the coronary arteries. Inflammation plays an important role in heart attacks by activating immune cells and increasing damage to the heart muscle. The heart tries to repair itself by forming connective tissue (fibrosis) in the damaged area. The alpha 7 nicotinic acetylcholine receptor (α7nAChR) is a receptor involved in the inflammatory reflex, which reduces inflammation in the body and requires α7nAChR to function. Activation of α7nAChR can reduce atherosclerosis by regulating immune responses. However, the effects of α7nAChR in myocardial infarction are not yet fully understood.

The study was conducted to investigate the role of α7nAChR in myocardial infarction in C57BL6 mice by using an agonist (PHA568487) and an antagonist (MLA) to activate or block the receptor. Sham group had their thorax opened without ligating the LAD (left anteior descending artery), control group was treated with saline, PHA group was treated with the α7nAChR agonist PHA568487 and MLA group was treated with the α7nAChR antagonist MLA. Control group, PHA groups and the MLA mice were treated before the myocardial infarction was induced and sacrificed one week later for histological analysis of the heart tissue. The results from Masson Goldner staining showed that there was no significant difference in the size of the myocardial infarction between the groups. In contrast, the mice treated with the α7nAChR antagonist MLA showed a significant increase in collagen in the area around the infarction compared to muscle fibers. This suggests that α7nAChR may have a histological impact in myocardial infarction, but further research is needed to fully understand its role in the course of the disease. The conclusion of this work is that inhibition of alpha 7nAChR increases the accumulation of connective tissue in the heart.

Glukoskoncentrationen skiljer sig mellan olika typer av trombocytkoncentrat och beror på vilken metod som används

By Hanan Al Masri

Bachelor thesis in Biomedical Laboratory Science performed at the component laboratory, Sahlgrenska University Hospital, 2023

Supervisor: Camilla Hesse, senior lecturer

Background: Platelets play an important role in the coagulation system, especially in hemostasis. Transfusion medicine has relied on platelet concentrates (PCs) to treat thrombocytopenia especially in patients at risk of severe bleeding and cancer. Research to develop the environment and methods for storing concentrated platelets has been made to ensure the effectiveness of platelets during and after the end of the storage period. According to European guidelines it is necessary to take precise measures to monitor quality and control metabolic parameters during the storage period. This study explores methods for measuring glucose - HemoCue, Alinity, and the ABL Blood Gas instrument - in three different types of platelet concentrates (pooled, apheresis and pathogen reduced).

Objective: This study aims to evaluate and compare the efficiency and reliability of three different methods for measuring glucose levels in three types of platelets concentrates at the end of the storage period and to evaluate measuring glucose as a quality marker compared to traditional markers such as pH.

Material and methods: In total 45 units were included in the study (25 pooled platelet units, 10 apheresis and 10 pathogens reduced). All units were analyzed on storage day 8 and for glucose measurements the HemoCue, Alinity and ABL Blood gas instrument were used. Analysis of pH and lactate was performed on the ABL instrument.

Results: The results indicate that there are significant differences in the concentration of glucose between the three devices for the different types of platelet concentrates. An increase in the glucose concentration was observed with the HemoCue device compared to the Alinity and ABL devices at the end of the storage period for all types of platelet concentrates included in this study. The metabolic parameters pH and lactate as analyzed with the ABL device were maintained, the glucose level was highest in apheresis units at the end of the storage period and lowest in pathogen reduced units. The practical handling differs between the methods used in the study. It is easiest to analyze the glucose concentration using the HemoCue, while it was perceived as the most complicated to fill the blood gas syringe correctly.

Conclusion: In this study, three different methods were used to determine glucose concentration and based on the results obtained, the practical handling and economic aspects, it is recommended to use glucose determination in FC-mix tubes on the instrument Alinity. The study shows that pH, which has traditionally been used as a quality marker at the end of the storage period is at an even level in all different types of platelet concentrates, which is probably since today's additive solutions are of a very high quality. However, the glucose concentration at the end of the storage time differs in the three types of platelet concentrates included in this study. In a future study, it would be interesting to study the reasons for this further.

Storage of whole blood on cooling plates after collection gives similar results on quality controls of blood components as storage of whole blood at room temperature.

By Ghosn Alban Alnsierat

Bachelor's thesis in Biomedical Laboratory Science performed at the component laboratory, Sahlgrenska University, 2023
Supervisor: Camilla Hesse, university lecturer/special BMA

BACKGROUND: Proper storage of blood bags prior to transfusion is important to procure and supply patients with high-quality blood components that are more effective for treatment. This means that a large part of routine work in transfusion medicine today is based on ensuring the quality of blood components by performing regular quality checks.

AIM: The purpose of the current study was to investigate whether various routine quality controls were affected if the whole blood was stored on a cooling plate or not after blood donation.

METHOD: To see if the quality of blood components is affected by whole blood being kept refrigerated or not, quality controls were performed on 79 units (24 whole blood, 24 erythrocytes, 24 plasma and 6 platelets) on days 1, 5 and 14 after blood donation. Whole blood was separated into blood components using Reveos. Advia was used for cell counting and measurement of haemoglobin and erythrocyte index. Hemolysis on erythrocytes was measured with the plasma low Hb method on HomeQue. Measurement of CD62 and leukocount was done on Lyric flow cytometer. Measurements of pH, glucose, lactate, potassium, and sodium were performed on the blood gas instrument ABL 800. Smears on erythrocytes and whole blood were performed on day 1 and day 14.

RESULTS: There was no significant difference between the values from chilled and room-temperature whole blood, nor on the values from the blood components in most of the results. A difference that could be seen in the pH values of platelets from day 1 and in the number of platelets in plasma from day 1. Blood smears from both groups were similar.

CONCLUSION: In conclusion, cooling does not have a great effect on the quality of whole blood or on the blood components in terms of the measured parameters, but some further measurements could have been made to obtain a more comprehensive result. Not using cooling plates can be an advantage as they can be expensive and take up space.

APOB/APOA-I RATIO, A POSSIBLE METHOD FOR DIAGNOSING PERIPHERAL ARTERY DISEASE

By Nina Alves-Martins

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of medicine, Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor: Caroline Schmidt, Docent

Introduction: Analysis of the apoB/apoA-I ratio is a useful method that gives an overall view of the total cholesterol concentration and is a strong indicator of the risk of developing cardiovascular disease. Few studies have studied the relationship between apoB/apoA-I ratio and perifer artärsjukdom (PAD), therefore the aim of this study is to investigate whether apoB/apoA-I ratio is a useful analysis method in the investigation and prognosis of PAD compared to traditional lipid status. **Method:** The study population consists of 757 subjects from the Gothenburg part of the MrOS study, Sweden. In this cross-sectional study the men were included based on available measurements of apoB/apoA-I ratio and ABI, as well as absence of current statin treatment. ABI was assessed bilaterally by dividing the posterior tibial artery systolic blood pressure by the upper arm blood pressure. Apolipoprotein B and A-I were measured using a turbidimetric method. **Results:** Statistically significant differences could be seen between the groups regarding apoB/apoA-I ratio, HDL and apoA. No statistically significant differences could be seen regarding total cholesterol, LDL, triglycerides, non-HDL and apoB. The men diagnosed with PAD were significantly older and had hypertension and diabetes to a greater extent than the men without PAD. Likewise, differences in the number of years of smoking were seen between the groups, where smoking is seen to be a risk factor for the development of PAD. ABI has a consistently weak correlation with investigated variables, where a very weak correlation was seen between ABI and apoB/ApoA-I ratio. **Conclusion:** The result of the study showed a very weak correlation between PAD and apoB/apoA-I. Despite a weak association, the tendency is that apoB/apoA-I can be a useful method also in PAD, but where more studies in the field will be needed.

Implementation of EZH2 antibody in the diagnosis of mesothelioma

By Raghda Asadi

Bachelor thesis in biomedical laboratory science performed at the clinical pathology, Halmstad Hospital, 2023
Main supervisor: Mohammad Mansour, cytologist, PhD student
Co-supervisor: Tomas Seidal, Cytopathologist, MD, PhD.

Malignant mesothelioma is a rare invasive tumor, which affects men more often than women, with a poor prognosis. The tumor arises from mesothelial cells in the serous cavities, mainly the pleura and the peritoneal cavity. The prognosis is poor, with a survival time of up to 6 months, and surgery may just be relevant in case of early detection, which can extend the survival time to 12 months. Immunohistochemistry (IHC) is used to find nuclear expression of Enhancer of zeste hemolog 2 (EZH2). EZH2 is a biomarker that can be used to diagnose mesothelioma earlier and can also be used as a diagnostic marker in the treatment which may include EZH2 inhibitors in the form of immunotherapy.

The aim of this project is to optimize EZH2 on biopsies from mesothelioma patients, to obtain an optimal protocol, to be used in the clinical diagnosis of mesothelioma by using IHC.

Positive and negative controls were used to sort the expression of EZH2 in different types of tissue. Five tissues were selected as controls and then EZH2 was optimized in these tissues. Different dilutions of antibody and pretreatment buffer in high and low pH were tested in two experiments. Then 9 patient samples were stained with the selected protocol for more reliable results.

The results showed good optimization at low pH and at an antibody concentration between 1:25 and 1:100 in the first attempt. In the second experiment, low pH was used with antibody concentrations of 1:50 and 1:75. The final protocol was optimized to obtain clearer EZH2 expression in the nuclei of the cells and less background in the cytoplasm. Finally, the final protocol was determined at low pH pretreatment buffer, and 1:50 dilution of EZH2 antibody, without changing other steps. Patient samples from individuals with a diagnosis of mesothelioma showed clear positive staining with the final protocol in some of the cases, and negative in others.

Early diagnosis of mesothelioma can prolong survival, and it is then required that EZH2 be optimized with the correct protocol, so that the antibody is allowed to be used in the clinical setting for mesothelioma diagnosis. The patient samples showed 56% positive expression for safer results. The final protocol showed good results and quality based on the study conducted, despite the low number of cases in this study.

Evaluation of the EnVision FLEX Immunohistochemical Visualization System for Detection of Primary Antibodies Used in Renal Diagnostics

By Nisreen Aziz

Bachelor thesis in Biomedical Laboratory Science performed at the clinical pathology, Sahlgrenska University hospital, 2023.

Supervisor: Ylva Magnusson, Biologist.

Glomerulonephritis is a complex kidney disease that is difficult to diagnose and treat. Two commonforms, IgA nephropathy and membranous nephropathy, are characterized by IgA, IgG and C5B9 deposition which cause inflammation in the glomeruli, which can cause a range of symptoms, including proteinuria and kidney failure. Immunohistochemistry (IHC) plays an important role in kidney diagnostics, and the EnVision™ system has been shown to be a versatile and effective method for detecting primary antibodies. The technique allows for rapid and accurate immunolabeling and improves sensitivity and specificity. Primary antibodies, against antigens such as IgA, IgG, IgG4, and C5B9, are important for visualizing antigens deposited in kidney tissue and facilitating the diagnosis of kidney disease. These antibodies are used to identify specific proteins and to understand disease mechanisms. The purpose of this study was to evaluate EnVision™ Flex for the detection of primary antibodies in kidney diagnostics, particularly for IgA nephropathy and membranous nephropathy. The goal was to standardize and improve the diagnostic process by using the EnVision™ Flex system as it is used for other immunohistochemical stainings in the immunology laboratory. In the study, kidney samples from patients with IgA nephropathy and membranous nephropathy were used. The material was obtained from Sahlgrenska Clinical Pathology and Sahlgrenska University Hospital. Immunohistochemistry and specific antibodies were used for diagnosis. Fourteen renal biopsies (5 IgA nephropathy and 9 membranous nephropathy) were included in the study. Positive tissue and controls were used for increased reliability. Section preparation and antigen retrieval were performed before the immunolabeling. The results were validated by scanning slides on the EnVision™ Flex and Dako EnVision FLEX systems. The results of this study showed that EnVision™ Flex is a reliable and easy-to-use method for the detection of primary antibodies in kidney diagnostics. The system was as good as the REAL EnVision™ system at detecting IgA nephropathy and membranous nephropathy. However, EnVision™ Flex was slightly better at detecting IgA nephropathy, while REAL EnVision™ was slightly better at detecting membranous nephropathy. Both systems can be used to provide a detailed picture of kidney inflammation. They are recommended for their reliability and flexibility.

Differences in the assessment of exercise induced myocardial ischemia, its degree, distribution and location in attenuation corrected versus non-attenuation corrected images.

By Pinelopi Bentley

Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2023

Supervisors: Anna-Karin Halldin MD. PhD, Sofie Ahlin MD. PhD

Background: Coronary artery disease is characterized by atherosclerotic plaque in the walls of the coronary arteries, which can limit the blood flow and lead to myocardial ischemia. Myocardial scintigraphy is the first exam that patients undergo when there is a high suspicion of coronary artery disease, as well as to find out the distribution and location of the ischemia. Despite the high accuracy of myocardial scintigraphy, its specificity is affected by attenuation artifacts. Therefore, attenuation correction is used for the assessment of the images to eliminate the artifacts. These characteristic attenuation artefacts can coexist with perfusion defects at the same location, which can lead to a perfusion defect being ignored simply because it resembles a characteristic attenuation artefact. This is risky as the perfusion defect might be caused by a significant coronary artery stenosis.

Aim: The purpose of this study was to examine the differences in the assessments between attenuation corrected and non-attenuation corrected images of myocardial scintigraphy regarding the assessment of exercise-induced myocardial ischemia, its location, degree, and distribution.

Method: This study included nine patients' myocardial scintigraphy examinations which were performed at Norra Älvborg hospital with the suspected diagnosis of exercise-induced myocardial ischemia. The images were shown to six doctors who assessed them without knowledge of whether they were attenuation corrected or not and without other clinical information about the patients. Cross-tabulations were constructed to visualise the assessment of attenuation corrected and non-attenuation corrected exams.

Results: The results showed that there were differences in the assessment of all the exams based on attenuation correction. However, the majority of these differences were not statistically significant.

Conclusion: This study showed that there were differences in the assessments of the occurrence, distribution, degree, and location of exercise induced myocardial ischemia in attenuation corrected and non-attenuation corrected myocardial scintigraphy images. However, other conclusions regarding how attenuation corrected affected the assessments cannot be drawn. Despite this, the differences in the assessments can be of great importance for the clinical management.

