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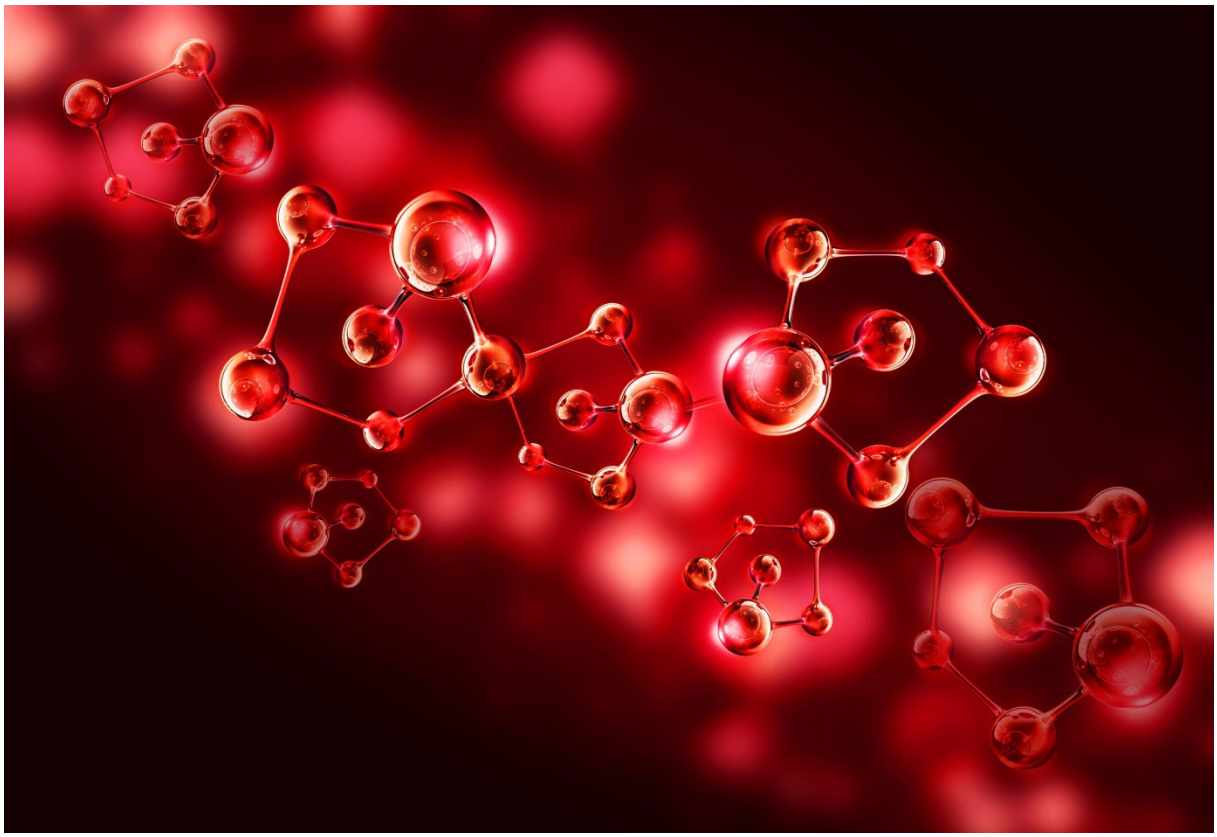

ABSTRACT BOOK 2016

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

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In vitro study of the effect of imbalanced dNTP levels on mitochondrial DNA replication

By Nour AL Muti

Bachelor thesis in Biomedical Laboratory Science performed at the medical biochemistry and cell biology department, Institute of Biomedicine, Sahlgrenska Academy, at University of Gothenburg, 2016

Supervisor: Anna-Karin Berglund, Post Doctor

The human mitochondria have a double stranded circular genome of 16.569 bp, which contains 37 genes, 22 of these genes encode tRNAs, 2 genes encode rRNAs, and 13 genes encode the protein subunits, which are all involved either directly or indirectly in the production of ATP. The human mitochondrial DNA is replicated by unique enzyme machinery, and the mechanism of replication is believed to occur by the strand displacement model. Many systems are responsible for maintaining the fidelity of mitochondrial DNA replication, and in turn protecting the genetic material. One of these systems is the balanced concentration of the overall mitochondrial dNTP pool as well as the individual dNTP levels. Too high and too low levels have been associated with many mitochondrial genetic diseases due to mtDNA depletion, deletions and point mutations. Therefore many studies indicate that imbalanced dNTP levels affect the mtDNA replication, but the particular effect is still unclear. This study aimed to clarify the specific effect of imbalanced dNTP levels on *in vitro* mitochondrial DNA replication.

In vitro reconstituted plasmids containing human mitochondrial DNA with normal (25µM) and high (500µM) dTTP levels were sequenced after many preparation steps. The results were inconsistent with our expectations; the mutations were only detected in the control samples that contained normal dTTP level. In order to explain this, the mtDNA replication was reconstituted again with normal and high dTTP levels.

In conclusion, no point mutations were detected due to high dTTP level; it was not known exactly the possible reason beyond this. Our results could not support the hypothesis that imbalanced dNTP levels might lead to site- specific point mutations. Although we repeated the *in vitro* replication, but we could not send the samples again to sequencing due to the colonies that were obtained in the heat inactivated control plate after the transformation of the rolling circle products, indicating that one or more of the rolling circle process' steps was not run accurately. We optimized the *in vitro* reconstitution replication system for further studies.

EVOLUTION™-FILTER GER INGA SÄKRA FÖRLUSTER AV KVALITÉ VID VÄRDERING AV PLANARA SKELETTSCINTIGRAFIER

By: Gustav Alrup

Bachelor thesis in Biomedical Laboratory Science performed at Uddevalla and Sahlgrenska hospital, University of Gothenburg 2016.
Supervisor: Martijn Van Hessen, physician.

Background and aim: Planar bone scintigraphy is an important tool to diagnose cancer and metastasis in patients. The image quality must be as good as possible while still maintaining low doses of radionuclides and time spent within the camera according to the ALARA-principle. One way to maintain quality while still decreasing time or dose is by the use of Resolution Recovery filters used after image acquisition. The aim of this study was to evaluate if these filters can maintain proper image quality while still enabling a reduction in time spent within the camera. **Method:** The study population consisted of 15 patients. The patients were investigated with a simulation program that reduced the amount of counts to 70% and then added the Resolution Recovery filter, called Evolution. The images were graded based on 4 different categories, their total points were calculated before and after the addition of the filter. The median of the total points remained 8 (P=0,417) both before and after the filter was applied. **Results:** The mean of the total points ± SD for images without the filter was 8,30 ± 1,80 and 8,17 ± 1,82 after the addition of the filter. **Conclusion:** Images treated with a Resolution Recovery filter after a planar bone scintigraphy did not suffer substantial quality losses.

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The automated determination of the phase III slope during a nitrogen washout test is not reliable for clinical routine

by Gianina Al Samarrai

Bachelor thesis in biomedical Laboratory Science performed at Clinical Physiology Östra, Sahlgrenska Academy, University of Gothenburg, 2016 Supervisor: Gert Hermansson (MD, PhD), Patrik Sundholm (MD)

Background: Inert gas washout tests are useful tools to detect an obstructive pathology of peripheral airways. Scond and Sacin are indexes of ventilation inhomogeneity that the analysis of the alveolar plateau (phase III slope) generate during a multiple breath inert gas washout. These indexes reflects the localisation of a lung injury and the disease severity. Because the manual adjustments are needed for the determination of the phase III slope and the manual calculation of Scond and Sacin is time-consuming and complex, the automated detection used in clinical practice needs to be reliable and unbiased. **Objective**: To compare the results obtained from a N_2 washout system to those calculated manual by a expert in lung physiology. **Methods**: The study population consisted of 111 patients. Automated generated Scond and Sacin indexes from Multiple breath nitrogen washout ($MBWN_2$) test was compared to the manual calculation. A smaller group (n=30) was then compared using only manual calculation. **Results:** The comparison of all data between the manual and automated calculation show a bad agreement and a poor correlation for Scond (r=0.6). The Sacin comparison show also a bad agreement between the methods. The coefficient of variance was 50 % for both indexes. For the smaller group (n=30), the comparison between the two manual analysis show a lack of agreement between the methods for Scond but a stronger correlation (r=0.87) and CV=35 %.The comparision for Sacin between two manual analysis shows good agreement and a strong correlation (r=0.99) and CV=9%. **Conclusions**: Although manual adjustments increases both the correlation and the agreement between the methods, the result indicate that only the automated determination of Scond and Sacin is not sufficiently reliable for use in clinical use since, in the current situation, the variation between the manual and the automated calculation was for each of the indexes 50 %.

Evaluation of anaerobic threshold, V'O_2peak and V'E/V'CO_2-slope obtained by cardiopulmonary exercise test as a prognostic value for heart transplanted patients.

By Gustav Andersson

Bachelor thesis in biomedical laboratory science performed at the section for clinical physiology, Sahlgrenska academy, University of Gothenburg, 2016

Background. Late stage heart failure is one of the most common reasons for heart transplantation. It's a major surgery where the survival rate in Sweden 2009 after one and ten years was 90% and 60% respectively. At the yearly follow-ups, amongst the examinations, an exercise test is performed. A cardiopulmonary exercise test with gas analyses gives us more valuable information than just the parameters we get in an exercise test. Parameters like respiratory exchange ratio, peak oxygen uptake, minute ventilation and anaerobic threshold can be obtained. These do not only provide us with more information but also give us physiological evidence if the exercise was well performed or not. **Aim.** The aim of the study was to see if anaerobic threshold, peak oxygen uptake and V'E/V'CO_2-slope could be of any value to the prognosis of heart transplanted patients. **Material and method.** We studied 10 patients that performed a cardio pulmonary exercise test and answered questions from a specialized survey to evaluate the quality of life pre versus post transplantation. **Result.** Anaerobic threshold did not show any difference between one year and five year post transplantation. Though both peak oxygen uptake and V'E/V'CO_2-slope showed deterioration from one to five years follow ups. **Conclusion.** Peak oxygen uptake and V'E/V'CO_2-slope could be of significant prognostic value for heart transplanted patients. This pilot study shows that a larger study group with further evaluation would give much needed information.

