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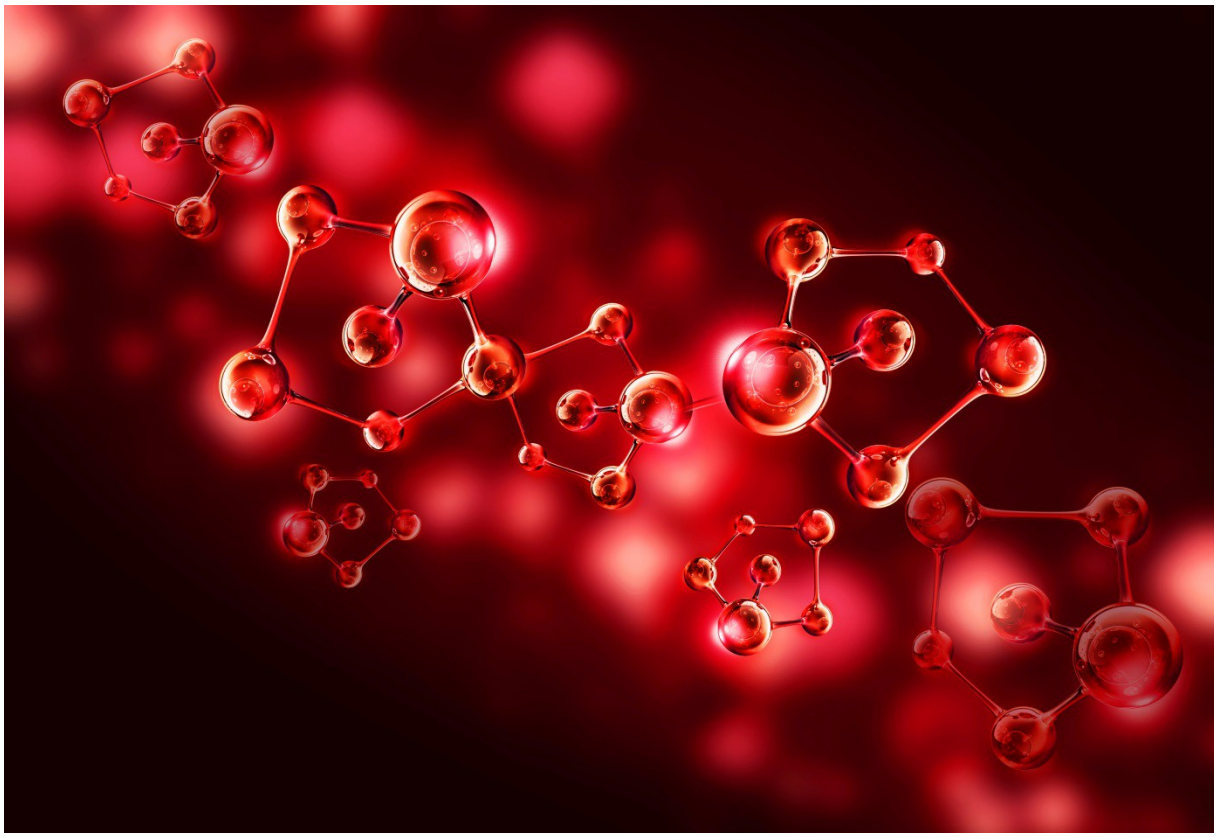

ABSTRACT BOOK 2017

Bachelor's Theses in Biomedical Laboratory Science

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ABSTRACT

Simple linear and areal dimensions can be used to calculate the total left ventricular myocardial mass

A cardiovascular magnetic resonance study

By Ameera Abusaif

Bachelor thesis in Biomedical Laboratory Science performed at the Departments of Cardiology and Clinical Physiology, Sahlgrenska University Hospital, University of Gothenburg, 2017.

Supervisor: Christian Lars Polte, MD, PhD, MSc
Co-supervisor: Odd Bech-Hanssen, MD, PhD

Background
Accurate assessment of the total left ventricular myocardial mass (LVM) is of utmost clinical importance. The first line diagnostic tool for the quantification of the total LVM is two-dimensional echocardiography (2DE). However, 2DE faces several limitations, as all available quantification methods as for instance the truncated ellipsoid technique are dependent on geometrical assumptions. Cardiovascular magnetic resonance (CMR), used as a second line diagnostic tool, is currently considered as the most accurate quantification method ("gold standard") to determine the total LVM using the so-called slice-summation technique. Nonetheless, CMR is more expensive and not as readily available as 2DE. Consequently, it is desirable to improve easier available 2DE methods in their diagnostic accuracy, which use linear and/or areal left ventricular (LV) dimensions for their calculations. However, the ability of linear and/or areal LV dimensions to predict and calculate the total LVM under optimal conditions such as in CMR is unknown.

Accordingly, the aims of this study were 1) to determine if simple, easily obtainable linear and/or areal LV dimensions as acquired by CMR can be used to determine the total LVM with acceptable precision and 2) to assess their respective inter- and intra-observer variability.

Methods
The study comprised a total of 20 healthy volunteers, 37 patients with aortic regurgitation and 37 patients with mitral regurgitation, which were subsequently divided into two subgroups (Derivation and Test group). CMR imaging was performed using balanced steady-state free precession sequences at a 1.5T scanner. LV linear (total length, length of the cylindrical part (CL) and length of the elliptical part (EL)) and areal dimensions (epicardial cross-sectional area, endocardial cross-sectional area and myocardial cross-sectional area (MCSA)) as well as the total LVM (according to the truncated ellipsoid technique, own regression equation (LVM = -196.3 + (32.5 x CL) + (9.4 x EL) + (7.8 x MCSA)) and slice summation technique (reference standard)) were obtained.

Results
Linear regression analysis (Derivation group) showed that a combination of the CL, EL and MCSA could best predict the total LVM as determined by the slice summation technique ($r = 0.97$, $p < 0.0001$). All three mass quantification methods determined overall a significantly different total LVM ($p < 0.0001$, Test group). The truncated ellipsoid technique overestimated the total LVM and our own regression equation underestimated the total LVM in relation to the slice summation technique (mean difference ± standard deviation 24 ± 24 g (limits of agreement -23 to 71 g) and -9 ± 16g (limits of agreement -40 to 22 g) respectively). Inter-observer variability was in general higher than intra-observer variability. Furthermore, linear LV dimensions had a lower variability than the obtained areal LV dimension.

Conclusion
In conclusion, our results show that simple, easily obtainable linear and areal LV dimensions can be used to obtain the total LVM with acceptable precision. Furthermore, our findings are of interest for the quantification of the total LVM by 2DE, but further studies are needed to evaluate feasibility and applicability.

Breathing is shown to have an effect on the quality of the myocardium images at D-SPECT

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2016.

Supervisior: Gert Hermansson, (JK, MP)

By Zahra Alhashemi

Abstract

Introduction: Coronary artery disease (CAD) is a disease caused by atherosclerosis and as a consequence of CAD, ischemia can develop. This disease can be diagnosed with myocardial scintigraphy. Recently, myocardial scintigraphy technology has been further developed. One of the new cameras is the D-SPECT camera. With the new technology there are certain limitations as artifacts can occur for various reasons. A suspected cause of the artifacts is physiological breathing and heart motions. The heart moves because of diaphragm movement associated with breathing. Patient movements can lead to artifacts that can be confused with ischemia both visually and quantitatively.

Purpose: The purpose of the study is to evaluate the effects of breathing motion on the image quality of D-SPECT, to provide a more reliable assessment.

Method: This study was based on a population of 10 patients. The patients underwent a standard rest study and two additional breathing images. Breathing images were taken during two different breathing types: chest and abdominal breathing. Then a weighted kappa test was made for comparisons of the effects of breathing motions in the different studies.

Result: The result of the weighted kappa for the standard vs breast study showed 78 % agreement. The weighted kappa value for standard vs abdominal was 77 % and 65 % agreement for breast vs abdominal. The mean value of obvious motion artifacts in the standard study was 1,8 , 2,2 during chest breathing and 1,9 during abdominal breathing.

Conclusion: Based on the results, it can be concluded that the image quality of the D-SPECT images may be affected by breathing motions, but that does not have a significant impact on the overall assessment. The assessment of severely sick patients remains the same and vice versa regardless of breathing movements.

Genotypic identification of clinically relevant species within the genus *Actinomyces*

By Ali Alshabeeb

Bachelor thesis in Biomedical Laboratory Science performed at CCUG Sahlgrenska Hospital, Sahlgrenska Academy, University of Gothenburg 2017.

Supervisor: Hedvig Engström Jakobsson, Post doctorial researcher.

Actinomyces is a genus belonging to the Actinobacteria fylle and consists of more than 30 species. *Actinomyces* are part of the normal bacterial flora. However, some species within the genus, such as *A. naeslundii, A. viscosus, A. oris* and *A. johnsonii*, have been shown to be involved in development of disease, for example in development of peridontal diseases and plaques. Identification of these species is today based on clinical microbiological laboratories using a range of biochemical and physiological tests as well as16S rRNA gene sanger sequencing. However, analysis of the 16S rRNA gene or by the use of conventional phenotypic tests is not sufficiently discriminatory for the most closely related spices.