MITOCHONDRIAL DNA CHANGES AND MITOCHONDRIAL FUNCTIONS IN MUSCLES FOR PRIMARY MITOCHONDRIAL MYOPATHIES AND YOUNG PATIENTS WITH INCLUSION BODY MYOSITIS

By Amanda Björklund
Bachelor thesis in Biomedical Laboratory Science performed at the Department of Pathology
Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor: Anders Oldfors

Background: Inclusion body myositis (IBM) is an inflammatory muscle disease with a general onset age of approximately 70 years. In addition to inflammation and inclusion bodies, the muscle tissue in IBM is characterized by mitochondrial changes. Because IBM affects older individuals, it has been speculated that these mitochondrial changes are an accelerated aging phenomenon.
Aim: The aim of the study was to investigate changes in mitochondrial DNA (mtDNA) and the effects of these changes on respiratory chain enzymes in muscle tissue in a group of young patients with IBM.
Materials and method: In the study, young IBM patients were compared with controls and primary mitochondrial myopathies caused by point mutations in the mt- $tRNA^{Leu(UUR)}$ gene (m.3243A>G or m.3252A>T) and large single deletions, respectively. Muscle biopsies from five individuals in each group were used. The methods included enzyme histochemistry, immunohistochemistry and western blot of respiratory chain complexes I, II and IV as well as deep sequencing of mtDNA.
Results: The result showed a high proportion of multiple deletions and duplications in mtDNA in IBM patients (average 5%), while the other three groups had very low levels. The group with primary mitochondrial diseases indicate that the proportion of mtDNA show an average of 43% single deletions and respectively 76% point mutations in $tRNA^{Leu(UUR)}$. The proportion of fibers with complex I and/or IV deficiency average were 0,2% in the control group, 5% in the young IBM patients, 7,5% in the single-deletion group and 13% in the point mutation group. All groups also show an enzyme histochemical cytochrome c deficiency in different proportions. Controls show 0,15%, IBM 2,6%, the group with the point mutations 3.9% and the single-deletion group 5.6%.
Conclusion: The conclusion that can be drawn are that young IBM patients suffer from large amounts of mtDNA deletions/duplications as well as those who got their diagnoses in an older age, which suggests that these mitochondrial changes are an essential part of the disease at all ages. The mtDNA mutations lead to defects in the respiratory chain that affects complex IV and even more complex I. Primary mitochondrial myopathies show a different distribution of fibers with complex I and/or IV deficiency.

The application of neutrophil myeloperoxidase in hematological diagnostics and research

By Emilia Börjesson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Alingsås Lasarett, Sahlgrenska Academy, University of Gothenburg 2023.

Supervisors: Huamei Forsman, PhD, Martina Sundqvist, PhD & Heléne Gustavsson, PhD & Biomedical Scientist.

Background

Neutrophilic granulocytes are the most common leukocyte in peripheral blood, neutrophils have a crucial role in immune response. The enzyme myeloperoxidase (MPO) is stored in the azurophilic granules and MPO is an essential component in the potent antimicrobial system MPO- hydrogen peroxide- halide system. The peroxidase content can be used to differentiate the leukocytes with the help of different hematology analyzers. In patients with suspected MPO-deficiency, methods commonly used in research can be utilized to study the enzymatic function of MPO.

The aim of this study was to investigate how MPO can be used in hematological diagnostics and research as well as to investigate the stability of the myeloperoxidase index over time after refrigerated storage in 2-8 °C.

Method

The study included 50 samples from patients in the intensive care unit, the emergency department, rheumatology- gastroenterology and hematology department. Samples were analyzed within an hour of sampling and after storage for 24-72 hours on the hematology analyzer ADVIA® 2120i. Thereafter, samples with a normal myeloperoxidase index, low myeloperoxidase index and an alarm for suspected MPO-deficiency were stained using a peroxidase staining method. A functional study was conducted on the sample with suspected MPO-deficiency as well as on a patient control sample.

Results

The results showed a variability in myeloperoxidase index after storage; however, the difference was not statistically significant. The results also showed that the two most common analytical flags after storage were presence of non-segmented neutrophils as well as presence of immature granulocytes. Peroxidase staining and functional analysis showed presence of peroxidase enzyme as well as enzymatic function, the functional analysis also showed no difference between the suspected MPO-deficient sample and the patient control sample.

Conclusion

In conclusion, the enzyme MPO can be used in both hematological diagnostics and research. However, it is important to remember that MPO-deficiency and conditions that resemble MPO-deficiency affect the results from hematology analyzers using the peroxidase to differentiate leukocytes.

Negative feelings are connected to a higher risk of cardiovascular events originating from both the heart and the brain, regarding initially healthy 58-year-old men.

By Moa Brinkhoff Diechle

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2023

Supervisor: Caroline Schmidt, PhD

Introduction: Mental health issues are connected to a higher risk of cardiovascular disease and stroke. The reason for this can be a higher risk of developing lifestyle factors that increase the risk of cardiovascular disease. Another reason can be the reactions that happen to the body during stress. Negative feelings have also been connected to a higher risk of stroke. Progression of intima media thickness in the common carotid artery has been connected to the feeling of discontent. This can indicate a faster progression of atherosclerosis which increases the risk of stroke. The aim of this thesis is to investigate whether there is a correlation between negative feelings and cardiovascular events, specifically stroke during a 10-year period for initially healthy 58-year-old men.

Method: The individuals included in the study were initially healthy 58-year-old men of Swedish descent. An MSE-questionnaire was conducted to assess feelings. Out of 24 questions only 15 were used and the symptoms were divided into three groups: contentment (mood, emotional concordence, aggressiveness, decisiveness, self-confidence, mental fatigue and general wellbeing), vitality (enthusiasm, initiative, endurance, concentration and responsiveness) and sleep (nocturnal sleep, quality of sleep and insomnia).

Results: Significant differences were discovered, for individuals with cardiovascular events, concerning a wider waist circumference, higher systolic and diastolic blood pressure, lower serum HDL, lower level of contentment and more specifically a lower level of mood, emotional concordance and general wellbeing. A higher risk of cardiovascular events from the heart was connected to a lower level of contentment (Exp (β) 1.036, 95% CI 1.001 to 1.071, p=0.041) and a higher risk of cardiovascular events from the brain was connected to a higher systolic blood pressure (Exp (β) 1.025, 95% CI 1.004 to 1.046, p=0.02) and a lower level of contentment (Exp (β) 1.044, 95% CI 1.003 to 1.086, p=0.033).

Conclusion: There is a correlation between negative feelings and a higher risk of cardiovascular events originating from both the heart and the brain, regarding initially healthy 58-year-old men.

Correlation between computed tomography and ultrasound in the assessment of Non-Alcoholic fatty liver disease, a comparison of non-invasive methods

By: Siri Ahlström

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of medicine, Sahlgrenska Academy, University of Gothenburg, 2023.
Supervisor: Caroline Schmidt, leg BMA, Docent

Background: Non-alcoholic fatty liver disease is spectrum of liver diseases. The disease is considered to occur when there is steatosis in more than 5% of the hepatocytes, together with little to no consumption of alcohol. The biggest risk for developing non-alcoholic fatty liver disease is the metabolic syndrome. Today is liver biopsy the standard to define non-alcoholic fatty liver disease, but because this carries other risks, liver biopsy recommended to be done routinely. Hepatic steatosis can be reliably identified by routine imaging techniques, but these have difficulty grading the amount of steatosis.

The aim of the study is to compare methods to assess the amount of liver fat in people with various non-invasive imaging techniques.

Method: Study participants from the municipality of Gothenburg were randomly selected. The methods studied and compared were computed tomography, ultrasound, and ultrasound elastography of the liver with acoustic radiation force impulse imaging, as well as analysis of various variables such as body measurements, metabolism, and various serum concentrations.

Results: The study shows the connection between the metabolic syndrome and non-alcoholic fatty liver disease. It also shows a high sensitivity, 0.947, and specificity, 0.888, between the methods computed tomography and ultrasound.

Conclusion: This study showed that visual classification of liver fat as present or absent reviewed from ultrasound images is very accurate and shows high values of sensitivity and specificity.

Method Development for Detection of Amyloids: A Comparison between Artisan Staining and Manual Staining with Congo Red and Sulfated Alcian Blue

By: Rawan Dadouch

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Pathology, Sahlgrenska Academy, University of Gothenburg, 2023.

Supervisor: PhD Ylva Magnusson.

Amyloidoses are a group of diseases characterized by the accumulation of amyloid proteins in tissues and organs, which can lead to organ failure and ultimately death. Congo Red (CR) is the most commonly used dye for the detection of amyloid deposits in tissue sections, but it has been associated with health risks such as mutagenicity and carcinogenicity. Sulfated Alcian Blue (SAB) is a dye that has been proposed as an alternative to CR for the detection of amyloids, but its effectiveness in detecting amyloid deposits has not been extensively studied.

The aim of this study was to develop a safer staining method in Artisan for the detection of amyloids and to compare Artisan staining with manual staining using Congo Red and sulfated Alcian Blue. The study was conducted on 14 randomly selected tissue samples containing amyloid deposits, including 5 heart specimens, 5 lung specimens, and 4 kidney specimens. The results showed a significant difference in amyloid deposits between manual staining and Artisan staining with Congo Red. Additionally, sulfated Alcian Blue showed to be an effective stain for visualizing amyloid deposits and can be used as an alternative to Congo Red staining. Overall, our study showed that Artisan staining with Congo Red is a safe and reliable method for detecting amyloids in tissue samples, which can improve the diagnosis of amyloidoses.

In summary, our study is an important contribution to the field of pathology and diagnostic medicine. By developing a safer staining method for detecting amyloids, our study can help to increase the reliability of the diagnosis of amyloidoses. Furthermore, the use of sulfated Alcian Blue as an alternative to Congo Red staining can open up new opportunities for diagnosis and research in this field. Moreover, our comparison of manual and automated staining with Congo Red in Artisan can contribute to improving the effectiveness and standardization of diagnostic methods in pathology.

Preclinical studies of the role of acetylcholine in the rewarding effect of alcohol

By Walaa Darraki

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Neuroscience and Physiology,
Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor: Anna Loften

Alcohol dependence is a significant problem with profound consequences for individuals and society. Despite existing treatment methods, many individuals still struggle with alcohol-related issues, highlighting the need for innovative and effective interventions.

Research has shown that cholinergic interneurons in the reward system play a crucial role in the rewarding effects of alcohol. Using an animal model, it has been observed that the reduction of cholinergic interneurons results in reduced alcohol consumption. Against this background, the overall aim of this project is to further investigate the role of these cholinergic interneurons in the rewarding effect of alcohol. More specifically, our study aims to restore the decreased alcohol consumption by administering Donepezil, an acetylcholinesterase inhibitor, to 43 rats. The rats received doses of either 1.5 or 3 mg/kg of donepezil. By exploring this interaction and its effects on alcohol intake, we seek to contribute to a deeper understanding of the molecular and circuit-related mechanisms underlying alcohol dependence.

The results showed that the administration of Donepezil at different doses did not have a significant effect on ethanol intake. However, an initial decrease in ethanol consumption was observed, emphasizing the need for further investigation into the underlying factors responsible for this effect. These results contribute to our understanding of the complex relationship between cholinergic signaling and alcohol consumption. Although the study did not demonstrate a direct effect of Donepezil on ethanol consumption, it lays the groundwork for future research to explore the intricate mechanisms involved in the interaction between cholinergic interneurons and alcohol reward. By gaining a comprehensive understanding of these mechanisms, we can develop targeted treatment strategies for alcohol dependence and thereby improve outcomes for individuals struggling with this disorder.

Effect of preanalytical heat treatment on FVIII-inhibitor assay

By Mohammed El baghdadi

Bachelor thesis in Biomedical Laboratory Science performed at the Specialkoagulation laboratory, Sahlgrenska Academy, University of Gothenburg, 2023

Supervisor: Anna Olsson, leg. Doctor

Hemophilia A is a inherited bleeding disorder characterized by a deficiency of factor VIII (FVIII). Patients with severe hemophilia A develop inhibitors, in the form of antibodies against FVIII, in approximately 20-40% as a result of FVIII deficiency treatment. The Nijmegen-Bethesda assay is the standardized method for diagnosing FVIII inhibitors, established by the International Council for Standardization in Haematology (ICSH). However, preanalytical factors such as manual sample handling introduce variations in the results between laboratories, with a CV ranging from approximately 30-60%. In an attempt to reduce this variation, ICSH has developed guidelines, one of which involves a preanalytical heat treatment at 56°C for 30 minutes for samples with FVIII levels <0.03 kIU/L. The aim of this study is to investigate whether this preanalytical heat treatment of samples with different FVIII concentrations affects inhibitor titers. Another aspect highlighted in this study is the importance of time in the analysis. Inhibitor titers are analyzed by calculating the residual activity of a known amount of FVIII after the influence of inhibitors. The calculation of residual activity is performed by measuring the FVIII activity in patient plasma and in a control mixture without inhibitors. This control mixture is analyzed before the first sample (K1) and after the last sample (K2). In this study, the control mixture is also analyzed a third time, 25 minutes after K1 (K3) to evaluate eventual changes in FVIII activity after 25 min. This pilot study consists of a cohort of patients (n=5) diagnosed with hemophilia A with inhibitors. Plasma samples from these de-identified patients undergo preanalytical heat treatment and are subjected to the Nijmegen-Bethesda assay to determine the inhibitor concentration. The results are then compared with patient plasma that has not undergone heat treatment. Aa mean value of 3.39 ± 2.04 Bethesda Units per milliliter (BU/mL) was calculated for the samples subjected to preanalytical heat treatment, and a mean value of 2.12 ± 1.32 BU/mL was calculated for the control group. Student's paired t-test did not show any significant difference between the heat-treated samples and their control group (p=0.55). To evaluate the importance of time, student's paired t-test was performed. Mean of K_1, K_2 and K_3 was determined to 0,39 ± 0,04 kIU/L, 0,4 ± 0,04 kIU/L and 0,29 ± 0,01 kIU/L. No statistical differences were observed between K1 and K2 (p = 0.11), while a statistically significant difference was found between K1 and K3 (p <0.05*). Therefore, this study did not demonstrate any significant differences with preanalytical heat treatment at FVIII concentrations <0.03 kIU/L. Further studies with larger sample sizes are required to draw conclusions about the effectiveness of heat treatment. The time aspect was found to play an important role, although larger samples may be necessary to draw definitive conclusions.

Towards a physiological understanding of cholestanol accumulation in Cerebrotendinous xantomathosis.

By Hanna Engblom

Bachelor thesis in Biomedical Laboratory Science performed at The Technological university Dublin. 2023

Supervisors: Steve Meaney, Sean Kennedy.

