Preface

The research work in the Institute for Surgical Research has progressed very well during the year 2011. This annual report gives a comprehensive overview on activities in the Institute during its 45th year of successful research.

The institute was founded back in 1966 based on generous donations given by the Norwegian Society for Fighting Cancer. Egil Amundsen, who was trained as a surgeon and in physiology, was appointed as the first leader of the institute. Several competent researchers quickly became connected to the institution and Amundsen's open-minded strategy provided for excellent research. The first doctoral thesis at the Institute for Surgical Research was completed already in 1969 by Sten Sander who later became a professor of urology at the University of Oslo.

Today the institute can look back on an impressive high number of doctorial dissertations altogether 148 at the end of 2011.

This year the orthopedic surgeons Ulf Eirik Wangsvik Sigurdsen and Stig Heir, the neurosurgeon Einar Osland Vik-Mo and the pediatricians Rønnaug Solberg and Marit Lund Dalen defended their doctorial theses from the institute.

SIGURDSEN, Ulf Eirik Wangsvik: Tibial bone healing: Experiments with external fixation and intramedullary nailing

HEIR, Stig: Focal Cartilage Defects in the Knee

VIK-MO, Einar Osland: Propagation and characterization of stem like cells from brain tumors - establishment of a clinical protocol for immunotherapeutic targeting of tumor stem cells in glioblastomas

SOLBERG, Rønnaug: Resuscitation of the newborn. An experimental study of toxic effects of supplementary oxygen in newborn piglets

DALEN, Marit Lunde: Hypothermia and room air resuscitation in NT2-N neurons, immature rats and newborn pigs.

In 2011, 20 original research publications have been released from the institute with an average impact factor of 4,78.

The Egil Amundsen lecture 2011 "Purinergic control of immune cell function " was held by professor Wolfgang G. Junger, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA. The lecture was a great success and very much appreciated both by clinicians and researcher at our hospital.

Our progress in the year 2011 would not be possible without the enthusiastic and hard work of all our staff. However the following members of the staff deserve to be particularly acknowledged for taking care of important in-house administration responsibilities: Jorunn H. Larsen (Secretariat), Per-Stian Støle (Administration), Signe Flood Kjeldsen (Laboratories), Vivi Bull Stubberud, Sera Sebastian, Aurora Pamplona, Roger Ødegård and Kristine Kloster-Jensen (Operating theatres), Shakil Ahmed, Biljana Stangeland, Espen Remme and Håvard K. Skjellegrind (Seminars).

During 2011 several groups from different departments in the hospital have performed their research activities in the institute. We are very thankful for their important cooperation, which are important links for the institute to the clinical departments.