In this study, we have investigated a representative collection of type and reference strains and oral isolates (n=50) and determined the partial gene sequences of three different housekeeping genes (*atpA, metG* and *secY*) which were described as good markers for species discrimination within the *Actinomyces* genus. These results were compared with 16S rRNA gene analysis as well as phenotypic data. These sequences identified the 4 closely related species and distinguish them. The partial sequences of *secY* and *metG* gave the best separation. The separation with *atpA* was not as good as others especially for *A. viscosus* and *A. johnsonii*.

The analysis of the phylogenetic trees for these housekeeping genes (*atpA, metG* and *secY*) for 4 closely related strains, observed a clearly separation between *A. oris, A. naeslundii, A. viscosus* and *A, jonsonii*. In this study, we could show that sequencing of the 16SrRNA gene is not sufficient to properly distinguish the closely related species. *AtpA, metG* and *secY* gave a good separation of the 4 closely related species and can distinguish them.

Proteolytic degradation of lubricin by synovial proteases

Towards understanding the loss of lubrication in ostearthritis

By Martin Angel

Bachelor thesis in Biomedical Laboratory science performed at the medical biochemistry and microbiology institute for biomedicine, Sahlgrenska Academy, at University of Gothenburg

Supervisor: Niclas Karlsson

Co-supervisor: Shan Huang

Lubricin, is a major component within synovial fluid and is believed to be contributing to the nearly friction free movement of the joint. An proteolytic degradation model of the osteoarthritis related loss of joint lubrication was developed and include optimization of conditions by altering incubation time and degrading enzyme concentration. The hypothesis was that MMP3 enzyme and other proteases in the synovial tissue are capable in destroying the boundary lubrication area of the joint by degrading lubricin on the cartilage surface. Our research will yield valuable insight into the initiation of osteoarthritis. The methods used to test the lubricin degradation by these proteases were SDS-PAGE, Western blot and mass spectrometry. The SDS-PAGE showed the fragmentation of lubricin and distinct bands representing degraded parts of lubricin could be analysed further by mass spectrometry. Western blot was also used to identify lubricin the cleavage-sites. Mass spectrometry was shown to be paramount to determine the content of the bands excised from the gel. Both MMP3 and cathepsin G seemed to cleave the lubricin at the C-terminal, possibly altering its lubricating function, but calpain 1 did not seem to be able to degrade lubricin. These findings are interesting and may shown to be significant for protelysis of lubricin as part of the pathology of OA. Further research is required for investigating Cathepsin G's and MMP3's involvement in the loss of joint lubrication experienced by patients with OA. ***In conclusion:*** Lubricin, which is one of three major component within synovialfluid, can be degraded by some of the proteases which are present within the SF and the surrounding area. MMP3 and Cathepsin G seems to cleave the lubricin at the C-terminal, possibly altering its biological functions. It remains unclear to what degree this is responsible for the loss of joint lubrication experienced by patients with OA, and it is still a long journey for us to explore if the enzymatic activity of these proteases is a part of the pathology of OA.

GENDER BIAS IN FOXP3 EXPRESSION IN HUMAN THYMIC TISSUE

By: Anton Arvidsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisor: Christina Lundqvist, Olov Ekwall

Introduction: World wide it is estimated that 5 - 10 % of the general population is affected by an autoimmune disease, and the numbers are growing. Females have an increased risk of developing autoimmunity with up to 9 times higher risk in some diseases. Two molecules have showed importance for the development of autoimmunity, AIRE in the medullar epithelial cells and Foxp3 in T_{reg} cells. In the thymus, the expression of AIRE has been shown to be increased in males compared to females but no such study has been made on Foxp3.

Aim: The aim of this study was to investigate if there is a difference in AIRE and Foxp3+ T_{reg} cells between male and female thymic tissue.

Material and method: Frozen thymus from 24 thymectomized children was sectioned in a cryostat and stained for AIRE and Foxp3. The samples were acquired with a confocal microscopy and the images were analyzed in ImageJ. Each sample was age and gender matched.

Results: No significant difference was found in AIRE expression between male and female thymic tissue. In Foxp3 expression levels, a significant difference was found with a p value of 0.03

Conclusion: This study found a significant difference between male and female in Foxp3+ T_{reg} cells, where females showed higher amount of Foxp3+ T_{reg} cells. Suggestively a new study should be made to determine why females show higher levels in thymus but lower levels in peripheral blood compared to males.

Analysis of growth factors in bone healing by MALDI spectrometry

By Olga Arvidsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Medical Chemistry and Cell Biology, Sahlgrenska Academy, University of Gothenburg, 2017
Supervisor: Håkan Nygren, Professor

Background. Bone has a remarkable potential for regeneration after injury and inducing of new bone formation is regulated by different growth factors. These compounds induce bone tissue building and reparation and blood vessel formation. BMP and TGF-β are signal transductions molecules from TGF- β superfamily. These proteins can bind various TGF-β receptors leading to recruitment and activation of SMAD family transcription factors and activate different genes. BMP2, BMP3, BMP7 are produced in bone and show osteogenic activity. VEGF is important for angiogenesis. Neuropeptides CGRP and substance P control bone formation and reparation. CGRP is a hormone which regulates a potent vasodilator calcitonin, which regulates of calcium and phosphorus metabolism. Substance P is an important signal peptide in nociception and tissue reparation. Mineralised bone is challenging to investigate. Bone and bone marrow are tightly associated anatomically and functionally and represent two sides of the same unit. Bone healing process enters bone marrow after bone injury and that is why bone marrow around the operation site can be used for analyse of growth factors. MALDI TOF MS gives a possibility to measure a physical property of analyte itself, its mass/charge (m/z) and is used for analysis of biomolecules. The aim of this work is to choose a suitable matrix for MALDI analysis of bone marrow proteins and to analyse growth factors associated with bone healing processes.

Materials and methods. Two male Sprague Dawley rats were used for experiments. One rat was operated (drilled tibia) and another one was untreated. Seven days after the operation both animals were harvested. Three different matrices (CHCA, SA and DHA) were tested in order to find the suitable matrix for MALDI measurement. Bone marrow proteins from these animals were investigated with this method. Mann-Whitney U test was used to compare some peaks with the same m/z for treated and untreated rats.

Results. DHA matrix was successfully used with MALDI analysis of bone marrow. All spectra obtained for the operated animal were more intense for detected peaks. Statistically significant differences were found for m/z 7929($p=0,029$), m/z 11055 ($p=0,029$), m/z 15206 ($p=0,029$) and m/z 15857 ($p=0,029$).

Conclusion. More intensive spectrum of bone marrow from operated animal can be result of higher growth factors concentration under bone healing process. It is possible that one of detected high peaks (m/z 15857) on treated rat spectrum is a BMP3 subunit. Further identification of this protein is needed.

Functional study of a disease-causing mutation in MGME1

By Malin Bengtsson

Bachelor thesis in Biomedical Laboratory Science performed at Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2017.
Supervisor: Jennifer Uhler, PhD; Co-supervisor: Maria Falkenberg, Professor

MGME1 is a recently discovered protein, which is encoded in the nucleus, but localizes to mitochondria. MGME1 has been shown to have nuclease activity with preference for single-stranded DNA. The function of MGME1 is not yet fully understood, but current knowledge supports a role in mtDNA maintenance and particularly in 7S DNA turnover. Previously, homozygous MGME1 mutations have been identified and shown to result in mtDNA depletion, increased levels of 7S DNA, and a deletion producing an 11-kb linear mtDNA fragment. In this project, a newly identified heterozygous MGME1 mutation was studied. The aim was to investigate if this new mutation has the same effect on mtDNA as the previously identified mutations. The study also aimed to investigate how MGME1 nuclease activity is affected by the heterozygous mutation.

Total DNA analysis by qPCR showed that patient fibroblasts had decreased levels of mtDNA and increased levels of 7S DNA compared to control fibroblasts. The results did not indicate the presence of the 11-kb linear mtDNA fragment.

The open reading frame of wildtype MGME1 with a His-tag was generated using PCR, and was used as a template to generate the mutant MGME1 by site-directed mutagenesis. Amplicons were ligated to the Pet17b plasmid and transformed into E. coli for protein expression. Optimization of wildtype MGME1 expression showed that the addition of 1 % glucose to LB media increased the amount of soluble MGME1. Wildtype MGME1 was purified with affinity and ion exchange chromatography. This resulted in a low protein yield and the degree of purity was insufficient for nuclease assays. Expression of mutant MGME1 was induced, but MGME1 could not be detected after the first purification step.

