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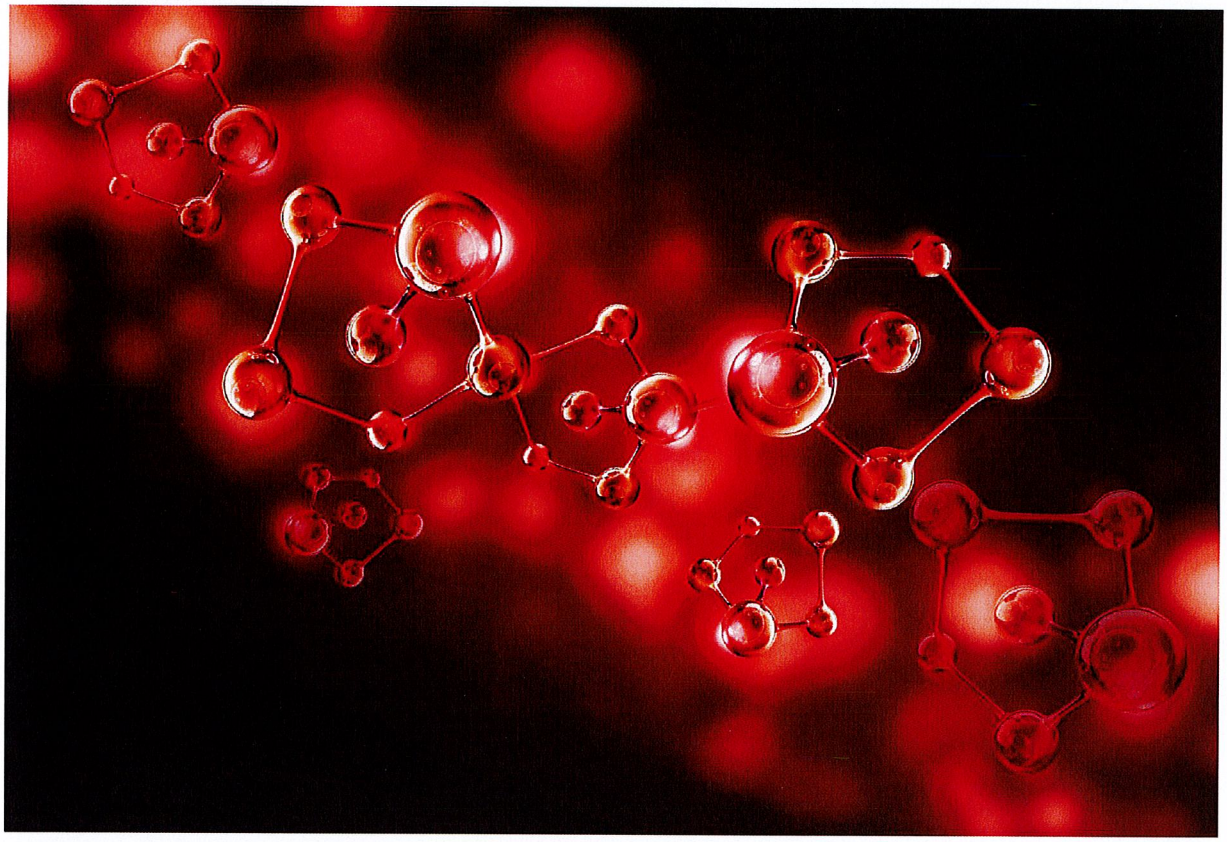

ABSTRACT BOOK 2020

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

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By: Hashim Abdul Aziz

Bachelor thesis in Biomedical Laboratory Science, University of Gothenburg, 2020
Clinical microbiology laboratory, at the regional hospital in Västerås, Sweden

Supervisor: Sandra Carillo Lopez, Process Manager Clinical Microbiology in Västerås, Daniel Heimer Chief Physician Clinical Microbiology in Västerås and Susanne Markström, Head of Clinical Microbiology in Västerås

Background

Helicobacter pylori (H. Pylori) is a fastidious, gram-negative, microaerophilic, spiral-shaped bacterium. It is generally acquired in childhood. About 50% of world's population are infected with it and it is associated with many stomach and intestine diseases like gastritis, peptic ulcer and adenocarcinoma. *Helicobacter pylori* is diagnosed by invasive and non-invasive methods. Each method has its advantages and disadvantages. Detecting of H. Pylori antigen in the stool is one of the non-invasive methods, which can be done by both ELISA and Turbidimetry method. The aim of the study is to evaluate and compare turbidimetry method with ELISA method in detection and determination of H. Pylori antigen in the stool.

Material and Methods

Fecal specimens were collected and stored at -20 ° C until analysis was performed by both methods. The samples were left at room temperature to thaw before analysis processes. In the beginning, feces specimens were tested with ELISA method (The Amplified IDEIA ™ Hp StAR ™) then twenty positive specimens and thirty negative specimens were taken and tested with turbidimetry method (H. pylori Turbilatex®). Two positive samples were rerepeated five times to determine the repeatability and precision of the turbidimetry method. Sensitivity, specificity, accuracy, positive and negative predictive values were calculated.

Results: The number of positivity with ELISA was 20 (40%) samples and the number of negativities was 30 (60%). The number of positivity and negativity in turbidimetry were 26 (52%) and 24 (48%), respectively. Results of the study using the turbidimetry method showed sensitivity of 85% (Negative predictive value 87,5%), specificity of 70% (Positive predictive value 65.3%) and accuracy of 76%. Standard deviation (SD) and coefficient variation (CV) for both two positive samples were 0.87,20 % and 6.33, 25,1 % respectively.

Conclusions

Turbidimetry method (H. pylori Turbilatex®) is a useful method to detect and determine H. pylori antigen in feces in screening cases (mass survey). It saves time and money but we need another method to confirm the disease with H. pylori.

Investigation of glycosylation pattern on platelets prior to transfusion

By: Maria Absim

Bachelor thesis in Biomedical Laboratory Science performed at the department of Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg 2020.

Supervisor: Camilla Hesse, Senior lecturer

Platelet concentrates are currently stored in room temperature for a maximum off 7 days, given that every unit undergoes a bacterial control. Cold storage would decrease the risk of bacterial infection and thereby the risk of complications due to transfusion of platelets. However, cold storage of platelet concentrates is not suitable since the cells become activated while being stored in a lower temperature. Activated platelets are not compatible for transfusion. The aim of this study was to investigate platelet glycosylation after storage in 4°C, after a few days in room temperature and after activation with the agonist TRAP.

Flow cytometry was used to detect different binding sites of the platelets, including sialic acid with two different linkages to galactose (alpha-2,3 and alpha-2,6) and N-Acetylgalactosamine, with the help of fluorochrome-labeled lectins. P-selectin was used as an activation marker. Two of the lectins and the platelet marker CD61 were used to stain platelets before examination using fluorescent microscopy.

Platelets stored for a few days in room temperature, in 4°C, or platelets stimulated with TRAP showed a significant higher level of the activation marker P-selectin. The lectins showed a higher fluorescens-intensity on platelets stored for only one day versus a few days. The non-stimulated platelets shows a higher fluorescent intensity on platelets stored in room temperature versus 4°C. Similar results were observed on the stimulated platelets except for one of the lectins. The fluorescent microscopy examination showed equivalent results. In conclusion, platelet glycoproteins undergoes changes while being stored in low temperatures, over time and after activation with TRAP.

Communication of human cells with bacterial via extracellular vesicles

By Mai Abu Deiab

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, 2020.
Supervisor: Hadi Valadi, PhD and Muhammad Nawaz, PhD.

Cell to cell communication reflects the ability of cells to interact with each other and to their external environment. There are different ways cells can communicate, one of them by sending biological messages via extracellular vesicles (EVs). EVs are nano- to micro- sized subpopulations of vesicles secret from all cells types including eukaryotes such as human or prokaryotes such as bacteria. EVs both from human and bacteria play an important part in intracellular communication as transporters of biomolecules between cells.The objective of the project was to examine the effect of the human EVs on bacteria by monitoring the bacterial growth pattern by treating them with human EVs.

Methods: Human cells (HTB-177) were cultured for 1, 2 and 4 days. EVs were isolated from day 2 and 4, and characterized by their total protein content. E.coli (MG1655) was inoculated in LB-media tube overnight. Then treated with human EVs in different concentrations each with two replicates. Optical density (OD) at 600 nm was measured for E.coli samples to compare the growth density between the samples treated with EVs. In parallel to this, E.coli were cultured on LB plate without Ampicillin after treated with EVs and examined visually.

Results: No significant differences were observed between E.coli treated with or without EVs derived from HTB cells based on the growth intensity, generation time of E.coli and visually examined E.coli cultured plate .

Conclusion: Human cells interact with each other via releasing EVs .the same communication occurs between prokaryotes, as well as communication of cross species such as human and bacteria. In this study, has shown no significant changes happened to bacteria treated with human EVs, however, the possibility of that can not be denied. Due to that, it has recommended for further experiments testing immune cells –derived EVs on pathogenic bacteria .

Keywords; cell to cell communication, extracellular vesicles, exosomes, E.coli bacteria, growth pattern, optical density.

EVALUATION OF CARDIOMYOCYTE TOXICITY IN RESPONSE TO DOXORUBICIN

By Russol Abushaia

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, 2020

Supervisor: Pegah Johansson, PhD, Principal biologist

Background: Cancer is a leading cause of morbidity and mortality globally. The most common ways to treat cancer is to use DNA damaging agents such as ionizing radiation and chemotherapeutic drugs. Doxorubicin is a widely used thermotherapeutic drug, the use is limited due to the severe side effects, such as cardiotoxicity. The mechanism of doxorubicin-induced cardiotoxicity is still not clearly understood.

Purpose: The purpose of this study was to investigate the doxorubicin-induced cardiomyocyte toxicity using three different flowcytometry based assays including the cell division assay, γ-H2AX assay and apoptosis assay. Understanding the mechanism of doxorubicin toxicity of cardiomyocytes may enable us to develop methods to protect the heart from damage due to the treatment.