Regulation of HER2 expression in breast cancer by Human Cytomegalovirus

By Hakan Babuna

Bachelor thesis in Biomedical Laboratory Science performed at the Söderberg Nauclér Group, Department of Medicine, Karolinska Institue.
Supervisor: Helena Costa, PhD.

Breast cancer is the fifth most common cause of cancer death and although the etiological factors of breast cancer are not completely clear, it is significantly affected by environmental factors, including viral infections. We aimed to study if Human cytomegalovirus regulates the expression of HER2 in breast cancer cells and by testing the role of Immediate Early HCMV gene and antiviral treatments to understand HCMV mechanism in HER2 positive breast cancer cells. The breast cancer celline SKBR3 and ovarian cancer celline SKOV3 was infected with HCMV VR strain with MOI5 and the effect of HCMV expression on HER2 and EGFR was studied in RNA level by real-time PCR at different timepoints. The protein level of HER2 and EGFR in SKBR3 was analyzed by western blot and the intracellular localization by immunofluorescence. The HCMV mechanism of HER2 regulation and the need of replication was tested by siRNA, ganciclovir treatment and HCMV exposure to UV-light. To study HCMV effect on HER2 amplification, in situ hybridization was performed. We showed that downregulation of HER2 and EGFR by HCMV occurs at mRNA and protein level in the breast cancer celline SKBR3. In the ovarian cancer celline our results showed a downregulation of HER2 and upregulation of EGFR at mRNA level. By siRNA and GCV treatment we also showed that the downregulation is not caused by HCMV IE72 or late viral genes and the viral gene expression is necessary, according to our UV-experiment. HCMV does not have an affect on HER2 amplification. All these are clinically relevant as HER2 downregulation by HCMV could convert HER2-type breast cancer into triple negative breast cancer, and thus anti-viral therapy should be considered in these cases.

Limited usefulness of global longitudinal strain in the assessment of left ventricular systolic function in clinical routine

By Melinda Bajra

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

Supervisior: Sinsia Gao (MD, PhD)

Background. Left ventricular ejection fraction (LVEF) is the most common parameter in the assessment of systolic left ventricular function. Global longitudinal strain (GLS), a deformation analysis in three apical longitudinal axes, is a quantitative method which has been developed in recent years and has been shown to detect subclinical left ventricular systolic dysfunction before the LVEF deteriorates in several pathological conditions including ischemic heart disease and during treatment with cardio-toxic drugs. However, the normal limit of GLS is still not established.
Aim. The aim of the study was to investigate the diagnostic value of incorporating GLS into clinical routine in a group of patients with normal echocardiographic findings including normal LVEF and to investigate the impact of using different normal limits of GLS.
Method. Patients with optimal echocardiographic image quality allowing LVEF calculation by Simpson´s rule and normal LVEF (n=201) were selected retrospectively from the Clinical Physiology Information System. After excluding 7 patients (3%) due to sub-optimal image quality or tachycardia, GLS analyses were performed in 194 patients. Clinical data were retrieved from the referrals. In order to clarify the lower limit of the normal range (LLN, -1.96 SD) of GLS, we performed a PubMed search.
Results. The mean age (± 1SD) was 54 ±17 years and 55% were females. Patients undergoing cardio-toxic treatments and patients scheduled for non-cardiac surgery were the two most common disease groups. Global strain was -18.4 ± 2.4% in apical long-axis view, -18.2 ± 2.5% in four-chamber view and -18.8 ± 2.4% in two-chamber view. Average GLS was -18.2 ± 2.7%. Twelve percent (n = 24) of the patients had reduced GLS according to the commonly used threshold value of -16%. Seven studies published between 2008-2016 with large healthy population (n > 100) were chosen and the LLN of GLS varied between -8.6 % and -17.6%. Applying to our patient group, 0 to 35% had reduced GLS depending on the definition of LLN.
Conclusion. In a patient population with normal LVEF the proportion with reduced GLS is highly dependent on the definition of the normal limits. The usefulness of GLS is hampered by the fact that the normal limits are poorly defined. Reduced GLS as a sole pathological finding should be interpreted with caution.

SEPARATE ABSTRACT

Hepatitis E Virus genotype 3 found in stem cell transplanted patients

By: Anton Berdenius

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Medical Microbiology and Immunology, Sahlgrenska Academy, University of Gothenburg, 2016.
Supervisor: Rickard Nordén, PhD

Hepatitis E Virus is a non-enveloped RNA virus that usually is self-limited with no or very few symptoms but can, in immunosuppressed patients, be fatal. We are therefore interested to examine the prevalence of hepatitis E antibodies and viral hepatitis E RNA in stem cell transplanted patients.

A total of 262 sera from stem cell transplanted patients, adults and children, in Sweden were collected and analysed with Dia.Pro ELISA kit for IgG and IgM antibodies. RT-qPCR was used to analyse hepatitis E virus RNA. Sera that were positive in RT-qPCR were analyzed with Sanger sequencing and assembled into a phylogenetic tree with known hepatitis E virus sequences to determine the genotype. We found 39 (14.9%) patients who were IgG positive and 3 who were IgM positive. Nine patients (3.4 %) were RT-qPCR positive for hepatitis E virus and we successfully sequenced two of the isolates. The result from the sequencing and the assembly of the phylogenetic tree revealed that we had two different genotypic strains. We found one strain (genotype 3Ia) that has been isolated from Swedish blood donors and transplanted patients, and one strain (genotype 3II) that have been reported to circulate among Swedish pigs and wild boars. This suggests that there are multiple ways to get infected after stem cell transplantation. The first one seems to have been infected during the transplantation and the second seems to have been infected from consuming infected meat.

SEPARATE ABSTRACT

TESTING OF COMPLETE BLOOD COUNT ANALYZER FOR POINT-OF-CAREACTIVITIES MEETS THE EXPECTED CRITERIA

By: Åsa Claesson

Bachelor thesis in Biomedical Laboratory Science performed at the Primary Care, Region of Västra Götaland, Sahlgrenska Academy, University of Gothenburg, 2016.
Supervisors: Sandra Weineland, MD, PhD

Introduction: Complete blood count is one of the most common analyses in laboratory diagnostics. Used as a point-of care analyzer it may lead to medical decisions made during the patient's appointment at health care. Before award contracts for the supply of instruments it is important to clarify the functional and performance needs that exist in the laboratories and to evaluate the market. The aim of this study was to make a comparison between Siemens Advia® 360 Hematology System and Medical Horiba ABX Micros ES 60 against the ADVIA 2120i with respect to accuracy, linearity and accuracy and instruments random measurement error in duplicate.
Method: The precision of the Siemens ADVIA 360 and Horiba ABX Micros ES 60 was determined using controls at three different levels. The linearity and accuracy were performed by analyzing 80 patient samples and comparing data with established laboratory based ADVIA 2120i analyzers. Instrumentation dispersion from duplicate samples was determined by analyzing duplicate and then Dahlberg's formula was used for calculations.
Results and discussion: The precision was sufficient for both of these instruments. The linearity and accuracy of both instruments are considered good. By evaluating the proliferation of duplicate samples it was confirmed that low values of LPK and the GRA should be checked on a reference instrument. Siemens ADVIA 360 was better than expected on low WBC and Gran and Horiba Micros ES 60 has good capacity to measure platelet counts, even in the high range.

PLATELET ADP RESPONSE DECLINES THROUGHOUT STORAGE, WHILE CD63 EXPRESSION INCREASES AND VASODILATOR STIMULATED PHOSPHOPROTEIN PHOSPHORYLATION SHOWS NO SIGNIFICANT CHANGE

By: Cambarta Cumar

Bachelors thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Camilla Hesse, Senior lecturer

Platelet concentrates are used to prevent and treat bleeding in thrombocytopenic patients. These concentrates can either be acquired with an apheresis technique or through the derivation of whole blood donations. Platelet concentrates can be stored up to 7 days and throughout storage there are changes in the structure and function of platelets, an event called platelet storage lesion. In vitro monitoring of platelet concentrates can be used to assess the platelet function. The agonist adenosine diphosphate plays a key role in the activation and function of platelets and is mediated by the receptors P2Y1, P2Y12 and P2X1. Vasodilator stimulated phosphoprotein is an intracellular actin regulating protein and measures if adenosine diphosphate is desensitising the P2Y12 receptor. CD63 is a marker for platelet activation and is increased on the surface of platelets after granule release. The aim of this study was to examine platelet adenosine diphosphate response in platelet concentrates from apheresis donors throughout the storage; day 0, day 1 and day 7. Multiple electrode aggregometry, a method based on impedance technique was used to assess platelet aggregation induced by adenosine diphosphate for donor (n=10) whole blood and platelet concentrate. Flow cytometry was used to measure vasodilator stimulated phosphoprotein (n=8) and CD63 expression (n=10). Results showed a significant decline in platelet aggregation and a significant increase in CD63 expression throughout the storage. The results also showed significant decrease in vasodilator stimulated phosphoprotein phosphorylation from day 1 to day 7. In conclusion, multiple electrode aggregometry and flow cytometry are useful tools to assess platelet function *in vitro* however the destiny of transfused platelet concentrates is highly dependent on the state of a patient. Further research is needed to better understand how *in vitro* assessment is related to *in vivo* transfusion.