In conclusion, the results suggest that the heterozygous mutation studied here gives rise to impaired MGME1 function, which has similar effects on mtDNA and 7S DNA levels as previously studied homozygous mutations. However, repeated experiments would be required to confirm this statistically. Neither wildtype, nor mutant MGME1 was successfully purified. This suggests that further optimization of expression and purification is needed. Also, it must be established whether the mutant MGME1 can be expressed in vitro or not.

Positive cardiovascular effects of dark chocolate intake in young and healthy adults

A randomized intervention trial

By Jacqueline Bergqvist

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical Physiology department, Coimbra Health School, Polytechnic Institute, Portugal 2017

Supervisor: Telmo Pereira, Senior Lecturer, Ph.D.

Introduction: Previous studies suggest that flavanol-rich chocolate can decrease the risks associated with cardiovascular disease. The purpose of this study was to evaluate and explore the benefits of long-term dark chocolate intake in young healthy adults by measuring cardiovascular function.

Methods: The study followed an intervention randomized and single blind trial design, comparing two objects of study; a control group assigned to chocolate with 55% cocoa content and an intervention group assigned to chocolate with 90% cocoa content. A baseline evaluation was performed before the participants ingested 20 grams of assigned chocolate type for 30 days in a row and a final evaluation was performed by the same means. The methods used for evaluation of the cardiovascular system were echocardiography, pulse wave velocity, pulse wave analysis and ventricular-arterial coupling.

Results: The baseline evaluation presented similar values within normal range in both groups. The positive vascular effects were overall more distinct in the group eating dark chocolate. In the total study group, we observed a significant increase in flow mediated slowing after intervention, from 6.57 ±3.21% at baseline to 11.77 ±5.33% ($p<0.001$). No structural modifications on the heart were found after intervention and cardiac function was improved on certain parameters, concerning 90% cocoa only. The ventricular-arterial coupling also displayed more enhanced values from intake of dark chocolate compared to less dark chocolate. We observed a significant improvement in the group of 90% cocoa after intervention, with coupling increasing from 0.674 to 0.719 ($p=0.004$), compared with a not significant change post intervention in the other group.

Conclusion: This study shows that a frequent consumption of dark chocolate has more beneficial effects of improving cardiovascular health in young and healthy adults, compared to chocolate with less cocoa content. Therefore, intake of dark chocolate can reduce the risks associated with cardiovascular disease but further research is needed to clarify all underlying mechanisms.

NO AGREEMENT BETWEEN INVASIVE AND NON-INVASIVE METHODS REGARDING FILLING PRESSURE AND DIASTOLIC FUNCTION IN HEART-TRANSPLANTED PATIENTS.

By Pär Bertilson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2017.
Supervisior: Entela Bollano (MD, PhD), Bente Grüner Sveälv (PhD)

Background: One of the most common causes of death after a cardiac transplant, is diastolic heart failure, which is characterized by elevated filling pressure. Cardiac catheterization is assessed to evaluate invasive diastolic function through measurement of left ventricular filling pressure. However, this procedure is stressful and exerts an increased risk for the patient. Furthermore, an invasive procedure is time consuming, expensive and requires highly qualified health care providers. In view of this, it is of the utmost importance to find a safe, non-invasive method, in order to measure the filling pressure.

Aim: The purpose of this study was to observe whether there is an agreement between invasive and non-invasive methods, and subsequent filling pressure and diastolic function on cardiac transplanted patients.

Method: 33 patients, all of whom have undergone a heart transplantation at Sahlgrenska University Hospital, and who have complied with the requirements for the echocardiographic examination, were selected to participate in the study. E and A from the mitral inflow, S and D from the lung flow, S, É, Á and IVRT from tissue Doppler were measured. Based on these values, a non-invasive pulmonary capillary wedge pressure (PCWP) was estimated using two different equations: $PCWP_{nagueh}$ and $PCWP_{richards}$. These were then compared with the PCWP value as measured by cardiac catheterization. Agreement was studied using a Bland Altman plot, and Spearman's rank correlation was performed in order to study correlation between PCWP from the cardiac catheterization and other measured variables.

Results: The result of cardiac catheterization shows that patients had an average $PCWP_{rest}$ of 5.94 ± 2.67 mmHg. The ultrasound measurements resulted in a $PCWP_{Nagueh}$ of an average 11.16 ± 3.55 mmHg, maximum value of 23.17 mmHg, and a minimum value of 7.63 mmHg. According to $PCWP_{richards}$ 19.99 ± 4.74 mmHg, with maximum and minimum values of 26.66 mmHg and 15.11 mmHg respectively. The Bland Altman plot showed that all values were within 2SD from the mean difference of the two measurements compared. However, high CV% was shown. The measurements of Spearman's correlation only showed two statically significant correlations between $PCWP_{rest}$ and PA and IVRT, but these were very weak.

Conclusion: The result of this study reinforces the fact that ecochardiographic parameters alone not can predict PCWP. We observed insignificant agreement between invasive and noninvasive methods with regarding the filling pressure and diastolic function in heart transplanted patients. In this present study invasive measurement is the gold standard to measure left ventricular filling pressure

Thermography can be used as an adjunct to ultrasound examination in diagnosis of venous insufficiency in legs

By Alexandra Bystedt

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

Sahlgrenska Academy, University of Gothenburg, 2017

Supervisor: Anders Thurin, MD

Background: Chronic venous insufficiency (CVI) is a disorder which affects a large part of the Western world, and is characterised by venous valvular dysfunction with manifestations ranging from telangiectasias to skin changes with active ulcer. Varicose veins are a common presentation of CVI and affects superficial veins in legs. The primary method for examination and preoperative mapping of veins is duplex ultrasound (DUS), but thermal imaging has recently become more accessible and has been discussed as a promising method that can give additional information in circulatory as well as in inflammatory disease.

The aim of the study was to investigate the utility of thermal imaging as an adjunct to duplex ultrasound examination of veins in examining superficial veins in the legs of patients with CVI.

Material and methods: In total, we included 49 legs in 42 patients, 24 females and 18 males with average age 58 years, scheduled for routine DUS venous exams. Thermography of the legs was performed before DUS in various positions (ventral, dorsal, lateral and medial sides), and in a subgroup of 26 patients (31 legs) thermography images were also taken after provocation (1 minute of cooling of the leg and 10 tiptoe movements). Recorded thermography images were visually compared with DUS reports by 4 judges, 3 of whom were experienced ultrasonographers.

Results: The main trunk of great and small saphenous veins can rarely be visualised by thermography, but superficial branches of these veins can often be seen. Severely incompetent perforator veins (by DUS graded as incompetent grade 2-3/3) were seen as “hot spots” (36,8% of cases) and missed in 52,6%. Weighted kappa value showed bad and less god agreement between 4 judges. The method of cooling legs (with a wet towel) did not seem to make significant difference (p=0.808) for the interpretations.

Conclusion: Thermography is a simple and fast method that can be used specially to locate incompetent perforator veins and document superficial branches in combination with ultrasound examination.

SEPERATE ABSTRACT

Optimization of fluorescence-activated cell sorting of leukemic populations for xenotransplantation and DNA/RNA co-extraction

By Jef Callaerts

Bachelor thesis in Biomedical Laboratory Science performed at Clincial Chemistry department, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisor: Pegah Johansson, PhD

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by undifferentiated and proliferative white blood cells, dominating the patient´s blood stream and bone marrow. In this bulk of leukemic cells, different subpopulations exist, of which some have relapse-initiating properties after surviving chemotherapy. However, little is known about the identity of these malignant cells. By sorting the subpopulations using fluorescence-activated cell sorting (FACS) and observing engraftment after injection into immuno-compromised mice, their identity can be studied. The aim of this project was to optimize FACS in order to sort viable populations for future engraftment studies. Viability of cells was studied for different sorting conditions using flow cytometric apoptosis and proliferation assays. Furthermore, we aimed to optimize co-extraction of DNA and RNA from low cell numbers, in order to genetically characterize sorted populations. The results suggested that, although cell death due to FACS was inevitable, viable populations could be sorted and kept alive for 24 hours prior to engraftment studies. We also found that during FACS, temperature, antibody staining and concentration of fetal bovine serum in medium affected viability. Additionally, DNA and RNA could be extracted from as little as 5000 sorted leukemic cells. These findings can contribute to future identification and genetic characterization of leukemic cell populations.