Method: The murine cardiomyocyte cell model, HL-1, were treated with doxorubicin to induce DNA damage. The cell division assay, γ-H2AX assay and apoptosis assay were optimized for these cells. These assays were then correlated to troponin T (TnT), the biomarker of heart damage used in the clinic today. TnT was measured in the medium of the treated cells

Result: The cell division assay showed that the cardiomyocyte sensitivity to doxorubicin is dose dependent. The γ-H2AX assay showed an increase in γ-H2AX signal with increasing doxorubicin concentration and the DNA damage was still not resolved 24 hours after treatment. The apoptosis assay showed that the level of apoptotic cells was dose dependent. There was not any clear pattern of TnT release from the treated cells in the medium that was measured.

Conclusion: In conclusion, this study shows that less cardiomyocytes are able to divide with an increasing concentration doxorubicin. It also shows that double strand breaks in cardiomyocytes are not repaired 24 hours after doxorubicin treatment and that apoptotic cell death is a consequence of doxorubicin treatment. The TnT measurements indicates that TnT cannot be used as a marker for doxorubicin-induced toxicity.

ABSTRACT

The meaning of placement for the EKG-electrodes *when assessing the respiratory curve*

By Josef Al Fayoumi

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2020.

Supervisor: Anita Persson (Leg. Biomedical scientist, PhD) and Angela Poller (Leg. Biomedical scientist)

Background: Pericardial effusion is an accumulation of fluid around the heart or in between the two serous layers of the heart. Fluid will assemble between the outer fibrous layer and the inner serous layer. Normally there is 10-50 mL of fluid there to lubricate the heart. But when the fluid builds up it can cause heart problems such as tamponade or in extreme cases "swinging heart", heart failure and other related diseases. When Pericardial effusion is present in the human body it may affect the heartrate and the normal passive breathing, this in turn will cause a respiratory variation. When a patient develops tamponade, it will alter the respiratory variation for the worse and it in turn will affect the hemodynamic in the body. Although the hemodynamic can be affected without tamponade present in the body. This differs between patients. The hemodynamics are investigated with echocardiography and a 3-lead EKG should be fitted, so that a respiratory curve is present to analyze the different stages of breathing. There are no guidelines for how the electrodes should be positioned during electrocardiography. The explicit goal of this study was to evaluate which 3-lead electrode position that was best for examination of the respiratory curve. We choose to try 3 different position, all from Einthoven's triangle. ***Method:*** All patients with potential pericardial effusion that had to undergo an echocardiography at Clinical physiology, Gothenburg, Sahlgren's University Hospital was included in the study. This ended up being a total of 15 subjects due to unexpected low influx of patients with pericardial effusion. Possibly the outbreak of COVID-19 had some impact on patient inflow. Nine images were taken with echocardiography for each patient, 3 per valve in the heart (Tricuspid valve, Mitral valve and the aortic valve). Each of these 3 images were taken with different 3-lead EKG positions, taken from Einthoven's triangle. These images were then assessed visually by 13 different medical staff from Sahlgren's University hospital, Gothenburg. The assessing staff ranked the curves from 1 to 3 in the best-worst order on all 15 patients. A Chi-square test with a null hypothesis was conducted to analyze the variables to get a significant result. ***Results:*** From 195 evaluated images 84 of 195 was assigned as best for Lead II at 43%. While Lead I only got 49 out of 195 at 25%. Lead III took a second place at 62 of 195 at 32%. These results also reflected in the ratings for worst position were Lead I took the advance at 89 votes out 195 with a likeminded 46%. Also Lead III came in second here which show a similar rating as for the best position approval. The Chi-square test got a significance level of α=0,01. ***Conclusion:*** Lead II was in this study the supreme position to use when evaluating breathingvariation when patients have pericardial effusion. The Chi-square test shows that there is a significant difference between the positions.

Saltin-Grimby's physical activity level scale identifies the Metabolic Syndrome among 64 years old women

By: Wendy Alanes Flores

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg Laboratory, Sahlgrenska Academy, University of Gothenburg, 2020.

Supervisor: Caroline Schmidt, Docent

Introduction: The metabolic syndrome (MetS) is composed by risk factors as abdominal obesity, dyslipidemia, hyperglycemia and hypertension. It is associated with diabetes type 2 and cardiovascular disease (CVD) which increases the probability of mortality. Women during and after the menopause are undergoing changes in their metabolism which could increase the MetS risk. Endurance training and improved cardiorespiratory fitness (CRF) have proved to improve the risk factors as well as higher levels of exercise.

Aim: The aim of this study is to examine if self-reported physical activity according to Saltin-Grimby physical activity level scale (SGPALS) is associated with the metabolic health in a group of 64 years old women.

Method: This cross-sectional study totally included 635 women at the age of 64. The diagnostic of MetS was based according the guidelines of National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). It meant that a score equal or higher than 3 indicated MetS. The physical activity level was assessed by SGPALS method.

Results: In this study 33 % of the sample met the MetS criteria. The group with MetS showed to have significant higher values for BMI, waist circumference, blood pressure, weight, waist-hip ratio, triglycerides, plasma insulin and glucose concentrations but had lower LDL and HDL concentrations compared to the group without MetS ($p<0,05$). In the group with MetS were significantly more women that spent their leisure time with sedentary behavior, however showed the group without MetS that significantly more practiced regular exercise ($p<0,001$).

Conclusion: In summary, it could be identified an association between self-reported physical activity and the metabolic health which were worse among women with lower activity level. The limitations of the study indicated that more studies were needed as well as follow-up to describe a stronger relationship between the metabolic health and physical activity and its development over the years.

Abstract

OPTIMIZATION OF POLYETHYLENIMINE TRANSFECTION OF HUMAN CELL LINES

By Ajeel Aldakhi

Bachelor thesis in Biomedical Laboratory Science at Clinical Chemistry laboratory, Institute for Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2020

Supervisor: Martin Lidell, PhD

Background: Transfection is a method that introduces foreign nucleic acids into cells to produce genetically modified cells. Transfection is a powerful analytical tool for studies of gene function and regulation and protein function. The introduced genetic materials (DNA and RNA) are found in cells either stably or transiently depending on the species whether the genetic material is integrated into the cellular genome or not. There are various transfection methods that include physical, chemical, and biological techniques; Some of the common transfection techniques include calcium phosphate precipitation, lipofection, electroporation and viral transfection. The aim of the work is to optimize transfection of human HEK-293 and HeLa cells with the transfection reagent polyethyleneimine (PEI) with respect to transfection efficiency.

Material and method: HEK-293 and HeLa cells were transfected with a plasmid encoding an erythropoietin (EPO) fusion protein and GFP. Different amounts of PEI and Lipofectamine 3000 were used as transfection reagents. Transfections were performed on different amounts of seeded cells and at different times after sowing of the cells (1 hour or 24 hours). The evaluation of transfection efficiency was done partly by fluorescence microscopy; where the proportion of GFP-positive cells was compared between the cells transfected with PEI and Lipofectamine 3000, and partly with western blot; where the intensity of the EPO-GFP protein detected with an anti-EPO antibody was compared between the transfections, and finally also with qPCR; where the relative expression of EPO was evaluated.

Results: The results of all transfection efficiency assays showed that the HEK-293 cells were transfected at least as efficiently with PEI as with Lipofectamine 3000; except when the lowest PEI amount was used. The transfection efficiency was just as good regardless of the amount of seeded cells, but transfection with PEI after only 1 hour after sowing the cells seemed to result in unwanted cell death, something we did not see the transfections after 24 hours. Unlike the transfections of the HEK-293 cells, the results from the HeLa cells showed that PEI gave a significantly lower transfection efficiency than Lipofectamine 3000.

Conclusion: The study shows that PEI is a suitable replacement for Lipofectamine 3000 in transfection of HEK-293 cells, which is advantageous because Lipofectamine 3000 is very expensive.

Evaluation of Two-tailed RT-qPCR of Epstein-Barr virus microRNA.

By William Apelberg
Bachelor thesis in Biomedical Laboratory Science performed at Sahlgrenska Cancer Center, Sahlgrenska Academy, University of Gothenburg, 2020.
Supervisors: Ka-Wei Tang (MD, PhD), Isak Holmqvist (Physician)

MicroRNA (miRNA) are short non-coding RNAs which regulate gene expression. They are associated with many diseases, including *post-transplant lymphoproliferative disease* (PTLD) which has specifically been associated with Epstein-Barr virus (EBV) miRNA. Monitorisation of EBV viral load has therefore been used to predict PTLD. This has been shown to not be a reliable method and instead direct quantification of EBV miRNA by RT-qPCR has been suggested. miRNA are, however, shorter than the common RT primer and so far variations of RT-qPCR have not yielded the reliability needed for clinical use. To address this, the method *two-tailed RT-qPCR* was developed. This method makes use of primers consisting of a hairpin structure and two tails, both complementary to their target miRNA, providing the sensitivity and specificity required in clinical diagnostics.

The aim of this study was to evaluate the functionality of 40 two-tailed primers targeted towards EBV miRNA. This was done as part of a larger project with the long term goal of evaluating the possibility of using EBV miRNA as biomarkers to predict patients' risk of developing PTLD.

Two-tailed RT-qPCR was performed. Synthetic miRNA and Namalwa cell line samples were used as positive controls, and no-template controls (NTC) as negative controls. Concidered parameters were Ct values, standard deviation between duplicates, alignment of melting curves between synthetic miRNA and cell samples, and positive results in NTC.

Out of the 40 tested primers, nine returned successful results, while 14 were deemed unsuccessful. The remaining 17 primers produced unstable results for one or more parameters, leaving their success debatable.

While unsuccessful primers should be discarded, successful primers can be moved along to be tested on patient samples. The remaining 17 primers need repeated testing before any conclusions can be made. Should their results remain indecisive, they should either be redesigned and tested again, or discarded.

Bachelor thesis in Biomedical Laboratory Science
Sahlgrenska Academy, University of Gothenburg, 2020
Department of Clinical Immunology and Transfusion Medicine
Supervised by Mats Bemark, Josefin Kanberg & Eva Hirsch
Student: Henrik Bejmar

A new method for flowcytometric crossmatch – evaluation of extraction, washing and analysing cells along with clinical validation.

Abstract

Background: Flowcytometric crossmatch is used clinically for detection of antibody mediated graft rejection within transplantation. Patients who has been transplanted without crossmatching is at much higher risk for suffering an acute graft rejection. In the procedure, lymphocytes are prepared from blood samples from the intended donor and then incubated with patient serum. Prior to analysis, fluorochrome-labeled antibodies reacting against the antigen CD3 (found on T cells), CD19 (on B cells) and human IgG are then added and then analyzed by flow cytometry. Between each step, the cells are washed thoroughly to avoid background binding.