Phenols influence the activation of Antisecretory factor and inhibit microorganisms isolated from patients with Cystic fibrosis

By Sali El-Ali

Bachelor thesis in Biomedical Laboratory Science performed at the Microbiology laboratory, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Ewa Johansson, Associate Professor

Cystic fibrosis (CF) is a genetic disease in which the battle between pulmonary infection and inflammation becomes the major cause of morbidity and mortality. The chronic infection, often with resistant and intractable microorganisms leads to an ongoing inflammation in the lungs that causes damage to the tissue. Therefore, it is very important to find new therapeutic strategies besides antibiotics to prevent pulmonary infections in this population. Antisecretory factor (AF) is an endogenous protein that regulates the fluid transport and inflammation and is a part of our innate and immune system. The transition from inactive to active AF is probably an important part of the natural defense at the normalization of the inflammation and fluid balance. AF is activated when the intestine is exposed to bacterial toxins, but it can also be activated by special food components, such as SPC-Flakes. There are special phenolic compounds in SPC-Flakes inducing active AF. These phenolic compounds have been described with many health-promoting properties such as antioxidant effect and antimicrobial effect. The aim of this project was to analyze the induction of active AF in rat after intake of phenolic rich extractions (such as sorghum) and also to analyze the inhibitory activity of AF or AF-inducing substances on CF pathogens. The plasma samples were analyzed with AF mAb43-antibody and antibody against complement C3c in enzyme-linked immunosorbent assay and the level was significantly increased after intake of brown or black sorghum ($p<0.05$ respectively $p<0.05$-0.0005). The inhibitory effects of AF or AF-inducing substances on nine different isolates of CF pathogens were tested in diffusion test method and broth cultures. The obtained inhibitory effects from the both methods were observed and data were interpreted. In conclusion, phenols were shown to have influence in the activation of AF in rats and may have some ability to inhibit microorganisms infecting CF patients.

Optimization of Real Time PCR for hepatitis C virus detection in samples from blood donor from Rwanda

By: Amina Elsiddig

Supervisors: Helene Norder, Professor and Marie Karlsson, Biomedical scientist

A thesis for partial fulfillment of the Bachelor degree in Biomedical Laboratory Science at the Department of Clinical Microbiology-Virology, Sahlgrenska Academy, University of Gothenburg, 2016

Abstract

Hepatitis C virus (HCV) is a virus that attacks the liver cells. It causes severe liver disease and it can even lead to hepatocellular carcinoma. The disease is considered to be one of the main reasons that necessitate liver transplantation and, unfortunately, no vaccine has been developed against it yet. According to world health organization in 2004, HCV infects about 170 million people worldwide. The prevalence of HCV in Sweden is about 500 cases every year according to Folkhälsomyndigheten.

HCV is a positive single strand RNA virus. Based on its genetic variability it is classified into seven genotypes, each further divided to different subtype (a-f). The transmission of HCV is blood-borne, i.e. via exposure to contaminated blood or blood derived fluids.

For the detection of any genome in biological materials by Real Time PCR, there are some important optimizing steps that have to be taken. This can be done by testing different concentrations of $MgCl_2$ or primers and probe, or different annealing temperatures.

The aim of the present study was to optimize a Real Time PCR for the detection of HCV RNA. The assay was to be used for detection of the virus genome in blood donor samples from Rwanda who were positive for anti-HCV antibodies. The Real Time PCR method was selected for detection of the HCV genome, because of its high sensitivity and specificity.

The material used for optimizing the assay was from a known Swedish chronic hepatitis C patient. The materials that were to be analyzed for HCV-RNA were from 78 anti-HCV positive blood donors from different regions in Rwanda.

Eighteen different master mixes with different concentrations of primers and probe were tested**.** The discrepancies in the amplification curves of these master mixes were rather small. However, there were five combinations in which all four serial dilution of the extracted positive control RNA (undiluted, 1/10, 1/100, 1/1000) were positive. One of these five combinations had in addition nearly overlapping amplification curves for each of the duplicate of each dilution. This combination was further used for analyzing the samples from the blood donor from Rwanda. The result showed that 13 (about 17 %) out of the 78 anti-HCV positive blood donors had detectable HCV-RNA, which means that the donors are carriers of the virus and that they may need treatment.

The results call for more work and survey of HCV in different regions in Rwanda. Gene sequencing is also needed in order to know what genotypes are dominating in Rwanda. That will contribute very much to the elimination of hepatitis C disease in Rwanda by setting the patients on the correct treatment because all genotypes do not respond equally well to antiviral treatments.

Skillnader i IgG-antikroppsnivån mot EBV gp350 och mässlingvirusantigenet N_{CORE} hos patienter med multipel skleros vid behandling med natalizumab och betainterferon

av Marcus Eriksson

Examensarbete i biomedicinsk laboratorievetenskap, grundnivå, vid klinisk virologi, Sahlgrenska universitetssjukhuset. Vårterminen 2016. Handledare: Linn Persson

Multipel skleros (MS) är en kronisk, neuroinflammatorisk sjukdom som i synnerhet drabbar unga vuxna och som kan ge allvarlig neurologisk funktionsnedsättning. Dagens behandlingar mot MS är ofta effektiva, men kan ge allvarliga biverkningar. Sjukdomsaktiviteten behöver kontinuerligt kontrolleras för att följa progressionen av sjukdomen och se till att insatt behandling har önskad effekt. Idag sker monitorering av sjukdomsaktiviteten främst genom kliniska undersökningar och resurskrävande magnetkamera-kontroller. De biomarkörer som används för att mäta sjukdomsaktiviteten vid MS analyseras i likvor och är inte specifika för sjukdomen. En biomarkör för MS i serum skulle underlätta provtagningen för patienterna och vara mer resurseffektivt för sjukvården. Tidigare studier har visat att det finns ett samband mellan mängden IgG-antikroppar mot EBV (Epstein-Barr-virus) och sjukdomsaktivitet i MS. EBV-antikroppsnivån kan därför vara en potentiell biomarkör för att utvärdera effekten av insatt behandling. Syftet med vår studie var att identifiera om mängden EBV-IgG-antikroppar i serum hos patienter med MS förändras av behandling med Natalizumab (NAT) respektive betainterferon (INFB). MS-patienter har förhöjd antikroppsreaktivitet även mot en del andra virus som mässlingvirus, så EBV-IgG-antikroppsreaktiviteten jämfördes dessutom med IgG-antikroppsreaktiviteten mot mässlingvirusantigenet N_{CORE}. Serumprover från 839 MS-patienter tagna före och under behandling med NAT analyserades avseende IgG-antikroppsreaktiviteten mot det EBV-specifika antigenet EBV-gp350 och mot mässlingvirus (MV)-antigenet N_{CORE} med enzymkopplad immunadsorberande analys (ELISA). En subgrupp av patienterna med MS (N=170) hade även två serumprover tagna tidigare under pågående INFB-behandling. Även dessa prover analyserades avseende IgG-antikroppar mot EBV gp350 och N_{CORE}. Resultaten i rapporten baseras på de parade prover som hann analyseras inom ramen för examensarbetet och som hade tillräckligt god analyskvalité. EBV-antikroppsnivån minskade signifikant under behandling med NAT (n = 401), men uppvisade ingen signifikant skillnad under INFB-behandling (n = 97). MV-antikroppsnivån minskade under NAT-behandling (n =466) respektive INFB-behandling (n = 76), dock i mindre utsträckning jämfört med EBV-antikroppsnivån. Våra resultat visar främst på ett samband mellan sjunkande EBV-IgG-antikroppsnivåer och behandling med NAT. Resultatet ger en indikation på att EBV-antikroppsnivåer i serum kan vara en potentiell biomarkör för behandlingseffekt, men detta behöver utredas vidare i framtida studier.

OPTIMIZATION OF A FLOW CYTOMETRY METHOD FOR MEASURING GAMMA-H2AX RESPONSE TO CHEMOTHERAPUTICS IN CULTURED MONONUCLEAR PERIPHERAL BLOOD CELLS

By Angelica Falk

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska University Hostpital, Gothenburg, Sweden, 2016
Supervisor: Pegah Johansson, PhD

Cancer patients receiving the same chemotherapeutic dose will suffer from various side effects, meaning that there is a variation in normal tissue sensitivity to DNA-damaging agents. Therefore there will be an advantage if the clinical response of DNA damage could be measured in each individual, so that the result can help guide the chemotherapeutic dose. Doxorubicin, etoposide, cytarabine and mitomycin C are all common chemotherapeutic drugs which induce DNA double-strand breaks (DSBs). DSBs will result in phosphorylation of histone H2AX on serine 139 (γ-H2AX), at sites flanking the DSBs. Using a fluorescently-labeled antibody directed against the γ-H2AX, the level of DSBs can be measured by a flow cytometer. The normal tissue sensitivity to chemotherapeutic drugs is not investigated in patients before receiving the treatment. Therefor we have optimized a flow cytometry method to measure the γ-H2AX level in cultured peripheral blood mononuclear cells (PBMC) in response to chemotherapeutics. Human peripheral blood was obtained from healthy patients. PBMC were prepared by density centrifugation and treated with chemotherapeutic drugs in different concentrations and incubation times. Treated cells were analyzed against controls (untreated cells) by a flow cytometer, detecting the level of γ-H2AX within each cell. We found that there is a correlation between the chemotherapeutic dose and the γ-H2AX signal, and that there is a variance in the γ-H2AX level for the same dose and time, among individuals. In conclusion, this method might be of clinical use to measure the drug sensitivity among individuals, but further validation is needed for this application.