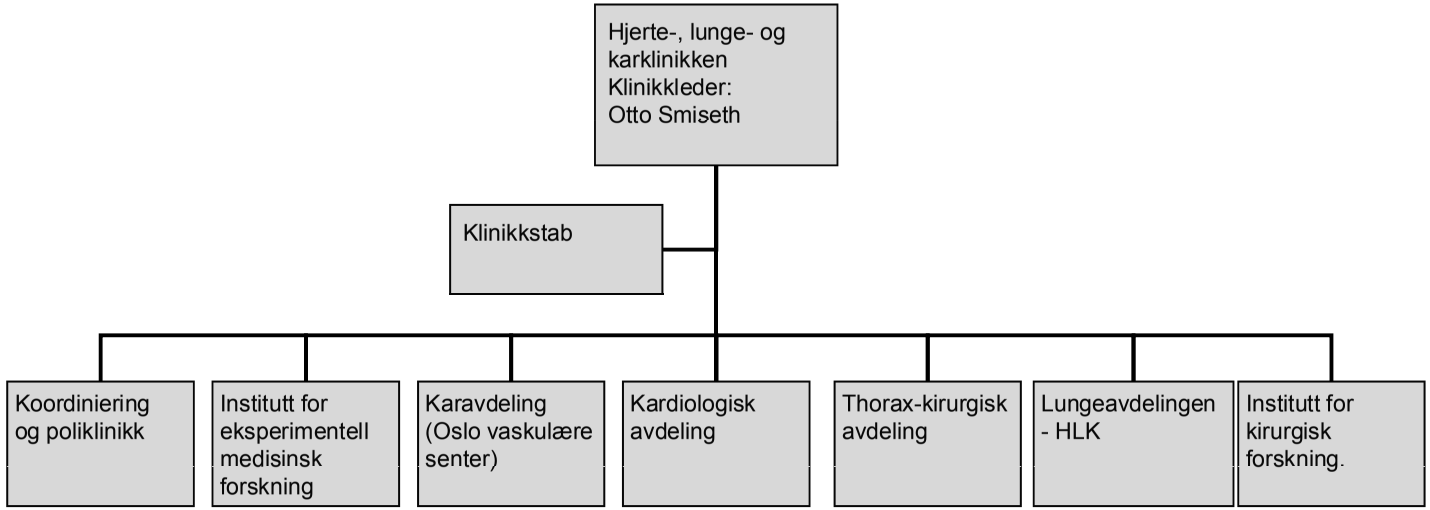
Institute for Surgical Research sincerely thanks Oslo University Hospital, University of Oslo and external institutions including the Norwegian Research Council, the Norwegian Advisory Committee for Cardiovascular Disease and the Health Authorities of South/Eastern Norway for important financial support.

This report was edited by Jorunn Hestenes Larsen, Per-Stian Støle, Håvard Attramadal and Ansgar O. Aasen.

Institute for Surgical Research, March 2012

Ansgar O. Aasen, Professor/Head of Institute

Hjerte-, lunge- og karklinikken



Institutt for kirurgisk forskning

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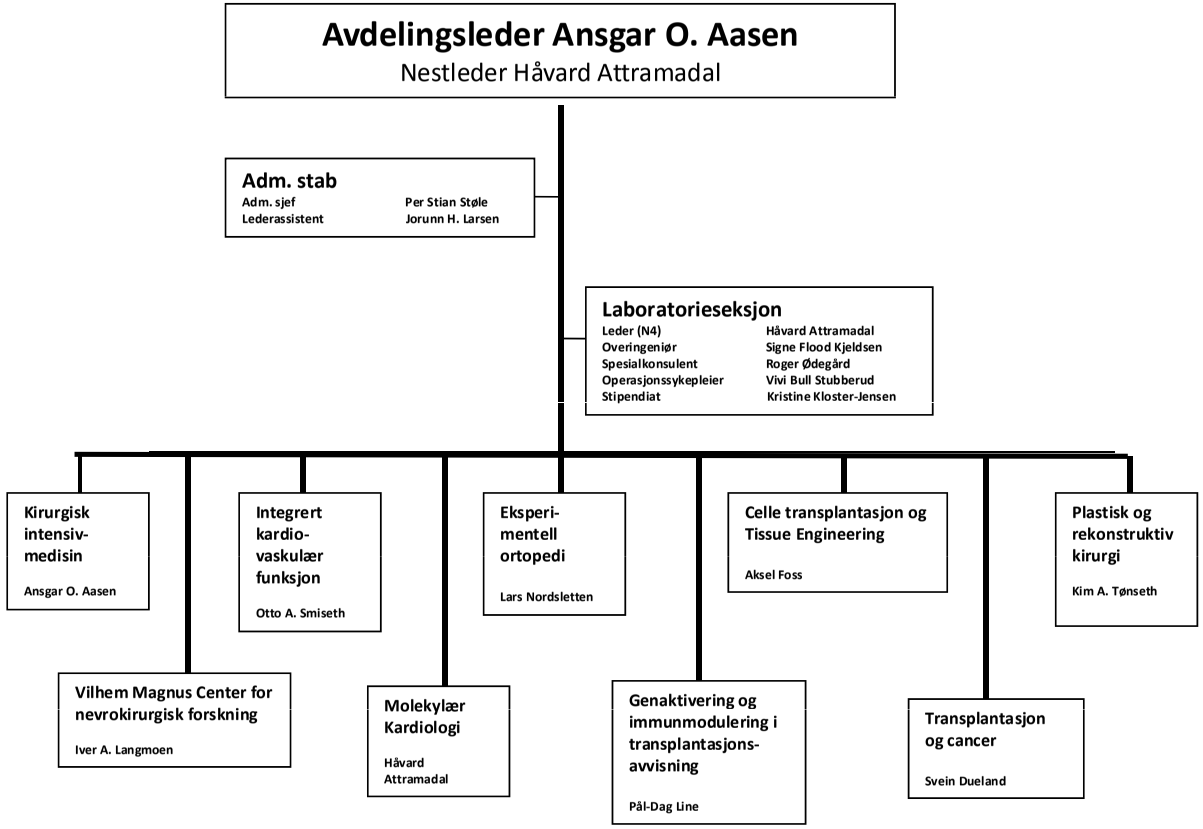
Forsknings-grupper