Method: By using the methodology currently used clinically for cross-testing, a new simplified protocol were developed for the preparation, washing and analysis of cells with the hope of achieving the same quality as the current method.

Results: The newly developed method for producing cells and analyzing these cells using a new type of flow cytometer gave comparable or better results than previous methods. When negative serum from healthy blood donors and positive serum with known reactivity were tested, it was found that the new washing method was less able to differentiate between negative and weakly positive samples.

Conclusion: The method as a whole meant several improvements, but the validation was not completely successful as the sensitivity decreased. The new cell production proved to be a particularly good method and the result of my study gives strong reasons to change the method for producing cells. However, the washing did not show reliable results and further testing of more variables that can be altered in the washing protocol is necessary before the methodology can be used clinically.

Interobserver variability for measurement of left ventricular volume and ejection fraction with biplane Simpson method and visually estimated ejection fraction

By: Pernilla Blåholtz

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2020
Supervisor: Sofie Ahlin, MD PhD

Abstract

Introduction: The biplane Simpson method is used in echocardiographic examinations to assess information on ejection fraction which reflects the systolic function of the heart and left ventricular volumes. Previous studies have shown large interobserver variability in the measurements of this method. Studies have highlighted the need to reduce the interobserver variability to minimize the risk of error when determining the patient's condition.
Objective: To study the interobserver variability in echocardiographic measurements of ejection fraction and left ventricular volumes assessed with the biplane Simpson method and visually estimated ejection fraction among the staff at the department of clinical physiology in the NU Hospital Group. Additional aims were to study differences in interobserver variability when experience from echocardiography differs, between different professions and between examinations with different image quality.
Method: Six echocardiographic examinations were chosen. Image quality was determined as good, intermediate or reduced. The staff determined the ejection fraction and left ventricular volumes using the biplane Simpson method and visually estimated the ejection fraction. Coefficients of variation were calculated.
Results: Thirteen observers (65% of the staff) participated. The lowest coefficient of variation was observed for visually estimated ejection fraction and the ejection fraction assessed with the biplane Simpson method with coefficients of variation of 3.30-17.20%. The largest coefficient of variation (7.65-25.5%) was observed for measurements of end diastolic volume. Examinations with good image quality displayed significantly lower coefficients of variation compared to examinations with poor image quality (p-value=0.026). No differences in coefficient of variation was observed between professions or between experienced or less experienced observers.
Conclusion: There is a large interobserver variability in the measurements of ejection fraction and left ventricular volumes among the staff at the department of clinical physiology in the NU Hospital Group. Poor image quality affects the interobserver variability.

Eye Lens Dose received by medical staff performing injection of 120mbq^{99m}Tc- NanoHSA prior to sentinel node screening.

By Hanna Camén

Bachelor thesis in Biomedical Laboratory Science performed at the section of clinical physiology and Radiophysics, Sahlgrenska Academy, University of Gothenburg, 2020

Supervisors: Johanna Dalmo, PhD; Jenny Ornestedt, Biomedical analyst

Introduction: In nuclear medicine screenings radioactive trace elements are injected into the body. The trace elements are then absorbed by the specific cells or organ for the screening. One of these screenings are called Sentinel Node, where the injected trace element is ^{99m}Tc- Nano albumin colloid (^{99m}Tc-NanoHSA). The trace element is injected a certain time before surgery at eventual spreading of cancer cells of breast cancer or malignant melanoma. At Sahlgrenska University Hospital the radiation amount was recently increased for Sentinel Node for patients injected the day before their surgery. The radiation amount was increased from 40MBq to 120 MBq for these patients. This increase will result in a higher radiation amount received by the staff carrying out the injection.

Aims: The aim of the thesis is to examine whether the dose received around the eye of the staff is at an high enough level to motivate a use of additional radiation protection.

Method: A mapping study of the staff working with injection of radioactive elements was made. Biomedical analyst/X-Ray nurse/doctor performs a sub-dermal injection 24 h to 2 h before the patient is sent to surgery. The procedure has been evaluated to increase radiation safety. The staff carried thermoluminescent dosimeters (TLD) in a head strap to estimate at what level the eye-lense dose received by a staff member injecting 120 MBq ^{99m}Tc- NanoHSA. A measurement of the dose received by the fingers and the neck area was also made with TLD. To receive reliable data from a TLD it needs to be exposed to radiation for a long period of time. The measurements were therefor made over a 5-week period, during which time the TLD was used by different staff. The TLD results will be evaluated per each task.

Results: A pilot study was made prior to the main study and showed results about how to place the TLDs and for how long the measurements needed to go on. The results showed no signs of high doses received by the eyes and thereby a low risk of eye damage. As expected, the hands received the highest dosage of radiation of the different measurement points, as they are closest to the source of radiation. If a lead syringe shield was used it was the assisting hand that received the highest dosage.

Conclusion: No additional radiation protection precautions needs to be implemented at Sentinel Node screenings at Sahlgrenska University Hospital when injecting 120 MBq ^{99m}Tc- NanoHSA. However, a rotating staff schedule to spread out the radiation dosage would be valuable.

Low content of long chain polyunsaturated fatty acids in breast milk from mothers delivering extremely premature babies indicates the need for extra supplementation

Written by Osman Cabdisamad Cismaan Supervisor: Ander K. Nilsson, Scientist, PhD

Bachelor thesis in Biomedical Laboratory Science performed at department of centrum for pediatric growth research, Östra hostpital , University of Gothenburg 2020

Aim: The main aim of this study is to analyze changes in fatty acids composition in breastmilk collected at three different stages during lactation from mothers with extremely premature born babies <28 weeks of pregnancy and to investigate if variation in breastmilk fatty acids composition depend on the mother's dietary habits under pregnancy. Many different diseases which effect these infants such as visual acuity and neurological problems are associated with deficiency of long chain polyunsaturated fatty acids (LCPUFAs).

Method: 305 breastmilk samples from mothers with extremely preterm born babies less than 28 weeks of pregnancy, collected at three neonatal wards in Sweden, Karoliniska hospital in Stockholm, Sahlgrenska university hospital in Gothenburg and Skåne university hospital in Lund, were analyzed. The milk samples were collected at different stages during lactation. Fatty acids were extracted, and composition of fatty acids were analyzed by gas chromatography- mass spectrometry.

Results: LCPUFAs showed a decrease during the period of lactation. From postnatal day 7 (PND 7) to postmenstrual age of 32 weeks (PMA 32) 60 % of all 32 identified and quantified LCPUFAs were reduced, including important fatty acids such as docosahexaenoic acid from 0,36 mol% (range 0,29 - 0,47) to 0,22 mol% (range 0,18 - 0,32) in medians. Half of the 32 analyzed LCPUFAs declined in concentration from PND 7 to PMA 40. The Investigation of if the fatty acids composition varies with mothers' dietary habits during pregnancy, observed no significant relationship between them.

Conclusion: At least half of the LCPUFAs were reduced in concentration during the lactation period in breastmilk from mothers delivering extremely premature babies. This finding urges the need for supplementation to these infants to get enough amount of these necessary fatty acids during a minimum time lasting as long as the third trimester of pregnancy.

Methods for overcoming interferences for detection of 1-3-Beta-glucan in patients with high risk of invasive fungal infection

By Mikaela Claesson

Bachelor thesis in Biomedical Laboratory Science performed at the department of serology, Clinical microbiology, Sahlgrenska university hospital, Sahlgrenska Academy, University of Gothenburg, 2020
Supervisor: Helena Hammarstrom, MD PhD, doctor at clinical microbiology and infection, SU

Introduction: 1-3-β-D-glucan is a polysaccharide, on the cell wall of many fungal species. This polysaccharide can be detected in serum from patients with invasive fungal infection. The mortality in patients suffering from these infections is high. Analysis of beta-glucan in serum samples is shown to be faster and more sensitive than the traditionally culture-based microbiology tests for the diagnosis of invasive fungal infections. A problem with the beta-glucan analysis is the relatively low specificity of the test. In one study, patients treated with the chemotherapy drug pegylated asparaginase, which has a known side-effect of triglyceridemia, showed false positive beta-glucan levels in serum. There was a strong correlation between the triglyceride serum level and the beta glucan-level of these patients.

Aim: The aim of this study was to study different methods to improve the specificity of the Beta-glucan analysis by determining if, lipaemic index correlate with false positive beta-glucan levels and by investigating different methods for reducing interferences in beta-glucan analysis. The hypothesis was that a high lipaemic index would give a high beta-glucan level. The best method for threating the lipaemia in the samples was evaluated.

Methods and materials: Serum samples from haematology patients that had been collected in an earlier study were used in this project. The hemolysis-, icterus- and lipaemic index, and the triglyceride levels of the samples were analysed. Three treatments for lipidemic samples were tested on some of the samples. The following three treatments for lipaemic samples were: heat-treatment with Na_2-EDTA, high speed centrifugation and Lipoclear®. The lipaemic index was thereafter once again tested to see the effectiveness of the treatment. Two of the treatments were later applied on the beta-glucan analysis to evaluate how the treatment affected the analysis.

Results: There was a correlation between beta glucan levels and lipaemic index, when the samples with lipaemic index over 200 were included ($r= 0,65$, $p= 0,12$). The most efficient treatment of lipaemic samples was the heat-treatment with Na_2-EDTA. There was a significant reduction in absorbance and consequently in beta-glucan-levels when the heat-treatment with Na_2-EDTA was applied.