Identification of the phylogenetic B2 sub-types in *Escherichia coli* obtained from the intestinal microflora of humans

By Mona Edin

Bachelor thesis in Biomedical Laboratory Science performed at the department of microbiology, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisor: Forough L. Nowrouzian

Introduction: *Escherichia coli* (*E.coli*) are a gram-negative facultative anaerobic bacterium that colonizes the human colon. The bacteria belong to the normal intestinal flora and serves as a commensal organism. Some *E.coli* can spread from their normal niche and cause extra-intestinal infections. Phylogenetic analyses have shown that *E.coli* can be divided into four different phylogenetic groups: A, B1, B2 and D. The phylogenetic group B2 consists of the most virulent strains and have been divided into ten different sub-groups (I-X). The most pathogenic B2 strains belongs to sub-groups I, III and IV and non-virulent strains often belong to sub-group VIII. The remaining sub-groups (II, V, VI, VII and IX) can be found to be both pathogenic and non-pathogenic. **Aim:** a) To set up a polymerase chain reaction (PCR) based method for detection of B2 sub-groups, b) to determine the distribution of B2 sub-types obtained from the intestinal microflora of three different human populations and c), to investigate whether particular B2 sub-types contribute to persistence of *E.coli* strains in the gut of healthy Swedish infants. **Methods:** A total of 157 B2 *E.coli* strains were investigated. The strains were obtained from the intestinal microbiota of Swedish schoolgirl's whit asymptomatic bacteriuria, healthy Pakistani infants and healthy Swedish infants. A recently described PCR method was optimized. DNA where extracted from the bacterial samples and two multiplex PCR assays were used to determinate the B2 sub-types. The PCR-products were separated using agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light. **Results:** Of the 157 strains tested, B2 sub-types could be determined among 114 strains (ca 73%). The B2 strains from Swedish infants mostly belonged to sub-groups II and IX were as sub-groups VII and IX where the most frequent in the Swedish schoolgirl´s. However, the majority of B2 strains from the Pakistani infants where undetermined. Furthermore, the *E.coli* strains from healthy Swedish infants the sub-group II and VI were more common in the long-time colonizers, but did not reach statistical significance. **Conclusion:** Our optimized method worked well and it gave reliable results when comparing with control strains in different experiments. The results suggests that B2 sub-types colonizing the intestinal microflora differs between different human populations and that strict pathogenic groups are not well adapted for de intestinal flora of infants.

Superficial vein thrombosis is common in PVC-Treatment

By Shamiran Yokhana

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Physiology Östra, Sahlgrenska Academy, University of Gothenburg, 2017.

Supervisor: Anders Thurin, MD

Introduction: Peripheral venous catheters (PVC) are widely used in health care to administer nutrition, liquids and drugs. PVC treatment is associated with many complications such as bruising, extravasation and infection, but the most common complication is thrombophlebitis. Superficial vein thrombosis is a common and benign disease, most often found in the veins of the lower extremities but occurs also in the upper extremity. Among risk factors for superficial vein thrombosis are endothelial damage.

Aim: The aim of this study was to investigate a group of patients with peripheral venous catheter in an arm or hand to determine whether small blood clots could be detected near a peripheral venous catheter during a ~ 24 hour follow-up.

Methods: The study population included 20 patients who during a two-day protocol of myocardial scintigraphy were investigated at three different occasions using ultrasonography; vein diameter at PVC site was measured with and without probe compression and a non-compressible vein was considered to contain a thrombus.

Results: Out of the 20 patients included, non-compressible veins were found in 17 patients at the first investigation with PVC duration around 4 hours. In the second investigation with PVC duration ~22 hours non-compressible veins were found in the entire study population and compressed diameter (thrombus size) was significantly increased ($p<0.02$) compared to first exam. In the last investigation with PVC duration ~26 hours non-compressible veins were found in 18 patients.

Conclusion: All of the patients had signs of superficial venous thrombosis during PVC treatment. It seems thrombosis often starts early and grows or stabilizes during the first 24 hours.

Information extraction out of echocardiographic answers works well, can be used in research and working monitoring

By Isabelle Enell

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Physiology, Östra hospital, Sahlgrenska Academy, University of Gothenburg, 2017.
Supervisor: Anders Thurin, Consultant MD.

Background: The function of the left atrium is to work as a reservoir for the pulmonary venous return, as a conduit for the pulmonary venous return and as a booster pump. An enlarged left atrium is often a result from an overpressure and/or a significant increase of the volume. It is also associated with atrial fibrillation which negatively affects the ventricular filling and the cardiac output. Thromboembolism is the most important complication of atrial fibrillation, and the most common cause of stroke in the elderly. Regular expressions is a method to specify search patterns, and can be used to extract information from medical records and reports. Such extracted information can be processed to for example, calculate reference values and population statistics.

Aim: The aim of this study was to test information extraction from echocardiographic reports from Clinical Physiology, Östra hospital with focus on atrial dimensions. We hope to relate this information to complications including mortality and presence of atrial fibrillation, heart failure and stroke.

Method: Computer scripts based on regular expressions were used to extract information from >38 000 textual reports to find data regarding left atrium, right atrium, left atrial volume index and ejection fraction. The extracted information was examined manually and extraction scripts improved in several recurrences.

Results: The quality of information extraction measured with F-value were for atrial dimensions 97,7 %, for left atrial volume index 99,9 %, for ejection fraction 99,8 % and for rhythm 93,3 %. Highly significant relations between atrial dimension and of age, gender, rhythm and ejection fraction could be shown using extracted data.

Conclusion: Regular expressions seems like a useful method to extract information from diagnostic reports, and for these searches in our material we reach high values of precision and recall. We demonstrate possible further analyses of data around the left atrium, and because of the large number of analysed cases, it’s easy to get highly significant relations.

Broth microdilution with sensititre, an alternative method for MIC-susceptibility testing of resistant gram-negative bacteria.

By: Sara Hagstedt
Bachelor thesis in Biomedical Laboratory Science performed at the institution of biomedicine, Department of infectious diseases, clinical microbiology, university of Gothenburg/ Sahlgrenska university hospital year 2017.
Supervisor: Erika Lindberg, PhD.

ABSTRACT

Extended spectrum beta lactamase (ESBL) producing bacteria are a worldwide problem which is growing and the increasing resistance to antibiotics is putting a lot of pressure on the different antibiotic susceptibility testing methods. Broth microdilution (BMD) is the method that's been classified as the "gold standard" that provides the most correct minimal inhibitory concentration (MIC) values.

The aim of this study was to evaluate a BMD method using a sensititre plate and compare it with the results from a reference laboratory. This 96 well plate has 17 different antibiotics in different concentrations and the MIC-value is determined in the well where the bacteria growth is inhibited.

The plates were read manually to reduce the minor, major and very major errors. A total of 55 isolates were analyzed and the majority of the bacteria where *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, but also a few other gram-negative species. The results were approved if the results varied no more than +/- 1 step from the reference laboratories results. The sensititre plate gave results that were approved in 94.5 % of the cases and only hade < 9% minor (**S/R** by one metod but **I** by the other metod using the SIR-system), major (**R** with sensititre but **S** by the reference) and very major errors (**S** with sensititre but **R** by reference). The approved percentage by the Clinical Laboratory Standard Institute (CLSI) for minor errors is 40 % and major errors and very major errors 10 %. There were some antibiotics what differed more than others, but overall the Sensititre method seemed to be an alternative method to E-test for antibiotic susceptibility testing of MIC.

Low reproducibility and a need for age-related reference values in assessing diastolic function

By Erika Hernlund

Bachelor thesis in Biomedical Laboratory Science performed at Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisor: Odd Bech-Hanssen, Associate Professor, chief Physician

Background: A routine echocardiography examination of the heart includes evaluation of the left ventricle's diastolic function. When assessing the diastolic function, early mitral inflow velocity (E) with pulsed Doppler (PD) and the early velocity (e') in the septal and lateral annular plane with tissue Doppler (TD) are important components.
Aims: The aim of the study was to, examine the reproducibility of the PD and TD measurements, in a prospective part. And to examine the distribution of E, e' septal, e' lateral and E/e' in a population considered normal as determinated by comprehensive echocardiography.
Method: We prospectively included patients (n = 26) in sinus rhythm. Two blinded investigators registered one set each of the required measurements. The first investigator (EH) performed measurements twice on the first registration and once on the second while the second investigator (OBH) performed measurements on the second registration. In the retrospective part 222 patients with normal echocardiographic findings were included.
Results: There was a significant difference in the measurement of e' lateral (P = 0.02) and near significant difference e' septal (P = 0.08) when 1 investigator measured on two different registrations. There was also a significant difference in measurement of E-velocity (P<0.0001), e' lateral (P <0.001), e' septal (P <0.0001) and the E/e' (P=0.002) when 2 investigators measured on the same registration. Among patients> 41 there were 28% respectively 44%, which have values below the thresholds for diastolic dysfunction in the parameters e' septal and e' lateral.
Conclusion: In the present study we found low reproducibility for parameters used in assessment of diastolic function that was due to differences in how the registrations and measurements were done. In individuals >40 years there are a large proportion with e' septal and e' lateral values that are below the recommended thresholds indicating diastolic dysfunction.