In the rare inherited disease Cerebrotendinous Xanthomatosis (CTX) the bile acid synthesis is impaired due to a mutation in the CYP27A1 gene coding for the enzyme 27α-hydroxylase. A novel metabolic pathway has been presented in patients with CTX and in this pathway cholestanol (5α-cholestan-3β-ol) cholestanol is synthesised from cholesterol. The aim of this study was to investigate the physiological reason for the accumulation of cholestanol in CTX. HepG2 cells were exposed to 4,6-cholestadien-3-one, 5-cholesten-3β, 7α-diol, 4-cholesten-3-one, cholestanol or lanosterol at different concentrations and for different times, and the MTT-assay, a cell viability assay, was then performed. Observation of the changes in HMGCR mRNA expression after treatment of cells with the substances was done with RT-qPCR. To observe the effects of the compounds on cell viability when CYP27A1 was temporary silenced, a small interfering RNA assay was performed and further a cell viability assay was executed. No significant differences in cell viability were seen between either time of treatment or concentration levels when the cells were treated with 4,6-cholestadien-3-one, 5-cholesten-3β-7α-diol. Significance was found between the treatment times, but not between the different concentrations in the cells exposed to 4-cholesten-3-one and cholestanol. Statistical significance between the different time periods was shown in the cells exposed to lanosterol. At a 48-hr exposure of the cells to cholestanol, a decrease in HMGCR expression was seen. However, an upregulation of HMGCR was shown when the cells were exposed to cholestanol for 72 hrs. Cells exposed to lanosterol for 24 hr showed a fold change of 2,6 in HMGCR expression, but exposure for 72 hrs gave a fold change of 0,39. When the cells were exposed to 4-cholesten-3-one for 72 hrs a 0,63-fold change in HMGCR expression was observed. However, when the cells were exposed to 4-cholesten-3-one for 24 hr or 48 hr, an upregulation of HMGCR was seen (a fold change of 4,0 and 7,0 respectively).

No conclusion could be made regarding the physiological reason for cholestanol accumulation in patients with CTX. However, an interesting finding was observed in cell viability when the cells were exposed to lanosterol for 72 hrs which contradicts findings of other earlier studies.

Validation of Tissue Microarray immunohistochemical analysis in simultaneous endometrial and ovarian cancer associated with endometriosis.

By Dila Fako

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Pathology, Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg, 2023

Supervisor: Claudia Mateoiu, PhD, Doctor at gynecological pathology, SU and Ylva Magnusson, PhD

Background: Tissue microarray assay (TMA) is a method that, in combination with immunohistochemistry (IHC), enables high-capacity analysis of multiple samples simultaneously on a single slide. Due to the high prevalence of mismatch repair deficiency (MMRd) in synchronous endometrial and ovarian cancer, screening for MMRd is performed as a predictive treatment marker. The MMR system is essential for repairing errors during DNA replication and involves four DNA repair genes (MLH1, MSH2, MSH6, and PMS2). Mutations in the DNA repair genes give rise to MMRd, which can be caused by epigenetic changes or inherited mutations underlying Lynch syndrome. Endometriosis-associated ovarian cancer (EAOC) primarily occurs as endometrioid (EOC) and clear cell ovarian cancer (CCC) and has a high prevalence of Lynch syndrome. Detection of MMRd and PD-L1 expression can select qualified candidates for anti-PD-1/PD-L1 immunotherapy.

The aime of this study was to validate the TMA method by comparing results obtained from IHC analysis performed on whole tissue sections with IHC performed on TMA sections. This study explains the importance of immunohistochemical analysis of MMRd and PD-L1 expression in synchronous endometrial and ovarian cancer and how such a testing protocol has been implemented.

Method: All cases diagnosed with synchronous endometrial and ovarian cancer (SEOC) expressing MMRd and/or PD-1/PD-L1 were selected for immunohistochemical staining of whole tissue sections. Out of a total of 12 cases of SEOC, 4 cases of EOC and 1 case of CCC were stained with MMR IHC, and 6 cases of EOC and 2 cases of CCC were stained with the monoclonal antibody PD-L1 22c3. IHC staining of both MMR and PD-L1 was validated by comparing results obtained from TMA sections with results obtained from IHC performed on whole tissue sections.

Results: This study demonstrated that the results from MMR IHC staining of TMA sections were consistent with the results from corresponding staining on whole tissue sections for 3 out of 5 patient cases. The difference in expression of MMR proteins was mainly observed as focal loss of MSH6 protein in whole tissue sections. The results also showed a significant difference in CPS score for PD-L1 expression detected in TMA sections compared to whole tissue sections, with slightly higher CPS scores noted for whole tissue sections.

Conclusion: Further research on the subject is needed to establish the relevance of the average CPS value for predictive treatment purposes. IHC analysis of TMA sections has significant prognostic value and is a valuable technique, however, it is necessary to supplement the sample results from TMA with the sample results from whole tissue sections to obtain the complete diagnosis.

Using microbroth dilution to determine minimum inhibitory concentration in different species of *Staphylococcus* for antibiotics linezolid, clindamycin, erythromycin and vancomycin

By Maria Finstad

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Microbiology laboratory, Sahlgrenska Academy, University of Gothenburg, 2023
***Supervisor**: Erika Lindberg PhD, senior lecturer.*

Background: About 30% of nosocomial infections are caused by species of staphylococci. They are seen as one of the biggest reasons for complications following implant surgery, mainly due to the fact that they can attach to materials, both organic and inorganic, and produce biofilm. *Staphylococcus* spp. can be split into two groups: coagulase-negative staphylococci (CoNS), containing over 50 different species, and coagulase-positive *S. aureus*. *S. aureus* is the more pathogenic of the two, possessing several virulence factors causing diseases and complications such as impetigo, meningitis, and sepsis. CoNS, although often seen as apathogenic, often possess resistance to several classes of antibiotics, making them difficult to treat when causing infection. Being able to determine the resistance of an isolate quickly and correctly could be crucial for a patient's wellbeing and survival.

Aim: The aim of this study was to verify microbroth dilution plate Sensititre® EUSTAPF for antibiotics linezolid, erythromycin, clindamycin, and vancomycin used in determining minimum inhibitory concentration in *Staphylococcus* spp.

Material and method: Altogether 26 isolates from 7 different species of *Staphylococcus* spp. were analysed 74 times by inoculating bacteria with a turbidity of 0,5 McFarland to wells in Sensititre® EUSTAPF microbroth dilution plate. Plates were analysed for the minimum inhibitory concentration. To test the robustness of the method plates were analysed after 18, 20, 22 and 24 hours incubation. The number of analyses with values within one value from referential value (essential agreement) and the number of analyses with the correct SIR-value compared to referential value (categorical agreement) was calculated. Passing limit was set to >90% for both.

Results: Essential agreement for linezolid, erythromycin, clindamycin, and vancomycin was calculated to 93,2%, 97,3%, 91,9% and 98,6% respectively. Categorical agreement was calculated to 100 % for linezolid and 95,9% to both erythromycin and clindamycin. Categorical agreement for vancomyin regarding *S. aureus* was calculated to 100%, and vancomycin for CoNS was calculated to 98,2%. MIC-value was consistent through the majority of readings regardless of reading time, within an 18–24-hour incubation period.

Conclusion: Percentage was calculated to >90% regarding both essential and categorical agreement, showing good precision and accuracy for the method. The robustness of the method was proven to be high by consistent readings of the isolates regardless of incubation time within the 18–24-hour span. Microbroth dilution plate Sensititre® EUSTAPF is thereby proven to be a reliable method for determining minimum inhibitory concentration in *Staphylococcus* spp. for antibiotics linezolid, clindamycin, erythromycin and vancomycin.

Regenerative mechanisms following cardiac arrest

Global hypoxia and its potential role in tissue regeneration

By Naya Gabrial

Bachelor thesis in Biomedical Laboratory Science performed at the department of biomedicine, Sahlgrenska Academy, University of Gothenburg, 2023.

Supervisor: Kristina Vukušic, leg BMA, PhD & Helen Jinton, leg BMA.

Background: Cardiovascular diseases are considered as one of the most common public diseases worldwide and bring great suffering and high healthcare costs. The regenerative capacity in the heart has always been in focus in the research community. However, current research suggests cardiac muscle cells may have the capacity for de-differentiation and regeneration, to some extent.

Aim: The main aim of this study was to investigate the expression of regenerative muscle cell markers and whether global hypoxia caused by cardiac arrest, can stimulate regenerative mechanisms, such as de-differentiation.

Method: For this study, a unique tissue material was used, including cardiac muscle biopsies from (N=9) healthy organ donors and (N=10) individuals who have suffered from cardiac arrest. These biopsies were taken from the left ventricle and left atrioventricular junction, previously suggested as a stem cell niche. By employing immunohistochemistry, tissue sections from both regions were stained with three separate combinations of biomarkers. These biomarkers featured regenerative muscle cellular markers: α-SMA, Perlecan, N-CAM, and the cardiomyocyte-specific cTnT. Fluorescence microscopy was used for further analysis of biomarker expression.

Results: The result revealed an expression of regenerative muscle cellular biomarkers in tissue sections from both healthy and post-cardiac arrest donors. The differences observed at the atrioventricular junction between these two groups were less pronounced compared to the differences seen in the left ventricle. Particularly in the left ventricle, a distinct subpopulation of cardiomyocytes exhibited a weaker expression of cTnT and an increased expression of α-SMA and N-CAM.

Conclusion: In summary, the results indicate the existence of cardiac muscle progenitor cells, also known as immature cardiomyocytes. However, the question still stands if these cells are a product derived by the de-differentiation or the regeneration of stem cells. Further exploration and research are imperative for a more comprehensive understanding of the complex mechanisms that govern the process of human heart regeneration.

Development of Multiplex PCR Panels as a Diagnostic Tool for Enterotoxigenic *Escherichia coli* (ETEC)

By Thea Galligani Vardheim

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Microbiology and Immunology, Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor: Astrid von Mentzer, PhD

Introduction: Enterotoxigenic *Escherichia coli* (ETEC) is a pathogen variant of *E. coli* which infects faecal-oral and is one of the major pathogens causing diarrhoea in low- and middle-income countries and it is also the primary cause of travellers' diarrhoea. ETEC is characterised by its toxins and colonisation factors (CF). The toxins are divided into heat-stable and heat-labile toxins, both increasing the efflux of negative ions and therefore water into the intestinal lumen, resulting in a watery, cholera-like diarrhoea. CFs are proteins expressed on the surface of ETEC that enable colonisation and are divided into groups based on sequence similarity of the major subunit. These CFs are detected using polymerase chain reaction (PCR). There are several multiplex PCR panels already designed for the detection of these virulence factors, but these do not cover all CFs and toxins. Moreover, several CFs cannot be detected by PCR due to lack of primers.
Aim: The overall aim was to design new panels for both CFs and toxins that are expressed in ETEC that infects humans and animals. The specific aim was to design primers targeting four CFs in the Class 1b group: CS27A, CS27B, CS28A and CS28B. The panels and primers were to be tested against ETEC strains available at the University of Gothenburg.
Methods: Two common primers, one for CS27AB and one for CS28AB, were designed using Primer-Blast from National Library of medicine and placed in a multiplex PCR panel together with primers for the CFs CS1, CS3, CS4 and CS12. A toxin panel was also designed using primers for LTh, STh, STp and STb. These primers and panels were tested against reference strains whose DNA had been extracted using a rapid boil technique. The panels were tested using PCR followed by gel electrophoresis and three different polymerases were compared for the CF panel; DreamTaq, ReadyMix and Q5 High-Fidelity and two for the toxin panel; DreamTaq and ReadyMix.
Results: The two primer pairs designed were able to detect CS27AB and CS28AB with the use of ReadyMix and when the working solution of the primers was used at a concentration of 10 pmol/µl. Two panels, one targeting CFs and one targeting toxins were tested and these created clear and specific bands of correct sizes when DreamTaq DNA Polymerase was used. ReadyMix gave clear bands for the CF panel, but not the toxin panel. The primer for LTIh annealed to a sequence in LTIp due to its sequence similarities at 99% in the genes *eltA-h* and *eltA-p,* respectively, and sequencing should be used to differentiate these to virulence factors.
Conclusion: Primers for CS27AB, CS28AB and two panels were successfully designed and evaluated. All primers worked when DreamTaq DNA Polymerase was used. Sequencing should be used to differentiate between LTh and LTIp, due to the unspecific bands created by the LTh primer. Several CFs still lack PCR primers, particularly in the Class 1b group, but these new panels and primers could be of great use in future surveillance studies.

LIPID OXIDATION INCREASES AND ATP DECREASES SIGNIFICANTLY DURING STORAGE OF ERYTHROCYTES FOR BLOOD TRANSFUSION

By: Adriana Ghasemi

Bachelor thesis in Biomedical Laboratory Science performed at Clinical immunology and Transfusion Medicine, Blood component lab at Sahlgrenska University Hospital, 2023

Supervisor: Camilla Hesse

Background: Blood transfusion is an important part of modern healthcare and means intravenous transfering of blood products to the circulation of a patient. Lipid peroxidation is a consequence of oxidative stress and is defined by the process in which oxidants attack carbon-double bonds in lipids. By-products of lipidoxidation have cytotoxic, mutagene and cancerogenic effects on nearby tissue. Adenosine triphosphate is the cell's energy value and is responsible for DNA synthesis as well as intracellular signaling and muscle contraction. Adenosine triphosphate progressively decreases in absence of glycolysis and oxidative phosphorylation within blood units stored in 2-6 °C. Measuring both lipidperoxidation and ATP within blood units is therefore relevant as they both play a role in patients' post transfusional recovery as well as chance for survival. The aim of the following study is to establish two working methods to measure lipidperoxidation and ATP in stored blood to discover potential differences in concentration between fresh and stored blood.

Materials and Methods: The study consisted of 20 units of erythrocytes from healthy donors from Transfusion medicine at Sahlgrenska university hospital. 10 units had been stored for one day and the remaining had been stored for five days in 2-6 °C. The amount of lipidperoxidation and ATP was measured by absorbance in a colorimetric assay. In addition common quality biomarkers such as hemoglobin content and hemolysis were measured.

Results: Two functioning methods were established to measure lipidperoxidation and ATP. The mean concentration of lipidperoxidation increased in blood on day five. On day one the mean concentration was 46,6 pmol/µL ± 0,71 SD. After five days of storage it increased to 51,5 pmol/µL ± 0,87 SD. The mean concentration of ATP had instead decreased during storage from 25,4 pmol/µL ± 0,36 SD on day one to 19,3 pmol/µL ± 0,38 SD day five.

Conclusion: Two methods using a colorimetric assay could successfully measure both lipidoxidation and ATP in erythrocyte units. A statistically significant increase of lipidoxidation- and decrease of ATP could be detected in blood units stored for 5 days. However the developed methods are part of a pilot study that would benefit from further testing with extended storage time and a larger cluster sampling.