Conclusion: The results of this study indicates that a high lipaemic index in serum samples may correlate to false positive beta-glucan results. Pre-treatment of the serum samples with Na_2-EDTA may be a useful method for overcoming these interferences and may result in an improved specificity of the analysis. However, the sample size used to study the correlation between lipaemic index >200 and beta-glucan was small, and the resulting p-value was not significant why we cannot draw any firm conclusions. Therefore, further studies including a larger sample size with larger number of samples with high lipaemic index and false positive beta-glucan level are needed. There is also left to investigate other interfering factors for the beta-glucan analysis, for example drugs that contains beta-glucan. The result of this work has led us further down the path of improving the specificity of the analysis.

qPCR, conventional PCR and sequencing shows that serologically D-negative pregnant woman from the Democratic Republic of Congo are genomically D-positive

By Yasmin Elghoz

Bachelor thesis in Biomedical Laboratory Science performed at the Department for Laboratory Medicine, Institute of Biomedicine
Sahlgrenska Academy, University of Gothenburg 2020
Supervisor: Camilla Hesse, Senior lecturer

Background: The Rh-blood group system is one the most polymorphic and immunogenic systems within blood group serology. The system consists of the two genes *RHD* and *RHCE* which produce several antigens including RhD and RhCE. Antibodies within the Rh system may lead to clinical complications such as destruction of transfused red blood cells during transfusion or hemolytic disease of the newborn. The D-antigen induces an immune response in 80% of D-negative patients upon transfusion with D-positive blood. The antigen is also responsible for 50% of hemolytic disease of the newborn immunization cases. The reason for the lack of D-antigen differs between ethnic groups and in African population D-negativity is often caused by pseudogenes, a common one being *RHDψ*. Serological testing is used in hospitals to determine the presence of antigens and antibodies. However weak or variants of the antigens or antibodies may evade the tests. Therefore, molecular methods are sometimes necessary.

Aim: The aim of the study is to analyze the *RHD* genotype of 20 pregnant women from the Democratic Republic of Congo using qPCR, conventional PCR and sequencing. Serologically, 12 of the women had previously been determined as D-negative and 8 as D-positive.

Method: DNA was extracted from blood samples. The samples were then analyzed using qPCR, conventional PCR and sequencing for detection of *RHD*. Conventional PCR and sequencing was done on exons 4, 6 and 7. Samples were chosen for sequencing based on the result from qPCR and conventional PCR. The sequences from the analysis were then compared with known sequences of *RHD* and *RHDψ*.

Result: qPCR revealed that all women but 3 had the *RHD* gene. With conventional PCR we were able to see that the 3 patients who had a negative qPCR result lacked exons 4 and 7. Aside from another patient who was also missing exon 4, all patients carried all three exons. Sequences were obtained from 9 out of 10 patients chosen for sequencing. However, sequences were not gathered for all three exons. One patient got a 100% match for exon 7 while two patients got 100% matches for exon 6. The remaining patients had an average of 82% match with 5,1% standard deviation for *RHD*. The percentage for *RHDψ* was either the same or lower than the percentage for *RHD*.

Conclusion: qPCR showed that out of the 12 serologically D-negative women, 9 had the *RHD* gene. Conventional PCR showed that all women had exon 6 while all but 4 had exon 4 and out of those, 3 also lacked exon 7. The sequencing results were not completely credible because of high background noise that disturbed the analysis as well as the result of low DNA concentration. It is plausible that the genomically D-positive women carry variants of *RHD*. Further analysis is required to determine which *RHD* variant the women carry.

Agglutinate in apheresis platelet concentrates associate with platelet activation and affect TRAP induced aggregability

By: Hanan Elsisy

Bachelor thesis in Biomedical Laboratory Science performed at Dep. Stem- cell and Component laboratory, Clinical Immunology and Transfusion medicine Sahlgrenska academy, University of Gothenburg 2020.

Supervisors: Helena Barreto Henriksson, associate professor, Camilla Hesse, senior lecturer

Platelet transfusion is crucial for modern health care. Platelet concentrates can be produced in different ways and one is by apheresis technique. Platelet concentrates yielded by apheresis sometimes arrive at the component laboratory containing agglutinate. These can sometimes be clearly visible macroscopically. In the daily clinical situation at Transfusion Medicine, there are relatively often few platelet units in stock and unnecessary disposal of platelet units should be avoided. Furthermore, an optimal platelet function is of great importance to the patient. The aim of this study was to investigate the quality of apheresis platelet concentrates containing agglutinate through measuring of the *in vitro* platelet aggregability and the expression of platelet activation marker CD62-P. Ten apheresis containing agglutinate (n=10) were assessed by macroscopic and microscopic morphological studies. The aggregation ability was tested on day 1 and 2 after donation via impedance aggregometry in response to various agonists such as arachidonic acid, thrombin receptor activating peptide (TRAP) and adenosine diphosphate. Furthermore, the activation marker CD62-P was measured by flow cytometry. Controls were fourteen apheresis not containing agglutinate (n=14). The main findings of the study were that apheresis platelets containing agglutinate had a higher activation rate and a worse ability to aggregate with TRAP. In conclusion, the results indicate that the presence of agglutinate affects the quality of apheresis platelet concentrates. Further studies are needed to evaluate the findings from this study.

Examination of drugX impact on equine chondrocytes production and arrangement of extracellular matrix in a *in vitro* pellet culture model.

by Mona Engström
Bachelor thesis in Biomedical Laboratory Science performed at the department 12 of Clinical chemistry, Sahlgrenska Academy, University of Gothenburg, 2020
Supervisor: Josefine Ekholm, PhD, Biology. Secondary supervisor: Susanne Nyström, PhD, Biology

Objective: The surface of joints is covered in hyaline cartilage which makes the joints move smoothly and without pain. Cartilage also exists in ears and epiglottis in the form of elastic cartilage and in intervertebral discs and meniscus as fibrocartilage. These 3 types of cartilage all consists of chondrocytes and extracellular matrix (ECM) and its different types and traits is determined by the composition of the ECM. The ECM mainly consists of osmotically active aggrecan and collagen and it is the type of collagen in ECM that gives the cartilage its attributes, hyaline cartilage has collagen II fibers while fibrocartilage has collagen I. Cartilage is avascular and aneural and gets it´s nutritional exchange from diffusion. This, in combination with the inability of the chondrocytes to migrate in the ECM, has a negative impact on the regenerating process. When hyaline cartilage is damage it tends to heal as scars consisting of fibrocartilage, if healed at all. This is a problem because this scar tissue does not have the smooth surface and also tends to break easily. Osteoarthritis (OA) is a common disease and occurs in as many as 10% of the adult population. Typical symptoms of the disease is pain, swelling and reduced function of the joint which in some cases can have a negative effect on the persons everyday life. The conventional treatment for OA is physiotherapy, lifestyle changes and pain relief but in severe cases arthroplasty can be necessary. There is a great need for less invasive and more effective treatment of OA. In this study we examined drugX impact on equine chondrocytes production and arrangement of ECM *in vitro* in pellet culture to try to determine it's use as a treatment for OA and other cartilage damage.
Design: Equine chrondrocytes collected from a joint with OA was differentiated into cartilage pellets and treated with 3 different forms of drugX in 5 different combinations and with high- (HG) or low glucose (LG) concentrations in the pellet culture medium. The pellet was treated for 3 days and thereafter grown in low glucose pellet culture medium for a total of 21 days with medium change every 3 day. The medium was collected for protein detection of COMP with Wes Protein simple. The diameter of the pellets was measured during the time and used to calculate the volume. On the 21 day 2 pellets from every treatment group was used for histological staining with Picrosiruis red + weigherts, Alcian blue + weigherts and immunostaining of aggrecan and collagen II. The other pellets were used for expression analysis of TLR4, BMP2, NGRF, COL2A1, Aggrecan, COMP, MMP13. ADDAMTS-4 and COL1 gene with QRT-PCR.
Results: The QRT-PCR analysis showed differences in gene expression between the groups treated with drugX and the LG and HG group. The pattern with drugX in LG and the same treatment in HG did not match each other. The histological staining showed an effect on the arrangement of ECM, collagen and chondrocytes with the drug treatment in both LG and HG groups. The groups with HG had a smaller area of pyknotic cells in the middle of the pellet and more collagen in the surrounding are. Collagene II was more prominent in the LG groups and hade a weaker signal in LG with combinations of drugX and all the HG groups.
Conclusions: drug X has an effect on gene expression, arrangement and content of the chondrocyte pellet but further studies is needed before conclusions on its application as a treatment for OA can be determined.

Interobserver variability in diastolic measurements and velocity time integral in the left ventricular outflow tract

By Anna Eriksson

Bachelor Thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, NU Hospital Group, Trollhättan, 2020

Supervisor: Sofie Ahlin, MD PhD

Background: The pulsed wave doppler technique is daily used in echocardiographic examinations in Swedish cardiac diagnostic. The technique can be used to quantify blood flow velocities and integrals of velocities over time. Reproducibility of measurements performed by different observers is important to ensure a safe diagnostic for the individual patient. Hence, it is important to have a low interobserver variability in assessment of the same variable between different observers.

Aims: The main aim was to study the interobserver variability among echocardiographic staff at the department of Clinical Physiology at the NU Hospital Group in measurements with pulsed wave doppler parameters in left portion of the heart. An additional aim was to study differences in interobserver variability in groups of different professions, in groups with varying experiences of echocardiography and between groups of examination with different image quality.

Material and method: Thirteen observers performed measurements of mitral inflow- and pulmonary vein inflow velocities and the velocity time integral of left ventricular outflow tract. The measurements were performed in images from six different echocardiographic examinations with offline software EchoPAC. For every examination each parameter was measured only once per observer. The coefficient of variation was used to quantify inter-observer variability. Paired and independent T-tests were performed to study differences in interobserver variability.

Results: There is interobserver variability for all of the measured variables, especially for pulmonary vein inflow velocities and the index of the velocities, with a coefficient of variation varying between 3,2-26,8%. The measurements of velocity time integral in the left ventricular outflow tract had the lowest interobserver variability with a coefficient of variation varying between 3,4-7,1%.

Conclusion: The coefficients of variation were within acceptable limits of 10% for most of the studied variables. No differences in interobserver variability were observed between groups of different professions, groups with different length of experience or between examinations with normal or poor image quality.

CNN-based AI-method for segmentation of subcutaneous fat and skeletal muscle using CT-images.

By Johanna Eriksson

Bachelor thesis in Biomedical Laboratory Science performed at Clinical physiology, Sahlgrenska Academy, University of Gothenburg, 2020. Supervisor: Reza Kaboteh, PhD.

Introduction

The depletion of muscle mass is the cause of several complications but most importantly it increases the risk of side effects when using a common form of cancer treatment, chemotherapy. The dose and number of treatment sessions are based on BSA that does not take the body composition into account. That evaluation can be done using CT images to further customize treatment, but it is very time-consuming to make a full-body assessment. The objective is to develop an AI-program for segmentation and quantification of subcutaneous fat and skeletal muscle in CT-images. The aim is that an AI will be able to segment and quantify equally well but significantly faster than a human.