Abbreviations

AHUS	Akershus University Hospital
CAST	Cancer, stem cell innovatin center
CIT	Clinical Islet Transplantation Consortium
FP 7, EU	Seventh Framework Programme, European Union
LO	Lovisenberg Diaconical Hospital
NCCD	The Norwegian Council on Cardiovascular Diseases
NRC	Norwegian Research Council
OC	Orthopaedic Centre, Oslo University Hospital
OUH	Oslo University Hospital
SENRHA	South-Eastern Norway Regional Health Authority
UIO	University of Oslo
VUSP	Valencia University School of Pharmacy

Research Groups

Surgical Intensive Care Medicine

Leader:

Ansgar O. Aasen, Professor, MD, PhD (UiO/OUH)

Deputy leader

Signe Flood Kjeldsen, Head engineer, MSc (UiO)

Scientific staff:

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Yngvar Gundersen, Senior scientist, MD, PhD (OUH)
Claus Danckert Krohn, Consultant, MD, PhD (OUH)
Johanna Samulin-Erdem, Senior scientist, PhD (OUH)
David Kunke, Senior scientist, PhD (OUH)
Kristin Bjørnland, Consultant, MD, PhD (OUH)
Yun Yong Wang, MD, PhD (OUH)
Claus Vinter Bødker Hviid, MD, PhD-student (OUH)
Krzysztofa Grezlak MSc (NAV)

Professor Ansgar O. Aasen

Research area

Infections following surgery or trauma continue to be a major clinical problem. Due to the immunological consequences of surgery, infections frequently develop into severe septic complications and multiple organ injuries. The problem in severe sepsis is a paradoxical and self-destructing inflammation leading to dysfunctional host defense and lethal injury to vital organ systems.

More than one million patients are expected to die annually from severe sepsis worldwide.

Aims

Our aim is to develop novel means to prevent or ameliorate the self-destructive inflammation in patients with infection. A major focus of our work is research into the cellular mechanisms involved and to facilitate translation of new knowledge from basic research into clinical practice.

Ongoing Projects in 2011:

Effects of CCN proteins in the development of sepsis-induced multiple organ dysfunction

Sepsis remains a leading course of death in the intensive care unit. A major predictor of its mortality is the presence of consecutive organ failure, as it is directly correlated to the number of organs failing. The extra cellular matrix (ECM) has received little attention in sepsis research. However, the matri-cellular cysteine-rich, angiogenic induced, 61 (Cyr61/CCN1); connective tissue growth factor (Ctgf/CCN2); and nephroblastoma overexpressed gene (Nov/CCN3) (CCN)-protein family has been attributed organ-protective properties.

