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Book of Abstracts

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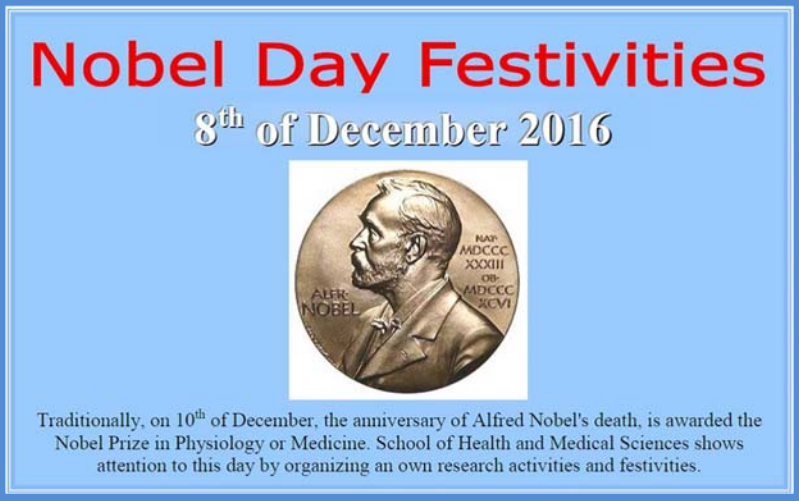

School of Medical-, & Health Sciences
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Book of abstracts, Nobel Day's Festivities 8th of December 2016

Preface

The"**Nobel Day Festivities"** were established 2009 by Biomedicine, Department of Clinical Medicine, School of Health and Medical Sciences at Örebro University, and is organized traditionally every year in order to notice the anniversary of Alfred Nobel's death (10th of December) with scientific activities including poster presentations and selected oral presentations by doctoral students, Postdocs/Researchers, which are documented in this *"Book of abstracts".* Nobel day's activities are open and scheduled so that all students and personnel can attend the scientific activities.

We warmly welcome you to enjoy all the good science that will be presented at Nobel Day Festivities, Örebro University 2016

The Organizers

Nikolaos Venizelos, Professor Em. (Chair)
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Magnus Grenegård, Professor
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Adherence to Swedish guidelines for pain treatment in tonsil surgery in pediatric patients

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The effect of interleukins 6 and 17A on vascular smooth muscle function during the development of atherosclerosis

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Objective: Atherosclerosis is a chronic inflammatory disease and the main cause of death and morbidity. The initial changes that lead to the formation of lesions of atherosclerosis begins in the endothelium as a response to endothelial injury. Injury to the endothelium usually leads to an increased lipid permeability, leucocyte recruitment and the released of inflammatory mediators such as cytokines and growth factors by both inflammatory and non-inflammatory cells which initiate multiple effects including phenotype switching of vascular smooth muscle cells (VSMC) from the quiescent contractile phenotype to the synthetic phenotype, where they can proliferate and migrate from media to the intima.
The aim of the project is to elucidate the effect of interleukins 6 and 17A on the vascular smooth muscle cells function.

Method: Commercially acquired human aortic vascular smooth muscle cells were stimulated with IL6, IL17A, soluble form of IL6 as well the combination IL6 and IL6 receptor, IL6 and IL17 and all the three together with concentrations ranging from (20ng/ml-50ng/ml) for 24 hours where the induction of the pro-inflammatory chemokines MCP-1 and GRO-α were measured using ELISA. Result: Our preliminary data shows the induction of MCP-1 and GRO-α in VSMC by both IL6 and IL17A.

Conclusion: Base on this preliminary result we want to conduct further experiment to block the two cytokines with specific antibodies.

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Subphenotypes of inflammatory bowel disease are characterized by specific serum protein profiles

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Background: There is a need for improved diagnostic biomarkers in inflammatory bowel disease (IBD). We wanted to identify serum proteins to discriminate between Crohn's disease (CD), ulcerative colitis (UC), and healthy controls (HC).

Methods: 91 inflammatory serum proteins from a discovery cohort of CD patients (n=54), UC patients (n=54), and healthy controls (n=54) were quantified using a proximity extension assay. We performed univariate analyses by Welsh t-test, and assessed false discovery rates. A sparse partial least-squares (sPLS) approach was used to identify additional discriminative proteins. Cross-validation error rates for discrimination were calculated. The results were validated in a replication cohort.

Results: By univariate analysis, 17 proteins were identified with significantly different abundances in CD and HCs, and 12 when comparing UC and HCs. Additionally, 64 and 45 discriminant candidate proteins, respectively, were identified with the multivariate approach. Correspondingly, significant cross-validation error rates of 0.11 and 0.19 were observed in the discovery cohort. Only FGF-19 was identified from univariate comparisons of CD and UC, but 37 discriminant candidates were also identified using the multivariate approach. Using univariate comparisons, 16 of 17 CD-associated proteins and 8 of 12 UC-associated proteins were validated in the replication cohort. The error rates for discrimination of subgroups increased when the sPLS model from the discovery cohort was applied to the replication cohort.

Conclusions: By investigating a panel of inflammatory proteins, we identified a number of discriminant candidate markers of subphenotypes of IBD, highlighting the potential of inflammatory serum proteins in diagnostic biomarker identification.

The role of the poly(A) tract in the replication and virulence of tick-borne encephalitis virus

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Objective: Tick-borne encephalitis (TBE) in humans results in symptoms ranging from mild flu-like symptoms to severe and long-lasting sequelae, including permanent brain damage. However, little is known about the underlying virulence determinants. I this study we investigated the role of an internal poly(A) tract and virus quasispecies in virulence of TBE virus (TBEV).

Methods: To characterize the role of the poly(A) tract in TBEV replication and virulence, we generated infectious clones of TBEV with a $(A)_3C(A)_6$ poly(A) tract sequence (Torö-6A) or with a modified $(A)_3C(A)_{38}$ poly(A) tract sequence (Torö-38A). In addition, we performed next generation sequencing to determine the quasispecies structure of TBEV isolated from cell culture and mouse brain.

Results: Torö-38A replicated poorly compared to Torö-6A in cell culture, but Torö-38A was more virulent than Torö-6A in a mouse model of TBE. Next-generation sequencing of TBEV genomes after passaging in cell culture and/or mouse brain revealed mutations in specific genomic regions and the presence of quasispecies that might contribute to the observed differences in virulence.

Conclusions: These data suggest that the length of internal poly(A) tract plays an important role in TBEV quasispecies diversity that in turn contributes to TBEV virulence in mice. In addition, these findings demonstrate host-specific differences in the quasispecies structure of TBEV.