Platelet role in Multiple Sclerosis: a study of regulatory genes in MS patients before and after treatment with Tysabri® associated with the immune response

By: Kristina Florentin

Bachelor thesis in Biomedical Laboratory Science performed at Department of Biomedicine, Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, 2016 Supervisor: Margareta Jernås, Associate Professor

Anucleated platelets participate in hemostasis and thrombosis but recent studies show that platelets also have important functions in inflammation and the immune response. Platelets contain many proinflammatory molecules and cytokines, they also express Toll like receptors which may be a link to the innate immunity. Other studies discuss platelets role in autoimmune diseases such as Multiple sclerosis (MS). MS is a chronic demyelinating disease of the central nervous system and the disease etiology is still unknown. The most effective treatment against MS today is Natalizumab (Tysabri®) but a deadly side effect is Progressive multifocal leukoencephalopathy. Can platelets be involved in the pathogenesis of MS, and potentially be a target for new medicines? In this study, three Tysabri treated MS-patients was studied before and after their treatment. Platelet count, platelet isolation, RNA preparation according to Chomczynski/Sacchi method, amplification using NuGEN Technologies and microarray analysis using Human Genome U133 Plus 2.0 array, Affymetrix, were carried out. We found 2 349 significantly regulated genes in platelets from MS patients before vs after treatment with Tysabri. Of these, we focused on 12 genes previously associated with platelet immune function and 7 genes associated as riskgenes for MS. In conclusion, platelets are important immune cells and may have a central role in the pathogenesis of MS. Further studies need to be performed to confirm such connection and results from this study need to be verified with additional methods such as real time-PCR and ELISA.

Differences in *Exophiala dermatitidis* specific immunoglobulin G subclasses in serum of patients with cystic fibrosis, where immunoglobulin G 2 were the most common subclass

By Nina Fransson

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Biomedicine, Department of Infectious Medicine, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Nahid Kondori, Researcher

Background: Cystic fibrosis patients often get colonized whit different types of bacteria and fungi in the airways. It has been shown that *Exophiala dermatitidis* colonizes respiratory tract of patients with cystic fibrosis and the fungus induces immune system to produce IgG antibodies.

Aim: The aim of this study was to analyze *Exophiala dermatitidis* specific IgG subclass antibodies (IgG 1-4) in serum of cystic fibrosis patients. The level of IgG subclasses to *Exophiala dermatitidis* in patients who were colonized were compared with patients who were not colonized with the fungus.

Methods: Serum samples from 91 Swedish cystic fibrosis patients were included in this study. An indirect ELISA method was used to analyze the patient serum to measure IgG subclasses (IgG 1-4). The patients were divided in two groups, one group who were colonized whit *Exophiala dermatitidis* and the other group who were not colonized with the fungus.

Results: The results showed that the levels of *Exophiala dermatitidis* specific IgG 1 and IgG 2 antibodies in serum from patients with *Exophiala dermatitidis* culture positive sputum samples were significantly higher compare with culture negative patients. IgG 3 and IgG 4 antibodies were expressed in low levels and could not be analyzed statistically.

Conclusion: *Exophiala dermatitidis* induces immune system to produce specific IgG subclass antibody, especially IgG 2, in serum in patients with cystic fibrosis.

Abstract

Different outcomes in step count between Actigraph wGT3X-BT and Polar A360.

By Rossana Fridlizius

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg laboratory,

Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Caroline Schmidt, Associate professor

Introduction: Sedentary behaviour and low levels of physical activity are correlated with an increased risk for cardiovascular and pulmonary diseases. Knowledge about these diseases and underlying factors are rapidly increasing and so also knowledge on how to estimate physical activity. Various methods can be utilized to measure the physical activity and accelerometer based activity monitors are frequently applied in clinical trials. However, there is no golden standard device that has proven to be optimal for this purpose. The knowledge on differences between monitor devices and factors affecting the accuracy, such as monitor placement, type of activity performed and device constructions, is still to be developed. **Aim:** The aim of this study was to investigate if there is a significant difference in detection and registration of step count between two different activity-monitoring devices. **Methods:** 19 participants were included in this study. Each subject wore an Actigraph wGT3X-BT accelerometer and a Polar A360 fitness watch simultaneously during four days and the registered step counts per day for each device were analysed and compared. **Results:** The outcomes of step counts between the devices showed to be significantly different, $p<0.001$. The Actigraph wGT3X-BT had a higher amount of registered steps per day with a median step count of 17503 steps to be compared to the median step count of 12071 steps for the Polar A360. **Conclusion:** This Study indicates a significant difference in step count outcome between the Actigraph wGT3X-BT and the Polar A360, where the former has registered a higher amount of steps. Suggestions for further research related to different explanatory factors are presented.

SEPARATE ABSTRACT

The effect of zoledronic acid during binding of Osteopontin to hydroxyapatite using an immunofluorescent method

By: Jeanette Grönborg Hansen

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Håkan Nygren, Professor

Titanium is a material frequently used in orthopedics as implants in bone tissue. Earlier research has studied various types of materials and modifications in order to obtain the best conditions for healing of the tissue. To understand the healing around implants and bone mineralization, several researchers have studied the various proteins involved. But further studies is necessary to fully understand the processes.

In the present study, an immunofluorescent method was used to study osteopontin, a protein in the family of SIBLING proteins with various functions involving the bone tissue mineralization. The aim of this study was to observe the binding of osteopontin to hydroxyapatite formed during incubation of titanium plates in cell culture medium. Also to study the effects of the bisphosphonate zoledronic acid during the binding of osteopontin to the hydroxyapatite.

Materials and methods: Plates of pure titanium were incubated in a cell culture medium. Zoledronic acid and osteopontin were added to the plates in two incubation steps. Anti-steopontin primary antibody were added to bind the osteopontin and the reaction was visualized by adding a FITC-conjugated secondary antibody and studied in a fluorescence microscope and by using the image analysis program ImageJ.

Results: The plates with added zoledronic acid had less bound osteopontin compared to the controls. However, the statistical tests did not present a significant difference between the groups.

Conclusions: The results indicate that the zoledronic acid is inhibiting the binding of osteopontin to the hydroxyapatite. This method can be used for further studies of metals as orthopedic implants and proteins involved in the bone mineralization.

Optimization of digital droplet PCR with plasmids for the standardization of Quantitative real-time PCR.

Evaluation of the protocol with samples from patients with Epstein-Barr virus, and cytomegalovirus infections.

By Kristine Gustafsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Medical Microbiology and Immunology, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Maria Andersson PhD student

Quantitative real-time PCR is the most common method used to determine virus-concentration in whole blood and serum/plasma, but the result may vary depending on where the analysis is performed. This is mainly because there are no real standardizations for the method. Droplet digital PCR (ddPCR) preforms a total quantification without standard curves and therefor provides less variation in the results. In this study ddPCR is optimized for plasmids with target sequences for five different herpes viruses. The optimized protocol provides a way to verify the calculated concentration of the plasmid and thereby obtain a standardization of the standard for real-time PCR. One of the target sequences is the Epstein-Barr virus (EBV) that is very important to quantify in case of EBV-induced post transplant lymph proliferative disease. Since no treatment is available, it´s important to follow EBV-DNA concentration to adjust the level of immunosuppression. The optimized protocol was used to compare real-time PCR to ddPCR by analyzing samples from transplanted patients with EBV-infection. The EBV-DNA concentration measured by real-time PCR had been varied and on several occasions no EBV-DNA was detected. On two of these occasions ddPCR was able to detect EBV-DNA, which indicates that ddPCR could be a more sensitive method. The opposite was shown when samples detected as slightly positive for cytomegalovirus and EBV by real-time PCR weren´t detected with ddPCR.

The conclusion drawn from this is that the optimized protocol can be applied to plasmids, which are of great benefit to the standardization of quantitative real-time PCR. But regarding patient samples further optimization is needed to achieve the best possible sensitivity for ddPCR.

Ablation therapy in children with heart rhythm disorders: mapping system reduces X-ray exposure.

By Hanna Hertzberg

Bachelor thesis in biomedical Laboratory Science performed at Cardiology laboratory, Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Lennart Bergfeldt (MD, PhD), Britt-Marie Jinhage Åberg (BMA), Monica Dahlin (BMA, MMED)

Background. Paroxysmal supraventricular tachycardia (PSVT) caused by an accessory pathway or dual AV-node physiology is fairly common in children. Wolf-Parkinson-White syndromes are due to an accessory pathway outside the normal conduction system and AV-node re-entrant tachycardia due to alternative pathway in or near the AV-node. Ablation therapy cures these tachycardia's in most cases but pharmacological treatment is an alternative until ablation can be used (the child is large enough). Severe complications occur in less than 1% of the adult cases. During ablation fluoroscopy is used but radiation exposure might be reduced by using anatomical mapping system. **Aim.** To study if an anatomical mapping system would reduce the X-ray exposure compared to conventional fluoroscopy guided catheter manoeuvring. The secondary aim was to study success and complication rates and number of radiofrequency applications used during ablation in children at Sahlgrenska University hospital 2005-15. **Methods and result.** 203 children age ≤ 17 years were studied retrospectively. Totally 214 ablation procedures were performed. Each patient was ablated using Seldinger technique for placing the catheters. Fluoroscopic use in minutes and dose were compared in ablation procedures with, versus without the use of anatomical mapping systems. We found a significant reduction in X-ray exposure in the first group both in exposure time and dose. The primary success rate was on average 92% and severe complications occurred in < 1 % of cases. We did not find any significant difference between the groups in number or time for radiofrequency applications. **Conclusion.** There was a statistically significant reduction in radiation exposure time and dose when using anatomical mapping systems. There was also a high primary success rate and low complication rate, but no difference in radiofrequency application time or number between the two groups Ablation therapy is an effective therapy with low risks including low radiation exposure in children with PSVT.