Method

Manual segmentation was performed on 62 CT-images collected at Clinical Physiology at Sahlgrenska university hospital in Gothenburg, Sweden. The material was acquired from the Nimsa-group patient database and the segmentation was performed in "RECOMIA", a cloud-based drawing tool for segmentation. The completed images were sent to mathematicians at Chalmers University of Technology, where an AI-method was developed using a CNN method. The results were obtained using a 4-fold cross validation technique and compared using the Sørensen–Dice coefficient.

Results

The overall average Dice-score is 87 % ± 9,1 %. If studied separate the Dice-score is 91 % ± 7 % in Nimsa_3 and 82 % ± 9 % in Nimsa_5.

Conclusion

The AI-method perform image segmentation considerably faster than any human, but not with equally good precision. To become a useful tool in healthcare the program needs further development.

Development of an analytical method for evaluation of an analytical interference caused by macro-Troponin I

By: Emma Erixon

Batchelor thesis in Biomedical Laboratory Science at the department of Clinical Chemistry, Sahlgrenska academy, University of Gothenburg, 2020.
Supervisor: Aida Muslimović PhD, chemist

Introduction: The cardiacspecific biomarker cardiac Troponin I (cTnI) is used for diagnosis of myocardial infarction. Today even high-sensitive cTnI assays are available on the market, detecting very low concentrations of cTnI in serum and plasma samples. This makes it possible to detect low risk patients and give them treatment in time. However, cTnI can form complexes with other molecules, especially IgG. The complex between cTnI and IgG is called macro complex or macro-TnI and is more resistant to degradation in the blood. Therefore, patients with macro complex are shown to have higher troponin I values even though no myocardial infarction has occured. To separate the macro complex from free cTnI protein A and G sepharose were tested due to their binding capacity to IgG.

The purpose of this study was to develop a simple spin column-based method using protein A and G sepharose with an IgG retention of ≥90%.

Methods: The development of the protocol was based on an already existing protocol for a commercially aviable protein G column. Both protein sepharoses were tested and optimised for sepharose gel volume, incubation time, sample and buffer volumes. The serum flow-through and elution were the main analytical outputs detected from the columns. The focus in the tests was to obtain a retention of ≥90% IgG and therefore only IgG was analysed in the majority of the flow-throughs.

Results: Both protein A and G sepharose showed a retention of IgG at ≥90% under the conditions where 250 µL sepharose and 350 µL serum sample were used with an incubation time of 10 minutes and then washed and eluted with the volume of 500 µL 0,2 M glycinebuffer pH 2,5. The results also showed that only about a third of the original IgG amount was eluted in the elutionbuffer. Repetitive usage of the sepharose gel showed that it can be used for at least 10 times and still give an IgG retention of ≥90%.

Discussion and conclusion: For both protein A and G sepharose in the repetitive test the IgG retention was stable at ≥90% which indicates that the mass can be used at least ten times. However, the method needs to be optimised further in order to be used for analysis of patient samples. To better the outcome of the analysed markers, additional steps before the elution could improve the results.

Analysis of thyroid-stimulating immunoglobulins compared with thyrotropin receptor antibodies associated with radioiodine treatment with Grave's disease patients.

By Sofie Esbjörnsson

Bachelor thesis in Biomedical Laboratory Science performed at the Thyroid Department Sahlgrenska University Hospital, Gothenburg 2020.

Supervisor: Helena Filipsson Nyström, Senior Consultant

Introduction: Hyperthyroidism is a disease affecting the thyroid gland to produce excessive amounts of hormones, causing the body's metabolism to increase. Graves Disease (GD) is the most common subtype and is an autoimmune disease caused by production of thyrotropin receptor antibodies (TRAb). In 95% of Graves disease TRAb can be detected and is enough to set a diagnose. An analyze of thyroid-stimulating immunoglobulins (TSI), the stimulating subtype of TRAb is common for GD patients and can help set a diagnos i the last 5 % of the cases.

Aim: The aim of the study was to investigate the clinical purpose of analyzing Thyroid-Stimulating Immunoglobulins compared to the conventional thyrotropin receptor antibodies in different clinical contexts and investigate the connection between radio iodine therapy (RAI) variables and TRAb/TSI levels. Patients with Graves disease and toxic nodular goiter (TNG) were investigated.

Method: A total of 68 blood samples was taken from patients, treated at the Clinic of Isotopes before or after radioiodine therapy and TRAb and TSI was analysed. The patients journals were examined to gather information about thyroid hormone levels, type of thyrotoxicosis, associated radioiodine variables, sex, age, endocrine ophthalmopathy and which context the TRAb/TSI was taken.

Results: TRAb and TSI both increased after radioiodine treatment signicaly for all as a group and for GD TRAb rose significantly. TRAb and TSI were unanimous, only one discordant value was observed. A strong correlation between TRAb and TSI was found pre and post RAI for GD and post RAI for TNG. The rise of TRAb and TSI-levels after RAI had a positive correlation to thyroid volume and radioactive iodine uptake regarding GD.

Conclusion: This study did not find any advantages in analysing TSI post RAI instead of the conventional TRAb. A high radioactive iodine uptake and a high thyroid volume predicts increased TRAb and TSI levels post RAI for GD patients.

The importance of different anticoagulants and sample handling for quantification of sphingosine-1-phosphate in blood samples

By Basema Hassan

Bachelor thesis in Biomedical Laboratory Science performed at the botanhuset/Deparmtment of Biology and Environmental Science, Sahlgrenska Academy, University of Gothenburg, 2020.

Supervisor: Anders Nilsson

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid and a ligand to five G-protein-coupled cell surface receptors. S1P regulates the basic biological processes and affects several cellular functions in blood vessels and the immune system. S1P is normally found in plasma and blood cells. S1P is mainly produced by platelets and erythrocytes. The purpose of this study was to the effect of different blood sampling systems and preanalytical treatment on S1P level in addition to evaluate the correlation between concentration of different blood cells and S1P level. Blood was collected from healthy individuals (n = 10) and different types of blood sampling tubes were used such as Lithium Heparin, Sodium Citrate K2EDTA, CTA Serum. The samples were analysed immediately after sampling or after standing for 24 hours at room temperature. After lipid extraction, S1P was quantified by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS). The results show that whole blood S1P correlates significantly with platelet count, but there was no association between plasma or serum S1P and cell number. S1P concentration was higher in serum than in plasma, due to platelets releasing of S1P during the coagulation process. In addition, the plasma S1P concentration was decreased immediately after sampling compared with that taken from blood at room temperature for 24 hours.

An increased Intima-Media Thickness predicts future Cardiovascular Events in a 13-year study

By Anna Holmkvist

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of Medicine Sahlgrenska Academy, University of Gothenburg, 2020.

Supervisor: Caroline Schmidt, Associate Professor

Background: Cardiovascular events is one of the most common cause of death's worldwide. Atherosclerosis is an inflammatory progressive process which increase the risk of cardiovascular events like stroke and myocardial infarction. Previous studies have shown associations between cardiovascular risk factors, atherosclerosis and intima-media thickness of the carotid.

Aim: The aim of the study was to investigate if an increased intima-media thickness predicts future cardiovascular events among middle-aged, Swedish men.

Material and Method: The Atherosclerosis and Insulin Resistance Study (AIR) include 396 healthy 58-year-old Swedish men. A variety of parameters such as blood pressure, lipids in the blood and BMI were collected from the participants. Intima-media thickness of the carotid artery on both sides were measured by ultrasonography. With a semi-automated method, a mean of IMT in the CCA and bulb were measured. Odds ratio were calculated with cardiovascular event as the dependent variable. Receiver operating characteristic (ROC) curve analysis were used to investigate which IMT variable and risk factor predict cardiovascular event.

Results: The association between apoB/apoA-I ratio, systolic blood pressure and cardiovascular events were significant. The IMT-variables were also significant associated with cardiovascular events. Mean IMT were higher in the group of men with cardiovascular event then without. The odds ratio for an increased IMT CCA is 2.2 (95% CI1.3 to 3.7; p=0.0021) and for IMT bulb 1.7 (95% CI 1.0 to 3.0; p=0.0288) to risk cardiovascular event.

Conclusion: An increased IMT in the CCA predict future cardiovascular events in 58-year-old men. IMT in the bulb were borderline significant with cardiovascular events.

Genomic blood group determination of patients with thalassemia and comparison between the methods ERY Q and ID Core XT

By Elin Jonsson

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2021
Supervisor: Camilla Hesse, Senior Lecturer

Introduction: A blood group is defined as a structure/antigen on the surface of an erytrocyte. There must also be at least one individual in the world that does not express the antigen but instead have formed antibodies against it. Blood groups play a critical role in transfusion medicine to prevent complications that can occur during and after blood transfusion. Traditionally a person's blood group is determined serologically but in recent years, new genetic methods have been developed. These methods can be more accurate and safer to use in patients that needs repeating blood transfusions.

Thalassemia patients often need blood transfusion as part of their treatment, and because of that there is a risk for them to develop alloantibodies against different blood group antigens. These antibodies can make it harder to find matching donor blood. In Palestine, there have not been many studies on blood group antigens and alloantibody frequencies in this group of patients.

The purpose of this study is to determine the blood group antigens of 100 thalassemia patients from Palestine with the genomic method ERY Q, and to compare the method with ID Core XT that is used in Sahlgrenska University hospital.

Material and Method: DNA from 100 thalassemia patients from Palestine and EDTA-blood from 4 individuals that have already been analysed with the ID Core XT method were used in this study. All samples were analysed with the three kits ERY Q RH, ERY Q KKD/MNS and ERY Q Rare from BAG Diagnostics.

Result: The most common antigens in the Palestine population was D (88%), s (90%), e (96%) and M (77%). 46% also had the gene Fy*02N.01.

When comparing the two methods ERY Q was easier and faster but could take less samples in one run. Two discrepancies were found that could probably be caused by gene variations.

Conclusion: ERY Q is an easy and fast genomic method to determine blood group antigens. The results are also matching the established methods that is used today. In the Palestine population, a large number of the patients had the allele Fy*02N.01 (Fya- Fyb-). This phenotype is common in African populations but has previously not been shown in large numbers in Palestine individuals.