LOS-dependent *Neisseria meningitidis*-induced caspase-1 activation in human innate immune cells

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Objective: Lipooligosaccharide (LOS) of Neisseria meningitidis is an endotoxin that is responsible for activation of immune cells and the release of proinflammatory cytokines. One of the most potent proinflammatory cytokines; interleukin-1β (IL-1β) is activated following caspase-1 activity in the intracellular multi-protein complex called inflammasome. Inflammasomes are activated by several microbial factors as well as danger molecules, but there are no data available regarding a role for inflammasome activation in meningococcal disease. The aim of this study was to investigate if N. meningitidis activates the inflammasome and if so, the role of bacterial LOS in this activation.

Methods: Human innate immune cells were subjected to wild-type FAM20 N. meningitidis and its LOS-deficient mutant (lpxA), and priming as well as inflammasome triggering was investigated.

Results: Whereas the wild-type strain significantly enhanced the caspase-1 activity in neutrophils and monocytes, the lpxA mutant was unable to induce caspase-1 activity. Both wild-type and LOS-deficient mutant induced IL-1β release but the levels were significantly higher when stimulated with wild-type FAM20 compared with lpxA mutant or unstimulated controls. Inflammasome priming detected as NLRP3 and pro-IL-1β expression was significantly increased in innate immune cells stimulated with lpxA mutant but even more by the wild-type strain.

Conclusions: We conclude that although non-LOS components of N. meningitidis, via a MyD88-dependent pathway, contribute to the priming of the inflammasome activity, LOS per se is to be considered as the central component of N. meningitidis responsible for both priming and triggering of inflammasome activation.

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NLRP3 is associated with uropathogenic *E. coli* adhesion and invasion of bladder epithelial cells

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Objective: Investigate the role of inflammasome activation in the immune responses in the urinary tract.

Methods: NLRP3 and Caspase-1 were knockedout/down with CRISPR-Cas9 in the bladder epithelial cell line 5637. Cells were then infected with the uropathogenic *E. coli* strain CFT073 and a type-1 fimbriae "ON" mutant and IL-1β secretion was measured by ELISA. We also analyzed bacterial adhesion and invasion by green fluorescent protein (GFP) expressing bacteria and by agar plating.

Results: We observed that knockout of NLRP3 and knockdown of Caspase-1 in bladder epithelial cells was associated with decreased IL-1β release upon an uropathogenic *E. coli* infection. NLRP3 was also observed to be associated with type 1 fimbriae mediated *uropathogenic E. coli* adhesion and invasion of bladder epithelial cells.

Conclusion: We conclude from these preliminary data that the NLRP3 inflammasome is not only important for the activation and secretion of IL-1β, but also for the colonization and persistence of uropathogenic *E. coli* in the bladder.

Aerobic fitness is associated with low CVD risk - The impact of lifestyle on early risk factors for atherosclerosis in young healthy individuals

The lifestyle, biomarker and atherosclerosis, LBA-study

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Background: The progress of cardiovascular diseases (CVD) and atherosclerosis is slow and develops over decades. In the Lifestyle, Biomarker and Atherosclerosis (LBA) study 834 young self-reported healthy adults 18.0-25.9 years have been studied to identify early risk factors for atherosclerosis.

Purpose: To assess selected cardio-metabolic biomarkers and carotid intima-media thickness (cIMT), as a marker of subclinical atherosclerosis and oxygen uptake (VO_2), as a lifestyle related indicator of aerobic fitness. Also present basic characteristics and associate selected cardio-metabolic biomarkers to food habits and VO_2. Furthermore, to identify subjects at risk for CVD using a risk score and to compare subjects at risk for CVD with subjects without risk.

Method: Blood samples were taken in fasting state and food habits were reported by questionnaire. The cIMT was measured by ultrasound and VO_2 by ergometer bike test. The risk score was calculated according to Wildman.

Result and conclusion: Carotid intima-media thickness 0.50 ± 0.06 mm (mean ± standard deviation). VO_2 37.8 ± 8.5; 42.9 ± 9.8 ml/kg/min, in females and males, respectively. cIMT was correlated to systolic blood pressure, waist circumference and VO_2 (L/min), $P<0.05$.

When using the definition by Wildman 12.1% of the subjects were classified as being at risk for CVD. Food habits did not differ between those at risk and the rest. Aerobic fitness measured as VO_2 (ml/kg/min) however differed, 46.5% of the subjects at risk had low aerobic fitness compared to 23.4% of the non-risk subjects, $P<0.01$.

In the studied population 15.3% were insulin resistant (HOMA-IR) before the age of 26 years. Of the females 35.1% and of the males 24.5% had low HDL-C according to Wildman's cutoff. Higher VO_2 was significantly associated with beneficial levels of HOMA-IR, HDL-C and body fat (%) indicating that higher aerobic fitness is protective in this age group.

Yeast-derived β-glucan reduced mast cell-induced hyperpermeability in human villus- and follicle-associated epithelium from patients with ileal Crohn's disease and control subjects

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Objective: Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by a chronic transmural inflammation which might originate from the stimulation of an exaggerated mucosal immune system by either a normal or dysbiotic commensal bacterial gut flora. It is known that patients with CD have increased gut permeability, and this might lead to increased passage of luminal bacteria, endotoxins and antigens, that in turn have further negative effects on the barrier function. Beta (β)-glucan from Baker's yeast has in several studies shown immune enhancing effects in both cancer therapy and, to some extent, resistance towards cold-induced infection but no study has demonstrated any beneficial effect on the human intestinal barrier function *ex vivo*. Our aim was to investigate whether a specific yeast-derived β-1,3/1,6 glucan could attenuate mast cell (MC)-induced hyperpermeability in follicle-associated epithelium (FAE) and villus epithelium (VE) of patients with CD ileitis and in non-inflammatory bowel disease (IBD) controls. Further we aimed to study mechanisms of β-1,3/1,6 glucan uptake in vitro.

Methods: Para- and transcellular permeability were investigated by mounting FAE and VE from 7 patients with CD and 8 non-IBD controls in Ussing chambers. Translocation of fluorescence labeled β-1,3/1,6 glucan was studied by immunofluorescence after 20 minutes stimulation in Ussing chambers. *In vitro* experiments were performed in Caco-2-cl1 monocultures and FAE co-cultures to investigate β-1,3/1,6 glucan uptake using endocytosis inhibitors.