Vascular axon reflex - Does it have a predictive value before treatment of trigeminal neuralgia?
An objective measure of nerve involvement preoperatively.

By Sergej Golubovic

Bachelor thesis in Biomedical Laboratory Science - Clinical physiology
performed at the Department of Neurophysiology,
Sahlgrenska Academy, University of Gothenburg, 2023

Supervisior: Linda Lundblad (docent, Leg. BMA)

Background: Trigeminal neuralgia is an extreme pain condition in the face, experienced as paroxysmal intense provoked or unprovoked attacks, that can involve more than one branches of the n. Trigeminus. The pathophysiology of Trigeminus is not fully established where some of the reasons are believed to be neurovascular conflict such as focal compression, secondary diseases as well as idiopathic unknown causes. Regardless of the cause, it is suspected that sensory fibers become hyperexcitable, as there is ephaptic crosstalk between these and thin pain fibers (Aδ- and C-fibers, so-called afferent polymodal C-fibers) in close physical connection. The activation of the pain fibers leads to vasodilatation, collectively called axonreflexmediated vasodilatation. For diagnosis, anamnesis and magnetic resonance imaging are widely used. As for pharmacological treatment, antiepileptic drugs such as Carbamazepine and Oxcarbazepine are the firsthand choice. Non-pharmacological treatment is in the form of ablative or non-ablative surgery.
Purpose: To see if there is an influence of axon reflex-mediated vasodilatation in trigeminal neuralgia using Laser Doppler flow measurement and if this can be applied as a unique diagnostic examination method in future diagnosis and treatment of the disease.
Method: Using a Laser Doppler flowmeter, the axon reflex-mediated vasodilatation after electrical stimulation is recorded for comparison of each half of the face (healthy and pathological), in three different points corresponding to the n. Trigeminal branches in four patients.
Results: Comparison of the data against the control group shows side differences that exceed the pathological normal limit in the forehead and cheek in two patients.
Conclusion: Currently, it is not possible to apply Laser Doppler flow measurement for evaluation of treatment or in the diagnosis of Trigeminal neuralgia. An axon reflex-mediated vasodilatation can be measured and the method is feasible in patients with the disease, but more studies of this kind need to be done.

Activation of the NF-κB signaling pathway by TLR-1, TLR3 and TLR7 affect gene expression of different types of γ-protocadherins in the heart. - Analysis of gene expression with qPCR.

By: Fredrika Gustafsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry at Sahlgrenska University Hospital, University of Gothenburg, 2023.
Supervisor: Victoria Rotter Sopasakis PhD.

Introduction: Heart disease is a significant cause of mortality globally. A chronic, low-grade inflammation can derive in heart tissue from both ischemic and non-ischemic causes. When confronted with a pathogen associated antigen different types of TLRs can be activated. TLR1, 3 and 7 have been shown to activate pathways including NF-κB and affect gene expression of PCDGH-genes in heart tissue. PCDGH-genes are coding a subtype of protocadherins which plays a role in the adhesion of cardiomyocytes. Those genes are showing a more deviant expression in patients with heart disease compared to healthy controls.

Aim: The aim of the study was to investigate differences in gene expression of genes included in NF-κB signaling pathways. We wanted to investigate the expression specifically in heart endothelial cells with certain TLRs activated by agonists. Also, we wanted to investigate if genes coding for subtypes of PCDHG was expressed differently in cells treated with different TLR-agonists.

Material and methods: The study was conducted in two parts. The first part included 16 samples of RNA eluted from cultivated cells treated with different agonists: TLR1-agonsit Pam3CSK4, TLR3-agonist PolyI:C, TLR7-agonist R848 and DSMO for controls. The samples were analyzed with Qiagen´s RT^2 qPCR array. The second part included 40 samples of RNA eluted from cultivated cells treated with different agonists: TLR1-agonsit Pam3CSK4, TLR3-agonist PolyI:C, TLR7-agonist R848, TLR4-agonsit LPS and DSMO for controls. The samples were analyzed with a TaqMan based qPCR-assay.

Results: The expression of PCDHG-coding genes where strongly down regulated in cells treated with the TLR3-agonist PolyI:C. Those cells also showed the most individual unique expressed genes compared to cells treated with other agonists. Activation of other TLR1, 4 and 7 either upregulated PCDHG-coding genes or did not affect them at all.

Conclusion: Signaling resulting from activation of TLR3 seems to be significant in the down regulation of PCDHG-gene expression in inflamed heart tissue in patients with heart disease. Heart endothelial cells seem to strongly contribute to that down regulation.

MICROBROTH DILUTION METHOD SENSITITRE FOR MIC-DETERMINATION OF *STAPHYLOCOCCUS* FOR TEICOPLANIN, RIFAMPICIN, TRIMETHOPRIM/SULFAMETHOXAZOLE AND VANCOMYCIN - *AN ALTERNATIVE TO ETEST*

By: Elena Hamid

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Microbiology Laboratory, Sahlgrenska Academy, University of Gothenburg, 2023

Supervisor: Erika Lindberg, PhD

Background: *Staphylococcus* spp is a part of the human microbiome and *Staphylococcus aureus* is a primary pathogen that can cause multiple infections, primarily in the skin and soft tissue. Coagulase-negative staphylococci is another branch of the *Staphylococcus* species in which *Staphylococcus epidermidis* stands for about 75% of all clinical isolates. Vancomycin is the first choice of treatment for resistant *Staphylococcus* infections in which it inhibits the bacterial cell wall synthesis. Teicoplanin is another antibiotic used to treat infections with *Staphylococcus aureus*. This antibiotic has a longer half-life which makes it possible for longer intervals in between dosage of the medicine. The microbrothdilution method is said to be a more reliable test to determine the minimal inhibitory concentration of antibiotic to treat an infection than Etest for certain antibiotics. To evaluate this statement, the aim of the following study is to verify the microbrothdilution method Sesititre for vancomycin, teicoplanin, trimethoprim/sulfamethoxazole and rifampicin in accordance with *Staphylococcus aureus* and Coagulase-negative staphylococci.

Materials and Methods: To verify this method, a total of 18 control strains and 8 clinical isolates of the *Staphylococcus* species, including *Staphylococcus aureus* and Coagulase-negative staphylococci were used. A Sensititer Gram Positive EUSTAPH plate including vancomycin, teicoplanin, trimethoprim/sulfamethoxazole and rifampicin was used to determine the antimicrobial susceptibility. Categorical and essential agreement were used to verify the accuracy and precision of the method.

Results: Essential and categorical agreement was found to be over 90% for rifampicin, trimethoprim/sulfamethoxazole and vancomycin. However, for teicoplanin, essential agreement was found to be 77% and 73% for the categorical agreement. There was a difference between the *Staphylococcus aureus* strains and the Coagulase-negative staphylococci strains in which Coagulase-negative staphylococci was found to be the reason for the low percentage of categorical and essential agreement.

Conclusion: The microbroth dilution method Sensititre is found to be approved for all strains of bacteria used in the study for all antibiotics used except for teicoplanin. The method in relation to teicoplanin was only approved for the *Staphylococcus aureus* strains and not for the strains of Coagulase-negative staphylococci. This would need further verification in order to get approved.

THE EFFECT OF SHORT-TERM STORAGE OF FORMALIN-FIXED NEEDLE BIOPSIES FROM THE PROSTATE IN WATER, FORMALDEHYDE, OR 70% ETHANOL

by

Samia Hassan

Bachelor thesis in Biomedical Laboratory Science at pathology laboratories Borås at Södra Älvsborgs Hospital, Sahlgrenska Academy, University of Gothenburg, 2023

Supervisor**: Roman Krenz,** PhD in medicine, **Pia Gabrielsson**, Biomedical analyst, and master's in medicine.

Background: Tissue handling and processing in clinical pathology require the use of chemicals such as formaldehyde. Fixation is the first step to stabilize the tissue and resist morphological changes. This permits pathological diagnostics for prostate cancer. Needle biopsies are taken from the prostate and are often fixated, dehydrated, sectioned, and then microscopically assessed by a pathologist. However, formaldehyde is a fixative that has many health effects on staff handling the samples and causes allergies, cancer and other serious health problems. Risk assessments and measures carries out to reduce exposure to formaldehyde. One of these measures is to store the sample material in water or 70% ethanol during tissue excision.

Aim: To investigate whether needle biopsies from the prostate that have been fixed in 4% formaldehyde are affected by being in water or 70% ethanol at the time of excision and whether the time that the sample remains in the respective solution has any effect for diagnostics.

Method: The methodology of this study follows the routine laboratory process that most clinical pathology laboratories have which begins with fixation, dehydration, embedding, sectioning and ends with staining and mounting. Needle biopsies from the prostate were examined macroscopically, where the length and number of bites were documented. The biopsies were stored in water, 70% ethanol, or formalin for 30 minutes and 60 minutes, respectively. The result was continuously evaluated during embedding and sectioning regarding how difficult versus easy it was to embed/section the sample. The morphology and immunohistochemistry were assessed by the pathologist.

Results: The study included 164 biopsies from 13 patients. The results from embedding, sectioning and morphology and immunohistochemistry assessment have shown equivalent results in all samples. Some deviations were shown in the results, but no significant association was found.

Conclusion: Finally, the study showed that pretreatment of needle biopsies from the prostate in water or 70% ethanol during excision does not have negative effects on tissue. Although the study has its limitations, the conclusion is considered useful within pathology laboratories in Borås.

BLOODPRESSURE CHANGEN COMPARED TO THE POSITION OF THE FEET

By Moa Hellström

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg laboratory
Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor: Caroline Schmitd

Background: According to American heart association the indirect measurement of blood pressure (BP) is one of the most poorly performed examinations in health care. At the same time accurate measurement of BP is essential for diagnosis of hypertension, BP greater than 140/90 mmHg, which is one of the leading causes of death worldwide. The indirect BP can either be measured auscultatory or half automated using the ocillometric technique, regardless of method, standardised protocols eliminating external factors effecting the BP variability is highly important. The present recommendation is to sit with feet flat on floor during the measurement referring to research that proves that BP increases with 5.9 mmHg in SBP and 2.3 mmHg in DBP while sitting with legs crossed at knee level compared to sitting with feet on floor.

Aim: The aim of the present study was to investigate if sitting with feet flat on floor compared to sitting with dangling legs is two equivalent methods.

Method: BP were measured while sitting on the edge of an examination table with legs flat on floor and with dangling feet, the mean values were compared using a paired samples t-test.

Results: A small but significant difference in SBP was found that 1.8mmHg ($p = 0.024$) greater while sitting with feet on floor compared to sitting with dangling legs. No significant difference in DBP were found.

Conclusion: In this study we were able to observe a small but significant change in SBP depending on the position of the feet but no difference in DBP. Consequently, this study shows the importance of following guidelines regarding measurement of BP.

Peter Herczegh

The disrupted glucoregulatory cell-cell signaling in islets of Langerhans of non-obese diabetic mice

Bachelor Thesis in Biomedical Laboratory Science

Institute of Neuroscience and Physiology

Sahlgrenska Academy, University of Gothenburg

Supervisor: Caroline Augusta Miranda Larsen, PhD, Assistant Professor

Abstract

Projected to reach over 700 million by 2045, the prevalence of diabetes globally is a concerning statistic that is expected to continue rising. The disease presents itself in two main forms, as type-1 and type-2 diabetes, both characterized by a disruption in regulation of blood glucose levels. Given the serious health complications and the need of lifelong treatment, information on the subject is valuable from both ethical and financial points of view. Research for a cure is ongoing and has come a long way in identifying multiple substances and signaling pathways that tightly regulate glucose homeostasis. The use of rodent models has been prominent in today's chains of experiments concerning diabetes, with the genetically modified non-obese diabetic mouse serving as a great tool in uncovering the mechanisms of disruption leading to disease progression. The aim of our study is to utilize both wild type-(WT) and non-obese diabetic (NOD) mice to shed light on differences in cell-cell communication of healthy and diseased pancreatic islet cell clusters. To achieve this, glucagon- and insulin secretion of multiple islets incubated at different glucose concentrations (1mM, 6mM and 20mM) with and without the receptor and gap-junction modulators CYN and carbenoxolone (CBX) was measured with direct sandwich ELISA. Live perfusion calcium imaging experiments were also conducted on single islets of both WT and hyperglycemic NOD mice. Multiple observations were made. Intracellular calcium oscillations, characteristic for each cell type and partly responsible for glucoregulatory hormone-filled vesicle exocytosis, readings in eu- and hyperglycemic NOD mice revealed reduced frequency compared to WT oscillations. Characteristic oscillation syncing seen in rodents between islet cells was also abolished in both types of NOD mice. Glucagon quantification by ELISA showed inhibition at lower glucose conditions between euglycemic NOD and WT mice. Insulin secretion of the euglycemic NOD mouse was reduced by 74% at high (20mM) glucose concentrations compared to WT. Our results hypothetically support the characteristics of insulitis, an autoimmune process in type-1 diabetes where lymphocytes infiltrate the islets of Langerhans and contribute to a decline in total β-cell count.

A study on sedimentations effect on the HbA1c analysis

By: Evelina Hurtig

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry at Sahlgrenska University Hospital and the Department of Laboratory Medicine at Kungälv Hospital

Supervisor: Ruth Wickelgren, PhD, Anders Olsson PhD, Annika Brude Biomedical Scientist

Introduction: The analysis of HbA1c measures the amount of glycated hemoglobin and the total amount of hemoglobin in whole blood. Today the analysis is used to diagnose and follow patients with diabetes in Sweden. When a sample has undergone sedimentation the HbA1c-results is elevated which has been noted in previous studies, resulting in that every sample must be mixed before analysis. The red blood cells in the whole blood weights the most and therefore they sink to the bottom of the sample which is the reason for sedimentation. The reason to the elevated results is not known but possible reasons are the extrapolation that today is done from the calibration curve or that it is the sedimentation itself that causes the effect. The method for creating the calibration curve in this study is built on measurements from two calibrators. If any results are over the highest calibrator the value is gained by extrapolation of the curve.

Aim: To investigate the reason to elevated HbA1c results after sedimentation, and to perform a material analysis of what's needed to analyze one sample for HbA1c and hemoglobin.

Method: Three methods were used in this study. The reason for elevated results of HbA1c when samples had undergone sedimentation was studied in two ways, both when whole blood samples had sedimented overnight (six samples) and after 1 hour, 2 and 4-6 hours (32 samples) of sedimentation. For the material analysis the amount and weight of every material used when analyzing HbA1c and hemoglobin was written down and calculated.