The protein family includes the six proteins CCN1 (Cyr61), CCN2 (CTGF), CCN3 (NOV), CCN4 (Wisp-1), CCN5 (Wisp-2) and CCN6 (Wisp-3), which functions contrast that of classical ECM proteins as they serve to modulate cellular responses to environmental stimulation rather than to sustain cell structure. Besides direct interaction with the inflammatory response, effects on critical aspects of endothelial and vascular cellular function, these proteins display organ-protective capabilities in vivo. Furthermore, the expressions of the CCN proteins are sensitive to environmental perturbations classical for sepsis but a potential role in sepsis remains elusive.

We have recently provided the first evidence that the CCN family members CCN1-CCN6 are regulated in the lung, liver, and heart of rats subjected to early stage experimentally-induced sepsis (accepted for publication in Innate Immunity 2011). Interestingly, a correlation was observed between CCN1 regulation and the expression of both pro-inflammatory, and anti-inflammatory cytokines, as well as chemokines. This relationship was recapitulated in vitro, as hepatocyte CCN1 mRNA responded in a specific and dosage-dependent way to TNF-α exposure. In conjunction, these data implies the eruption of cytokines/chemokines as a potential mechanism in the regulation of CCN1 expression in sepsis (Figure 1). Furthermore, a tissue-specific CCN2 regulation and a uniform CCN3 mRNA regulation were observed. The underlying mechanisms of CCN2 and CCN3 regulation seem however, diverse from those for CCN1. The regulation has been further characterized in a long-term