Results: β-1,3/1,6 glucan significantly attenuated both paracellular and transcellular hyperpermeability caused by mast cell degranulation in patients with CD and controls. *In vitro* studies showed an increased passage of β-1,3/1,6 glucan through the FAE co-culture compared to Caco-2-cl1, $p<0.05$, and passage was attenuated by the lipid raft inhibitor methyl-β-cyclodextrin, $p<0.05$. Fragments of β-1,3/1,6 glucan in different sizes were found both directly under the epithelium but also further down in the lamina propria. Immunofluorescence revealed β-1,3/1,6 glucan co-localising or in close proximity to mast cells, macrophages and dendritic cells.

Conclusion: This study demonstrated that yeast-derived β-1,3/1,6 glucan had a beneficial effect on the intestinal barrier function by inhibiting stress effects on the epithelium. Further, *in vitro* studies showed increased β-1,3/1,6 glucan passage through the FAE model that seemed to be dependent on lipid raft formation. Our results provide important and novel knowledge, and highlight the possible application of yeast-derived β-1,3/1,6 glucan in health disorders and diseases characterised by intestinal barrier dysfunction.

Activation of inflammation by respirable quartz or respirable non-specified dust in the work environment

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Objective: Inhalation of respirable quartz is a well-known hazard and a risk factor for severe lung diseases at high exposures. However, it is possible that repeated occupational exposure to respirable quartz also could cause a chronic systemic low-grade inflammation and contribute to the development inflammatory diseases, such as cardiovascular diseases. The objective of the study is to establish a possible link between occupational exposure to respirable quartz and/or respirable non-specified dust and activation of inflammation. The results of this study will contribute to our understanding of respirable particle toxicity and could be used as a reference to future revisions of the occupational exposure limits to respirable quartz or respirable non-specified dust.

Methods: Occupational exposure to respirable dust and quartz during an 8 h shift was measured on 84 individuals working in Swedish iron foundries, and their exposure to respirable dust and quartz was correlated to plasma levels of several cytokines, including the inflammasome-activated cytokines: interleukin (IL)-1 and IL-18, and their anti-inflammatory counterparts IL-37, IL-1 receptor antagonist and IL18 binding protein.

Results: Cytokine analyses are planned to be carried out before the end of November, in time to present some of the data on the Nobel Day Festivities.

Conclusion: No conclusions can be drawn before results are acquired.

Effect of Faecal Microbiota Transplantation on Anxiety and Depression in Irritable Bowel Syndrome patients

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Investigation of the molecular mechanisms by which butyrate affects gut-brain axis function

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The gut-brain axis is a bidirectional communication system comprised of both neuronal and humoral pathways that links gastrointestinal (GI) function and the central nervous system (CNS). The GI tract is colonized by an extensive and complex community of microbiota that has recently been shown to have a substantial impact on host physiology. Alterations in microbiota not only affect GI homeostasis and digestion, but recent evidence also supports a role for microbiota in brain development, CNS function, and behavior. One of the mechanisms by which microbiota affect host physiology and health is the production of short-chain fatty acids (SCFAs), which are generated by bacterial fermentation of fiber in the gut. Butyrate, a microbial metabolite that serves as an energy source, has been shown to possess anti-carcinogenic and anti-inflammatory properties, modify visceral sensitivity and intestinal motility, and affect barrier function. In addition to its role in the gut, butyrate has also been shown to affect neurological function when administered systemically in models of depression and neurodegenerative disease.
Together, these findings suggest that butyrate is a key mediator of communication between the gut and the brain and that altering gut-derived butyrate may have therapeutic effects in neurological disease.

Because of its potential in treating neuropsychiatric disorders, it is of great interest to understand the molecular mechanisms by which gut-derived butyrate affects CNS function.
In our lab, we assess the effect of butyrate on the function of the tryptophan transport system, a biomarker for neuropsychiatric disorders, using skin-derived fibroblasts obtained from healthy volunteers. Perturbed transport of tyrosine and tryptophan (precursors for monoamines) has been demonstrated in several studies using fibroblasts from patients with various neuropsychiatric disorders. Preliminary findings from our lab indicate that both pro-inflammatory cytokines and oxidative stress, two perturbations implicated in the pathogenesis of several neuropsychiatric disorders, can cause disturbed transport of tyrosine and tryptophan across the fibroblast cell membranes.

We demonstrate that butyrate is able to rescue oxidative stress-induced perturbations of the tryptophan transport system in a dose-dependent manner. At Örebro University, we have access to a biobank of fibroblasts obtained from patients with depression, schizophrenia, autism, and Parkinson's. Now that we have established fibroblasts as a model system in which we can assess the effects of butyrate on tryptophan transport, we plan to assess how this process is altered in neuropsychiatric disorders.

Together, our findings suggest that altering tryptophan transport is a potential mechanism by which butyrate affects CNS function.

Resource efficiency in ambulance care
The importance of referring care seekers to the right level of care

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Fibroblasts in chronic wounds – what is wrong?

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Chronic wounds are generally defined as wounds that do not heal within three months. These wounds are difficult to manage and the underpinning mechanisms of aberrant repair are only partly understood. Crucial factors likely to contribute to poor healing include poor blood supply with concomitant insufficient oxygen levels, persistent inflammation and free radical load.

Fibroblasts are key players in repair process both for generating an appropriate extracellular matrix and for regulating the inflammatory response. Some studies suggest impaired functional properties and signs of oxidative senescence of chronic wound fibroblasts. In this study we aimed at analyzing fibroblasts from a limited number of patients with chronic wounds and to compare these with normal controls.

Fibroblasts were cultured and subjected to analyze of whole proteome, growth rate, migration capacity, and proteasome activity.

Proteomics suggested limited number of dysregulated proteins in chronic wound fibroblasts, e.g. cellular retinoic acid binding protein 2. Proliferation and migratory capacity was not altered significantly, while some proteasome activities were impaired in these fibroblasts.

This calls for further studies of fibroblasts from distinct areas of the wounds in order to confirm dysfunctional niches in the tissue.

IL6/IL6R induce slow and sustained tyrosine phosphorylation of STAT3 in human platelet mitochondria

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Introduction. Platelets are non-nucleated cells that play crucial roles in hemostasis and thrombosis. Platelets also contribute to acute inflammation by releasing a number of cytokines and chemokines. Conversely, several inflammation mediators act on surface receptors and modulate platelet functional responses. The transcription factor STAT3 is a key downstream signaling molecule of interleukin IL6-activated cells. Herein, we characterized the molecular and cellular effects of IL6 on human blood platelets with particular emphasis on STAT3. As platelets lack nucleus, they may represent an excellent cellular model to study non-genomic actions of STAT3.

Methods: Human platelets isolated from healthy volunteers were exposed to IL6 in the presence or absence of the soluble IL6 receptor (IL6R). Platelet aggregation and ATP secretion was monitored by using a lumino-aggregometer. Expression and release of IL6R were analyzed using a commercial ELISA kit. Phosphorylation of STAT3 at Tyr705 and Ser727 residues was analyzed using Western Blot and Ca2+mobilization was analyzed by using the fura-2 technique. Mitochondria's were isolated using a commercial kit.