Expression of androgen receptor, midkine and runt-related transcription factor 2 in prostate cancer bone metastases

By Helen Hosseini

Bachelor thesis in Biomedical Laboratory Science performed at The Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisors: Karin Larsson, PhD and Karin Welén, PhD

Prostate cancer is the most common type of cancer among Swedish men. When the cancer is localized in the prostate, the prognosis is good. But yet there is no cure once the disease has metastasized. Androgen deprivation is the standard therapy for metastasized disease, but although most patients respond, all inevitably develops castration-resistant prostate cancer. To be able to treat lethal metastatic prostate cancer it is important to know how regulatory proteins and putative drug targets are expressed in the bone metastases.
The aim of this project was to analyze the expression of proteins in bone metastases in patients with hormone-naive prostate cancer and castration-resistant prostate cancer. Androgen receptor, midkine and runt-related transcription factor 2 expressions were studied in tissue from bone metastases with immunohistochemistry. The evaluation of the result was performed with semi-quantitative scoring system.

Androgen receptor was expressed in nucleus cell and cytoplasm. Androgen receptor expression was higher in the castration-resistant prostate cancer group compared to the hormone naive group, which shows a significant difference. There was no significant difference in expression between the groups for the proteins midkine and runt-related transcription factor 2.

In conclusion, the significant difference for Androgen receptor expression indicates that hormone therapy has an effect on the protein expression. Further studies need to be performed for the understanding of midkine and runt-related transcription factor 2 roles in advanced prostate cancer.

Arterial stiffness in adolescents is associated with a low family socioeconomic status

By: Sandra Isaksson

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of Medicin, the Department for Molecular and Clinical medicine, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Yun Chen, associate professor. Peter Friberg, professor

Abstract

Introduction Few studies have been linking adolescent stress and health to cardiovascular disease in adult life. It is important to early detect individuals at high risk for cardiovascular disease. Arterial stiffness is one important predictor, it is therefore crucial to establish what other factors can contribute to the development of arterial stiffness.
Aim To investigate which factors correlates with pulse wave velocity, a measurement of arterial stiffness, in healthy school children.
Method We examined 150 children (80 girls) with the mean age of 13,3 years. We measured different health parameters including anthropometrical variables, blood pressure, pulse wave velocity and hand grip strength. We investigated the level of physical activity, stress, sleeping hours, psychosomatic problems and family socioeconomic status using a questionnaire.
Results Girls had statistically significant higher levels of BMI and psychosomatic problems than boys, while boys had higher levels of systemic blood pressure, height and reported higher levels of physical activity than girls. After adjustments for different co-variables, the socioeconomic status of the family household was the strongest predictor for variation in pulse wave velocity among healthy school children.
Conclusion There are significant differences in health parameters between girls and boys. The family socioeconomic status is the strongest predictor for arterial stiffness. Children with a low family socioeconomic status had higher levels of pulse wave velocity and therefore a higher degree of arterial stiffness than children with high family socioeconomic status.

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Does the ratio of IMMUNOGLOBULIN KAPPA- AND LAMBDA-POSITIVE CELLS Vary between DIFFERENT B cell SUBSETS?

By Rehana Khan

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Rheumatology and Inflammation,

Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Alessandro Camponeschi, MSc and Ola Grimsholm, PhD

ABSTRACT

Individual B cells express a B-cell receptor (BCR) that contains either an immunoglobulin (Ig) kappa (κ) or lambda (λ) light chain but not both. The ratio of κ: λ expressing B-cells is about 60% κ and 40% λ in peripheral blood of healthy humans. Transitional B cells are immature B cells that have just migrated from the bone marrow and can be divided into transitional1 (T1), transitional 2 (T2), transitional 3 (T3), based on their maturity and surface expression markers. It has been recently observed (in our preliminary experiments) that T1 B cells express λ to a higher extent than T2 and T3 as well as naïve and memory B cells. The aim of this study was to investigate if the κ/λ ratio varies between different B cell populations.

To address this question, peripheral blood samples from seven healthy donors were acquired on a flow cytometer. Frequencies of different B cell subsets were analysed on FlowJo software. Our results demonstrate that T1 B cells express λ to a higher extent than T2 and T3 B cells as well as naïve and memory B cells. Furthermore, data from peripheral blood samples were compared to one bone marrow sample that showed a higher frequency of λ + than κ+ cells, thus resembling the T1 B cells.

No studies have been found on κ: λ expression in transitional B cells in human peripheral blood. The ratio of κ: λ in mature cells is known but the altered κ: λ ratio in immature B cells and transitional B cells has to be further explored.

Our results supported our hypothesis that T1 B cells express λ to a higher extent than T2 and T3 B cells as well as naïve and memory B cells.

Abstract

Introduction of analysis of IgG-antibodies to cardiolipin and β_2-glycoprotein-I to diagnose APS

By Malin Larsson

Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Chemistry Halland, Hallands sjukhus Halmstad, 2016
Supervisor: Maria Held, MD, PhD

Antiphospholipid syndrome gives an increased risk of thrombosis and obstetric events. The cause of the disease is due to the presence of circulating heterogeneous antiphospholipid autoantibodies such as anticardiolipin antibodies and antiβ2-glycoprotein antibodies. The aim of the project was to assist in the implementation of the analytical methods for anticardiolipin antibodies IgG and β2-glycoprotein-I IgG antibodies. They are assessed along with Lupus anticoagulant to decide if the patient has antiphospholipid syndrome. The methods were verified and compared with another laboratory so that the Clinical Chemistry Halland could decide whether to set up the analysis. EliA Cardiolipin IgG and EliA β_2-Glycoprotein-I IgG from Thermo Fisher Scientific Inc. is a fluorescence enzyme immunoassay. For the study we used **Visu**Con™ normal donor set and 63 plasma samples which were positive for Lupus antikoagulans. The methods were verified and compared with another laboratory so that the Clinical Chemistry Halland could decide whether to set up the analysis. The strength of agreement for anticardiolipin antibodies between the laboratories became with Cohen's k test 0.60, which is moderate. A cut-off, recommended by the *Eleventh International Congress on Antiphospholipid Antibodies, Sydney, 2005*, (> 40 GPL) was k = 0.84. Analysis of IgG antibodies against cardiolipin and β2-glycoprotein-I is a valuable addition to lupus anticoagulant. Positivity in several criteria gives an increased risk of thrombosis. An established analysis of anticardiolipin and anti-β2-glycoprotein-I will give Clinical Chemistry Halland greater certainty of finding patients with antiphospholipid syndrome. It only remains to determine the cut-off level to use.

Establishment of a rapid gas chromatographic method for analysis of faecal short-chain fatty acids – An altered short-chain fatty acid pattern in children with newly diagnosed inflammatory bowel disease

By: Sandra Larsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Infectious Diseases, Institution of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Fei Sjöberg

Background. Inflammatory bowel disease (IBD) is characterised by a chronic inflammation of the gastroenterological tract, and primary includes Crohn's disease and ulcerative colitis. The cause of the disease is still unknown. During the last decades there has been a rapid increase in the incidence of IBD. According to the hygiene hypothesis, the increase may be due to a higher standard of living including a reduced microbial exposure, which could affect the maturation of the microflora and immune system in children. Short chain fatty-acids (SCFAs) are metabolic end products formed by bacterial fermentation in the colon and have been suggested to play a potential role in the development of IBD.

Aim. The aim of the study was to establish and validate a rapid gas chromatographic method that makes it possible to quantify nine different SCFAs in faecal samples. After validation, faecal samples obtained from children with IBD will be analysed in order to compare the SCFA pattern with healthy children.

Materials and Methods. A standard solution containing acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, caproic acid and heptanoic acid were prepared and diluted into serial concentrations. For each SCFA an individual standard curve was formed. The gas chromatographic analysis was performed using a column with free fatty acid phase that minimized the pretreatment of the faecal samples. Validation of the method was performed by measuring three parameters; relative error, repeatability and reproducibility. Faecal samples were collected from children diagnosed with Crohn's disease (n=11), ulcerative colitis (n=19), diseased controls (n=20) and healthy controls (n=9). The samples were homogenised in water, acidified and spiked with an internal standard before analysis with gas chromatography.

Results. Regarding the validation of the gas chromatographic method, six of the SCFAs showed low relative errors (-0,2 to – 9,0 %), indicating good accuracy. Acetic acid, propionic acid and heptanoic acid had relative errors of 14,2 %, 15,5 % and 61,6 %, respectively. Small variations were observed in both repeatability and reproducibility test for most of the SCFAs, coefficients of variation (cv) from 0,9 % to 7,5 %. In repeatability test, acetic acid, propionic acid and butyric acid showed a lower precision (cv=14,8-25,8%). Heptanoic acid showed a lower precision in the reproducibility test (cv=26,8 %).

Children with ulcerative colitis had significant lower diversity in the SCFA pattern ($p \leq 0.01$) and had significant lower levels of faecal acetic acid ($p \leq 0.05$), propionic acid ($p \leq 0.05$), isobutyric acid ($p \leq 0.01$), isovaleric acid ($p \leq 0.0001$), valeric acid ($p \leq 0{,}0001$) and caproic acid ($p \leq 0.0001$) comparing to healthy children. Children with Crohn's disease showed lower amount of isobutyric acid ($p \leq 0.05$), isovaleric acid ($p \leq 0.01$), valeric acid ($p \leq 0.0001$) and caproic acid ($p \leq 0.001$). Diseased controls showed significant lower amount of isobutryic acid ($p \leq 0.05$), isovaleric acid ($p \leq 0.001$), valeric acid ($p \leq 0.001$) and caproic acid ($p \leq 0.01$) comparing to healthy children.