Optimized qPCR detected high frequency of hepatitis E virus RNA in serum of Swedish blood donors

By Veronica Amaya Johansson
Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2017
Supervisor: Heléne Norder, Professor and Marie Karlsson, medical laboratory scientist

Background Hepatitis E virus (HEV) is a non-enveloped RNA virus infecting both humans and animals. There are five genotypes of HEV (HEV1-4, and HEV7) that infect humans. HEV1 and 2 infect only humans while HEV3, 4 and 7 also infect animals. HEV1 and 2 are prevalent in Asia and Africa and may cause large water borne outbreaks with high mortality in pregnant females. HEV3 and 4 have zoonotic transmissions mainly from domestic pigs and wild boars. HEV3 is endemic in industrialized countries and may cause acute hepatitis which can develop into chronic hepatitis in immunocompromised patients. These patients may get infected by HEV infected blood components. The prevalence of HEV RNA in blood products from four European countries has been shown to vary between 0.02-0.05%.

Aim The aim of this study was to optimize a qPCR for HEV RNA detection and compare assays for extraction of nucleic acids in sera, to use these techniques to investigate the frequency of HEV RNA in sera from Swedish blood donors.

Methods The most sensitive detection method for HEV RNA was optimized by analyzing different concentrations of primers and probe in the qPCR. Two commercial assays for nucleic acid extraction were compared. The most sensitive techniques were used for analyzing sera from blood donors collected weekly during 2016. A total of 1 111 sera from blood donors were used in pools of 5 sera each in the analyses.

Results The most sensitive concentration of primers and probe detecting a low level of HEV RNA in the RT-qPCR was 20 µM in all primers and probe A higher amount of HEV RNA were detected in all serum concentrations with Circulating Nucleic Acid (Qiagen). The most sensitive techniques analyzed HEV RNA in 225 pools in which HEV RNA was identified in six serum pools and additional six pools showed a weak reactivity for HEV RNA. If one serum in each pool is considered as HEV RNA positive, the frequency of HEV RNA was shown to vary between 0.5 to 1%, which is high compared to the findings from other European countries.

Conclusion The results of this study showed that optimization of the techniques made it possible to detected HEV RNA in low concentrations. The frequency of HEV RNA in Swedish blood donors was higher than expected. However, further investigations are needed to certify that there was no false reactivity and to determine the number of positive blood donors in each serum pool. It is also important to sequence the blood donor strains to identify possible blood borne transmissions by comparing these HEV strains to those from infected patients.

Evaluation of circulating calprotectin (S100A8/A9) levels in a prospective cohort of children with Juvenil Idiopathic Arthrithis

By Hanna Kaup
Bachelor thesis in Biomedical Laboratory Science performed at Clinical Immunology, Sahlgrenska University Hospital, University of Gothenburg, 2017
Supervisor: Rille Pullerits

Background: Calprotectin is a complex consisting of two subunits, S100A8 which has a molecular weight of 8 kD and S100A9 with a weight of 14 kD. Its growth-inhibiting and death-inducing activity tells us that calprotectin has a role in the inflammatory process. Calprotectin has been found in elevated levels in many inflammatory diseases including juvenile idiopathic arthritis (JIA). JIA is a broad term for arthritis with unknown cause which begins before the age of 16 and is divided into seven subgroups.
Aim: The aim of this study was to evaluate serum calprotectin levels in JIA patients to determine whether measuring the levels of calprotectin might be useful in clinical praxis as routine analysis and whether it reflect the extent of inflammation in JIA patients.
Method: Serum samples from 109 Estonian children with JIA were included. A 10 year follow-up group with 22 samples from the initial cohort were also analysed. A control group consisted of 36 children who were investigated for suspected allergy. An enzyme-linked immunosorbent assay was optimized and used for calprotectin detection. Calprotectin levels were analysed with respect to JIA subgroups, inflammatory markers and disease characteristics.
Result: We observed a significant positive correlation ($p=0.004$, $\rho=0.464$) between calprotectin levels and erythrocyte sedimentation rate (ESR). This shows that calprotectin might have a role in inflammation. There was a statistically significant difference ($p=0.017$) in calprotectin levels between HLA-B27 positive (median1404 ng/mL) and HLA-B27 negative (553 ng/mL) JIA patients. No differences in calprotectin levels were seen between different JIA subgroups. Surprisingly, JIA patients had significantly lower ($p<0.05$) serum calprotectin levels (median 888 ng/ml) compared to disease controls (median 1382 ng/mL).
Conclusion: Calprotectin levels seem to reflect the extent of inflammation in JIA and might be clinically useful as an inflammatory marker. However, calprotectin levels had no additional value for discrimination between different JIA subgroups in this study cohort. Further investigation is needed using healthy controls and larger patient cohorts with more patients in different subgroups in order to evaluate the usefulness of calprotectin in JIA diagnosis.

THE LEVEL OF FACTOR VIII IN PLASMA FOR TRANSFUSION DECREASE DURING STORAGE AT 4 DEGREES CELSIUS

By: Lisa Kylbring

Bachelor thesis in Biomedical Laboratory Science performed at the laboratory for immunohematology & component preparation and the laboratory of clinical chemistry, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisors: Camilla Hesse, Senior lecturer. Inger Fagerberg Blixter, BMA. Lena Lyxe, physician.

Introduction: Factor VIII (FVIII) is one of the most important coagulation factors. The level of FVIII differs depending on multiple variables such as gender, blood group and infection. According to European guidelines plasma for transfusion shall contain a minimum of 70IU/100mL (0,7kIE/ml) of FVIII. There are mainly two ways to measure FVIII today, a one-stage clot method and a chromogenic assay. The clot-time analysis is based upon APTT and the chromogenic is based in hydrolyzation of a substrate and photometrical detection. At the blood bank at Sahlgrenska thawed plasma is stored for 14 days in 4°C before it is outdated.
Aim: The aim of this study was to compare the two methods for FVIII measurements and to investigate for how long FVIII is sustainable when stored in 4°C. Also, the study aimed to investigate the impact of freezing on the FVIII levels.
Materials and methods: Samples were collected from a total of 20 plasma units before freezing. New samples were collected when the plasma unit was thawed after three weeks and then after 7 and 14 days. Samples were run with the one-stage clot method and the chromogenic assay at the coagulation laboratory at Sahlgrenska university hospital according to the manufacturer's instructions. Wilcoxon's signed rank test was used for comparison between groups. The statistical test was also used in comparisons between days, gender and blood group.
Results: No significant difference could be seen between the two methods used for measurement of FVIII. The FVIII activity decreased over time, after 7 days the level had decreased with 39-51% compared to baseline values. There was a significant difference between the baseline and the level directly after thaw, 24 hours, 7 and 14 days. However the difference directly after thaw was not so pronounced. According to the EU guidelines about 35% of units were below the cut-off level after 24 hours and only 10 % of units were above the cut of level after 7 days. No correlation could be seen between FVIII and gender or blood group in this material.
Conclusion: The two methods compared in this study resulted in similar results and therefore, the one-stage clot method can be used for quality evaluation of plasma for transfusion. After 7 days of storage at 4 ºC the level of FVIII had significantly decreased and therefore this study indicates that plasma should be used as soon as possible after being thawed.

En analys av retrovirala expressionsvektorers lämplighet för transgent uttryck av FOXC2-BioID2 fusionsprotein

Av Emma Larsson

Examensarbete i biomedicinsk laboratorievetenskap utfört på institutionen för biomedicin, avdelning medicinsk kemi och cellbiologi, grundnivå, Sahlgrenska akademi, Göteborgs universitet, 2017
Handledare: Martin Lidell

I djurförsök har man visat att transkriptionsfaktorn FOXC2 har förmåga att omvandla vit energilagrande fettvävnad till brun energikonsumerande fettvävnad, vilket skyddar mössen mot fetma. Hur FOXC2 orsakar detta är oklart men proteinet är ett möjligt mål för läkemedel mot fetma. För att kunna utreda mekanismerna för hur FOXC2 åstadkommer sina effekter är det viktigt att identifiera proteinets co-faktorer; proteiner som reglerar FOXC2:s funktion. Syftet med detta projekt var att utreda vilka retrovirala expressionsvektorer som lämpar sig för fortsatta studier ämnade att identifiera co-faktorer till transkriptionsfaktorn FOXC2 med hjälp av BioID2 tekniken. I denna teknik uttrycker man sitt protein som ett fusionsprotein till BioID2, ett biotinligas som i närvaro av biotin kan biotinylera proteiner i sin närhet. De biotinylerade proteinerna kan sedan renas upp och dess identitet bestämmas med masspektrometri. Metoder som användes i projektet var transfektion/transduktion av celler, proteinanalys med gelelektrofores och Western blotting, samt genutrrycksanalys med qPCR. Utfallet av studierna visar att fusionsproteiner mellan FOXC2 och BioID2 bildas och fungerar som förväntat i de två vektorsystemen, pBabe-Puro och pRetroX-TetOne-Puro, samt att båda vektorsystemen lämpar sig för virusproduktion och transduktion av celler.

Automatic segmentation and quantification of liveruptake on PET/CT-studies in patients with lymphoma

By Erica Lind.