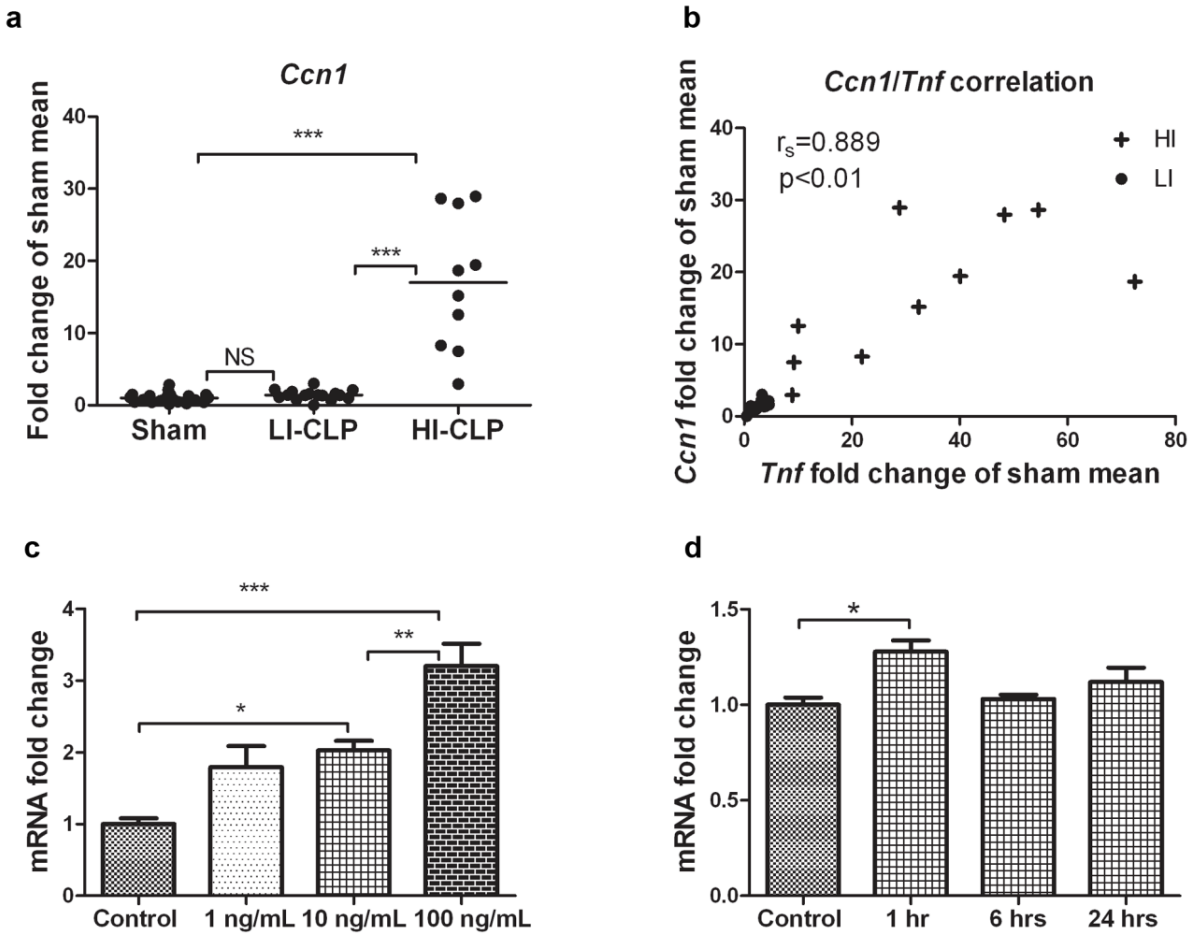


Figure1.
CCN1 mRNA induction (a) and its correlation to TNF-α gene expression (b) in rat livers following 18 hours of sepsis. The septic rats divided in two groups, one with excessive (HI-CLP) and one with limited (LI-CLP) inflammatory activation. This pattern was observed also in CCN1 mRNA induction (a). Statistical analysis revealed a strong correlation between CCN1 gene expression and inflammatory gene activation (b). The molecular basis for this correlation was further approached in hepatocytes in vitro. Upon TNF-α exposure, a dosage-dependent CCN1 mRNA response was observed (c). The specificity of this response were confirmed in a subsequent pulse-chase experiment (d).

CLP model in collaboration with Professor Redl and Pofessor Bahrami at the Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in Vienna. This study indicates CCN1 as an especially interesting candidate gene in the regulation of sepsis-induced multiple organ dysfunction. To further elucidate the functional role of the observed CCN1 regulation in experimental sepsis and to investigate the underlying molecular regulatory mechanisms, in vitro studies have been initiated.

Thrombin Generation: Its use as a marker for inflammatory changes.

Several animal models have been developed to study aspects of the pathophysiology of sepsis with the cecal ligation and puncture (CLP) model becoming the "gold standard". Whilst it reproduces the pivitol clinical characteristics of human sepsis the assessment of the inflammatory process is usually measured and monitored by the assessment of individual inflammatory markers such as IL6 and TNF-α. These markers do not always correlate well with the inflammatory process and can fail to clearly differentiate different animal groups.

Inflammatory changes found in conditions such as septicaemia, are known to increase the release of microparticles that are associated with the production of Tissue factor (TF). The release of such particles markedly influences haemostasis often leading to an increase in Thrombin production.

In our most recent study in rats following surgical intervention (n=30), we have measured Thrombin generation, Thrombin's natural inhibitor Antithrombin III and Thrombin/Antithrombin III complexes to assess differences in the inflammatory processes. Peak Thrombin was found to be significantly decreased (Fig 2, p=0.015) in the CLP group of rats compared to both the Sham group and Control (no surgical intervention) group.

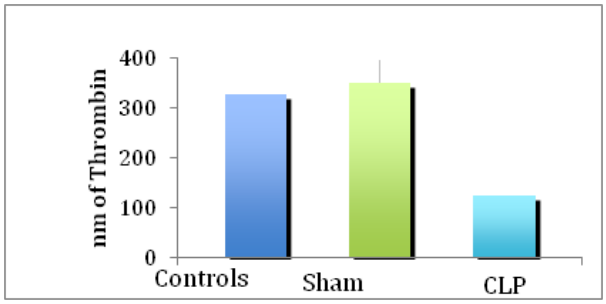


Figure 2. *Changes in Thrombin generation.*

Along with these changes in thrombin, the natural inhibitor, Antithrombin III decreased markedly (Fig 3, p=<0.0005).