Results: Exposure of human platelet to IL6, soluble IL6R (sIL6R) and the combination of the two did not influence on platelet aggregation, dense granule secretion and Ca2+ mobilization. However, it was found that platelets expressed a releasable fraction of sIL6R. Furthermore, IL6 alone induced a minor phosphorylation of STAT3 at Tyr705 residue whereas sIL6R alone had no effect. Combined treatment of platelets with IL6/sIL6R induced a pronounced and long-lasting Tyr705 phosphorylation of STAT3. Finally, tyrosine phosphorylated STAT3 was detected within the mitochondria of platelets.

Conclusion: IL6/sIL6R-induced STAT3 phosphorylation was not accompanied by classical platelet functions like aggregation and secretion. Instead, STAT3 may participate in the regulation of platelet mitochondrial genome transcription, energy metabolism or other manifestations related to mitochondrial functions.

The role of CARD8 in NF-kB activation in HUVECs

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Objective: CARD8 belongs to the proteins that constitute the NLRP3 inflammasome. Its expression is highly increased in atherosclerotic lesions and its inhibition reduces the levels of atherosclerosis- involved chemokines like MCP-1 [1]. Moreover, there is evidence that CARD8 has a suppressive effect on NF-kB activation in 293T cells [2]. The aim of this study is to investigate the effect of CARD8 on NF-kB activation in HUVECs, considering its aforementioned role in the atherosclerotic process.

Methods: HUVECs were specifically knocked down for CARD8 using RNA interference. Cells were, then, treated with TNFα or not for 30' and 24h. Western blot was used to determine the expression levels of CARD8, phospho-p65, phospho-p38 and p-c-Jun in cell lysates. The former is a subunit of the activated NF-kB whereas the two latter represent the MAPK and AP1 signaling pathways. These pathways crosstalk with the NF-kB activation pathway. Finally, ELISA was used to detect MCP-1 secretion in control and CARD8 knocked down cells.

Results: CARD8 was successfully knocked down in HUVECs. The protein expression levels of phospho-p65, phospho-p38 and p-c-Jun were not affected by CARD8 knock down with or without TNFα treatment compared to control cells. Moreover, MCP-1 secretion levels were significantly reduced in CARD8 knocked down cells upon 1day TNFα treatment compared to the same treatment in control cells.

Conclusions: According to the findings of this study, no suppressive effect of CARD8 on NF-kB activation was detected in HUVECs. However, CARD8 has an enhancing effect on MCP-1 expression in the same type of cells.

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Synthetic fucoidan-mimetic sulfated glycopolymers provoke molecular and cellular responses in human blood platelets

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Introduction: The marine sulfated polysaccharide fucoidan displays superior ability to induce platelet aggregation compared to other sulfated polysaccharides. Therefore it is an attractive tool for studying molecular and cellular responses in activated platelets. Its heterogeneous structure poses a problem in such applications as chain length, branching, saccharide composition and degree of sulfation affect its platelet activating properties.

Methods: Fucoidan-mimetic glycopolymers with various chain lengths and defined degree of sulfation were synthesized. Platelet dense granules secretion and aggregation were determined using a Chrono-log model 700 instrument. Platelet surface changes were analyzed by flow cytometry and protein-tyrosine phosphorylation was assessed by immunoblotting.

Results: We found that the chain length determines the potency of fucoidan-mimetic glycopolymers. Synthetic long-chained fucoidan glycopolymer and natural fucoidan (Fucus vesiculosus) were equipotent in causing granules secretion, integrin $\alpha_{IIa}\beta_3$ activation, and platelet aggregation. Synthetic glycopolymers with average chain lengths of 329, 34, and 13 fucose molecules induced full platelet aggregation with EC50 values of 0.015 µM, 0.20 µM and 0.88 µM, respectively. Furthermore, the non-sulfated derivatives of the polymers were biological inert in terms of platelet functionality.

Conclusions: Synthetic fucoidan-mimetic glycopolymer are full agonists inducing protein-tyrosine phosphorylation, granules secretion, integrin $\alpha_{IIa}\beta_3$ activation, and platelet aggregation. However, the chain length determines the potency of the compounds. Multivalent binding and/or facilitated formation of signaling clusters may be the underlying reason for high potency of long-chain sulfated fucose polymers. We propose that synthetic fucoidan-mimetic glycopolymers are unique tools for elucidating platelet activation caused by carbohydrate molecules.

Bacteriocins as therapeutical agents to combat periodontal infections

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Background: The complications in healthcare systems associated with antibiotic-resistant microorganisms have resulted in an intense search for new effective antimicrobials. Attractive substances from which novel antibiotics may be developed are bacteriocins, such as the two-peptide bacteriocin PLNC8 αβ. These naturally occurring peptides are considered to be safe and efficient at eliminating pathogenic bacteria. *Porphyromonas gingivalis* is a key pathogens in the development and progression of the chronic inflammatory disease periodontitis.

Methods: Binding of PLNC8 αβ on the bacteria was determined using surface plasmon resonance analysis and membrane permeabilization was investigated by DNA staining with Sytox Green. Liposomal systems and transmission electron microscopy (TEM) were acquired to verify bacterial lysis. Proteomics analysis of human gingival fibroblasts was performed to determine differentially expressed proteins after stimulation with *P. gingivalis* and PLNC8 αβ, alone and in combination.

Results: The two-peptide bacteriocin PLNC8 αβ efficiently bound to *P. gingivalis* and caused permeabilization of the membranes. The antimicrobial activity of PLNC8 αβ was found to be rapid (1 min) and visualized by TEM to cause cellular distortion through detachment of the outer membrane and bacterial lysis. PLNC8 αβ was not cytotoxic and observed to counteract *P. gingivalis*-mediated apoptosis of fibroblasts. These findings were verified by profiling the proteome of gingival fibroblasts infected with *P. gingivalis*, in the presence or absence of PLNC8 αβ.

Conclusions: Soluble or immobilized PLNC8 αβ bacteriocins may be used to prevent *P. gingivalis* colonization and subsequent pathogenicity, and thus supplement the host immune system against invading pathogens associated with periodontitis.

Biological monitoring and dermal uptake upon exposure to cobalt in a Swedish hard metal production industry: Does cobalt contribute to uptake?