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2017.
Supervisior: May Sadik (Biomedical scientist, PhD)

Background. Lymphoma is a cancerous disease that affects lymph nodes. The disease can be divided into two main groups: Hodgkin's lymphoma and non-Hodgkin's lymphoma. PET/CT has a high sensitivity (> 90%) to detect the most common types of lymphoma. Deauville five-point scale was reported in Deauville, France in 2009 as a recommended tool to standardize PET interpretations in patients with lymphoma. With Deauville five-point scale, the patient's therapy response is evaluated by visualizing a value (1-5) that represents how well the treatment has worked. A value between 1 and 3 means that the treatment is successful, a value of 4 means that the patient has to be observed further, and a value of 5 means that the treatment has to be changed. The long-term goal of the project is to standardize the interpretation and develop a computer software that automatically evaluates the therapy response. The specific purpose of the report is to develop a computer program that automatically segments the liver on CT images in lymphoma patients and calculates standard uptake values (SUV) on the PET images.
Method. All remitted lymphoma patients (80 patients) who had undergone a PET/CT-examination at Clinical physiology, Gothenburg, Sahlgrenska University Hospital between 2008-2010 were included in the training group. Segmentation of the liver were performed on all patients in the training group regardless of other factors. The liver was segmented on the transaxial CT images. The liver was also monitored visually in coronal and sagittal CT images. The segmentations were presented to convolutional neural network that learned how the liver looked like. To test how well the automated method worked Dice index were used as a measure. Two radiologists manually segmented the liver for the testgroup consisting of 6 new patients. Statistic tests were performed on the material to find out how well the automated program worked in comparison to the manual. Liver volumes, Dice index, Precision, Recall and median SUV were calculated.
Results. Liver volumes were calculated to average 1752 ml by the automatic software. Liver volumes were manually calculated to 1757 and 1768 ml by the two radiologists. The difference between the automatic segmentations and the manual segmentations performed by Radiologist A and Radiologist B was calculated to average 0.95 for Dice index. The median of the SUV was calculated to average 1.85 for the automated software. The SUV was manually calculated to average 1.83. The difference SUV between the automatic and manual segmentations were < or equal to 0.1.
Conclusion. An automated computer software that automatically segmented the liver on PET/CT images was achieved. Dice index was calculated to mean 0.95 for the automatic segmentations, which means that these were 95% correct. The difference SUV between the automatic and manual segmentations were minimal and were calculated to < or equal to 0.1.

Validation of SNP-assays for cell-free DNA with droplet digital™ PCR

By Karina Mattsson

Bachelor thesis in Biomedical Laboratory Science performed at Klinisk Kemi, Sahlgrenska Academy, University of Gothenburg, 2017.
Supervisors: Anne Ricksten, Associated Professor, Carina Wasslavik, Biomedical Scientist

Abstract

Background Sometimes a heart-transplant (HTX) is the only alternative for treatment of acquired or inherited disease. In order to take action in the event of rejection, check-ups are performed with repeated endocardial myocardial biopsy (EMB). EMB is a risky, invasive and stress-inducing method and ultimately a subjective assessment. It has long been known that cell-free DNA (cfDNA) from the donor can be found in the plasma of the recipient after an organ transplant. DNA from the graft in the recipient's blood secondary to cellular damage makes these molecules potential biomarkers for the graft's health. The method droplet Digital™ PCR (ddPCR, Bio-Rad) have sufficiently high specificity and sensitivity to quantify these small amounts of DNA. To differentiate between donor and recipient DNA single-nucleotide polymorphisms (SNPs) can be of use. When donor and recipient both are homozygous for a SNP but for different ones DNA can be differentiated. A significant number of analyzes of SNPs are needed to ensure finding such a difference.

Aim Optimizing a number of SNP-assays with regard to annealing-temperature. Some assays would be validated for limit of detection at concentrations of DNA corresponding to the low levels of ddcfDNA present in the recipient after HTX. HTX patients participating in the study would be genotyped within this project. To evaluate the efficiency in number of analyzes, a frequency analysis of the SNP analyzes used in genotyping was done.

Material and Method Assays were performed with droplet DigitalTM PCR (ddPCR, Bio-Rad) using genomic DNA (gDNA) in concentrations corresponding to the amount of cfDNA in plasma.

Results Analyzes showed significant difference between false positive values and low allele frequency in the range 0.5-1% of total gDNA. No significant difference was found between false positive values and low allele frequencies in the range 0.05-0.1% of total gDNA.

Conclusion Analyzed assays in this work can measure corresponding low levels of ddcfDNA in recipients below pathological rejection values. For sufficient probability to find different homozygous for at least three SNPs more assays are needed.

FÖR ATT UPPNÅ EN VENTILATIONSDOS PÅ 25-30 MBQ VID V/P SPECT BÖR EN RÄKNEHASTIGHET PÅ 2000-2500 COUNTS/SEKUND EFTERSTRÄVAS

Felicia Harming Mellander

Bachelor thesis in Biomedical Laboratory Science performed at Uddevalla hospital and Sahlgrenska hospital, University of Gothenburg 2017. Supervisor: Johan Svalbacke

Introduction: Pulmonary emboli is one of the common diseases that can cause sudden deatth. It occurs when a venous thrombosis emigrates to the lung and party or completely occludes a pulmonary artery. The most common risk factors associated with venous thrombosis are malignity, heredity, immobilization and surgery. V/P SPECT is a method with higher sensitivity and specificity than the traditional planar pulmonary scintigraphy. In order to maintain high quality examinations certain guidelines regarding dosage and total counts during the ventilation and perfusion study must be followed. To achieve the right ventilation dose, the count rate can be used as a guide. **The aim** of the study was to make a follow-up of the V/P SPECT method by evaluating the ventilation dosage relative to the count rate before the acquisition starts and also to compare the total counts in the ventilation and perfusion study with recommended intervals. Also the ventilation dosage was evaluated relative to body size. **Methods:** The study population included 31 patients who underwent V/P SPECT. Out of the 31 patients in the study population, total counts from the ventilation and perfusion study and the perfusion dose from 14 patients was retrieved from old examinations. From the 17 patients in the study population who underwent V/P during the time this study was conducted, beyond the previously mentioned parameters, also count rate, height, weight, and thorax circumference was retrieved. **Results:** The ventilation dose was within the correct interval in 16% of the patients. Out of the 31 examinations, 7 (23%) ended up within the desired intervals for both the ventilation and perfusion study. There were a significant correlation between the ventilation dose and the count rate. There were also a significant correlation between BMI and thorax circumference, but not between these two compared to ventilation dose/countrate. **Conclusion:** The chances of maintaining a correct ventilation dose (25-30 MBq) is higher if a count rate of 2000-2500 counts/s is achieved. There is no significant correlation between patient size and ventilation dose.

Temperatur hos helblodspåsar, från blodgivning till process, visar inget samband med låga trombocytantal

Av Karl-Oskar Nordberg

Kandidatuppsats i biomedicinsk laboratorievetenskap utförd på laboratoriet för immunhematologi och komponentframställning, Sahlgrenska Universitetssjukhuset, Göteborgs Universitet, 2017
Handledare: Camilla Hesse, universitetslektor

Bakgrund

Transfusioner av trombocyter utförs i dagens sjukvård rutinmässigt som terapi vid olika sjukdomstillstånd. Genom olika metoder går det att framställa trombocytenheter separerade från övriga komponenter i helblod. För ändamålet används på Sahlgrenska Universitetssjukhuset det helautomatiska blodsepareringssystemet Reveos 3, vilket har kapacitet att processa fyra helblodspåsar samtidigt och dessutom lämna ut ett, för Reveos specifikt, platelet yield index-värde (PYI) för varje framtagen trombocytenhet. De erhållna värdena är ibland så låga att enheterna blir obrukbara och får kasseras. Vad som ligger till grund för de låga värdena kan variera, men en orsak skulle kunna vara att helblodspåsarna under förvaring på kylplattor innan de processas i Reveos har en temperatur som påverkar trombocyterna negativt.

Syfte

Syftet med studien var att undersöka hur temperaturen i helblodspåsar varierade under förvaringen av dem innan de processades i Reveos och om eventuellt avvikande temperaturer, utanför rekommenderat intervall, hade ett samband med låga PYI-värden. Vidare undersöktes om PYI-värden erhållna från Reveos motsvarade de trombocytpartikelkoncentrations (TPK)-värden som mättes i enheter poolade med Reveos-trombocyter.

Material och metod

Temperaturvariationer för 56 helblodspåsar följdes med temperaturmätarsystemet QTA Tracer System®, från det att tappning utfördes på Droppen i Nordstand i Göteborg till dess att de processades i Reveos på Sahlgrenska Universitetssjukhusets komponentavdelning. De erhållna temperaturvärdena granskades och bearbetades för att kunna utröna om en korrelation fanns till låga PYI-värden. På 10 poolade trombocytenheter utfördes TPK-kontroller genom impedansmätning med CELL-DYN®. TPK-värdena jämfördes därefter med erhållna PYI-värden från Reveos.