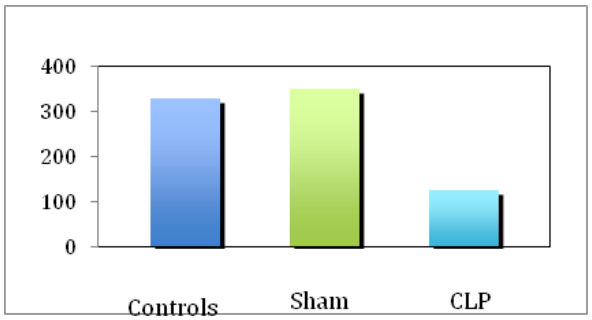


Figure 3. *Changes in AntithrombinIII values*

Thrombin/AntithrombinIII complexes were significantly increased (Fig 4., p=0.049) in the CLP group compared to the Sham group.

These results show significant haemostatic disturbance, possibly due to disseminated intravascular coagulation, resulting in the consumption of proteins and inhibitors involved in haemostasis.

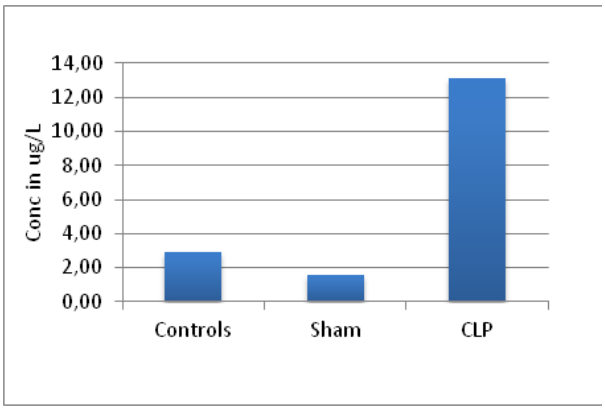


Figure 4. *Changes in Thrombin/antithrombin complexes.*

This preliminary study gives a clear distinction between the different animal groups studied and may offer a more cost effective way of monitoring the effects of inflammatory changes within the CLP group of animals.

Comparison between laparoscopic and open antireflux surgery on cellular immune responses in children.

Laparoscopic procedures are increasingly being performed in children. In adults, several randomized studies have shown that laparoscopic surgery is safe, have at least as good results as open surgery, and cosmesis, influence of the immune system, need of postoperative analgesics, and recovery are superior as compared to open surgery. In children, there are very few randomized studies comparing open and laparoscopic surgery. Because of the rapid introduction of and the enthusiasm for laparoscopic procedures, it has been difficult to arrange randomized trials to validate laparoscopy in children. Antireflux surgery (fundoplication) is one of the most commonly performed gastrointestinal procedures performed by pediatric surgeons. In contrast to adult patients operated with fundoplications, most children referred for antireflux surgery have several co-morbidities. As compared to results from adult series, recurrence rates after fundoplication seem to be higher in children than in adults. Particularly children with severe gastrointestinal dysmotility (neurologically impaired children) have the highest recurrence rates. Furthermore, children seem to have longer convalescence time than adults. To compare results of open and laparoscopic fundoplication, a prospective, randomized study has been performed in 88 children. Various results such as complication rates, recurrence rates, hospital stay and convalescence will be compared between the methods. In addition, we will assess if the influence on cellular immune responses vary between laparoscopic and open operations, and if clinical parameters are related to