Results: Sedimentation overnight led to a maximum of 1,15% difference from the mixed 0-samples result for HbA1c. Sedimentation for 1 to 6 hours resulted in a difference up to 8,5%. The mixed end samples did not have a statistically significant difference from the 0-samples in either case. For the 0-samples extrapolation of the calibration curve was found for total hemoglobin (THb), the values measured was above the highest calibrators results. For glycated hemoglobin no extrapolation of the calibration curve was found, however the extrapolation that was found for THb was correct since the results followed the calibration curve. Calculated THb, which was calculated with the HbA1c-results from the 0-samples and the glycated hemoglobin, did not follow the calibration curve when the samples had undergone sedimentation for two hours or more. The material analysis showed that cardboard, plastics in different forms, and reagents dominated the material use.

Conclusion: Inaccurate extrapolation of the calibration curve hade the biggest impact on the HbA1c results after sedimentation, however an effect from sedimentation alone could not be ruled out. Additional calibration levels are needed in order for the analysis of sedimented samples to give more accurate results.

Measurements in vascular axon reflexes of the trigeminal nerve in the face of individuals without facial pain to create a reference material

By Tina Khorasani

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Neurophysiology at Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg 2023.
Supervisor: Joakim Strandberg, Med. Doctor, PhD and Linda Lundblad, Docent, Leg BMA, PhD

Background
Trigeminal neuralgia is a severe condition that results in facial pain which can be described as short, yet sharp attacks of unilateral pain. The ethology is unknown though there are factors that provoke the pain in brushing your teeth, touch and chewing food. The neurogenic mechanism behind pain is mediated by unmyelinated C-fibers and thin myelinated A-delta fibers. Pain mediated C-fibers will contribute to the creation of neurogenic inflammation which is a response to increased blood flow. There are no clinical quantitative tests to implement to diagnose individuals with trigeminal neuralgia in the present time, but by analyzing blood flow with a laser-doppler perfusion imaginer it can be possible to indirect measure pain.

Aim
Analyze the vascular axon reflexes of nervus trigeminus on healthy people, by collecting data referred as normal data to create a reference material to represent the healthy population. The main question of this study is, what is the view of the vascular axon reflex for people without facial pain and how does the intraindividual and interindividual variability look like between the left and right side of the face.

Method
With the help of a laser-doppler perfusion imaginer investigate 30 healthy people over 18 years without facial pain. The attempted areas are all the main trigeminal nerve branches on left and right side of the face, which is the forehead, cheek and chin. A light electric impulse will be given to create a neurogenic inflammation and at the same time apply the laser-doppler perfusion imaginer to measure the possible increase of blood flow.

Result
There was no significant side difference between left and right face half with p-values between 0.27-0.89. The upper normal limit for the absolute side difference for the measured areas are, forehead: max answer 69 percentage points and total answer 64 percentage points, cheek: max answer 68 percentage points and total answer 66 percentage points, chin: max answer 66 percentage points and total answer 58 percentage points.

Conclusion
A normal data has been created from 29 healthy subjects. The normal data can later be used to examinate if the method can be established as an objective diagnostic measurement for individuals with trigeminal neuralgia.

Role of free fatty acid receptors in regulating intestinal goblet cell intrinsic defense

By: Viet Linh Le

Bachelor thesis in Biomedical Laboratory Science performed at Mucin Biology Groups, Sahlgrenska Academy, University of Gothenburg, 2023.

Supervisors: George Birchenough Ph.D. Akshi Singla Ph.D.

Background: Inflammatory bowel diseases (IBD) are characterized by high or low chronic inflammation in the gastrointestinal tract. The cause of IBD is multifactorial and microbiota is one such factor. It is known that diet plays a role in the shaping of microbiota composition and might be a critical factor in the pathogenesis of IBD. Through diet, undigested dietary fibers metabolize into free fatty acids by the microbiota in the intestine. Free fatty acid receptor, Ffar2/Ffar4, stimulation has shown to decrease intestinal inflammation and regulate intestinal homeostasis. A specialized type of cell named goblet cells partakes in upholding intestinal homeostasis with the main function of creating a mucus layer that separates the microbiota from the epithelium wall. There is no prior evidence linking goblet cell specific free fatty acid receptors with microbiota-host signaling of goblet cells to regulate their defense functions.

Aim: The aim of this study is to investigate any difference in the microbiota and its composition, inflammation activity and colonic morphology between wild type mice (WT) and goblet cell specific Ffar2/Ffar4 knock out-mice (KO) at healthy state.

Method: At least eight goblet cell specific Ffar2/Ffar4 knock-out mice and eight wild type mice were employed for each analysis. PCR-amplification targeting Muc2-iCre gene and gel electrophoresis was used to determine the mice genotype of WT and KO. The inflammation activity was assessed via the inflammation marker Lcn-2 in fecal content using ELISA-method. Common bacteria taxa in microbiota were quantified with RT-qPCR. The colonic microbiota was spatially, quantitatively, and qualitatively assessed by 16S rRNA fluorescence in situ hybridization (FISH) as well as mucus production with WGA and UEA1 stain from colon tissue sections.

Result: Significant increase in quantity of *Bacteroides/ Bacteroidetes* and decrease of *Akkermansia* and *TM7* was observed in cohoused female Ffar4 KO-mice. Cohoused WT male mice had significant increase in *Gammaproteobacteria* compared to their Ffar4-KO littermates. Cohoused Ffar2 KO male mice had significant lower amount of total bacteria concentration than their WT counterpart.

Conclusion: The study shows no differences in crypt morphology, goblet cell count, inflammation, bacterial community structure nor mucus production between WT and Ffar2/Ffar4 KO mice. Data suggests goblet cell specific FFARs might affect the microbiota composition via unknown mechanism as part of goblet cell defensive functions but further research is needed.

Screening the variation in treatment response in patient-derived glioblastomas treated with a combination of radiation and a novel DNA-repair inhibitor

By Josefine Ljungberg

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Chemistry
Sahlgrenska University Hospital
Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor and Assistant Supervisor: Pegah Johansson, PhD. Aditi Banerjee.

Background: Glioblastoma multiforme (GBM) is the most prevalent malignant brain tumor in adults. Even when under treatment, the estimated median survival is about 15 months after diagnosis. The standard therapy includes surgical resection of the tumor followed by a combination of chemotherapy and radiation. However, alternative combination therapies are in the uprising. Ataxia-telangiectasia mutated kinase (ATM)-inhibitors have been developed to inhibit DNA-repair pathways in treated tumor cells in order to increase the treatment efficacy. This study aims to identify the individual treatment response of patient-derived GBM tumor cell to traditional and new therapies using the Cell Division Assay (CDA), with hope that the information can be used to identify biomarkers that enables development of individual treatment strategies.

Methods: Five patient-derived GBM tumor cell lines were treated in vitro with chemotherapy, radiation, two different ATM-inhibitors and a combination of radiation and ATM-inhibitors and then analyzed using the CDA. After treatment, the cells were incubated for 48 hours (24 hours when optimizing the assay) and there after the thymidine analog 5-ethynyl-2′-deoxyuridine (EdU) was added and the cells were incubated for an additional day. The dividing cells that had incorporated EdU were visualized using a Click-it protocol and analyzed in a flow cytometer. The cell sensitivity was then calculated by counting the EdU-positive cells in a treated sample and divide it with the number of EdU-positive cells in a non-treated sample from the same patient.

Results: The tumor cell lines showed heterogeneity in phenotype and response to treatment. Also, one ATM-inhibitor showed a trend to be more effective than the other. Though, because of the studies limitations regarding the CDA protocol and low replicate numbers, the experiments need to be repeated and the CDA further optimized in order to provide trustworthy conclusions.

Conclusion: This study highlights the heterogenous nature of GBM-derived tumor cells and their heterogenous response to treatment. Furthermore, the CDA is showing potential in order to assess treatment response which could be used to identify genetic biomarkers of treatment sensitivity for developing tailored treatment for GBM. To address the limitations of the study, further optimizations of the assay to obtain better precision is required.

Development of an LC-MS/MS method for quantification of Platelet Activating Factor in serum

By Petra Lundqvist

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor: Anders Nilsson, PhD

BACKGROUND: Lipids are essential for cellular functions and contribute to the cell's structure, serve as energy storage, and can act as mediators of signaling pathways. Platelet activating factor (PAF) is a potent lipid mediator produced by platelets, endothelial cells, macrophages, monocytes and neutrophils. Under normal circumstances PAF is maintained at a low quantity, but its synthetis peaks as a reply to inflammatory response. The activity of PAF is controlled by PAF acetylhydrolase (PAF-AH) which inactivates PAF.

Inflammation is a key component in Retinopathy of Prematurity (ROP) which affects retinal vascularization, primarily in premature infants born before 30 weeks of pregnancy. ROP is considered to be one of the leading causes of visual impairment and blindness in premature infants. Results from previous research has indicated that levels of PAF-AH and the severity of ROP is correlated.

AIM: The project aimed to develop a method for liquid chromatography in combination with mass spectrometry (LC-MS/MS) for quantification of PAF in serum. The ambition is to be able to measure PAF in low sample volumes of serum from premature infants to study the involvement of PAF in the development of ROP.

METHOD: The project started with optimization of the analyt fragmentation, using an Agilent 1260 Infinity LC system connected to an Agilent 6470 triple quadruple MS. The development of the method included both reversed phase and normal phase chromatography, evaluation of three different lipid extraction methods, and the creation of a standard curve. Sample materials included standard of PAF C-16 and PAF C-16d_4, plasma samples from adults and lipid free serum.

RESULTS: Normal phase chromatography presented better results in the separation of PAF compared to reversed phase chromatography. Evaluation of lipid extraction methods showed that Bligh & Dyer was the optimal method for PAF. During the project a contamination of PAF was discovered in the instrument which led to some results being considered unreliable.

CONCLUSION: The developed LC-MS/MS method cannot be considered as a functioning method for measuring PAF in serum. Further optimization is necessary before this method can be justifiably used for patient samples.

Effect of Ursodeoxycholic Acid on Skin Fibroblasts from Patients with Systemic Sclerosis

By Linnéa Lundström

Bachelor Thesis in Biomedical Laboratory Science Performed at the Department of Rheumatology & Inflammation Research, Sahlgrenska University Hospital, University of Gothenburg, 2023

Supervisor: Cristina Maglio, MD, Associate Professor, and Pradeep Kopparapu, Post-Doc

Background: Rheumatic diseases, including systemic sclerosis, pose significant challenges for patients and healthcare providers. Fibrosis, a hallmark of systemic sclerosis, results from aberrant activation of fibroblasts and excessive extracellular matrix deposition. Ursodeoxycholic acid is commonly used as treatment in liver disease and has shown promising anti-fibrotic effects in various tissues. Understanding the mechanisms by which ursodeoxycholic acid influences fibroblasts may lead to the development of novel therapeutic strategies for managing fibrosis in systemic sclerosis patients.

Aim: The current project aimed to determine if ursodeoxycholic acid has anti-fibrotic effects on human skin fibroblasts isolated from systemic sclerosis patients.

Material and methods: The project study was conducted in the frame of the Western Sweden Systemic Sclerosis Project. Skin biopsies were obtained from a subset of participants (two patients and three healthy donors) and later cultured to passage five, at which point they were stimulated with transforming growth factor beta 1 alone or in combination with ursodeoxycholic acid. Assessment of fibrotic markers (collagen type 1 alpha 1 and alpha smooth muscle actin 2) in fibroblasts was carried out by TaqMan gene expression assay and quantitative polymerase chain reaction.

Results: Stimulation with transforming growth factor beta 1 alone or in combination with ursodeoxycholic acid did not show any effect in neither patient fibroblasts nor healthy fibroblasts in terms of collagen type 1 alpha 1 and alpha smooth muscle actin 2 expression.

Conclusion: The small sample size and the heterogeneity of the samples cannot allow to draw any conclusion regarding the possible anti-fibrotic effect of ursodeoxycholic acid on skin fibroblasts from patients with systemic sclerosis.

Immunohistochemical staining patterns of P53 in ovarian endometrioid carcinoma

By Nabila Monir

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical pathology laboratory, Sahlgrenska University Hospital, Gothenburg, 2023.
Supervisor: Claudia Mateoiu PhD, Senior Consultant in Gynecological Pathology, Clinical Pathology, Sahlgrenska University Hospital.

Epithelial ovarian cancer, or ovarian cancer, is the second leading cause of death among all cancers affecting female reproductive organs. It is often incurable due to delayed diagnosis, with three-fourth all of cases being diagnosed in advanced stages. Epithelial ovarian cancer encompasses five distinct histotypes according to the fifth edition (2020) of the WHO classification: high-grade serous carcinoma, low-grade serous carcinoma, mucinous carcinoma, endometrioid carcinoma, and clear cell carcinoma.

Among these various genital tumors, endometrioid carcinoma and clear cell carcinoma are part of this classification. Endometrioid ovarian cancer accounts for 10% of all ovarian cancer cases. One primary cause of the development of endometrioid ovarian cancer is endometriosis, a condition where the growth of the uterine lining spreads to the pelvic region, fallopian tubes, uterine surface, or other pelvic organs. Another cancer associated with endometriosis is clear cell carcinoma.

The WHO categorizes endometroid carcinoma in the uterus into four molecular subtypes: POLE mutated, MMR deficient, no specific molecular profile, and P53 abnormal, which are associated with disease prognosis and treatment selection. Similar efforts are underway for endometroid carcinoma in the ovaries due to histological similarities with the uterus.

The aim of this study was to investigate the clinicopathological correlation of P53 abnormality, which is linked to *P53* tumor suppressor gene alterations, in ovarian endometroid carcinoma. The expression of the P53 protein was analyzed by immunohistochemistry using Tissue Microarray from 61 different archived samples of ovarian endometrioid cancer. Moderate variation in P53 expression was categorized as normal or wild type, and no expression, overexpression, or cytoplasmic expression as P53 abnormality.

In this study, a significant correlation was identified between P53 status and tumor grade ($P < 0.01$), as well as a trend to a variation in cancer stage depending on P53 status. In conclusion it was found that P53 abnormality tends to cause medium-/low-differentiated tumors in cancer stage II/III which corresponds to an aggressive and difficult to treat characteristic of ovarian endometroid carcinoma.

In conclusion, combination of immunohistochemical analysis of expression of the P53 protein with histological grading enables a correct diagnosis of ovarian endometrioid cancer but large-scale study is needed to confirm the highlighted data.