Evaluation of electrode placement for VEP recording

(Visual Evoked Potentials)

By Teres Kalyun

Bachelor thesis in Biomedical Laboratory Science performed at the Dept of clinical neurophysiology, Sahlgrenska Academy, University of Gothenburg, 2020

Supervisor: Josefin Nilsson, Co-supervisor: Linda Lundblad

Abstract

Background: Visual Evoked Potential (VEP) is an electrophysiological examination method that records signals from the visual system. An evoked potential is a response to a visual stimulus, in this case a pattern reversal stimulus (pVEP) which stimulates the retina. pVEP is used to diagnose different types of disorders that affect the visual system, mainly the function of the optic nerves and the optic tract. The signals are recorded using EEG electrodes over the visual cortex (occipital lobe) while simultaneously displaying visual stimuli.

Purpose: There are different protocols for positioning of electrodes when recording VEP. The aim of this study was to find out if the height of the Oz-electrode from the occipital inion affects the result.

Materials and methods: pVEP-responses were recorded to a lack-white checkerboard pattern stimulus. Two different Oz locations were used, in order to induce visual blur testing glasses were also added to each condition. Twelve participants were examined, 3 men and 9 women.

Results: The height of Oz gave no significant differences in amplitude or latency of VEP responses. Men had longer latencies compared to women. Adding visual blur showed no significant difference in amplitude compared to without, although there was a tendency for lower amplitudes with lower visual acuity (e.g. with testing glasses).

Conclusion: The results showed no significant differences in amplitude or latency between the recordings from normal or higher Oz position. Men had significantly longer latency than women, but the group is very small and there is an age difference between the genders in our material. The examination with artificially reduced visual acuity also did not show significant differences, but there was a tendency for lower amplitudes and longer latency, which is expected. The absence of significant differences may be due to the size of the material.

INTRODUCTION OF AN IMMUNOHISTOCHEMICAL DOUBLE STAIN WITH CYTOKERATIN AND DESMIN IN DETRUSOR INVASIVE UROTHELIAL CANCER

By **Melisa Karabulut**

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Pathology, Sahlgrenska Academy, University of Gothenburg, 2020

Supervisors: Martin Johansson, Professor/Chief of Physician, and Anders Bergström, specialist in Urologic Pathology.

Background. Urothelial cancer (UC) is one of the most common malignancies worldwide and originates from the superficial cells of the bladder wall, the urothelium. 75 - 80% are diagnosed with non-invasive UC that is confined to the lamina propria, and 20 - 25% with invasive UC, in which tumor invades the detrusor muscle of the bladder. Non-invasive UC is usually treated conservatively, but a slight invasion into the outer layer of the detrusor muscle dictates towards more aggressive treatment with radical cystectomy, chemotherapy or radiation therapy. The diagnostic difficulty lies in the pathologic grading of detrusor invasion from transurethral bladder resection (TURB) specimens, which causes a major challenge for pathologists since bundles of detrusor muscle can be mistaken for muscularis mucosae of lamina propria. The aim of this study was to introduce an immunohistochemical double stain in TURB specimens with cytokeratin (CK) and desmin using two chromogens, which will increase the diagnostic precision and aid in the pathologic grading of detrusor invasive UC, which will further prevent a potential overdiagnosis of the patient.

Method. Formalin-fixed, paraffin-embedded blocks of TURB from 10 cases with UC were sectioned and double stained according to the immunohistochemical visualization system Dako EnVision™ FLEX using two chromogens, DAB and magenta. DAB visualized CK AEI/AE3 of both normal and neoplastic tissue, and magenta visualized desmin in smooth muscle cells.

Results. The double stain with DAB and magenta successfully visualized CK AEI/AE3 and desmin in all 10 cases. The staining intensity of desmin in detrusor muscle and muscularis mucosae did not significantly differ from each other. In specimens where morphology was more intact, desmin seemed to facilitate the differentiation of detrusor muscle from muscularis mucosae. The double stain with CK AE1/AE3 also aided in the assessment of the histopathological relationship between cancerous tissue and smooth muscle cells.

Conclusion. Desmin is a very useful marker for facilitating the visualization of both detrusor muscle and muscularis mucosae in TURB specimens along with CK AE1/AE3. However, additional studies are required in order to identify an antibody or antigen that will act as a differential marker for either the smooth muscle cells of detrusor muscle or muscularis mucosae, which may visually distinguish them in TURB specimens.

Hemolytic interference – a comparison of the manufacturers recommendations and results obtained with increasing hemolysis for ferritin, haptoglobin, iron and total protein

By Lina Karlstad

Bachelor thesis in Biomedical Laboratory Science performed at the section of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg 2020.
Supervisor: Ruth Wickelgren, PhD.

Introduction: The most common pre analytic reason for tests being invalid is hemolysis. The sensitivity to hemolytic interference depends on which analyte being detected and which methods and instruments being used. Therefore, each laboratory should perform their own interference-studies instead of copying the manufacturers recommendations.
Aim: The aim of this project is to investigate the influence of hemolytic interference while detecting ferritin, haptoglobin, iron and total protein on Alinity ci.
Method: Patient samples was collected in different concentrations and stored in -20°C. Erythrocytes from EDTA-blood was isolated and frozen on dry ice to extract hemolysate. After thawing and centrifugation the supernatant was collected and diluted in seven different concentrations. Plasma/serum was thawed, centrifuged, and divided in eight aliquots before spiked with hemolysate (diluent was used to obtain a blank for each level). All samples were analyzed on Alinity ci-series.
Results: Lower concentrations of iron and ferritin showed a rapidly increase due to an modest increase in hemoglobin, while within the upper part of the biological reference range they showed no or small increase in hemoglobin levels up to 1,1 respectively 2,5 g/L. Total protein showed an increase in 7,8 – 12,6 % at hemoglobin levels of 2,5 g/L. A variation between -13,4 and 16,7 % was seen for haptoglobin at the first level of hemoglobin (0,6 g/L), a variation that was relatively unaltered as the hemoglobin was increased.
Conclusion: All analytes showed an increase/variation in concentration due to an increase in hemolysis. Since the results for ferritin and iron do not agree with previous known results, these will undergo further investigations that will ruling out a potential individual variation of intracellular ferritin and iron for the hemolysate being used. For haptoglobin ant total protein, currently used limits could be changed to the manufacturer's upper limits of 20 and 2,5 g/L.

Framingham risk score can predict cardiovascular events in healthy middle-aged men

By Tuba Kazemi

Bachelor thesis in Biomedical Laboratory Science performed at Wallenbergslaboratoriet, Institute of Medicine, Sahlgrenska Academy, and University of Gothenburg 2020

Supervisors: Caroline Schmidt, Docent

Introduction: Cardiovascular disease (CVD) is one of the leading causes of death in the world, and by early identification of the risk factors that is associated with CVD it can reduce both mortality and morbidity. Stroke, myocardial infarction or peripheral vascular diseases are some examples for cardiovascular diseases. CVD increase exponentially with age and the risk of suffering is greater in older populations. For many years, many risk functions have been developed to predict cardiovascular risk. In this study, Framingham risk score has been used which is one of the most functional indicators.

Aim: The aim of the study was to predict cardiovascular disease by using Framingham risk score in a group of healthy middle-aged men.

Method: This prospective observations study have included a total of 396 men, aged 58 years and free of cardiovascular disease and other clinical diseases. The recruitment of these men was randomly from the council register.

Results: Analysis showed that 17.2% of the subjects were developing a cardiovascular disease during 12.6±3.6 year's follow- up. It was shown that 69 % of subjects had high risk, 31% moderate risk of 10 year risk of cardiovascular risk. None of the subjects had low risk (<10%) for cardiovascular risk. The study showed that the high-risk group according to Framingham risk score had significantly increased risk of suffering a cardiovascular event during follow-up compared to the moderate- risk group.

Conclusion: Framingham risk score can predict cardiovascular events in healthy middle-aged men.

Analytical ultracentrifugation of troponin T from cell medium and plasma in sucrose gradients

By Elin Larsson

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Chemistry laboratory, Sahlgrenska Academy, University of Gothenburg, 2020

Supervisor: Ola Hammarsten, Professor, chief physician

Background: Cardiovascular disease causes 3.9 million deaths annually in Europe. One such disease is acute myocardial infarction, an important diagnosis to confirm or rule-out when a patient presents to the emergency room with chest pain. When damage is caused to cardiac muscle cells, troponin is released, which is a common biomarker for myocardial infarction. Troponin T is also elevated in patients with kidney disease. The aim of this study is to compare the size of the troponin T fragments in cell media from living and necrotic heart cells as well as in plasma after myocardial infarction.

Material and Method: Cell medium from living and necrotic HL-1 cardiomyocytes and plasma from a patient with confirmed myocardial infarction were added to linear sucrose gradients. By using analytical ultracentrifugation the present proteins were separated by fragment size. By doing the same protocol to a series of proteins with known molecular weights and making a standard curve of their data, the approximate molecular weight for the troponin T in the samples could be calculated.

Results: In cell medium from living cells, troponin T occurred in a large complex of about 202 kDa. The cell medium from the necrotic cells mainly contained Troponin T of three different approximate molecular weights, 12 kDa, 43 kDa and 148 kDa. In the plasma sample from the patient with myocardial infarction, there were only small Troponin T-fragments of roughly 9 kDa.

Conclusions: This study both shows new findings and confirms previous studies of troponin T degradation. Since this study shows that different lengths of protein occur, the size of troponin T fragments could potentially be analyzed to assess whether the patient has an ongoing myocardial infarction, if the elevated levels of troponin T is due to a previous heart attack, or if the elevation is due to kidney disease. This can potentially improve the analysis of troponin T for the diagnosis of myocardial infarction.

APPROXIMATELY 50% OF PATIENTS MEDICATED WITH BETABLOCKERS DOES NOT REACH 85% OF THEIR MAXIMAL HEART RATE

Improving the diagnostic accuracy for patients with betablockers at the exercise test.

By Rebecka Larsson

Bachelor thesis in Biomedical Laboratory Science performed at Klinisk fysiologi at Östra sjukhuset, Sahlgrenska Academy, University of Gothenburg, 2020

Supervisors: Patrik Sundholm MD, Hannele Korhonen BMA and Caroline Schmidt Associate Professor.