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Background: Occupational exposure to cobalt can occur in the hard metal manufacturing industry and from the use of hard metal tools. Cobalt is a known sensitizer and can induce allergic contact dermatitis as well as asthma, hard metal lung disease, cardiovascular diseases and cancer. The primary route for uptake of cobalt is via inhalation and the relation with cobalt concentrations in blood is well established. However considerable uptake via the skin is known to occur, yet the relation between skin exposure, skin penetration and blood concentrations is not substantially investigated.

Objective: To investigate if skin exposure to cobalt contributes to blood concentrations in a hard metal manufacturing industry to determine a possible uptake route through the skin.

Methods: Measurements on skin, inhaled air and biological sampling of cobalt in blood were performed on workers engaged in different departments in a hard metal production industry. The amount of cobalt was determined in the samples and skin and air concentrations were correlated to cobalt blood concentrations.

Results: Correlating (Spearman´s rho) cobalt on skin concentrations to cobalt in blood and inhalable air, significant correlation was seen for all. Linear regression analysis of the same data showed significant correlation for cobalt in blood and inhalable air, but not for cobalt on skin and in blood.

Conclusions: Our results suggest a co-variation between the amount of cobalt absorbed via the skin and the amount of inhaled cobalt. A model demonstrating the influence of skin absorption of cobalt on the blood concentration is presented.

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Aspergillus niger-derived enzyme efficiently degrades gluten in the stomach of gluten-sensitive subjects

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Objective: *Aspergillus niger*-derived prolyl endoprotease (AN-PEP) has been shown to degrade gluten into non-immunogenic compounds in an *in vivo* setting in healthy subjects in which AN-PEP was added to a liquid, intragastrically infused meal[1]. The current study investigated the efficacy of AN-PEP to degrade gluten in a physiological meal setting in gluten-sensitive subjects.

Methods: In this randomized placebo-controlled cross-over study, 18 self-reported gluten-sensitive subjects attended three test days. Subjects consumed a porridge containing 0.5 g gluten as well as two tablets either containing 160 000 PPI of AN-PEP (high dose), or 80 000 PPI (low dose) placebo. Gastric and duodenal content was sampled over 180 minutes and analysed for gluten epitopes using the Gluten-Tec® ELISA. The 180-min areas under the curve of epitope concentration were calculated using curve fitting.

Results: AN-PEP significantly lowered the gluten concentrations in the stomach and in the duodenum compared to the placebo in both high and low dose. In the stomach, gluten levels were reduced from 218 ± 155 µg/ml (mean ± STD) in the placebo to 31 ± 24 µg/ml in the high dose (p=0.001) and to 31 ± 22 µg/ml in the low dose (p=0.001). In the duodenum, gluten levels were reduced from 65 ± 88 µg/ml in the placebo to 12 ± 13 µg/ml in the high dose (p=0.019) and to 8 ± 5 µg/ml in the low dose (p=0.015).

Conclusions: Even in a physiological meal setting, AN-PEP significantly degraded most gluten before it entered the duodenum in self-reported gluten-sensitive subjects.

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The Role of αvβ5 integrin in the function of intestinal macrophages

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Macrophages *(mϕ)* are highly abundant in the healthy intestine, where they reside close to the epithelium and are thought to be important in preventing invasion by commensal bacteria. We have shown recently that resident intestinal *mϕ* are replenished continuously by circulating monocytes that differentiate locally into anti-inflammatory cells characterised by high levels of CX3CR1, MHCII and IL10 production.
Using microarray and phenotyping, we show here that maturing macrophages acquire several scavenger receptors, tissue remodelling metalloproteases (MMPs) and receptors associated with clearance of apoptotic cells, including the αvβ5 integrin crucial for clearance of effete rods and cones in the retina.
Functional studies confirm that αvβ5 integrin mediates phagocytosis of apoptotic cells in colon macrophages *in vitro*. Bone marrow chimera studies demonstrate that monocytes are reduced in both colon and small intestine of β5 KO ----> WT chimeric mice. QPCR analysis of intestinal *mϕ* sorted from β5 KO recipients shows significantly reduced expression of IL-10, MMP2, MMP9 and MMP13. In parallel, KO chimeric mice are more susceptible to DSS induced colitis than wild type chimeric mice.

Altogether these data suggest that αvβ5 integrin is important for intestinal *mϕ* to maintain local homeostatic functions such as apoptotic cell clearance and tissue remodelling.

Development and validation of a skin fibroblast biomarker profile for schizophrenic patients.

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Objectives: Gene expression profiles of peripheral tissues through microarray technology could be used in schizophrenia studies, adding more information to the results from similar studies on postmortem brain tissue. The ultimate goal of such studies is to develop accessible peripheral biomarkers.

Methods: We performed supervised machine learning methods, in order to examine if the gene expression signature from skin fibroblast cells classify schizophrenia subjects. A dataset of skin fibroblasts gene expression profile from schizophrenia patients was obtained from Gene Expression Omnibus. After applying statistical criteria, we concluded to genes that present a differential expression between the schizophrenic patients and the healthy controls. Based on those genes, functional profiling was performed with the BioInfoMiner web tool.

Results: After the statistical analysis, 63 genes were identified as differentially expressed. The functional profiling revealed interesting terms and pathways, such as mitogen activated protein kinase, cyclic adenosine monophosphate signaling pathways and immune system related mechanisms. A subset of 16 differentially expressed genes from fibroblast gene expression profiling that occurred after Support Vector Machines Recursive Feature Elimination could efficiently separate schizophrenics from healthy controls.

Conclusions: These findings suggest that through the analysis of a fibroblast based gene expression signature and with the application of machine learning methodologies we might conclude to a diagnostic classification model in schizophrenia.

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Development of an edible TBEV vaccine

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Introduction*:* The genus ***Flavivirus*** includes more than 70 members, of which several members are important human pathogens with significant number of patients each year worldwide. The clinical manifest as a mild acute febrile syndrome, but in many cases patients can develop severe encephalitis, hepatic or haemorrhagic disease. Flavivirus is mainly transmitted to their host by mosquitoes or ticks, including tick-borne encephalitis (TBEV) dengue (DENV), Japanese encephalitis (JEV), yellow fever (YFV) and West Nile (WNV) viruses. Due to the lack of an antiviral drug against flavivirus, vaccination remains the most important tool for prevention. Despite the availability of vaccines for some of the flavivrus members (YFV, TBEV and JEV), the efforts are insufficient to prevent infection. During the last few decades' researchers has provided important tools for vaccine development, such as the use of plants as antigen production platform. In this study, a development of an edible TBEV vaccine, part of the ScandTick project funded by "intrerreg", an EU regional development fund we aim to express the structural genes (CME) of TBEV in *Nicotiana benthamiana (*Tobacco*) and Tetragonia tetragonoides (*New Zeeland spinach*)*. A successful expression of the structural protein in the plant system generate Virus like particles (VLPs) that can be harvested and used as vaccine candidate for TBEV. The benefit with tobacco plants is fast growing and yielding large quantities of biomass in a short period of time. However, the advantage by using New Zeeland spinach as a plant platform to express the TBEV VLPs, is to produce an edible TBEV vaccine.