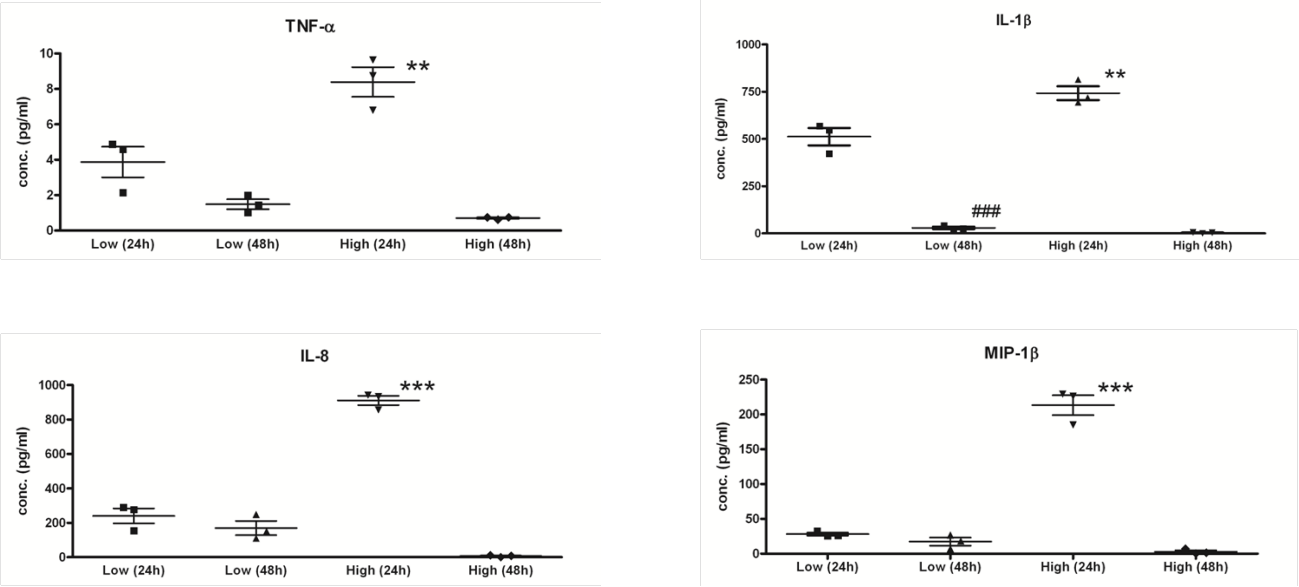
immune response. For studies on cellular immune responses, blood was taken preoperatively and on the first and second day postopera tivly. Plasma levels of several cytokines will be measured by ELISA (multiplex). Furthermore, blood was incubated in a whole blood model. The blood was first anticoagulated with heparin (25 U/mL) and incubated at 37°C with slow rotation in the presence of either Lipopolysaccaride (10 ng/ mL blood), Peptidoglycan isolated form Staphylococcus aureus (1 µg/mL blood) or saline, respectively. At 0,1,4,6,12 and 24 h, plasma was obtained by centrifugation and stored at -70°C. Levels of cytokines will be measured to further assess inflammatory responses after laparoscopic and open surgery. Clinical data are recorded prospectively during hospital stay and at follow-ups 6, 12 and 24 months postoperatively.

The study is now closed and results being processed.

Determination of P.aeruginosa virulence.

The nosokomial *Pseudomonas aeruginosa* (PAER) is an opportunistic bacterium which seldom causes disease in healthy individuals. But, the increasing number of immunocompromised individuals has provoked a rise in PAER infections. In vitro determination of PAER virulence is complicated, and the current gold standard is a C. elegans killing assay. The killing assay measures virulence of PAER serotypes, but a major draw-back is that it is time consuming and resource demanding. The *C. elegans* killing assays optimized by Reza Assalkhou, PhD, (Dep. of Infection Prevention) showed that this assay is highly reproducible and can differentiate between strains of PAER with high, moderate and low virulence. In collaboration with Department of Infection Prevention headed by Egil Lingaas, MD, PhD ,we investigate markers of the innate immune-response as an improved alternative to the established assay and detect the molecular interaction between the bacteria and the immune system.