The frequency of extended-spectrum beta-lactamase-producing Escherichia coli from blood cultures at Södra Älvsborg Hospital is similar compared to other Swedish regions and lower compared to Southern Europa

By Shadi Moradi Sohi

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Microbiology, Södra Älvsborgs Sjukhus, 2023

Supervisor: Erika Lindberg (PhD certified biomedical analyst), Annika Ljung (physician in clinical microbiology)

BACKGROUND: Antimicrobial resistance is a growing global health problem that threatens the effectiveness of antibiotics. Antimicrobial resistance occurs when microorganisms such as bacteria develop the ability to adapt and survive in the presence of drugs that previously affected them. Antibiotic resistance and the occurrence of extended-spectrum beta-lactamase in *Escherichia coli* have caused major challenges in healthcare and agriculture. These strains have been found to be resistant to several classes of antibiotics.

AIM: This study aimed to analyze the development of antibiotic resistance and production of extended-spectrum beta-lactamase in *Escherichia coli* from blood cultures during one year at Södra Älvsborg Hospital and compare the results with other regions in Sweden and different parts of Europe.

METHOD: A total of 160 *Escherichia coli* isolates were tested. Levels of antibiotic resistance were determined by the disk diffusion method with antibiotic disks containing cefotaxime, ceftazidime, tobramycin, piperacillin/tazobactam, ciprofloxacin, trimethoprim sulfate, and meropenem on the Mueller-Hinton agar plate. Susceptibility levels were determined according to NordicAst (Nordic Committee on Antimicrobial Susceptibility Testing) species-related zone breakpoints. The *Escherichia coli* strains resistant against cefotaxime and/or ceftazidime were tested for ESBL by double disk diffusion test with antibiotic disks containing ceftazidime, cefotaxime, amoxicillin/clavulanic acid, cefepime and cefoxitin on the Mueller-Hinton agar plate. Synergy was investigated between amoxicillin/clavulanic acid and cephalosporins and the zone of inhibition around cefoxitin was measured. In addition, the Cloxacillin synergy test was used for the detection of the presence of ESBL M with antibiotic patches containing cefoxitin, cloxacillin, and ceftazidime on the Mueller-Hinton agar plate. Synergy was investigated between cloxacillin, ceftazidime, and cefoxitin.

RESULTS: The results show that the prevalence of antibiotic resistance and extended-spectrum beta-lactamase -producing *Escherichia coli* from blood cultures in Borås (8.7%) are similar to other parts of Sweden, the Västragötaland region (8.5%), Skåne (7.5%) and in Sweden as a whole (7.5%). On the other hand, a higher proportion is noted in the Stockholm region with cephalosporin-resistant *Escherichia coli* (19%) compared to other regions. The proportion of resistant *Escherichia coli* is close to the levels observed in central European countries such as Germany (9.4%), France (8.4%) and Austria (8.3%). In southern Europe, the resistance rate is higher, Italy (24.2%), Greece (23.3%) and Spain 14.8%.

CONCLUSION: This study highlights the global challenge of antimicrobial resistance, particularly focusing on the prevalence of antibiotic resistance in *Escherichia coli* strains isolated from blood cultures. The results underline that despite an increasing frequency of resistance to third-generation cephalosporins, it is still lower in Sweden compared to southern Europe. There are also some differences within Sweden. This suggests a common concern and highlights the need for joint efforts to address antimicrobial resistance.

Keywords: Antimicrobial resistance, *Escherichia coli*, blood infections, Extended-spectrum β-lactamases.

AUTOMATED AI-CALCULATION OF METABOLIC TUMOR BURDEN IN NEWLY DIAGNOSED PATIENTS WITH DIFFUSE LARGECELL B-CELL LYMFOMA

-A comparative study of the difference between AI- and manually calculated tumor volume.

By: Johanna Mörk

Bachelor thesis in klinical fysiology, preformed at Sahlgrenska Acadamy, Gothenburg University 2023. Supervisor May Sadik, Professor

Abstract

Background:

Total metabolic tumor volume (MTV) has been shown to be a good prognostic tool to asses progression-free and overall survival of different tumor diseases. MTV is not used in any greater occurrence in daily clinical work, due to lack of accurate methods. A tool that uses artificial intelligence (AI) could be of great value here. An AI-based tool for automated quantification of MTV in patients with Hodgkins lymfoma (HL) has been developed earlier. The aim is now to investigate whether this tool can be used to calculate MTV in patients with diffuse largecell B-cell lymfoma (DLBCL).

Methods

70 newly diagnosed and untreated patients with DLBCL, who had undergone stadging with FDG-PET/CT at Sahlgrenska University Hospital between 2019 and 2022 were included. Mean age was 55 years (range 17-90 years) and 39% were women. A biomedical analyst and a biomedical analyst student preformed manual tumor-segmentation in PET/CT-scans and calculated MTV.

The AI-tool then did the same segmentations and calculations and the results were compared.

Resultat

The results showed a significant ($p<0,001$) difference between manuel and AI-calculated MTV. Mean value for the manuel segmentation was 412 cm^3 (IQR 125-745 cm^3) and mean value for the AI-segmentation was 247 cm^3 (IQR 106-470 cm^3). In 46 (66 %) of the cases, the AI-tool calculated a smaller MTV then the manuel. The difference in calculated MTV increased withe the size of the total MTV.

Conclusion

The AI-tool developed for HL can also be used to segment and calculate MTV in patients with DLBCL. It underestimated tumors to a grater extent and the difference between AI- and manuel calculated MTV grew bigger with increased total tumor volume. A large tumor burden is often associated with a complex picture and high background uptake that makes interpretation more difficult.

In order for an AI-tool to be successful it needs to be trained on a large number of pictures with a great variation. The 70 patients in this study will be included in the training material but it will require a greater number of cases to reach a desired accuracy.

Method comparison between the automated urine particle analyzer UF-5000 and manual microcopy

By Emelie Ng

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, University of Gothenburg, 2023.

Supervisor: Ruth Wickelgren, PhD.

Background: Different urine particles for example erythrocytes, leukocytes and hyaline casts can be observed in microscopic analysis of urine sediment. An increased number of one or more different types of urine particles in urine sediment can be an indication of kidney disease. In the microscopic analysis of urine sediments there is for example an intra and inter-observer variation. With the development of automated instruments for particle analysis based on different types of technology, this has been reduced. The purpose of this study was to study the agreement between the fully automated urine particle analyzer UF-5000 and manual microscopy with respect to the results obtained from both methods, to provide a basis for whether UF-5000 can replace manual microscopy at Clinical Chemistry, Sahlgrenska University Hospital and to evaluate the performance of UF-5000.

Method: A total of 79 samples were analyzed for erythrocytes and leukocytes and for hyaline casts 68 samples were analyzed. The samples were analyzed in both UF-5000 and using a microscope. An evaluation of the intermediate precision of UF-5000 was performed. The statistical analysis used were sign test, confusion matrix, weighted kappa coefficient and calculation of the coefficient of variation.

Results: Based on the statistical analysis, 65% of 79 samples had exact agreement in UF-5000 and manual microscopy performed by a licensed biomedical scientist for erythrocytes, 67% of 79 samples had exact agreement for leukocytes and 75% of 68 samples had exact agreement for hyaline casts. The degree of agreement between both methods was moderate for hyaline casts and substantial for erythrocytes and leukocytes. When analyzing the intermediate precision of UF-5000, the coefficient of variation was high, between 50-96% for UF-CONTROL-L for both erythrocytes and leukocytes. While for UF-CONTROL-H the coefficient of variation was lower, between 6-18%. There was a skewed distribution and very large scatter among the measured values for intermediate precision.

Conclusion: In conclusion, the results indicate that the UF-5000 cannot currently replace manual microscopy and that further studies need to be done, where the analysis methods are standardized. In addition, a more thorough evaluation of the UF-5000 needs to be carried out, including analysis of carry over, linearity and intermediate precision.

Development of antibody assays for endemic human coronaviruses and their potential cross-reactivity to SARS-CoV-2

By Sarah Noory

Bachelor thesis in Biomedical Laboratory Science performed at the department of infectious diseases, institution of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2023.

Supervisor: Linn Persson Berg, MD, PhD

Abstract

In 2019, a new coronavirus called Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was detected which caused a global pandemic. This virus caused coronavirus disease 2019 (Covid-19), which has caused nearly 7 million deaths and more than 764 million confirmed cases reported by World Health Organization (WHO). SARS-CoV-2 belong to a broader group of coronaviruses and prior to 2019 the endemic human coronaviruses were considered to be -HCoV-NL63, -229E, -HKU1 and -OC43, which cause upper respiratory tract infections. SARS and MERS coronaviruses are known to cause severe respiratory disease in human but are much less prevalent as compared to the endemic human coronaviruses that are globally ubiquitous. The aim of this study is to identify whether antibodies directed against the S1 subunit of the spike protein of HCoV-NL63, -229E, -OC43 and -HKU1 cross-react with the S1 subunit of the spike protein of SARS-CoV-2.

The spike-1-protein (S1) of HCoV-NL63, -229E, -OC43 and -HKU1 were produced at the Mammalian Protein Expression core facility at Sahlgrenska Academy. Two SARS-CoV-2 antigens were also produced, one in Chinese hamster ovary (CHO) cells and one in immortalized human embryonic kidney cells (HEK 293). Serum samples from patients with previously polymerase chain reaction (PCR) confirmed infection with NL63 (n=10), 229E (n=10), OC43 (n=10) and HKU1 (n=10) were analyzed by indirect ELISA with the six produced S1-antigenes. In addition, 10 samples that had previously become anti-SARS-CoV-2 IgG-positive were also analyzed in the same manner.

Patients with previous HCoV PCR-confirmed infections (n=40) and the IgG seropositive SARS-CoV-2 patients (n=10) showed with our new ELISA methods antibody responses against the infectious viruses. Most patients also showed IgG reactivity to the other endemic coronaviruses, which was quite expected since HCoVs are commonly found in upper respiratory tract infections in humans.
In total, 38/40 analyzed serum samples from patients with previous PCR-confirmed infection against HCoV (NL63, 229E, OC43 and HKU1) showed no reactivity against the two SARS-CoV-2 antigens. The last two serum samples came from two patients with previous PCR-confirmed OC43 infection. These two samples showed little reactivity in the first assay but no reactivity when reassayed. Further analysis is needed to determine whether these samples are reactive or not. The results of our study indicate that S1-specifik antibodies show poor cross-reactivity between the S1 domain of the spike protein of HCoVs and SARS-CoV-2.

MESURMENT OF SERUM INSULIN LEVELS IN PATIENTS WITH ARTHRALGIA AND RHEUMATOID ARTHRITIS

By Rohullah Norozi

Bachelor thesis in Biomedical Laboratory Science performed at The Department of Rheumatology and Inflammation Research, Sahlgrenska academy, University of Gothenburg, 2023
Supervisors: Maria Bokarewa, professor

INTRODUCTION: Production of insulin by beta cells in the pancreas is necessary for glucose homeostasis and regulation of the energy balance in the body. Lack of insulin signalling can cause diabetes mellitus. Insulin resistance is a known risk factor of cardiovascular disease and diabetes type II. Cardiovascular disease is the main morbidity and mortality of patients with rheumatoid arthritis (RA). RA is a systemic inflammatory autoimmune disease that mainly effects the synovial joints and is characterised by immune cell infiltration in the joint.

AIM: This study was performed to get to know whether serum levels of insulin between patients with arthralgia (ALG) and RA differs, to study the relationship between insulin levels and RA development.

METHOD: Insulin levels were measured in a total of 669 patients with RA, referred by doctor for analysis of the arthritis-specific autoantibodies (ASAK), rheumatoid factor and/or anti-citrullinated peptide antibodies to the laboratory for clinical immunology at Sahlgrenska University Hospital the years 2012-2013 and 2018-2019.

RESULTS: Levels of insulin were significantly ($p < 0.0001$) higher in patients with RA as compared to ALG, and men had higher insulin levels among patients with ALG and RA. No differences in insulin levels were found between patients with ALG and RA with two different ASAK. Lowest insulin levels were detected in patients with ALG in pre-RA with no ASAK. However, pre-RA patients with two different ASAK had as high insulin levels as patients with RA.

CONCLUSION: The result of study showed that patients with RA had a higher level of insulin than ALG-patients in pre-RA stage. High insulin levels could indicate a pre-RA phase.

Association between malnutrition and gastroenteric microorganism in under five years old children from Rwanda, whit real-time PCR

By Alva Olsson

Bachelor thesis in Biomedical Laboratory Science performed at Department of Clinical Microbiology, Sahlgrenska University Hospital, 2023.

Supervisor: Maria Anderson, Senior Biomedical Laboratory Scientist, PhD

Introduction: The association between malnutrition and intestinal parasitic infections is asserted. A connection where the undernourished children is at greater risk at being infected and the infections can lead to malnutrition. Addition to parasitic infections, can viral and bacterial infections cause gastroenteritis and may correlate whit malnutrition.
Aim: The aim of this thesis is to validate real-time PCR systems for helminths also too compare treatment whit proteinase K before DNA extraction whit DNA extraction whiteout proteinase K treatment, for detection of giardia. Subsequently use this and other real-time PCR systems to investigate the association between malnutrition and microbes.
Method: Rectal swabs from children under fem years old from Rwanda was collected, 72 from malnourished children and 72 children whiteout malnutrition matched in age and gender. The incidents of different microbes were detected whit real-time PCR, whit also was used to validate different real-time PCR systems for helminths and to compare treatment whit proteinase K before DNA extraction whit DNA extraction whiteout treatment whit proteinase K for detection of giardia.
Result: In total nine different real-time PCR systems for helminths was tested, seven systems could be validated. The sample that was treated whit proteinase K had a higher cycle threshold value than the sample which DNA was extracted from whiteout treatment whit proteinase K. The association of malnourished children and prevalence of microbes was not statistically significant.
Conclusion: To conclude the malnutrition in under five years old children from Rwanda is not caused by a specific microbe. Other determents such as living conditions will lead to reinfections of the different microbes whit the malnutrition is a consequence of.

Quantitative PCR of intestinal biopsies from children with and without celiac disease reveals differences in gene expression.

By Ida Olsson

Bachelor Thesis in Biomedical Laboratory Science preformed at Clinical Genetics, Sahlgrenska Academy, University of Gothenburg 2023
Supervisor: Åsa Torinsson Naluai, Docent

Celiac disease is a common autoimmune disease, affecting about 1% of the population. Unfortunately, a lot of people are undiagnosed, especially in developing countries. In Sweden 2% are estimated to be undiagnosed. With previous research it has been found that the disease has a strong genetic association and that HLA DQ2 and HLA DQ8 essential to develop the disease. However, HLADQ2/8 cannot take full reasonability of the genetic background. Still, the complete pathogenesis and the reason to why people develop celiac disease has not been determined. In previous research it was found that the genes CXCL11 and IL17A was upregulated in intestinal biopsies from celiac patients compared to a control group.