Resultat

Sammanlagt fick 4 trombocytenheter framtagna med Reveos kasseras till följd av låga värden och/eller röd färg/aggregat. Av de 56 helblodspåsarna var det ingen som understeg den

minimumgräns som rekommenderas. Den korrelationskoefficient som erhölls mellan PYI-värdena och temperaturerna innan processning i Reveos låg på 0,072 och hade ett p-värde på 0,599. TPK-värdena för de 10 poolade trombocyterna varierade kraftigt, 199,6 x 10^9/enhet – 285,3 x 10^9/enhet. Samtliga låg under det PYI-värde som Reveos lämnade ut.

Slutsats

Samtliga helblodspåsar höll en temperatur över rekommenderad minimumgräns under förvaringen och en korrelation gick inte att finna mellan de temperaturer som uppmättes och låga PYI-värden. Av de TPK-värden som analyserades på 10 poolade trombocytenheter var det inget som motsvarade de PYI-värden som erhölls från Reveos.

High endoparasitic burden in horse herds in Västra Götaland County (Sweden) but a single anthelmintic treatment is still efficient

By: Malin Olsson
Bachelor thesis in Biomedical Laboratory Science performed at Husaby Hästakut, Skara, 2017.
Supervisor: Erika Lindberg, Senior lecturer

Background: The struggle against endoparasites in horses by using anthelmintic has been going on for many years in Sweden. The goal has always been to keep the levels down and to control the egg-shedding. Not too long ago it was routine to buy anthelmintics over-the-counter at the Pharmacies. However in the last couple of decades many helminth species worldwide have developed resistance against many of the drug products. Swedish veterinarians and the The National Veterinary Institute have urged to use these products with restrictions since the lesser use of wormers will hold the resistance down as well as the spreading of it. To emphasize the importance of these requirements Swedish government decided in 2007 to restrict anthelmintics for horses with prescription-only usage, which requires a diagnosis for each case of treatment. Though the resistance is not widely spread in Sweden, it is absolutely accurate to use the ones that are effective with care since there aren't any new substances available in the nearest future.
Aim: The major aim was to study the prevalence of endoparasites at some Swedish horse farms in the county of Västra Götaland and the effect of single-treatments in those individuals who were in need for deworming. The selection of horses was made on healthy animals in all ages, genders and breeds to see if any connections could be linked between age and/or localisation and high occurrences of parasites.
Method: The analysis was performed in the laboratory at Husaby Hästakut in Skara by using the McMaster-technique. The McMaster is a well known quantitative method based on a faecal suspension made from a known weight of faeces. The suspension are being examined microscopically by using a counting chamber and the number of detected eggs are then being calculated into eggs per gram faces (EPG) by a simple conversion factor. The modified procedure that was used in the study had a sensitivity of 50 EPG.
Result: The result in the study showed bloodworms to be the most common helminth species but *Parascaris equorum* and *Anoplocephala perfoliata* were also found. The anthelmintics used in the study contained the substances *ivermectin, moxidectin* and *pyrantel*. By using the selective treatment-programme instead of medicate all horses by routine the number of anthelmintic-treatments were reduced by 42%.
Conclusion: As a conclusion most of the horses, 65%, seemed to be infected at some level by bloodworm. However, no significant correlation could be linked between the animal's age or localisation and high helminth infections. Treatment were given to 11 horses and the administrated anthelmintics was considered to have a good treatment effect.

THE COMMERSIALLY AVAILABLE METHODS; GLUCATELL AND FUNGITELL SHOWS A SIGNIFICANT CORRELATION WHEN DETECTING THE FUNGAL ANTIGEN ß(1-3)-GLUCAN IN SERUM FROM PATIENTS WITH SUSPECTED INVASIVE FUNGAL INFECTION

By: Elvira Pettersson

Bachelor thesis in biomedical laboratory science performed at the clinical department of bacteriological serology, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisor: Nahid Kondori, Associate Professor

Background: Immunocompromised patients are at an increased risk of contracting opportunistic infections and among these invasive fungal infections (IFI). IFI can be defined as the presence of mold or yeast fungi in organs or the bloodstream. The symptoms for IFI are non-specific and diagnosing the decease is difficult. At present time, the gold standard for diagnosing an IFI is based upon cultivation and microscopic examination which is time consuming and has a low specificity. Therefor there is a great need for new methods that can detect the fungi faster and with a higher specificity and sensitivity to enable faster treatment that could decrease the morbidity and mortality for IFI and thus also resistance development in fungi. In this study two methods for detecting the fungal cell wall antigen ß(1-3)-glucan in serum are compared (Glucatell and Fungitell). The principle for both assays is based on a modified form of *Limulus* Amebocyte Lysate (LAL) pathway in which ß(1-3)-glucan in patient serum can activate a coagulation cascade that results in a chromophore that can be measured spectrophotometrically.

Aim: The aim of the study was to compare two commercially available methods (Glucatell® and Fungitell®) for detecting the fungal cell wall component ß(1-3)-glucan in serum from patients with suspected IFI. In addition to comparing the two methods different pretreatments for the serum samples were evaluated and also the thermal stability of ß(1-3)-glucan.

Materials and Methods: Serum samples, n=111 from patients with suspected IFI were analyzed using the assay kits Glucatell and Fungitell. The results were obtained by spectrophotometric measurement using an end-point procedure with Glucatell and a kinetic procedure (optical density over time) with Fungitell. In addition, six other serum samples were used, five for the pretreatment trial and one for the examination of ß(1-3)-glucan's stability.

Results: ß(1-3)-glucan in serum was detected using the Glucatell and the Fungitell assays. Out of the 111 serum samples 47 (42%) received a positive result with Glucatell while 56 (50%) samples received a positive result with Fungitell. The results from the two assays had a significant correlation of r=0,891. The ß(1-3)-glucan molecule showed stability at room temperature for up to three days. The different pretreatments for serum samples did not show any significant difference.

Conclusion: Both assays looks promising as an aid in diagnosing IFI, Fungitell as a FDA-approved method for diagnostic use may provide safer results.

Rheumatoid Arthritis and Hyperuricemia

By Noor Sabateen

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Rheumatology and Inflammation Research, Institute of medicine, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisor: Maria Bokarewa, MD, PhD

Objectives: To understand the frequency of hyperuricemia in rheumatoid arthritis (RA) patients compared to patients with arthralgia, patients with other types of arthritis and patients with other rheumatic diseases. Another objective is to see whether hyperuricemia plays a role in the presence of RA-specific autoantibodies (RF and anti-CCP) and survivin or not. ***Background:*** The coexistence of hyperuricemia with RA-specific autoantibodies and survivin is poorly studied. Early researches suggested that hyperuricemia acted like an immunosuppressant protecting patients from rheumatoid arthritis, such studies were then falsified; some reports show that there is a considerable proportion of patients who exhibit rheumatoid arthritis and hyperuricemia concurrently. ***Methods:*** Serum levels of uric acid were measured in 342 first-visit patients to rheumatology clinics during 12 consecutive months between October 2012 and October 2013. Serum levels of autoantibodies and survivin were also measured at the same occasion. 119 of them have Rheumatoid Arthritis, 87 with Arthritis other than RA, 62 with Arthralgia and 43 with gout as well as 31 other Rheumatic diseases patients. The results were then analyzed using MS Excel and IBM SPSS software version 24. ***Results and conclusions*** Frequency of mild hyperuricemia showed no significance differences when compared between gout group and each of the rest patient groups, while severe hyperuricemia showed statistical differences. The frequency of female patients with higher levels of uric acid was over-represented among RA patients. Hyperuricemia may be connected to the presence of RF but not to anti-CCP or survivin.

Abstract

Identification of the early colonization of Enterococci on species-level and Streptococci on group-level in premature infants in relation to the delivery path.

By: Laila Salama
Bachelor thesis in Biomedical Laboratory Science performed at the Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska academy, University of Gothenburg 2017.
Supervisor: Fei Sjöberg, Ph.D., Postdoctoral researcher

Background: The microbiota is established during the first year of life of a human being and starts in conjunction with childbirth where the vertical transmission of the mother's microbiome to her child is the most important source of microorganisms. However, the entirety of the establishment of the microbiota is not yet completely understood. The first intestinal colonizers are *E. coli, Klebsiella spp., Enterococcus, Streptococcus* and *Staphylococcus* whereas the first oral colonizer and most dominant are of the genus *Streptococcus.* Children born with caesarean section may not inherit their mothers' vaginal and fecal flora and instead acquire bacteria from surrounding milieu. Children born preterm may not develop a healthy microbiota. The composition of the microbiota may have significance for the risk of premature children developing necrotizing enterocolitis (NEC) and other inflammatory diseases. Therefore it is of interest to know the early colonization patterns in preterm infants with relation to delivery path with the aim of identifying Enterococci and Streptococci in the oral-, epidermal- and fecal flora.