Conclusions. We established a rapid and reliable gas chromatographic method for analysis of faecal SCFAs in children with IBD. Our result showed an altered SCFA pattern in Crohn's disease and ulcerative colitis comparing to healthy subjects. In accordance to the hygiene hypothesis, our results indicate a less mature microflora in patients with IBD that may contribute to the development of IBD.

DNA MICROARRAY ANALYS AV REGLERADE GENER HOS TROMBOCYTER UNDER FÖRVARING

Associerat med platelet storage lesions effekter

By Annie Lilja

Bachelor thesis in Biomedical Laboratory Science performed at the department for Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, 2016.
Supervisor: Margareta Jernås, Associate Professor

Background. Platelets are the second most common cell in the blood and are found to be involved in many processes beyond coagulation such as inflammation, angoigenesis and tumor growth e.g. Megakaryocytes form and releases platelets without a cell nucleus, they contain only a small amount of mRNA which is derived from the megakaryocyte. Platelets transfusion is an important part of treatment of many different conditions such as thrombocytopenia and other platelets disorders. Platelets undergo changes in morphology and physiology during storage and the phenomenon is called platelet storage lesion. Platelet storage lesion is believed to be involved in transfusion complications. The aim was to study the genome expression in platelets during storage and compare day 1 to day 7 and identify significantly regulated genes to increase the knowledge of the mechanisms behind platelet storage lesion.

Materials and Method. Platelets during storage were isolated day 1 and day 7 from five patients, RNA was prepared, amplified and hybridized to DNA microarrays. Using bioinformatics analysis, significantly regulated genes were identified.

Results. The microarray analysis showed a mean value of 21% activated genes day 1 and 13% day 7. There were 2573 significant regulated genes (*P*-value <0,05) between day 1 and day 7 and the ten most regulated genes and also apoptosis-associated genes were selected.

Conclusion. The findings in this study indicate that the platelets during storage day 7 are in a less functional condition compared with day 1. Retention of platelet function is of greatest importance as this may sustain storage. Our analysis identified regulated genes that can be involved in the mechanism behind platelet storage lesion, however further studies are needed.

Forssman antigen negativitet påvisad hos en population av afrikanska kvinnor och i vävnad från magsäck.

By Caroline Lindberg

Bachelor thesis in Biomedical Laboratory Science performed at the Institute for Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Camilla Hesse, Ali-Reza Moslemi

Forssman antigen is a carbohydrate antigen expressed on the erythrocytes of some mammals. It has long been belived that it is not present in human tissues. So far three families in the UK have been found to have the mutation that gives rise to the expression of Forssman antigen. It´s not known much about the antigen or how it may affect in transfusion and transplantation. Several studies show that the antigen is present in the gastrointestinal tract in both normal and cancer tissue. Immunological methods have earlier been used to identify the antigen.
The purpose of the project was to sequence the gene for the enzyme giving rise to the Forssman in a female population from DR Congo in Africa and tissues from a few Swedish patients with stomach cancer. Also, the project aimed to develop an immunological staining method for tissue sections from stomach, to study the expression of the Forssman antigen.

DNA from blood samples was recovered from African population and from the tissues from the stomach for PCR and sequencing. Tissue sections from the stomach were immunologically stained an IgM antibody against the Forssman antigen. Sudan Black B was used to reduce autoflurescence.

Forssman antigen could not be detected in a population of African women or tissue from the stomach, neither by sequencing nor with immunohistology, in this study. Further studies need to be done to ensure the results.

SEPARATE ABSTRACT

Identification of *Candida* using multiplex TaqMan real-time PCR - an evaluation of primers and probes

By: Paulina Lindberg

Bachelor thesis in Biomedical Laboratory Science performed at Klinisk Mikrobiologi, Hallands sjukhus Halmstad, 2016.
Supervisor: Milton Karlsson, PhD, Molecular biologist.

Fungal infection of the nail, onychomycosis, caused by various *Candida* species is an emerging problem in the world. To be able to give patients right diagnosis and treatment, it is vital to use a well-developed and rapid method with the capacity of analyzing more than one fungi species at the time.
The aim of this work is to evaluate and compare two sets of primer pairs for the analysis and identification of *Candida* species using a multiplex TaqMan real-time PCR. The goal is to improve the sample flow in everyday clinical work in the laboratory. We therefor extracted DNA from six different *Candida* species followed by a multiplex TaqMan Real-time PCR. From six serial dilutions, standard curves were made to determine the reactions efficiency and level of detection.
The results showed a difference in the specificity of the two sets of primer pairs. The first set to be analyzed gave no cross reactions therefor proved to be the best choice for identifying *Candida* species.
A multiplex TaqMan Real-time PCR is definitely a method worth investing in to be able to identify more than one species at the time.

Immunoglobulin kappa:lambda light chain ratio shifts between transitional and naïve B cell subsets in patients with common variable immunodeficiency

By Anna Lyytikäinen

Bachelor thesis in Biomedical Laboratory Science
Performed at Ospedale Pediatrico Bambino Gesù, Rome
Sahlgrenska Academy, University of Gothenburg, 2016
Supervisor: Ola Grimsholm, PhD

Introduction: Common variable immunodeficiency (CVID) is a group of disorders where the genetic background is largely unknown. The common hallmark is low serum immunoglobulin levels, but approximately 20 – 40 % of patients also present with autoimmune disease. This has been considered a paradox as immunodeficiency and autoimmunity represent lack of immune function and immune hyperreactivity, respectively [1]. Normally, autoreactive B cells are censored by receptor editing and anergy; a change in these mechanisms could be reflected in the B cell surface immunoglobulin light chain ratio (Ig kappa:lambda ratio).

Aims: To examine possible changes in the Ig kappa:lambda ratio on different B cell subsets of healthy donors and patients with CVID, as well as possible correlations between Ig kappa:lambda ratios and two B cell survival markers: B cell activating factor (BAFF) and B cell activating factor receptor (BAFF-R).

Method: Peripheral blood mononuclear cells from 19 patients with CVID and 16 healthy blood donors were stained for markers specific to different B cell subsets, analysed by flow cytometry and gated into transitional, naïve and memory B cell subsets in order to calculate Ig kappa:lambda ratios. BAFF-R expression was investigated for 14 patients and 9 donors. Plasma was gathered from 25 patients with CVID and 13 healthy volunteers, and BAFF was measured with a commercial anti-BAFF enzyme-linked immunosorbent assay (ELISA).

Results: The Ig kappa:lambda ratio decreased significantly between transitional and naïve B cells within patients with CVID. BAFF-R and BAFF levels did not correlate with the Ig kappa:lambda ratio of any B cell subset, though BAFF was significantly increased in patients with CVID compared to healthy donors. Notably BAFF-R in transitional cells was variable and could be split into two populations according to BAFF-R and CD24.

Discussion: Regardless of clinical phenotype, the Ig kappa:lambda ratio of patients with CVID decreases between transitional and naïve B cell subsets, but does not appear to correlate with BAFF. In contrast to earlier studies, BAFF-R expression was not found to differ between healthy donors and patients with CVID, although within virtually all individuals BAFF-R expression increased in naïve and decreased in memory B cells. Whether the Ig kappa:lambda change holds stable over time or is related to autoimmunity remains to be studied, but this study has allowed for pinpointing the change toward early B cell checkpoints (transitional – naïve) instead of peripheral ones (naïve – memory) and provides a possible stepping stone for a larger study.

En face immunohistochemistry suggests blood flow-mediated regulation of fibrinolysis in mouse aorta

By Jenny Lövestam

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg laboratory, Sahlgrenska Academy, University of Gothenburg, 2016

Supervisors: Pia Larsson and Per Fogelstrand

BACKGROUND AND AIM: Cardiovascular disease is the leading cause of death. One major subgroup is thrombotic cardiovascular disease, when a blood clot obstructs a vessel. To avoid excessive blood clot formation, the body has a mechanism responsible for the continuous breakdown of blood clots, the fibrinolysis. Upon clot formation, the key fibrinolytic enzyme tissue type plasminogen activator is instantly secreted into the lumen of the vessels by endothelial cells. Tissue type plasminogen activator catalyzes the conversion of plasminogen to plasmin, which breaks down fibrin that holds the blood clots together. *In vitro*, tissue type plasminogen activator has shown to be regulated by shear stress caused by blood flow. Since there has been no method for viewing the whole endothelium, this has not yet been assessed *in vivo*. After the development of a novel method, *en face* immunohistochemistry this is now possible. Since tissue type plasminogen activator is a releasable protein, a low level of protein in the endothelium could either be due to a high secretion or to a low gene expression. The aims of this study is to assess the endothelial expression and localization of tissue type plasminogen activator *in vivo* in mouse aorta and to see if the levels correlate with blood flow pattern.

METHODS: In this study we stained aortic arches from mouse with *en face* IHC, using specific antibodies for tissue type plasminogen activator, intercellular adhesion molecule-1 and CD-31. Then, the fluorescent signals were quantified, and the results between an area with turbulent blood flow and an area with laminar blood flow was compared. To assess the expression of tissue type plasminogen activator mRNA in the same vessels, different protocols for QuantiGene viewRNA ISH cell assay was tested on *en face* samples.