Material and Method: Codon optimized TBEV-CME was cloned into a pEAQ.HT vector and transformed to *Agrobacterium tumefaciens* strain for co-agaroinfiltration for VLP expression in 4-6 weeks plants. Non-Infiltrated leafs, infiltrated GFP-pEAQ.HT and CME-pEAQ.HT was harvested 7 days' post infiltration (dpi). Harvested leafs were grounded in liquid nitrogen to a fine powder and extracted in RIPA buffer for western blotting.

Results: In progress, we have successfully agroinfiltrated the tobacco plant with CME-pEAQ.HT and the control vector (GFP- pEAQ.HT)). Next is to analyze the expression of CME and quantify the produced VLP. The TBEV VLPs was also visualized by TEM.

Conclusions: We are in the start line of producing an edible TBEV vaccine by using two different plant platforms. The CME-pEAQ.HT infiltrated plants are under process of being analyzed for CME expression and quantification of VLPs. However, plant molecular farming is of great potential for the cheaper, more rapid, scalable manufacture and foremost far safer to make and use than inactivated live vaccines. In the case of viral vaccines, it has proved possible to make functionally assembled VLPs and immunoglobulins that can either provoke protective immune responses as shown in animal models.

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Pro-inflammatory Impacts of IL-17A and IL-6 on Human Endothelial Cells

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The IL-6/IL-17A axis has been suggested to be involved in the development of atherosclerotic plaques and the onset of myocardial infarction. IL-17A is a potent pro-inflammatory cytokine produced mainly from Th17 cells. It contributes to vascular and systemic inflammation mainly by inducing release of other cytokines such as IL-6. IL-6 plays a central role in inflammation by controlling differentiation, proliferation, migration and apoptosis of targeted cells. Recent mice studies showed synergistic effect by IL-17A and IL-6 to induce a massive amplification of the inflammatory response [1, 2]. Therefore, the aim of this study was to determine if such effect could occur on human endothelial cells.

First, we looked at the expression of receptors of both cytokines on HUVECs using qPCR and ELISA. We found that IL-17A receptors (IL-17RA and IL-17RC) were expressed and similarly, IL-6R and the signal transducing molecule gp130 were detected on HUVECs. Then, we treated HUVECs with IL-17A, IL-6 and sIL-6R and investigated pro-inflammatory responses using ELISA and qPCR.

We showed that IL-17A induced release of GRO-α and MCP-1 from HUVECs. IL-6, combined with sIL-6R, also induced release of MCP-1. When we stimulated HUVECs with combination of IL-17A and IL-6/sIL-6R, an amplified release of GRO-α, but not MCP-1 was detected.

Taken together, our data suggest that IL-17A and IL-6/sIL-6R synergize to induce release of GRO-a from human endothelial cells and the synergy seems to be specific to amplify IL-17A induced signaling pathways.

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The burden of non-communicable diseases in the two Palestinian entities

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Objective: Chronic Non-Communicable Diseases (NCDs) are a major health concern to Palestinian health sector, which challenge the system in many different ways. In this study, we focus on the magnitude of reported NCDs in two main Palestinian entities (West Bank and Gaza Strip).

Methodology: Disability Adjusted-Life Year (DALY) approach was employed in this study. The data extracted from different sources, the health survey 2010 and Ministry of Health materials and annual reports for the same year as well. We calculated Years Life Lost (YLL) due to premature mortality and Years Lived with Disability (YLD), and then summation of both values provided DALY.

Results: The study elucidates that the DALYs lost due to frequently reported chronic NCDs were 5700/100,000 in Gaza Strip vs 6000/100,000 in West Bank. Each one DALY lost is thought of as one lost year of optimum healthy life. The study shows that Ischemic heart disease and hypertension contributed to the greatest numbers of DALYs lost due to chronic NCDs among both men and women, followed by cancer (breast cancer in women and lung cancer in men).

Conclusion: Cardiovascular disease and cancer remain the major burden of NCDs epidemic and are still constituted a big challenge to Palestinian healthcare sector despite the ongoing intervention programmes by many actors in Palestine. Our study recommends that basic and more comprehensive intervention strategy is important to tackle the epidemic of chronic disease and its risk factors.

Keywords: DALY, chronic disease, non-communicable disease, burden of disease, West bank, Gaza strip, Palestinian territories.

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The magnitude and nature of war-related injury in the Gaza strip, Palestine

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Objective: Frequent wars are a major cause of injury, disability and mortality in the world as well as the Palestinian Territories. This study aims to explore the nature and magnitude of war injuries in the Gaza Strip counties during 2014 war.

Methods: The current study was conducted using the Gaza War 2014- related injuries data. We selected a representative random sample of about 420 (317 men vs 103 women) out of 11000 injured, in this study.

Results: The current study demonstrates that men more than women were observed 75.5% vs 24.5% with a ratio 3.1:1among injured, almost half of them 49.5%, aged between (20-39) followed by youngers and children (31.5%). Upper body regions were mostly injured due to explosions. Study identifies multiple body shrapnel-related wounds and burns (39%), followed by multiple organs injury 24%, all types of fractures 14%, bleeding and internal organs injury 10%, amputation related to injury 5%, superficial wounds, lacerations and contusions 4%, hearing or vision loss or both 2% and respiratory problems 2%. Almost 26% of injured peoples had disability. Finally, the study indicates that nearly half of injured peoples need follow up.

Conclusion: This is first study of its kind to explore war injuries. A rabid increasing of war injuries in the Palestinian territories raise alarm and remain a major health concern to the Palestinian health system and the community as a whole. Additional large scale research studies are warranted to address the problem more different aspects on a large scale.

Keywords: injuries, war injuries, nature of injury, disability, Gaza war 2014, Gaza strip.

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”Meal is an activity involving at least two persons” – experiences of meals by older persons

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Objective: Studies often focus on older persons’ nutritional status. Few studies describes older persons’ experience of meals e.g a prerequisite related to their nutritional status. Distribution of food and meals in elderly care is frequently tailored to surrounding social organisation, not to the older person’s needs and wishes. This study describes meals and meal-related situations from older persons’ perspective.

Methods: Semi-structured interviews with 18 older persons with an average age of 83 years and with different needs for support from social and health care were conducted. Thematic analysis was used.