Background: The exercise test is one of the most common procedures at Klinisk fysiologi. It is used for diagnosing ischemic heart disease and for evaluating treatment of heart and lung diseases. During exercise the demand of blood flow to the coronary arteries increases and ischemic symptoms may appear. During the exercise test the patients should reach at least 85% of their maximal heart rate. The heart rate can be affected by a lot of things, such as sick sinus nod, lung disease and the medicine betablocker. A diagnostic exercise test should be performed without any medicine that can affect the disease being searched for. Betablockers lower the heart rate and blood pressure both at rest and exercise. Lowering the heart rate and blood pressure result in a reduced demand for oxygen and blood flow to the heart. However, this can affect the diagnostics by hiding both symptoms and signs of ischemia at the electrocardiogram (ECG).

Purpose: The purpose of this study was to evaluate if there is a difference in the quantity of patients not reaching 85% of their maximal heart rate depending on if they are medicated with betablockers or not.

Method: A total of 1381 patients underwent an exercise test. Of these 1381 patients there were 937 patients that did not reach the criteria. Of the remaining 444 patients, 119 of them were medicated with betablockers and 325 patients were not.

Result: In the group with betablockers there were 44,5% of them that did not reach 85% of their maximal heart rate compared to the group without betablockers, where only 6,5% of them did not reach 85% of their maximal heart rate.

Conclusion: There was a big difference when comparing the two groups; 44,5 % vs. 6,5% ($p<0.001$). One significant fact to keep in mind is the reason for doing the exercise test, which decides whether the betablockers are to be used before the exercise test or not. Considering the result, I do think that the guidelines should change in order to maintain or even strengthen the sensitivity and diagnostic accuracy of the exercise test for patients with betablockers.

DIABETES MELLITUS TYPE 2 IS ASSOCIATED WITH A THICKER INTMA-MEDIA THICKNESS I A. CAROTIS COMMUNIS

A Study made on 64-years old women in Gothenburg, Sweden

By: Ylva Magnestam

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg laboratory for cardiovascular research, Sahlgrenska Academy, University of Gothenburg, 2020
Supervisor: Caroline Schmidt, Leg BMA, Associate Professor

Introduction: Diabetes mellitus is one of the most common endemic diseases in Sweden and is associated with a greater risk to suffer from cardiac disease like atherosclerosis. Women with diabetes have shown to have a greater incidence for cardiac disease than men. The intima-media thickness in a. carotis communis is used as a marker for the evaluation of atherosclerosis. The aim of this study was to investigate if there is an association between intima-media thickness in a. carotis communis for women at the age of 64 with diabetes mellitus and women with normal glucose tolerance.

Material and methods: The material consist of a database including 639 individuals. Anthropometrical test and blood values like cholesterol, triglycerides, LDL, HDL and HbA1c was evaluated. The intima-media thickness was measured with ultrasound. A mean value of intima-media thickness was calculated in a. carotis communis and the carotid bulb from both the left and right artery. An independent T-test was used to evaluate the difference between the group's diabetic subjects and non-diabetic subjects.

Results: The intima-media thickness was measured in 226 subjects with diabetes and 188 subjects with normal glucose tolerance. The independent T-test showed a significant difference in the intima-media thickness in both a. carotis communis and the carotid bulb between the groups. The subjects with diabetes had a greater intima-media thickness than the non-diabetic subjects and had a greater progress of the intima-media thickness during the six year follow up period.

Discussion: The conclusion of this study was that the results agreed with the hypothesis and a conclusion that the intima-media thickness in a. carotis communis is greater in women at the age of 64 with diabetes mellitus type 2 than in healthy women with normal glucose tolerance in Gothenburg can be made.

Survey of echocardiographic examinations prior to non-cardiac surgery.

By: Josefin Nyberg

Bachelor Thesis in Biomedical Laboratory Science performed at Clinical Physiology, Salgren's Academy, Universeiy of Gothenburg 2020.

Supervisor: Anita Persson Phd, leg BMA & Angela Poller leg BMA

Introduction: At the echocardiographic section of Clinical Physiology, Sahlgren´s University Hospital, echocardiography is performed on patients before surgery. There is a great variety of referral text and issues that underlie the echocardiography examination of patients that will undergo a non-cardiac surgery. The referral often lacks information regarding cause, previous findings and issues. The purpose of this study is to survey patients who have been examined with an echocardiography prior to a non-cardiac surgery. Proximally one-third of deaths postoperatively are caused by cardiovascular complications. Severe aortic stenosis and mitral stenosis and EF below 40% indicate high risk for patients undergoing surgery. The risk of a healthy patient having perioperative complications regardless of risk classification is 0.1%.

Method: 162 patients who underwent an echocardiographic examination at Clinical Physiology, Sahlgren´s University Hospital prior to a non-cardiac surgery were included in the survey. A protocol was designed in Excel with various categories such as gender, age and referral question. Mean, amount and percentage of the different categories were analyzed. The correlation between the effect of echocardiographic result of surgery and other categories analyzed with Chi-square test and Fisher´s exact test.

Results: 78.0% of patients had one or more comorbidity and 48.8% had an incomplete referral. 138 patients had a pathological examination result. The connection between the effect of echocardiographic result of surgery and the categories, if cardiac disease occurred during the anesthetic assessment and whether prior echocardiographic examination had occurred was significant with $p = 0.02$.

Discussion: Data from a patient that underwent an echocardiographic examination at another clinic were excluded because it could not be found in the computer system used. Excluding data affects the credibility of the connection. The connection may also have been affected by the low proportion of patients with an echocardiographic examination result that affected surgery.

Conclusion: Further analysis of connections between different categories and with the assistance of anesthesiologists is needed.

Microbroth dilution method is a better alternative than gradient test of resistance determination for *Streptococcus pneumoniae*

By Abu Osman

Bachelor thesis in Biomedical laboratory Science at clinical microbiology laboratory, Sahlgrenska hospital, University of Gothenburg,2021.
Supervisors : Erika Lindberg, PhD, Associate Professor

Determination of antibiotic resistance is used e.g to decide the minimum inhibitory concentration (MIC) of any antibiotic that are used to treat bacterial infections. Clinical microbiology laboratories use different methods to determine resistance of antibiotics, but The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommends microbroth dilution method as a reference method to determine MIC-values of antibiotics against *Streptococcus pneumoniae*, instead of gradient test (E-test). In this study, resistance determination for *S. pneumoniae* was validated by microbroth dilution method with Sensititre system.

Ten control strains with known MIC-values from EUCAST and 10 clinical isolates which were analysed at the national reference laboratory for antibiotic resistance determination in Växjö, were used. The Sensititre plate SEMST7 contained 9 various antibiotics with different concentration ranges. At the validation, precision, accuracy, method comparison, robustness, and also essential agreement and categorical agreement were analysed. The Sensititre plates were read by using VIZION, Trek Diagnostics.

The precision was validated by measuring the method's ability to repeat the results at different days, runs and incubation times. The essential agreement for both the control and clinical isolates was 100% for all antibiotics that were tested. The accuracy was tested by calculating the categorical agreement for strains according to the SIR-definition. The microbroth dilution method gave a categorical agreement of 90 -100% for the control strains and 67-100% for the clinical isolates.

The microbroth dilution method can be an acceptable alternative to gradient test (E-test), especially with labour efficiency and cost. In summary, the microbroth dilution method will be used to determine resistance for *S. pneumoniae* in clinical microbiology laboratory, Sahlgrenska University Hospital after further validation.

Optimization and evaluation of molecular diagnosis for *Helicobacter pylori*

-PCR on biopsies

By Anna Palm

Bachelor thesis in Biomedical Laboratory Science at department of molecular biology, Unilabs Skövde, 2020
Supervisor: Helena Enroth, Molecular biologist, PhD, Adjunct professor
Laboratory supervisor: Maria Andersson, BMA

Background: *Helicobacter pylori* infects over half of all adults in the world. The bacteria are associated with several diseases of the gastrointestinal tract. One problem with eradication of *Helicobacter pylori* is its development of antibiotic resistance, mainly against chlaritromycin. Several methods are available for detection. The present methods used at Unilabs are stool antigen test, immunohistochemistry on fixated tissue and culture of biopsies.

Aim: The purpose of this study was to investigate if diagnostics of *Helicobacter pylori* can be optimized, by evaluating whether PCR is an alternative to culture.

Method: Twenty-four patient isolates plus one reference isolate were cultured and 140 biopsies were pretreated. Isolates and biopsies were extracted, and a qualitative multiplex real-time PCR was performed with the commercial kit Amplidiag *H. pylori+Clari*^{R} Kit, from Mobidiag Espoo, Finland.

Result: Twenty-two isolates and the reference isolate grew in culture and those were also detected by PCR. Ten of the isolates were resistant against chlaritromycin, detected by both previous culture and PCR. Culture detected 37/137 (26,6%) positive biopsies for *H. pylori* and 12/137 positive for chlaritromycin. PCR detected 49 (35,3%) positive results for *H. pylori* and 14/137 positive for chlaritromycin. This means that the positivity rate increased by 8,7% from culture to PCR. The biopsies that only were positive in PCR generally had a higher Ct-value, indicating low number of bacteria. No false negatives were detected.

Conclusion: The kit was easy to apply, time efficient and showed a high sensitivity and specificity. Additional methods are needed to detect false positive results. The study suggests that detection of *Helicobacter pylori* with the kit Amplidiag *H. pylori+Clari*^{R}can optimize diagnostics for biopsies at Unilabs.

RETAINED SYSTOLIC AND DIASTOLIC FUNCTION OBSERVED THREE YEARS AFTER HEART TRANSPLANTATION

By Michaela Paulsson-Febo
Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2020
Supervisors: Bente Grüner Sveälv PhD, Entela Bollano MD, PhD, Pär Bertilson BSc

Introduction: Mortality amongst heart transplant recipients has been an issue since the first successful heart transplantation in 1967. The survival rate has increased successively, today 90 % of the patients are alive after 1 year and 60 % after 10 years. Despite the accomplishments made in the field, survival after cardiac transplantation is limited. Diastolic dysfunction is a known risk factor of increased mortality and therefore the patients require regular follow-ups. The aim of this study was to investigate if any early changes, with respect of systolic and diastolic function in the transplanted heart, could be detected from 1 and 3 years after heart transplantation regarding parameters from echocardiography and right heart catheterization. ***Methods:*** 61 heart transplant recipients were included in this study. Measurement data from echocardiography with doppler and right-sided cardiac catheterization were included from patients who underwent 1-year and 3-year follow-ups, respectively. The parameters of interests were blood pressure, heart rate, echocardiographic measurements from mitral inflow (E/A-ratio; deceleration time, DT), tissue doppler measurements (E/e'-ratio; isovolumetric relaxation time, IVRT; S', systolic velocity), ejection fraction (EF), left atrial volume index (LAVI) along with pulmonary capillary wedge pressure (PCWP) and PAP (pulmonary artery pressure). Due to several related parameters, a p-value less than 0.01was considered as statistically significant. ***Results:*** Using a paired student's t-test, we observed from the heart catheterization a decrease in resting heart rate (85 ± 11 to 79 ± 11, p value = 0.002), a trend to increased systolic blood pressure (123 ± 14 vs 130 ± 14, p value = 0.042); diastolic blood pressure (74 ± 9 vs 78 ± 9, p value = 0.051) and from echocardiography an impaired E/A-ratio (2.2 ± 0.6 vs 2.4 ± 0.8, p value = 0.029). ***Conclusion:*** The systolic and diastolic function are preserved 3 years after cardiac transplantation in adults. Between 1 and 3 years it is possible to observe a tendency to early change in systolic blood pressure and E/A-ratio. However, these results have to be interpreted with caution. If these changes have any significance for the development of diastolic/systolic dysfunction in the future is still uncertain. Additional studies with a longer term follow-up are required to confirm this conclusion.