RESULTS: The results of this study suggests that the tissue type plasminogen activator levels in the greater curvature is higher than in the lesser curvature of the aortic arch and that the levels of the flow marker intercellular adhesion molecule-1 show anti-correlation with the tissue type plasminogen activator levels. For mouse aorta, neither of the QuantiGene viewRNA protocols for adherent cells, combination protocol for adherent cells and tissue or tissue protocol for frozen sections was optimal.

CONCLUSION: This study shows that there is a possible correlation between tissue type plasminogen activator levels and blood flow patterns, though in need of testing on a bigger population, and that QuantiGene viewRNA ISH assay needs further optimization for studying tissue type plasminogen activator mRNA in mouse aorta.

Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method in HILIC Mode for Quantification of Myo-Inositol

By Emina Muminovic

Bachelor thesis in Biomedical Laboratory Science performed at AstraZeneca, Gothenburg

Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Tasso Miliotis, PhD

Biomarkers are known as characteristic, objectively measured and evaluated as indicators of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. Performed research and development in biomedical and translational science indicates that the discovery, verification, and validation of different kind of analysis for quantification of biomarkers can lead to individualized diagnosis and treatment and hereby revolutionize personalized medicine. Diabetes mellitus is among other diseases, a condition where these types of biomarkers would be of huge importance in order to stratify these patient populations.

It is of importance that the characteristics of a biomarker are carefully considered before its potential usefulness is determined. Since myo-inositol is a compound necessary for development of infants, it must be supplied in in the diet by inositol-enriched milk or formula when it cannot be administered the normal way, through breast milk. Different kind of rapid and simple methodologies to determine myo-inositol are desirable since it is important to not exceed the permitted levels of the substance in formula.
The limitations of recent performed studies with the analysis of myo-inositol are in need of very time consuming analysis (up to 60 minutes). The performed analysis also include complicated sample preparation procedures which are very time consuming.
The aim of this study is to evaluate some properties of the HILIC-mode chromatography combined with MS/MS and develope a minimum sample preparation, only adding an organic solvent to the sample before injection onto the LC-MS/MS system. The plasma precipitation will lead to a simplified preparation and provides samples compatible with the technique of LC-MS/MS, in HILIC mode, described herein.
Evaluating retention data for myo-inositol, significant changes in both selectivity and peak shape were observed between the three evaluated HILIC columns. Improvement of the conditions for the quantification of myo-inositol resulted in optimal resolution (RS of 4.72), retention time and separation of the substance using a ZIC-cHILIC column.
The calibration curve was linear between concentrations of 0.5 and 20 µM. The inter-assay precision resulted in a coefficient of variation of 12% and the recovery was determined to 90%. The results indicates that the method described herein is robust and reproducible and could be implemented for future analysis of myo-inositol in plasma.

KEYWORDS: Myo-inositol, LC-MS/MS, HILIC

Absolute quantification of the fusion transcript KMT2A-MLLT3 with droplet digital PCR as an alternative method in clinical practice

By Daniel Palmér

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Genetic Analysis, Clinical Chemistry, Sahlgrenska University Hospital, 2016
Supervisor: Julia Asp (Docent), Angel Cheng Pettersson (Medical laboratory scientist)

Abstract

Background and aims: Translocations to the *KMT2A* gene is a common genetic rearrangement associated with acute myeloid leukaemia in children where the most frequent partner gene is *MLLT3*. For diagnose the fusion transcript, *KMT2A-MLLT3,* is quantified by real-time PCR. A new quantitative assay comparable to quantitative PCR is the droplet digital PCR. This method performs an absolute quantification with no requirements for comparison against a calibration curve. The aim of this study was to investigate droplet digital PCR ability to quantify the fusion transcript and the reference gene *GUS* with the DNA-binding dye EvaGreen®. The aim was also to optimize and evaluate the method on the basis of its capacity to amplify longer amplicons and differentiate them based on fluorescence intensity.

Material and Methods: Optimization of droplet digital PCR and quantification of the fusion transcripts was performed on patient samples and positive controls. To cover the fusions breakpoints a set of multiple primers were used. The fusion transcripts were also analysed with various primers to generate amplicons of varying base pairs lengths. The reference gene *GUS* was also quantified for comparison results between droplet digital and quantitative PCR.

Results: Optimization showed that a dilution of samples (cDNA) is required for quantification because undiluted material provides a poor separation between positive and negative droplets; this separation is required for calculation of positive droplets and by extension quantification of the target. Without a dilution of the sample highly expressed reference genes also saturated the reaction. Quantitative results from optimization had a coefficient of variation below 8% regardless of different primer concentrations. An optimum separation of the droplets was identified when adding 150-200 nM primers. Quantitation of patient samples showed that 7 out of 9 previous positive samples could be quantified by droplet digital PCR based on assay limit to 1 copy/μL. The fusion was detectable in all samples. Droplet digital PCR ability to quantify longer amplicons was found to be limited to 627 bp. Our result results also showed a correlation between amplicon length and fluorescence amplitude of positive droplets. We were able to distinguish products with a length of 248 and 135 bp. This correlation decreased with amplicons longer than 248 bp.

Conclusions: In this study we have demonstrated that the fusion transcript *KMT2A-MLLT3* can be quantified with droplet digital PCR with a high sensitivity for transcript with a low concentration. We also observed that the assay has a high capacity for detection of longer amplicons and this suggests that the number of primers used for detection of the fusion transcript can be reduced.

Keywords: PCR, ddPCR, EvaGreen, KMT2A-MLLT3

SAHLGRENSKA AKADEMIN

Elderly men with high blood pressure are overrepresented among people with atrial fibrillation, first degree-atrioventricular block and bundle branch block

By Johanna Rickardsson

Bachelor thesis in Biomedical Laboratory Science performed at the Institute of Medicin, Sahlgrenska Academy, University of Gothenburg, 2016 Supervisor: Maria Rosvall, associate Professor in Social Medicine.

Aim: The aim of this study was to examine whether there is a link between ECG changes and socio- demographic factors (age, sex , education) and lifestyle (BMI , smoking, physical activity, alcohol consumption).

Method and results: This study analyzed the epidemiological data sets including survey responses, as well as ECG from the InterGene study where ECG was performed on 3232 randomly selected adults in Gothenburg and its surroundings. The results showed that 0.9% of the subjects had atrial fibrillation. These subjects were more often male, older, had a lower level of education than university education, higher systolic blood pressure, and more often experienced financial stress than people without atrial fibrillation. It was also more common for people with atrial fibrillation to have a history of a previous heart attack and they were more likely to have diabetes than people without atrial fibrillation. 1,2% of the study participants had first degree-atrioventricular block (AV blockage I). These subjects were more often male, older, had a higher BMI, higher systolic blood pressure, and drank on average more alcohol than people without AV blockage I. 2,3% of the study participants had a bundle branch block. These subjects were more often male, older, had less often university education, were less often stressed at home, had a higher BMI and higher systolic blood pressure than people without bundle branch block. It was also more common for people with bundle branch block to have diabetes and to have had a heart attack or stroke.

Conclusion: The prevalence of atrial fibrillation, AV-blockage I and bundle branch block varied between 0,9% to 2,3% in a population-based sample ages 24-74 years living in Gothenburg or its surroundings. Generally, male sex, older age and a higher systolic blood pressure was more common among subjects showing such arrhythmias. Neither smoking nor a sedentary leisure time had a significant association with any of the arrhythmias, while alcohol consumption was higher among those with AV-blockage I compared to those without such blockage.

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DETECTION OF VIRULENCE-ASSOCIATED GENES IN *STAPHYLOCOCCUS AUREUS* STRAINS FROM PATIENTS WITH COMPLICATED BACTEREMIA

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Bachelor Thesis, spring term 2016

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ABSTRACT

Background: *S. aureus* is a gram positive cocci bacterium that belongs to the family of Staphylococcaceae. Although *S. aureus* is a member of the normal microbiota, this bacterium has also the potential to act as a versatile pathogen causing severe invasive infections such as bacteremia, endocarditis, etc. *S. aureus* is one of the most common causes of bacteremia, of which the incidence is increasing, particularly the nosocomial acquired bacteremia. *S. aureus* possess a great range of virulence associated determinants including toxins with superantigenic activity, toxins without superantigenic activity and adhesins that contribute to *S. aureus* pathogenicity and trigger infections.

Aim: The aim of the current study was to 1) identify virulence gene carriage rates in *S. aureus* strains (n = 42) obtained from 42 patients suffering from complicated bacteremia, and 2) compare pathogenic characteristics in invasive *S. aureus* strains with those from healthy controls.

Materials and methods: The carriage of 35 virulence genes in *S. aureus* strains obtained from patients with complicated bacteremia was determined using a molecular-based method, termed polymerase chain reaction (PCR). *S. aureus* strains were subjected to several PCR approaches for detection of virulence genes.

Results: Majority of *S. aureus* strains (69%) carried genes encoding superantigens SElM and SElO. The second common group of toxin genes was *sea, sec, sel* and *tst* (14-21%). Approximately, all *S. aureus* strains carried adhesin genes *clfA, clfB and fnbB*. More than 50% of strains (60%-81%) possessed *ebp, fib, fnbA* and *lbp* genes. Prevalence of *cbp* and *bsp* adhesin genes was less than 50%. Further, virulence gene carriages between invasive strains and those from healthy controls were compared. All toxin genes except *sec* were roughly higher in invasive *S. aureus* than strains from healthy individuals. However, any significant differences were not observed. In general, adhesin genes for *cbp, ebp, bsp, lbp* and *fnbA* were more common in *S. aureus* strains from healthy controls, whereas *fib* gene was more frequent in invasive strains. When comparing virulence gene carriage rates in *S. aureus* from survivor and non-survivor patients, the virulence profile of *S. aureus* strains from survivor patients tended to be similar to those from healthy controls.