Results: For older persons, the meal is of great importance of various reasons, not only as a source of energy but also as a social meeting place between at least two persons. The meal-related situation was described as an enabler for activity, self-image and identity. Five themes were identified through the analysis: *The meal as activity and identity*; *Home-cooked food versus meals on wheels*; *Getting to set own meal schedule versus staff directed; Eating together in peace and comfort*; and *Meals are related to habits and traditions.*

Conclusion: The results suggest that improvements may be possible based on common sense, respect, and reprioritization by the health care professionals’ managers as well as politicians with responsibility for care of older persons. Therefore, education, discussions, and new routines based on ethical principles are needed for maintaining older persons’ autonomy during meals and meal-related situations.

Bactericidal Effects of Gallidermin on *Staphylococcus epidermidis* and *Staphylococcus aureus*

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Objective: Bacteria secrete ribosomally synthesized cationic antimicrobial peptides, so called bacteriocins. Gallidermin from *Staphylococcus gallinarum* belongs to lantibiotics which is a subgroup of bacteriocins that contain lanthionine and/or 3-methyllanthionine. This study aim to evaluate the bactericidal effects of gallidermin on *Staphylococcus epidermidis* and *Staphylococcus aureus.*

Methods: Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined for four strains of *S. epidermidis*; biofilm-negative ATCC 12228, biofilm-positive RP62A, strain N15 isolated from a healthy person, and strain 117 isolated from an infected prosthetic hip, and two strains of *S. aureus;* methicillin-sensitive MSSA ATCC 29213 and methicillin-resistant MRSA CCUG 35601. Gallidermin were diluted in a 96-well microtiter plate with concentrations ranging from 100 to 0.19 µg/ml. Bacteria (10^6 CFU/ml) were added and incubated at 37°C for 24 h. MIC correlated to the lowest concentration that inhibited bacterial growth. For MBC, 10 µl of bacterial suspension was streaked on blood agar plates and the lowest bactericidal concentration was determined.

Results: The MIC value of gallidermin for all four strains of *S. epidermidis* was 6.25 µg/ml. MBC differed, 25 µg/ml for ATCC 12228 and RP62A, respectively, and 6.25 µg/ml for both N15 and 117. For MSSA, both MIC and MBC was determined to 12.5 µg/ml. Interestingly, gallidermin was highly efficient towards MRSA with MIC ranging between 0.39-1.5 µg/ml and MBC 1.5 µg/ml.

Conclusions: Gallidermin is a promising candidate for treating infections caused by *S. epidermidis* and *S. aureus*, including MRSA.

CARD8, a protein of innate immunity regulates the release of inflammatory cytokines in human endothelial cells.

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Objective: Atherosclerosis is a chronic inflammatory disease of large and medium-sized arteries characterized by altered immune response in the atherosclerotic lesion. The activation of the endothelium is an important phenomenon during atherogenesis, promoting the migration of leukocytes into the vessel wall. Caspase activation and recruitment domain 8 (CARD8), a component of innate immunity, was previously found to be over expressed in atherosclerotic lesions and the *CARD8* variant (C10X polymorphism), encoding a truncated CARD8 protein, has been associated with lower expression of *CARD8* in plaque and to lower levels of CRP and MCP-1in serum[1]. The present study aims to investigate the role of CARD8 in modulating the expression of cytokines and chemokines in endothelial cells.

Methods: CARD8-specific siRNA was used to knockdown CARD8 in HUVECs with and without LPS treatment. Quantitative RT-PCR, ELISA and western blot were used to analyze the targets at gene and protein expression level, respectively. The release of chemokines and cytokines in the culture supernatant of HUVECs was semi-quantitatively assessed using human cytokine expression array panel.

Results: HUVECs and smooth muscle cells were found to constitutively express CARD8 in the arterial wall. The knockdown of CARD8 in HUVEC could reduce the secretion of IL6 and the chemokines RANTES, IP10, MCP-1 and ICAM-1. The CARD8 mediated regulation of *IL6*, *RANTES*, and *IP10* was found to occur at the transcriptional level.

Conclusion: CARD8 is required for the expression of cytokines and chemokines, suggesting that CARD8 is an important component of endothelial activation entrained by pro-inflammatory stimuli.

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Effects of sequencing depth and pipeline tuning on microbiome analysis

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Random shotgun sequencing of DNA obtained directly from the environment has revealed profound microbial novelty and diversity. Culture-independent Next Generation Sequencing method presents an exciting means to elucidate microbial dynamics, which invariably determine human health and global biogeochemical processes [Franzosa, E.A. et.al.,2015]; making it an important tool for microbiome analysis, regarding composition and functional prediction.

Even though deep sequencing microbiome data is a possible input for tools predicting protein and metabolite functional levels, the validity of these estimates remains to be demonstrated. However, variation in sequencing depth could possibly affect the analysis results with high distinction, with possible consequences both for comparability across studies, as well as affecting usability in clinical classification tasks [Ritchie, M.D. et. al., 2015]. NGS pipelines use different sources for quality check, assembly, alignment, and a variety of databases en route to analysis and prediction

Our dataset comprises fecal microbiome NGS data as well as LC/GC-MS proteomics and GC-MS metabolomics profiles for three groups of elderly subjects with different degrees of gut functional disturbations.

We try to establish an optimal combination regarding choice of pipeline and sequencing depth, to achieve both a good resolution in compositional and functional details, as well as good classification power for our study dataset.

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"It helps if you coax with them": Ethical challenges during the provision of home health care service

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Objective: To describe ethical challenges during the provision of community home health care services.
Background: As the population ages, the provision of home health care services increases, and home health care services are seen as being a desirable option. Health care personnel encounter ethical challenges when providing home health care services. There is lack of studies describing ethical challenges using non-participant observation techniques

Methods: An ethnographic approach with non-participant observation were used to describe everyday ethical challenges for personnel including registered nurses (*n*=8), and nurse-assistants (*n*=4) who are providing home health care services. Personnel providing community health services in older people's homes were followed for 21 days (*n*=21 days; hours n=148).

Results: The result described one main category: To coax the patient in order to provide a justified care, with four subcategories: 1) To coax the patient with the intention of providing good care, 2) To coax the patient in order to provide care which satisfied the next-of kin, 3) To coax in order to provide equal care, 4) To coax for better relationships and care of the patients with addiction issues.

Conclusions: Coaxing was performed in order to provide good care based either on good intention, pressure from the next of kin, factors such as inequality of care provision or as lack of a relationship with patients with addiction issues.

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Patients' and Staffs' Experiences of Quality of Forensic Psychiatric Care: a four year longitudinal cohort study in two clinics.