In this project we examined differences in gene expression of the genes LPL, CXCL9, CXCL3, IL17A, CXCL11, LCP1, SLAMF7, KCNJ10, SAA2, LYPD5, TP73, TSN4, ARHGEF3 of 190 intestinal biopsies from children. The children were included in five different groups depending on their diagnosis; celiac disease, latent celiac disease, treated celiac disease, Crohns disease and controls. qPCR with ΔΔCT method was performed with YWHAZ, ACTB and HPRT1 as reference genes. The purpose was to see if any significant differences in gene expression between the groups could be found.

With Bonferroni corrected significance level, it turned out that CXCL9 was upregulated by 218% in celiac patients compared to the control group. CXCL11 was 565% more expressed in celiac patients compared to controls, LPL was 806% more expressed in celiac patients compared to controls and SLAMF7 was 47% more expressed in celiac patients compared to controls.

Atp10a and its role in cardiomyocyte function

By Duha Omra

Bachelors thesis in Biomedical Laboratory Science performed at Wallenberg Laboratory for Cardiovascular and Metabolic Research within the Department of Molecular and Clinical Medicine, Department of Medicine, University of Gothenburg

Supervisors: Linda Andersson, PhD and Malin Levin, Professor

BACKGROUND: Cardiac remodeling involves maladaptive processes in the heart, which can result in heart failure. In heart failure, there are changes in the metabolism, and sphingolipids, especially glycosphingolipids that affect heart function. By increasing the understanding of genetic factors that affect cardiomyocyte function, we can obtain a deeper insight into the mechanisms underlying heart failure. Preliminary results within the research (Levins group) identified *Atp10a* as an interesting candidate gene that potentially affects cardiomyocyte sphingolipid levels. It has previously been shown that *Atp10a* is a gene that codes for a protein in the P-type ATPase family and the protein is involved in phospholipid transport across the cell membrane. It is not yet known how Atp10a affects the function of cardiomyocytes**. AIM:** The aim of this study is to clarify the mechanisms behind how the *Atp10a* gene affects cardiomyocyte function.

METHOD: To study the function of Atp10a in cardiomyocytes, a cultured cardiac muscle cell line (HL-1-cardiomyocytes), which contract and retain phenotypic characteristics of the adult cardiomyocyte, were used. The expression of the *Atp10a* gene was inactivated using Atp10a-siRNA for 48 h (n=4 for Atp10a-siRNA and n=4 for negative control-siRNA). The qPCR-TaqMan method was used to study how efficient the inactivation of the *Atp10a* gene was and then markers for cardiomyocyte function were analyzed using qPCR-TaqMan and western blotting. Sphingolipids were analyzed using mass spectrometry.

RESULTS: Inactivation of the *Atp10a* gene by siRNA showed an 89.3% reduction of Atp10a-expression in cultured HL-1-cardiomyocytes. Mass spectrometry results showed no significant change in phosphatidyl choline and lactocylceramide, but a significant decrease in sphingomyelin, ceramides, and dihydroceramide, as well as a marked increase in glucosylceramide by 348% after *Atp10a* inactivation. The gene expression of *Ugcg*, the gene encoding the enzyme that synthesizes glucosylceramide, increased by 101.3%. Studies of the cardiac markers Nppa and Nppb showed a significant increase, indicating an impact on cardiomyocyte function. Western blot results showed a significant change in the ratio of the proteins Bax/Bcl-2, which are important for apoptosis. In addition, the results showed a significant impact on ULK and of p-p70S6K, which activated the autophagy signaling pathway.

CONCLUSION: Atp10a plays a role for cardiomyocyte function. The increase in glucosylceramide and changes in cardiac markers indicate deterioration of cardiomyocyte function upon inactivation of *Atp10a*. We saw the impact on the mTOR signaling pathway, which is relevant for cellular growth and survival. We also found activation in apoptosis and hypertrophy signaling. The researchers are investigating the mechanisms further, which can contribute to a more comprehensive understanding of Atp10a function in the heart and its influence on various cellular processes.

Evaluation of cDNA synthesis
A partial step in the diagnosis of acute myeloid leukemia

By Emma Borg

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Genetics and Genomics, Sahlgrenska University Hospital, 2023

Supervisor: Julia Asp, Docent and Assistant Supervisor Ulrika Odén, Biomedical Scientist

Background: AML is a form of blood cancer and is characterized by uncontrolled clonal expansion of hematopoietic precursor cells. Genetic changes, such as fusion genes, are a causative factor for the disease, which are important to identify to determine the prognosis and find a suitable treatment. For quantification of fusion genes, the qPCR technique is used to analyze RNA in leukocytes. Then the RNA needs to be turned into cDNA, which is done with a reverse transcriptase. The process is called cDNA synthesis.

Aim: The purpose of this study is to compare two different cDNA synthesis kits with the kit that is currently used for diagnostics of AML, at Genetics Laboratories at Sahlgrenska University Hospital, to see if more sensitive detection can be achieved in routine diagnostics.

Methods: Thirty patient samples were obtained and analyzed for inv16A, *NPM1* or t(8;21). RNA extraction was performed, and the samples were pooled based on the genetic change into six pools of five samples each. Two cDNA synthesis kit IV VILO (SuperScript IV VILO Master Mix) and TATAA (TATAA GrandScript cDNA Synthesis Kit) were compared with the cDNA synthesis kit VILO (SuperScript VILO cDNA Synthesis Kit) currently used in routine diagnostics. Each cDNA kit was tested with all pools in three rounds. qPCR was performed for each pool and a comparison between the reference gene *ABL1* for the three different cDNA kits was made based on gene expression. A statistical test was performed to detect any differences between the kits for each pool. An optimization was performed where two pools were used and where the kit's protocol was adjusted regarding time and temperature.

Results: The result showed that the currently used kit in routine diagnostics, VILO, had the highest gene expression of the tested kits for all pools but was the worst choice from time- and labor perspective. VILO was closely followed by IV VILO while TATAA had very low expression relative to the other kits. Optimizing of IV VILO showed an improvement but was not expressively better than the VILO kit.

Conclusion: The study has shown that the cDNA kit VILO clearly has the best yield, but that IV VILO with an optimization reaches similar levels. A change of kit in the routine diagnostics of AML can thus be considered from a time- and labor perspective if further tests show similar results.

Comparison of Tensol70 and epoxy resin as casting media - aims to optimize the perfusion corrosion technique

By Viktoria Redjepagic

Supervisor Diana Martins, PhD

Bachelor Thesis in Biomedical Laboratory Science performed at School of Health Technology of Coimbra, institute Biomedicine.

Preservation of organs is essential for studying the anatomy and for us to gain better understanding and knowledge of its structure like branching patterns and internal architecture in use for research and teaching. Corrosion casting technique have been discovered as a new preservation method and the current study aims to optimse and implement the technique as a long-term preservation method of cardiovascular- and kidney system by comparing different casting media. Perfusion corrosion is a method which enables a three-dimensional cast of the blood vessels of organs and tissue to be formed by injecting a casting media into the vessels. The replica of the vascular structure can be visualized through corrosion of the surrounding tissue. In previous study, Tensol70 and two different epoxy resins were used as casting media and potassium hydroxide as corrosion solution. Specimen such as bovine heart and piglet heart and kidneys were studied.

Casts obtained from Tensol70 were hard and detailed, vessels such as right coronary artery, left coronary artery, circumflex branch, anterior interventricular branch, and the finer vessels at apex were observed, nevertheless renal artery, and renal urine complex. Injection with epoxy resin (La Parajita) resulted in softer and less detailed replica. Meanwhile, the epoxy resin (Barbot) was not resistant to potassium hydroxide which contributed to maceration of the cast.

In conclusion, perfusion corrosion is a good technique to study structures in both normal- and pathological specimens, moreover use as preservation method. Tensol70 is recommended as casting media if the purpose is to study smaller specimens since it polymerizes within an adjustable time period and is resistant to potassium hydroxide, which is an effective corrosion solution. However, the method must be developed to be able to use the technique to obtain a cast of the venous system.

VERIFICATION OF A LC/MS/MS METHOD FOR DETECTION OF BENSOYLECGONINE IN URINE

By Sara Sandberg

Bachelor thesis in Biomedical Laboratory Science performed at the Norra Älvsborgs länssjukhus with in NU Hospital Group, Sahlgrenska Academy, University of Gothenburg, 2023

Supervisor: Johanna Rudbäck, Chemist with a PhD

Kamil Slupecki, Biomedical Scientist

Introduction: Cocaine is a stimulant of the Central nervous system and is strongly addictive. Cocaine can after intake give symptoms like delusion, tremor and ailment. In case of higher doses other more severe symptoms like psychosis can occur. Cocaine is metabolized to various substances in the human body, most of the consumed cocaine metabolized to benzoylecgonine. This procedure starts directly after intake and therefore is it more common to use one of cocaine's metabolites as an analyte, because it can be detected under a longer period. Clinical drug analysis is performed in two steps, first screening and then verification of positive tests which are analysed with a quantitative method. The most common verification method for drug analysis is LC/MS/MS.

Aim: In this study the purpose was to verify a analyse method for benzoylecgonine as a verification for detection of cocaine in urine with LC/MS/MS instrumentation. Two Agilent 6470 Triple Quadrupole 1290 instruments.

Materials and method: In the study two Agilent 6470 Triple Quadrupole 1290 instruments were used equipped with Waters Acquity UPLC HSS columns. Initially a method was developed with an optimization. Later tests for precision, accuracy and stability were performed. Tests that were also included were precision test, method comparison, carry over test, LLOQ and Ion suppression test. The matrix used in this study was urine. The sample consisted mainly of calibrators diluted to different concentrations, blank urine and 25 de-identified patient samples.

Result: The result indicates that the created method had good precision, accuracy and stability. The correlation between the old and the new instrument was 95,6% for the whole selection of 25 de-identified patient samples which is within the designated acceptable value of 95%. Correlation for samples within the measurement interval that the method was calibrated for was 97,5%.

Conclusion: The method created in the study is verified and can be used on the new instrument.

THE GAP JUNCTION-MEDIATED ELECTRICAL SIGNALING BETWEEN BETA-CELLS AND DELTA-CELLS:
Investigating cytoarchitectural alterations during onset of type-1-diabetes

By Lamija Sefer

Bachelor thesis in Biomedical Laboratory Science performed at the University of Gothenburg
Sahlgrenska Acadamy, University of Gothenburg, 2023
Supervisor: Caroline Miranda, postdoctor

Background: Islets of Langerhans in the human pancreas consists of beta-cells which secrete insulin, alpha-cells which secrete glucagon as well as somatostatin-secreting delta-cells. Type-1-diabetes (T1D) is an autoimmune disease that causes the destruction of beta-cells due to genetics and environmental factors playing a part. Although, in order to cure diabetes, studies have been focusing on beta-cells only and not interactions with these other cells, which can play a big role with T1D. One way for cells to communicate is through gap-junctions and the proteins forming them are called connexin (Cx), where six Cx molecules make up a hemisphere. This hemisphere, also called connexon, when connected to another connexon on a different membrane make up the intercellular channel. In the pancreatic islets, the most prominent Cx protein is Cx36 and is expressed by insulin-producing beta-cells.

Aim: The goal of this study was to investigate cytoarchitectural changes during onset of T1D. It was also a goal to find out what happens with the gap-junction coupling between beta- and delta-cells at the same time.

Methods: Three different types of islets of Langerhans were stained with immunofluorescence. Those were non-obese diabetic (NOD) mice, wild type (WT) mice and human type 2 diabetes islets. After staining with different antibodies, images were taken with a laser scanning confocal microscope which picked up on fluorochromes depicting different targets of structure like insulin, somatostatin and Cx36.

Results: There was strong colocalization between Cx36 and beta-cells, indicating communication through gap junctions. The findings support the prominent association of Cx36 with beta-cells compared to delta-cells, highlighting intercellular communication within the beta-cell population. Furthermore, the cell numbers decreasing in T1D islets confirms the impact of the disease on beta-cell populations.

Conclusion: Gap-junction mediated form of communication was expressed the most between beta-cells but occurs in delta-cells as well.

Development of a finger-prick test for dementia

By Nellie Simonsen

Bachelor's thesis in Biomedical Laboratory Science performed at the department of Psychiatry and Neurochemistry, Institution of Neurology and Physiology, Sahlgrenska Academy, University of Gothenburg, 2023.

Supervisor: Nicholas J. Ashton, Assistant Professor and Postdoctoral Research Associate.

Co-supervisor: Hanna Huber, Postdoctoral Researcher.

BACKGROUND: There has been recent progress made in measuring neurodegenerative biomarkers in blood, but with sampling through finger-prick cards that do not require centrifugation or transport in cold storage, inexpensive remote collection of dry-plasma spots for large-scale studies and early diagnosis of dementia could become possible.

AIM: The purpose of this work was to test the potential of measuring the neurodegenerative biomarkers p-tau181, NFL, GFAP, Aβ40 and Aβ42 in dry-plasma spots (DPS) from finger-prick tests and optimize the plasma extraction method for analysis on Single-molecule array (Simoa).

METHOD: Noviplex Duo cards were spotted with whole blood and tested in different storage conditions of room temperature (RT), 4C and 37C for up to 4 months. Elution with different buffers were tested to see if processed DPS eluates correlated with the sample's plasma concentrations measured with two commercial Simoa assay kits: Neuro 4-plex (N4PE) and p-tau181. IgG concentrations were measured in eluate with Nanodrop to investigate heterophilic antibody interference. Statistical analysis of change in concentration over the storage time was performed with repeated measures one-way ANOVA and Wilcoxon signed rank test. Associations between buffers, reference plasma and IgG were tested with linear regression analysis and the two-sided significance set at $P<0.05$ for all statistical analyses.

RESULTS & CONCLUSIONS: All analytes except for Aβ42, which fell below the detection limit, could be measured in DPS after storage in all tested temperatures, but some variation in concentration could be seen for Aβ40 and GFAP over the 4 months. Although p-tau181 was stable in the storage tests, an association with standard plasma measurements was not seen for the analyte in the buffer tests, so the storage effects of it could not be concluded. As for the N4PE analytes, significant associations between plasma and eluate was found for NFL, GFAP and Aβ40 with the N4PE assay diluent in a 1st buffer test, but were only repeated for NFL in a 2nd buffer test and thus further optimization would be required for consistent results. In the buffer tests, only some analytes showed an association with standard plasma measurements after elution with PBS and the Homebrew buffer C, D and E. However, a correction of IgG concentrations in p-tau181 eluted with Homebrew buffer E yielded results which associated with plasma and could potentially be used as an elution buffer instead of the p-tau181 assay diluent. In conclusion, a common buffer was not found for the N4PE and p-tau181 assays, however analysis of NFL, GFAP and p-tau181 in DPS is possible with this method using the N4PE assay diluent for the N4PE assay, and Homebrew buffer E for the p-tau181 assay, though optimization is required to yield consistent results that associate with standard plasma measurements.