Conclusion: We have identified virulence gene carriage rates in *S. aureus* strains obtained from patients with complicated bacteremia. Carriage rate of several genes was more common in invasive strains than those from healthy controls. However, the differences did not reach statistical significances. Further, virulence patterns of *S. aureus* from survivor patients tended to be similar with those from healthy controls. It might suggest that some of virulence genes enriched in *S. aureus* strains from survivor patients are likely involved primarily in bacterial colonization rather than infection. Furthermore, the host factors including clinical data were not evaluated as part of the study. Thus, the impact of virulence genes on *S. aureus* colonization and infection remains to be explored.

Abstract

Serum estradiol concentrations in prepubertal boys born small for gestational age (SGA) – A comparison between extraction-RIA and GC-MS/MS

By Sofia Scherwin Morichetto

Bachelor thesis in Biomedical Laboratory Science performed at Tillväxtlaboratoriet, Department of Paediatrics, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Carina Ankarberg Lindgren, Biomedical Scientist, Doctor in Medicine

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Background: Estradiol plays a key role in development and maturation in children and adolescents. To be able to accurately study the low levels of serum estradiol in children there is a need for a sensitive and valid method like Gas Chromatography-tandem Mass Spectrometry (GC-MS/MS). The ultra-sensitive extraction-radioimmunoassay (RIA) that is used today will be phased out and therefore has to be replaced with a new method. Earlier determinations of serum estradiol in children born SGA has shown a higher concentration than expected at an age of 5 years. Children born small for gestational age (SGA) has a higher risk of developing cardiovascular and metabolic diseases like obesity, high blood pressure, type 2 diabetes and dyslipidemia as adults.

Aim: In this study we evaluate the comparison between the two methods in samples from children and in a study group of boys born SGA and the possibility to transfer the reference intervals developed for the extraction-RIA to the GC-MS/MS.

Materials and Methods: Serum estradiol determined by extraction-RIA and GC-MS/MS with negative chemical ionization. The material in this study were 40 serum samples from children (pooled and single samples from routine analytical methods for determination of serum estradiol), earlier determined with extraction-RIA method and 28 serum samples from boys born SGA, 5 years of age.

Results: Limit of detection (LoD) was estimated to 2 pmol/L for GC-MS/MS compared to 4 pmol/L for ultra-sensitive extraction RIA. The GC-MS/MS method also showed better reproducibility compared to ultra-sensitive extraction RIA. Linear regression analysis showed a correlation between the two methods ($r = 0.97$). GC-MS/MS bias of the study was set to -5.5%. All serum estradiol concentrations for the study group, boys born SGA 5 years of age, analysed with GC-MS/MS got a value below LoD, whereas they had been determined to values between 5.2 and 22.4 pmol/L previously with ultra-sensitive RIA

Conclusion: The GC-MS/MS-method is a sensitive method with a good reproducibility. There is a strong correlation between the two methods. More investigation is necessary to ensure the correlation in the lower concentrations. The study group, boys born SGA 5 years of age, differed from the rest of the samples since they all showed levels under LoD with GC-MS/MS, which is considerable lower than the measurements performed before with ultra-sensitive RIA, probably due to some interference in the RIA. This is interesting findings that has to be further investigated.

A method for cloning and purifying the mitochondrial proteins FASTkd2 and FASTkd3

By Thomas Schwartz

Bachelor thesis in Biomedical Laboratory Science performed at
the Dept of Medical Biochemistry and Cell Biology,
Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Maria Falkenberg, Professor
Viktor Posse, PhD Student

Summary

The FAST proteins are important for the post transcriptional processing of genes from the mitochondrial genome. Even though recent publications have established causal links between most of the FAST proteins and specific RNA targets *in vivo*, *in vitro* experiments to identify the molecular mechanisms have not yet been published. This paper describes methods for cloning two of the FAST proteins, FASTkd2 and FASTkd3 which will enable future *in vitro* experimental procedures to examine their function more fully. We present working primers and plasmid combinations for the assembly of protein expression plasmids and identify Rosetta pLysS cells as superior to Arctic Express cells for the production of both proteins. The efficiency of the procedure is confirmed through a bulk volume culture of each protein construct and a Western Blot analysis using protein-specific antibodies. This information should greatly reduce the burn-in time of future work *in vitro*.

Suppressed Diversity of Survivin Splicing in Active Rheumatoid Arthritis

By Minna Turkkila

Bachelor Thesis in Biomedical Laboratory Medicine

Sahlgrenska Academy, University of Gothenburg, Spring 2016

Abstract

Objective. Alternative splicing is generally viewed as a mechanism distinguishing normal and cancer cells. Survivin is a tumour antigen, high levels of which recognize patients with severe therapy resistant rheumatoid arthritis (RA). Here we assess clinical relevance of alternative splicing of the survivin gene in leukocytes of peripheral blood (PBMC) and bone marrow (BM) in RA patients.

Methods. Transcription of the survivin wild-type (survivin-WT), survivin-2B and survivin-ΔEx3 splice variants was measured in an observational cohort of 67 RA patients and in 23 patients before and after B cell depletion with rituximab. Splice variants were analysed in relation to disease activity, anti-rheumatic treatment and serum levels of rheumatoid factor and survivin.

Results. Survivin-WT was the dominant splice variant equally expressed in T and B cells of the peripheral blood, while survivin-2B and survivin-ΔEx3 were higher in B cells. High clinical activity of RA was associated with an excess of survivin-WT and low ratios between survivin-2B/WT and survivin-ΔEx3/WT in PBMC. Anti-rheumatic immunosuppression increased the survivin-2B/WT ratio. Depletion of B cells caused by rituximab was associated with a decrease in survivin-WT in PBMC, and an increase of the ratios between survivin-2B/WT and survivin-ΔEx3/WT in BM. This increase in alternative survivin splicing was inversely related to a reduction in number of CD19+ BM cells, serum levels of rheumatoid factor, and the activity of arthritis.

Conclusion. This study demonstrates that the suppressed diversity of survivin splicing in leukocytes is attributed to adverse self-recognition in RA. Depletion of autoantibody producing B cells improves a balance in survivin splicing.

Abstract

Measurement of DNA damage induced apoptosis in patients' peripheral blood mononuclear cells

By: Talle Van Campfort

Bachelor thesis in Biomedical Laboratory Science, Sahlgrenska Academy, University of Gothenburg, Spring 2016

Supervisor: Pegah Johansson, PhD

Background: Ionizing radiation is used as a treatment for cancer. Radiation treatment induces damage in the normal tissue and there is a difference in individual sensitivity to radiation. The damage of radiation leads to DNA double-strand breaks and this induces apoptosis. The level of apoptosis in patient cells may help predict their sensitivity to radiation treatment. Therefore, we optimized a method to measure apoptosis after radiation in peripheral blood mononuclear cells.

Methods: Peripheral blood mononuclear cells were isolated from healthy individuals by density centrifugation. The cells were radiated to induce apoptosis and incubated for different time periods. The apoptotic cells were labeled with annexin V and measured with flow cytometry.

Results: An incubation of PBMC at 24 h gave similar results as a 72 h incubation. The Annexin V assay was able to detect an increase in apoptotic PBMC when there was an increase in radiation dose. The Annexin V assay was able to find a variation in radiation induced apoptosis of PBMC isolated from healthy individuals. The Annexin V apoptosis assay correlated with another known assay (cell division assay) in predicting sensitivity after irradiation.

Conclusion: Our results indicated that the Annexin V assay is a fast and reliable method to measure apoptosis after a DNA damage was induced by radiation. A relationship has found between the Annexin V assay and the EdU cell division assay.

Using small molecules to differentiate induced pluripotent stem cells into chondrocytes

By Hanna Zoric

Bachelor thesis in Biomedical Laboratory Science performed at the Institution of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2016

Supervisor: Itedale Namro Redwan and Stina Simonsson

Osteoarthritis (OA) is the most common joint disease in the world and causes severe pain for the afflicted patients as well as being a significant socioeconomic burden in developed countries. The cause of OA is the degeneration and loss of the articular cartilage in the joint. Articular chondrocytes are the major components of this type of cartilage, and due to the avascular nature of the tissue, articular cartilage lacks any significant repair capacity making it at high risk for damage when put under degenerative conditions.

A widely used treatment method for OA is the autologous chondrocyte implantation (ACI) technology aimed to replace the damaged articular cartilage. However, efficient cell replacement therapies require the generation of a correct and reproducible cell type. Previous studies have comprised the use of human embryonic stem cells and mesenchymal stem cells for differentiation purposes. However, these come with ethical concerns as well as the disadvantageous possibility to result in hypertrophic cells. Thus the use of induced pluripotent stem cells (iPSCs) shows a promising alternative. The aim of this thesis was to use small molecules to direct the differentiation of iPSCs towards the chondrocyte fate following a modified version of the protocol described in a Nature Biotechnology paper published by Craft, A.M. *et al* in 2015 and comparing degrees of differentiation by monitoring the expression of chondrocyte progenitor markers *PAX1* and *MEOX1* using Reverse Transcriptase Quantitative Real Time Polymerase Chain Reaction (RT- qPCR). In this study, we identify small molecules that demonstrate an increase in the expression of the *MEOX1* gene when compared to the published procedure.

The use of small molecules for differentiation purposes is considered as promising alternatives to growth factors and transcription factors, regularly used in previously reported protocols, since batch-to-batch variations are eliminated. The identified small molecules will serve as a starting point for further development and optimisation of differentiation protocols leading to chondrocytes, in the long run giving rise to new treatments for osteoarthritis.