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Background: High quality of care is linked to both patient satisfaction and work satisfaction and is considered to be a right of all patients and a responsibility for staff. Quality of care is however a wide concept and considered to be context specific. Therefore, it can be a challenge to choose the most effective strategies and also to identify relevant outcomes in order to improve the quality of care. Benchmarking has been widely used as a way to improve quality of mental health care as well as other health care specialties. Through repeated comparison with another care deliverer, preferably those representing best known practice, benchmarking project participators can evaluate their own efforts and improvements over time. One of the clinics in this study was considered to have an ongoing care of high quality while the other was planning improvements the coming years.

Objective: To describe variations of quality of care over time, rated by patients and staff in two forensic psychiatric care clinics.

Design: It is a four year longitudinal cohort study in two clinics.

Methods: Patients and staff at the clinics were invited once a year to complete a questionnaire (QPC-FIP/QPC-FIPS). This instrument measures the experienced level of quality of care in forensic psychiatry.

Results: We found that there are differences in how staff and patients experience the quality of care and that it is important to consider both perspectives in improvement work. It is also important to follow developments over time, since external factors like organization, management and facilities might have an impact on how the quality of care is experienced.

Relevance: This study is expected to give a deeper understanding of how patients and staff in forensic care perceive the quality of care and to give decision makers and staff knowledge to develop forensic psychiatry.

Bariatric surgery will not decrease use of opioid analgesics

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Objective: There is little known regarding the post-operative use of opioid analgesics in bariatric surgery patients. Raebel et al., recently published an article where 77 % of the pre-operative chronic opioid users, one year after surgery not only continued, but increased their use of opioid analgesics, concluding that these patients were not receiving optimal pain management [1]. The Roux-en-Y gastric bypass (RYGB), which is by far the most used technique in Sweden, has been shown to significantly increase the rate of morphine absorption and exposure[2], raising concerns regarding a potentially increased risk of side-effects and developing substance misuse disorder (SUD). In this study we will describe the use of opioid analgesics in Swedish bariatric surgery patients.

Methods: Patients were identified using The Scandinavian Obesity Surgery Registry (SOReg) and cross matched with the Swedish Prescribed Drug Registry (SPDR). Data on all dispensed opioid analgesics from two years before surgery until two years after surgery was collected.

Results: The study population included 36 353 patients undergone bariatric surgery; this included 1639 (4.5%) high volume consumers, and 34 714 (95.5%) low volume consumers. There was a statistically significant increase in mean daily use postoperatively ($p < 0.0005$) in the total population and in the low volume consumers group. In the high volume consumers group there was not a statistically significant change in mean daily use. From the 1639 patients classed as high volume consumers preoperatively 1213 (74%) continued using high volumes of opioid analgesics postoperatively.

Conclusion: In this large population based study, bariatric surgery did not decrease use of opioid analgesics. There was an increased opioid analgesics use after bariatric surgery in the low volume consumers group. In the high volume consumers group, post-surgery mean daily use was not statistically increased.

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Omitting radiotherapy in women ≥ 65 years with early breast cancer and favorable histopathology after breast-conserving surgery, sentinel node biopsy and adjuvant endocrine therapy is safe

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Background: The benefit of radiotherapy in older women with endocrine responsive early breast cancer treated with breast-conserving surgery and endocrine therapy is unclear. The aim of this study was to verify if omission of radiotherapy in a predefined cohort of patients with good prognosis early breast cancer after breast conservation is safe.

Methods: Eligibility criteria were: consecutive patients with age ≥ 65 years, breast-conserving surgery (sector resection + sentinel node biopsy), clear margins, unifocal T 1 N0, Elston grade 1 and 2, estrogen receptor-positive. After informed consent adjuvant endocrine therapy, either tamoxifen or an aromatase inhibitor, was prescribed for 5 years. Primary endpoint was ipsilateral breast tumor recurrence (IBTR). Secondary endpoints were contralateral breast cancer, recurrence-free survival (RFS) and overall survival (OS).

Results: Between 2006 and 2012, we included 603 women from 14 Swedish centers. Two patients did not fulfill the inclusion criteria and were excluded from the analysis. Median age was 71 years (range 65 to 90). At a median follow-up of 59 months (range 2 to 110) 13 IBTR (cumulative incidence at five years, 1.3% (95% CI, 0.6% to 2.7%), 4 regional recurrences (one combined with IBTR), 2 distant recurrences both without IBTR or regional recurrence and 11 contralateral breast cancers was observed. Twenty-nine patients were diagnosed with tumors of other origin. Seven of them were endometrial cancers. There were 39 deaths. Only one of the deaths (2.6%) was due to breast cancer and 11 (28.2%) were due to other cancers (2 endometrial cancers). Five-year overall survival was 93.9% (95% CI, 91.4% to 95.7%).

Conclusion: This study demonstrates, with a median follow-up of 59 months, that breast-conserving surgery and endocrine therapy without radiotherapy is a safe treatment option in women with early breast cancer and favorable histopathology aged ≥ 65 years. The risk of IBTR is comparable to the risk of contralateral breast cancer. The low rate of breast cancer deaths indicates that breast cancer mortality is of secondary importance in this subset of women.

Do age and gender affect the NLRP3 inflammasome activity in human neutrophils?

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Introduction: Balanced and controlled inflammation is the beneficial immune response initiated by cells of the innate immune system in response to noxious stimuli, including irritants or pathogens. Dysregulated inflammation, on the other hand, is detrimental to the host, *e.g.* by failing to clear invading pathogens, and can lead to systemic and chronic diseases. One of the most potent proinflammatory mechanisms known include activity of multiprotein complexes called inflammasomes. Discovered some 15 years ago, still little is known about how inflammasomes are controlled and regulated. Recently, a chronic, low-grade inflammation termed "inflammaging" has been described in elderly people and speculations of factors causing this include impaired cellular and organelle components, free radicals from oxidative stress and metabolites, such as fatty acids and extracellular ATP; all of these being potential activators of the NLRP3 inflammasome and could therefore induce an inflammatory response.

To date, nothing is known concerning natural variations and how inflammasome activity may vary during homeostatic conditions. The aim of the present study is to investigate if the NLRP3 activation in circulating blood immune cells differ corresponding age and gender. This will provide a better understanding of the NLRP3 activity in the healthy population and specifically how inflammasome activity may vary in the population due to age and gender.

Material and methods: Inflammasome activity will be determined by measuring genetic upregulation of inflammasome components (qRT-PCR), caspase-1 activity and cell viability (flow cytometry) as well as released proinflammatory factors, such as IL1β, IL18, IL37 (ELISA and multiplex) produced during inflammasome activity in human innate immune cells in peripheral blood.

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