EVALUATE IF CHANGES IN INTER-CRYPT GOBLET CELLS OCCUR DURING INTESTINAL INFLAMMATION DEVELOPMENT

By Rebecka Simre

Bachelor thesis in Biomedical Laboratory Science performed at the Lundberg laboratory, Sahlgrenska Academy, University of Gothenburg, 2023
Supervisors: Prof. Dr. Malin Johansson and Dr. Francesco Suriano

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Background: The human gastrointestinal tract (GI) is covered by mucus which acts as the interface between the host and the gut microbiota. MUC2 and Fcgbp proteins are the two major mucus components. The intestinal mucus is secreted by a heterogenous population of mucus-producing goblet cells (GCs) and a newly identified population, fast mucus-producing inter-crypt goblet cells (icGCs), creates one of the two different layers protecting the colon. The importance of icGCs, the mucus component Fcgbp and of the gut microbiota is still not thoroughly investigated. **Aim:** Define the importance of icGCs, the mucus protein Fcgbp and the gut microbiota in the protection of the intestinal epithelium in colitis-mouse models. **Method:** Distal colonic tissue samples from IL-10 and Fcgbp deficient mice were collected for ApoMuc2, MUC2, and Fcgbp immunostaining and imaged with confocal and light microscope. Additionally, distal colon tissue from IL-10 mice and their respective controls was fixed and analyzed as tissue wholemounts. Fcgbp deficient mice body weight were measured before, and during, receiving treatment with DSS. After finalized treatment, colon length was measured and distal colonic tissue samples were collected. To further investigate the importance of gut bacteria, a bedding transfer experiment was performed for 4 weeks. Thereafter, the mice were treated with DSS, followed by measurement of colon length and distal colonic sample collection. All handling of the mice in this study was performed by competent and educated personnel. **Results:** A significant decrease in icGC number was observed in 8-week-old IL-10 deficient mice compared to the control group. Fcgbp deficient mice showed no difference in MUC2-expression compared to the wildtype. The DSS-treated Fcgbp deficient mice showed no significant difference in initial body weight. However, a significant decrease in body weight and in colon length during the DSS-treatment were observed, along with an increase in DAI-score compared to the control group. The bedding transfer resulted in a significant reduction in the colon length in male mice only. **Conclusion: Altogether, our results underlined (i) the number of icGCs is impaired during colits development, (ii) the importance of Fcgbp protein in the protection of the intestinal epithelium, and (iii) the lack of Fcgbp might be associated with changes in the gut microbial communities.**

Effects of caffeine on cognitive function and cortical activation patterns in young adults with and without sleep deprivation - A randomized controlled trial

By: Julia Sjögren

Bachelor thesis in Biomedical Laboratory Science performed at Coimbra Health School, Polytechnic Institute of Coimbra, 2023
Supervisor: Telmo Pereira, Senior Lecturer, Ph.D.

Introduction: Caffeine is a mild psychostimulant commonly known to enhance cognitive function in various contexts. However, its effectiveness on task performance remains a topic of debate, as previous studies lack consistency in results. The aim of this study was to evaluate the effect of caffeine on cognitive function and cortical activation patterns in participants under different sleep conditions. In turn, the cognitive benefits of this habitually used substance could be determined.
Methods: A randomized and controlled trial was conducted using a within-and between subject crossover study design. Fifteen young adults performed four increasingly demanding tasks of the Double-Stroop test before and after caffeine consumption, once after normal sleep and once after sleep deprivation. Reaction times and accuracy rates were used to analyze the behavioral neuromotor performance, and event-related potentials and event-related spectral perturbations from the EEG recordings were used to analyze the cortical activation patterns.
Results: Results showed a significant effect of caffeine on cognitive function in the sleep-deprived condition, specifically in the more cognitive demanding tasks of the Double-Stroop test. Participants had faster reaction times and higher accuracy rates after caffeine consumption, with a corresponding increase in cortical activation patterns observed through EEG recordings. No significant beneficial effects were observed in the normal sleep condition.
Conclusions: These findings suggest that caffeine can improve cognitive performance in sleep-deprived young and healthy adults, particularly in more cognitively demanding tasks.

Neuronal calcium binding protein 1 expressing the functional role of dorsal horn neurons in touch and movement

By: Mimmi Sjöstedt

Bachelor thesis in Biomedical Laboratory Science performed at the department of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, 2023
Supervisor: Line Löken, PhD

Background

Sensory stimuli are processed in parts of the dorsal horn, where nerve fibers transport stimuli into the spinal cord. Stimuli of various forms are associated with different types of nerve fibers. Neuronal calcium-binding proteins 1(Necab1) are expressed in neurons thought to be involved in sensory stimulation in the dorsal horn. Knowledge of the neural circuits involved in making touch stimuli painful after nerve damage is still limited. In order to map neural circuits and increase the understanding of these neural circuits in the dorsal horn, they need to be studied more closely.

Aim

This study is part of a larger project on the subject of chronic pain conditions. To investigate and to understand the functional role of whether Necab1 expressing neurons are activated by touch and movement.

Methods

In order to study neuronal activity in relation to different stimuli, immunohistochemistry is utilised to detect the expression of Necab1 in the dorsal horn. By using inducible tamoxifen transgenic mice with endogenous fluorescent markers for c-Fos, one can show whether a neuron has been active by emitting a fluorescent light. The Necab1 antibody labels the outside of the neuron and the neuronal activity after tamoxifen injection is expressed inside the neuron. Using these techniques together, one can detect whether a activated neuron belongs to the group of neurons expressing Necab1, focusing on a normal pattern of activity in the mouse.

Results

This study confirms that Necab1 expressing neurons are localized in the dorsal horn of the mouse. They also showed one clear colocalization and that there were 9 indications of overlap between Necab1 and the activity of movement and touch stimuli in the same neuron.

Conclusion

Further study is needed to explore the connection between activated neurons and Necab1 expressing neurons. While this study shows a possible connection, futher studies are needed to confirm this result.

A COMPARISON BETWEEN TWO GENERATIONS OF HLA-GENOTYPING,

an evaluation of LABtype™ SSO PCR contra AllType™ FASTplex™ NGS

By Golenoush Talebzadeh

Bachelor thesis in Biomedical Laboratory Science performed at the Tissue Typing Laboratory,

Sahlgrenska Academy, University of Gothenburg, 2023

Supervisor: Pauline Isakson, PhD

Abstract

Human leukocyte antigen (HLA) complex is the most polymorphic gene cluster that codes for the surface antigens found on most human cells and can be recognized by the body's immune system, among other things, to distinguish between the body's own and foreign cells and presents foreign antigens. The great variation it causes among the population and its role in the immune system make HLA genotyping necessary before transplants to ensure that the recipient receives tissue (stem cells/organs) whose HLA genotype is as similar to theirs as possible. This is to minimize the risk of transplant rejection and transplant complications. HLA genotyping performed using methods that provide a higher resolution has been shown to improve transplant efficacy and recipient survival. Next Generations Sequencing (NGS) is a very promising method that can sequence HLA genes with a high resolution at the allele level. Whether the method can eventually be applied in routine operations is therefore relevant. During the study, an NGS-based method, AllType™ FASTplex™ NGS, was compared with a well-established molecular biology method, LABType SSO-PCR, which is routinely used for HLA genotyping. HLA genotyping of 5 loci (HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ) was performed on 16 samples using both methods. The result showed that the NGS-based method succeeded in resolving several ambiguities that had arisen with SSO-PCR and importantly NGS resulted in high resolution genotyping. However, the method is relatively new and several comparisons with a larger number of samples are needed before validation for use in routine operations.

Studies of the measurement of Breslow thickness in primary cutaneous malignant melanoma: variation in digital and analog measurement and variation in z-direction

By Isaac Joel Torres Muller

Bachelor thesis in Biomedical Laboratory Science perfomed at the department of clinical pathology at Sahlgrenska University Hospital, 2023.
Supervisor: Iva Johansson, PhD.

Background: Malignant melanoma of the skin is a very aggressive tumor disease in which even small primary tumors can lead to a metastatic process and death. The incidence increases by about 5% each year in the light-skinned populations. In early disease (clinical stage I-II), the prognosis is primarily determined by the thickness of the primary tumor, measured according to a classic method described by A. Breslow in 1970. Breslow thickness is measured under a microscope or on digitally scanned images, forming the groundstone for staging. Correct measurement is critical as the tumor stage then controls the entire continued treatment and follow-up of the patient. Preparation techniques in the pathology laboratory also play a central role in producing adequate histological images and, thus, a key function in the diagnostic process. As the tumor is a three-dimensional structure and histological sections provide a two-dimensional image, the Breslow thickness can vary depending on the position of the examined sections within the tumor.

Aim: The purpose of this study was to compare the measurement of Breslow thickness under a microscope and digitally. Furthermore, to investigate where within the tumor the measurement point of Breslow thickness is usually located and to analyze the variation of the Breslow thickness in a hypothetical z-direction.

Method: 80 primary cutaneous melanoma cases were identified (SNOMED code malignant melanoma M87203) in pathology archives. The cases were not previously immunostained. Following exclusion, a total of 55 cases were included in the study. For each included case, one original slide with the thickest tumor representation was scanned. A new Breslow measurement was performed for all cases by an experienced senior consultant in pathology, both under the microscope and digitally. Furthermore, the variation of the Breslow thickness in z-direction was studied by a digital measurement within a 3 µm wide band-shaped area centered around the point with the Breslow thickness.

Results: The point for Breslow thickness meassurement was located in the central portion of the tumor in 96% of the cases. Analog and digital measurements resulted in change of T stage in 20% and 23%, Cohen´s kappa 0,726 and 0,626, when compared to the original diagnosis. The intra-observer kappa was 0,739 with changes of T stage in 19%. In z-direction, 31% of the cases changed T stage.

Conclusion: In our cohort, the measurements of Breslow thickness showed a good agreement between different pathologists if performed under a microscope. Measurements with digital tools can result in a significant differences compared to measurements under a microscope and cause a change of tumor stage. Breslow thickness can vary significantly in z direction within a block section with change of T stage in 31% of the cases if another slide section was randomly chosen.

Stability study of five selected analytes in plasma or serum stored on separator gel in room temperature after centrifugation

By: Emmelie Zarén

Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Chemistry, Södra Älvsborgs Hospital, Borås, 2023
Supervisor: Tommi Tallheden, PhD. Co supervisor: Christina Suhonen, Biomedical scientist

Introduction: That a sample is analysed directly after sample collection is desirable but not always possible. There are several factors that could delay the analysis process and in those cases it becomes important to know the stability of the analytes.

The aim of this study was to investigate the stability of cortisol, procalcitonin, total PSA, free PSA and TPO-antibodies in plasma or serum when they were stored on separator gel in room temperature after centrifugation.

Method: The sample material used for the study consisted of plasma or serum that remained after routine analysis at Södra Älvsborgs Hospital. Ten samples were used for each analyte besides for TPO-antibodies where two samples were included. A reference sample was collected and frozen at -80 ℃ two hours after the blood was collected from the patients. The sample was then kept in room temperature for up to 72h. Aliquots was collected and frozen after 4h, 24h, 48h and 72h. All samples were then thawed and analysed in batches for each analyte. The results were then normalized and the average percentage change at each time point was compared to a calculated total change limit (TCL) that was set to be the acceptable limit for stability. If the average percentage change at any time point exceeded the TCL, the change interpreted as clinically significant, and the analyte considered to be unstable. TCL is a combination of the analytical variation and the intra individual biological variation.

Results: When the average percentage change of the different time points were compared to the calculated TCL for each analyte it was seen that the result of cortisol, total PSA and TPO-antibodies did not exceed the TCL at any time point. For procalcitonin the average percentage change at the time point 24h exceeds the TCL and for free PSA TCL was exceeded at the time point 4h.

Conclusion: Cortisol, total PSA and TPO-antibodies could be analysed up to 72 hours, procalcitonin up to 24 hours and free PSA up to 4 hours after the samples were collected from patient without any clinically significant change in concentration. All the analytes in the study where stable on separator gel in room temperature longer than what was known before.

Species Identification of New Marine Bacteria from Baumann's Collection Using Next-Generation Sequencing

By Amjad Zaytoun

Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Microbiology, Sahlgrenska University Hospital, University of Gothenburg, 2023.

Supervisors: Erika Tång Hallbäck, PhD & Liselott Svensson Stadler, PhD.

In 1971, Paul and Linda Baumann conducted a study to examine marine bacteria, isolated from the Pacific Ocean around Hawaii, using chemical, physiological, and morphological analyses. In 2022, a student at CCUG (Nikolai Basic) conducted a study by 16S rRNA sequencing of 95 strains to verify if Baumann's species identification still held true. Six strains could not be identified and were suspected to be new. The purpose of this study was to use next generation sequencing to for species identification of these bacterial strains. Two different methods were used and compared: Ion Torrent and Illumina sequencing. The analysis of the results employed Average Nucleotide Identity on the JSpecies platform and digital DNA-DNA hybridization on the Type Strain Genome Server platform. Phylogenetic trees based on whole-genome sequencing and the 16S rRNA gene were also compared for all strains.

Strain CCUG 16168 had a high matching percentage against *Psychrobacter sanguinis*, but it was excluded from the Illumina sequencing due to a high level of contamination in its sequence. The results from Illumina and Ion Torrent sequencing for the other strains were identical, although Illumina exhibited lower coverage compared to Ion Torrent, but still sufficient. Illumina was shown to be more sensitive in determining the number of contigs. None of the strains showed a sufficient match to any strain in the reference databases; therefore, all strains were considered new. CCUG 16159 and 16160 were identical and were found to be the same species, showing similarity to several genera but most closely related to *Leisingera*. The results also indicated that CCUG 16165 and 16166 are identical and belong to the same species as CCUG 16023, within the genus *Marinobacterium*.

In conclusion, based on this study, all strains, (the contaminated strain CCUG 16168 not included), are new species or could even represent new genus, and have not previously been identified. For further species or genus identifications, additional analysis for confirmation would be required such as performing Long Read Sequencing and core genome comparison with closely related type strains.