A COMPARISON OF PLATELET FUNCTION AND QUALITY BETWEEN PLATELET CONCENTRATES MADE FROM FRESH AND OVERNIGHT STORED WHOLE BLOOD

By Isabelle Persson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical immunology and transfusion medicine, Sahlgrenska University Hospital, Gothenburg.
Supervisor: Helena Barreto Henriksson, Docent

Background: Platelets are cells in our peripheral blood and arrive at sight of vascular injury to create a platelet plug and thus prevent bleeding. Their function is dependent on their ability to adhere, activate and aggregate, which is regulated by receptors such as CD62p and CD6 and agonists. The quality of the PC is important, with requirements of a platelet count $>200x10^9$/unit and a white blood cell count (WBC) $<1x10^6$ /unit. Patients with low platelet count need platelets transfusions. Platelet concentrates (PC) are used for this and they can be produced by separating whole blood (WB) into its components by centrifugation. This can be done on fresh or overnight stored WB. One of the resulting components are interim platelet units (IPU). A PC can be created by pooling four IPUs of the same blood group together with platelet additive solution (PAS).

Aim: The aim of this study was to investigate if there is a difference in platelet count, WBC count, CD62p and CD61 activity and agonist triggered platelet function between PCs from fresh WB and PCs from overnight stored WB.

Method: Platelet count and WBC count was analyzed in 30 PCs made from fresh WB and 30 PCs made from overnight stored WB using flow cytometry and an automated cell counter. Platelet function stimulated by agonist ADP, TRAP-6 and ASPI was measured using Multiplate Analyzer, activity of CD62p and CD61 was measured and visualized using flow cytometry and immunohistochemistry in 10 PCs made from fresh WB and 10 PCs made from overnight stored WB.

Results and Conclusion: A significant difference was found, with platelets made from overnight stored WB, having a greater platelet count, activity of CD62p and platelet function stimulated by agonist TRAP and ADP. This information can be used to further optimize the workflow and provide patients with better PCs.

Abstract

Analysis of nanoparticles impact on induced pluripotent stem cells

By Hana Rahmani

Bachelor thesis in Biomedical Laboratory Science at Clinical Chemistry laboratory, Institute for Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2020

Supervisor: Stina Simonsson

Background: Tre dimensional (3D) bioprinting is a technique that can be used to print 3D structures of organ and tissues which mimic the natural living organ and tissues. 3D-bioprinting using induced pluripotent stem cells (iPSCs) in a bio-ink manufactured of 80% nanocellulose and 20% Alginate can be used to repair damaged cartilage of osteoarthritis patients.

The aim of this study is to measure the viability of iPSCs when they are exposed to different types of nanoparticles and nanoparticles that have different arthritis medicine bound to themselves. Nanoparticles that will be used in this study are produced by EU project RESTORE. A long-term goal is to transplant 3D-bioprinted cartilage tissue with ability to heal in OA-patient's defect side.

Material and method: Human induced pluripotent stem cells (iPSCs) cultured in Defined Culture System (DEF-CS). Cultured iPSCs were used for three dimensional (3D) bioprinting and analysis of nanoparticles impact on iPSCs. A bio-ink manufactured of 80% NFC and 20% A for 3D- bioprinting. Nanoparticles impact on iPSCs was analyzed through measurement of iPSCs viability that have been exposed to various nanoparticles for 24 hours with The NucleoCounter NC-200 with Via1Cassette. IN CELL Analyzer 6000 was also used to take confocal microscopic images of living/dead iPSCs that have been exposed to the various of nanoparticles. Live/Dead stain (ThermoFisher Scientific) 5µl Calcein AM and 20µl Ethidium homodimer-1 (EthD-1) were used to detect viability of the iPSCs.

Results: The analytical methods used in this research were difficult to handle in 96 well plate. Large dispersion of cell counting results was noticed. Despite these results more living cells were achieved from wells exposed to nanoparticles for 24 hours than wells without nanoparticles.

Conclusion: The conclusion shows that the nanoparticles had a positive effect rather than a negative effect on iPSCs.

Weak correlation between left atrial strain and left ventricular filling pressures in heart transplant recipients

By Anton Schubert

Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg 2020

Supervisors: Bente Grüner Sveälv PhD, Magnus Johansson PhD, MD, Entela Bollano MD, PhD, Hanna Siegmund, leg. BMA

Background: Heart transplant patients (HTx) require frequent heart catheterisations in order to monitor the pulmonary capillary wedge pressure (PCWP), which is important in order to be able to detect diastolic dysfunction and possible allograft rejection. The frequent routine catheterisation is a cause of risk and discomfort for the patient. An attractive alternative would be to use echocardiographic parameters as a non-invasive way to predict PCWP.

Aim: The purpose of this study was to find a correlation between deformation imaging by 2D speckle-tracking echocardiography (STE) in the left atrium and invasively measured PCWP in HTx recipients.

Method: 46 patients that had undergone heart transplantation at Sahlgrenska University Hospital were selected to participate in this study. As part of their routine surveillance they were examined by heart catheterization and echocardiography 1 and 3 years after their surgery. We measured atrial STE and strain-rate on the echocardiographic images in the reservoir, contraction and conduit phase and compared it with the invasively measured PCWP.

Results: Strain measured in the contraction phase of the left atrium correlated with PCWP at both the 1- and 3-year check-up (r -0.48, p=0.01 and r -0.43, p=0.04, respectively) The coefficient of variation (CV%) within two observers were: Reservoir strain (20.2%, averaged for 1 and 3 years). CV within one observer was: Reservoir strain (18.8% averaged), Contraction strain (25.5% averaged) and for strain rate (30.5% averaged).

Conclusion: In this study, we found a correlation between PCWP and strain-parameters measured in the left atrium. However, the results should be interpreted with caution as no patients with high PCWP were included in this study, the chosen method of measuring strain proved to be difficult to use, and CV for inter- and intravariability were above recommended values.

Abstract

Kinetic release of extracellular vesicles in a function of time

By Rahaf Zeer

Bachelor thesis in Biomedical Laboratory science performed at the Rheumatology department, Sahlgrenska Academy, University of Gothenburg, 2020.
Supervisors: Hadi Valadi, PhD and Muhammad Nawaz, PhD

Extracellular vesicles (EVs) are heterogeneous population of vesicles, which are variable in size and morphology and are different in their biogenesis. EVs are secreted from both prokaryotic and eukaryotic cells. Exosomes, microvesicles and apoptotic bodies are considered as EVs. They carry generic material and play important roles in intercellular communication by sending biological messages between cells. Because of cargo they carry and deliver, EVs are biologically important to study the normal physiology and diseases, and can be applied as diagnosis and therapeutics. In this study was to study kinetic release of Extracellular vesicles (EVs) in a function of time. The release of EVs is necessary to study, especially after knowing their biological and therapeutic importance. For the study, Human lung epithelial cells (HTB-177) were cultured for four days to produce EVs. Cells were harvested (cells and supernatants) at different time points to examine the EV release at function of time. EVs were isolated from supernatants at each time point (day 1 - day 4). Total EVs (proteins) and cells were counted and total RNA was isolated both from EVs and cells. Results showed that cells release EVs at varying amounts increasing with the passage of time and as cell growth proceeds. However, the overall, number of EVs remains more or less constant, which indicates that cells not only release EVs but also uptake the secreted EVs, possibly for communicating the messages.

Multiplex Real-time PCR with BD MAX-system for detection of enteric viral pathogens

A pilot study to the validation of BD MAX enteric viral panel using E-swab tubes

By Lars Åsfeldt

Bachelor thesis in Biomedical Laboratory Science performed at Klinisk mikrobiologi, Norra Älvsborgs Länssjukhus, Trollhättan, 2020

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

Viral gastroenteritis is an intestinal infection primarily caused by Noro-, Rota-, Adeno-, Sapo and Astroviruses and can cause illness in humans of all ages. Today, Norra Älvsborgs Länssjukhus (NÄL) uses multiplex real-time PCR (GeneXpert) for the analysis of Norovirus and immunochromatography for the analysis of Rota- and Adenovirus while Sapo- and Astrovirus is sent for analysis to Sahlgrenska Universitetssjukhus (SU). BD MAX enteric viral panel for BD MAX™-system offers the possibility to analyze all of these viruses in parallel. The aim of this study was to begin the validation of BD MAX enteric viral panel at NÄL using faeces samples collected in E-swab tubes.

The study included 38 samples whereas 30 were positive and 8 were negative. The distribution of the positive samples between Noro-, Rota-, Adeno-, Sapo and Astrovirus was 18, 2, 5, 3 and 2 respectively. The results showed a 100% sensitivity for Noro-, Rota-, Sapo and Astrovirus but only 60% for Adenovirus. The specificity for Noro-, Adeno-, Sapo-, and Astrovirus was 100% while Rotavirus showed 90% specificity. The low sensitivity at 60% regarding Adenovirus was the result of two false negatives and Rotavirus specificity at 90% was the cause of one false positive.

The conclusion of the study is that the method shows good potential in its ability to detect each of the viruses except Adenovirus, but given the inadequate number of samples, a fair evaluation of the method at this point is not possible. Further analyses are needed before a real assessment can be made of the methods reliability.