Methods: With traditional cultivation and matrix assisted laser desorption ionization time of flight mass spectrometry (Maldi-TOF MS) it is possible to identify microorganisms based on their ribosomal proteins, separated by their time-of-flight to identify enterococcal and streptococcal isolates to species and group level.

Results: The enterococcal isolates showed a strong dominance of *E. faecalis* over *E. faecium* in all sources and both vaginally delivered infants and caesarean-section delivered infants had a similar colonization pattern. The streptococcal isolates showed *S. mitis* as the most prevalent group in all sources however the oral flora had the most isolates compared to the other sources. No statistical significance was found.

Conclusion: No difference was found between the two groups in the colonization pattern of Enterococci and Streptococci in all sources. For further investigation of how colonization patterns relate to the different pathways of birth and prematurity, further studies are necessary for the understanding of these topics.

Affibody SYM73 binder till C-terminalen på β-amyloid i cerebrospinalvätska

- En jämförande studie med solanezumab

Av: Emma Sjons

Examensarbete 15 hp, grundnivå. Biomedicinska analytikerprogrammet 180 hp.

Klinisk kemi Enhet 7 Neurokemi, Sahlgrenska Universitetssjukhuset, Mölndal.

Handledare: Kina Höglund, docent, 1:e kemist och Malgorzata Rozga, postdok.

Plack i hjärnan bestående av peptiden β-amyloid är ett kännetecken vid Alzheimers sjukdom. I forskning och kliniska fas III-studier har olika β-amyloid-bindande molekyler använts, till exempel antikroppen solanezumab (m266). I den här studien studeras bindningsprofilen till β-amyloid för den syntetiska affibody SYM73, för att få mer kunskap om molekylen och huruvida den kan vara en läkemedelskandidat. Jämförande analyser har genomförts med m266 som är en molekyl med känd bindningsprofil. Immunoprecipitation med SYM73 och m266 användes för att isolera β-amyloid från cerebrospinalvätska och fragmenten analyserades med LC-MS/MS och MALDI-TOF-MS. Resultatet visar att alla peptider som SYM73 binder till innehåller aminosyra 36. Detta innebär att epitopet för SYM73 troligen är vid C-terminalen på β-amyloid i cerebrospinalvätska.

IMMUNOHISTOKEMISK DETEKTION AV FORSSMAN ANTIGEN I FRYSSNITTAD LUNGVÄVNAD FRÅN TUPP OCH CELLER FRÅN C2C12-CELLINJE

By Isabell Stångberg

Bachelor thesis in Biomedical Laboratory Science performed at the Institution for biomedicine, Sahlgrenska Academy, University of Gothenburg, 2017
Supervisor: Camilla Hesse, Doctor, Ali-Reza Moslemi, Docent

In 1911, an antibody was discovered with the capability of lysing erythrocytes. The antigen on the erythrocytes was later called Forssman (Fs). Species have been categorized as Fs-positive or negative. Humans have been categorized as Fs-negative, although 3 families have been found to be Fs-positive. Fs is a carbohydrate antigen, usually expressed on a lipid structure. Catalyst Fs glycolipid synthase is the enzyme responsible for the last step in the synthesis of Fs. In humans, mutations in the gene for Fs glycolipid synthase seem to have caused the Fs-negativity. In recent studies the Fs antigen has been associated with certain kinds of cancer tissues. Patients with these types of Fs-positive cancer tissue has been seen with lower serum levels of Fs antibody, in comparisons with healthy individuals with higher serum levels. In some cases, the Fs-antibody could have a part as an index of recurrence in this type of cancers. Therefor the detection of the Fs antigen and Fs glycolipid synthetase can be of importance. In this study, the Fs antigen and Fs glycolipid synthetase were analyzed with immunohistochemistry in the C2C12-celline and lung tissue from rooster. This study showed that Fs antigen is absent in C2C12-celline but expressed in rooster lung tissue. The Fs glycolipid synthetase was missing in both the cell line and the tissue. Further studies are needed to determine the expression of Fs glycolipid synthetase, also, additional studies are needed to optimize the method for Fs-antigen detection.

PHENOTYPIC CHARACTERIZATION OF THYMIC B CELLS IN HUMAN

By Hillevi Sundin

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, 2017

Supervisors: Alessandro Camponeschi, Dr; Christina Lundqvist

Among the lymphocytes in the thymus there is a subset of B cells. Previous studies have shown that B cells from this site have phenotypes different from the B cells in the peripheral blood. It has also been shown that they harbour an ability to present self-antigens, which suggests a role in negative selection of autoreactive T cells. Although, their purpose is not yet thoroughly understood. Most studies on thymic B cells have been performed in mice and less is known about these cells in humans. In this project thymic samples from infants were analysed by flow cytometry with in total, nineteen different markers. Compared to peripheral blood, the results revealed a high concentration of mature cells and displayed differences not described before.

Left atrial strain is often possible to analyze but is dependent on several factors

By Jonna Helena Wessmark Tränk

Bachelor thesis in Biomedical Laboratory Science performed at Clinical physiology, Östra sjukhuset, Sahlgrenska Universsity Hospital, University of Gothenburg, 2017. Supervisor: Magnus Johansson, Associate Professor and Doctor

Background: The left atrium has three major functions; to work as a reservoir for pulmonary venous return, work as a conduit for pulmonary venous return and work as a booster pump that augments ventricular filling. Strain is a common method to measure the ventricular, and more recently the atrium function by assessing deformation (%). Diabetes mellitus type 1 results in increased risk of atherosclerosis, coronary disease, stroke and peripheral vascular disease. **Aim:** The main aim of this study was to examine how often it is possible to analyze left atrial strain and what factors do not allow strain to be performed in standard echocardiographic images. **Method:** 201 patients were included in this study, 151 patients had diabetes mellitus type 1 since at least 20 years and 50 individuals were an age-matched control group, without diabetes. Strain was measured in the atrium using both two chamber view and four camber view. In the generated curves the average curve was measured at two points, peak atrial longitudinal strain and peak atrial contraction strain. A second measurement was performed in 34 randomly selected patients to control reproducibility. A Student's t-test and Spearman's rho correlation test was used to analyze data. **Result:** Of the 402 images that was used, 132 (32.8 %) was not able to be analyzed due to too low frames per second, <4 out of 6 segments was approved by the software, poor image quality, the whole atrium was not included in the picture or the image was in a distorted projection and did not show the true atrium size. There were no statistical significant between strain values measured in diabetics compared with the control group. The strongest correlation was found between age and peak atrial longitudinal strain, with a p-value <0.001. **Conclusion:** It was possible to analyze 79.1% of patients in four chamber view and 55.2 % in two chamber view. It was found that the main reason for not being able to analyze images from the study was because the software could not accept enough segments when measured. The software was thus a shortcoming in the method but also the image capturing was at fault. Based on our results in this study, it can be concluded that atrial strain can usually be analyzed in standard echocardiographic images, but better image quality is required in the two chamber view. Further research is required to create more knowledge of, for example, which segments in the atrium are most useful.

Low reproducibility of left atrial volume index by echocardiography can be an important limitation of diagnostic performance in clincal routine

By Tea Zoraja

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2017.

Supervisor: Sinsia Gao (MD, PhD)

Background Left ventricular diastolic dysfunction and increased left ventricular filling pressure cause symptoms in patients with many cardiac diseases, including hypertension, aortic stenosis, ischemic heart disease, infiltrative myocardial disease, cardiomyopathy and heart failure. Left atrial volume index (LAVI), an indicator of left ventricular filling pressure, can be assessed by echocardiography and is an independent predictor of cardiovascular risk. **Aims** The present study aims to evaluate reproducibility of LAVI in clinical routine, identify sources of variability in order to standardize investigation and measurement procedures and improve reproducibility. **Methods** This study included 30 patients undergoing routine echocardiography with good image quality of left atrium in 4 and 2 chamber views. In the end of the investigation a separate dataset of 4 and 2 chamber views was added by another investigator. LAVI was calculated according to Simpson´s rule by 2 independent observers to assess intra-observer variability, combined intra-observer and test-retest variability as well as combined inter-observer and test-retest variability. Bias (= mean difference between 2 measurements), coefficient of variation (CV=standard deviation / means of two measurements, %), limits of agreement (LOA=1.96 * CV, %) were calculated. **Results** Bias / CV / LOA of LAVI for intra-observer variability was 0 ml/m^2 / 6% / 12%. The corresponding for combined intra-observer and test-retest variability was -7 ml/m^2 / 18% / 36%, and for combined inter-observer and test-retest variability was -8 ml/m^2 / 18% / 35%. **Conclusions** Test-retest variability contributes significantly to the variability of LAVI assessment in clinical routine. Low reproducibility reduces diagnostic performance of LAVI in evaluation of diastolic function according to the current guidelines. Standardization of the investigation procedure is crucial to improve reproducibility.