In pilot experiments two clinical isolates of PAER from patients with blood stream infection were subjected to comparative evaluation in both the established *C. elegans* killing assay and a well-established human whole blood. To study the inflammatory response the *ex vivo* whole blood model were utilized to measure the cytokine production of the leukocytes. Due to variation in individual response in human whole blood model we decided to use a cellular model: THP-1 cell line. The viability assay confirmed the differences in virulence of the strains tested on *C. elegans* and the highly virulent strain for *C. elegans* reduced significantly the viability of the cells during the assay. For screening the innate immunological response in the infected cells an antibody microarray (Multiplex 25, with 25 different antigen) was used. (Figure 5



** High (24h) vs High (48h)*
 # Low (24h) vs Low (48h)

Figure 5: *Multiplex assay: testing media from PAER infected THP-1 cells, harvested 24 and 48 h postinfection*

For high virulent strain was shown inflammatory dependent and statistically significant effects by decrease of pro-inflammatory (TNFa, IL- 1b), anti-inflammatory (IL-8) cytokines and chemokines (MIP-1b).

To confirm the obtained data from the bioplex (multiplex) experiments as well as to simplify the future experiments we are currently utilizing the semi quantitative and easy reproducible ELISA tests (TNFa, IL-1b, IL-8 and MIP-1b)) for measuring the immune response of THP-1 cells after being infected with PAER. Using a statistically relevant number of different PAER blood stream infection isolates, 5 virulent and 5 nonvirulent strains whereas the virulence was assessed by C. elegans killing assay, were selected for further study of cytokine production in THP-1 cells. Samples were harvested at 2, 6 and 24 hours postinfection. The selected cytokines produced by THP-1 cells was measured for each sample using ELISA tests. The 24 hours incubation was the only sample with a detectable level of cytokine production.

Nucleotides play a role as crucial regulator of inflammatory and immune response. ATP can act as extracellular signaling molecule through the activation of plasma membrane receptors (Di Virgilio et al 2001). In our preliminary experiments we have tried to investigate influence of extracellular ATP on the survival of THP-1 with a highly virulent strain of PAER. We are currently investigating the impact of such a variation both on THP-1 cells viability and the survival of the PAER after infection as well. The experiments are under optimization using a virulent strain of PAER.

Studies on possible effects of a hemapheresis filter on cytokines, hematologic- and hemodynamic parameters during experimental endotoxinemia in pigs.

Cytokines are thought to be important factors in the pathophysiology of sepsis. A few publications exist on possible effects of using hemofiltration filters in humans during severe infections. Earlier *in vitro* studies have indicated that titanium has the ability to remove the cytokine IP 10 from blood. A new filter (TM 100) has been developed by TicoMed AB, Sweden, containing titanium. In co-operation with prof. Ståle Petter Lyngstadaas at the University of Oslo and representatives from TicoMed AB we have accomplished altogether 17 experiments in a porcine model of endotoxemia. After induction of anesthesia, a tracheostomy was performed for mechanical ventilation. Central veins and arteria jugularis were cannulated for blood sampling. The animals got infusions of different doses of LPS (range 4-15 µg/kg/h) into either vena cava or a mesenterial vein. During the last three experiments the animals received initially 10 µg/kg LPS/20 min until the mean pulmonary artery pressure (MPAP) increased 50%. Thereafter LPS was reduced to 4 µg/kg/h through a mesenterial vein. A Swan Ganz catheter was passed from a femoral vein to a pulmonary capillary wedge position for MPAP monitoring. Arterial and central venous blood sampling were performed, and hemodynamic parameters (CVP, AP, MAP, PAP, MPAP) were registered. Arterial and venous blood gas analyzes were followed regularly. Filters containing different amounts of titanium were connected to the vena cava inferior via a femoral vein. Full heparinization was performed in all animals. The prototype of the filter has continuously been improved upon during the experimental period, but still there is need for further improvements. The animal model developed during these experiments seems suitable for further studies on hemofiltration in experimental endotoxinemia.

Collaborators :

Prof. Heinz Redl, Prof. Soheyl Bahrami and
Senior Scientist Martin F. Osuchowski, DVM, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria
Prof. Michael A. Rogy, Vienna, Austria
Prof. Christoph Thiemermann, The William Harvey Research Institute, London, UK
Prof. Irshad Chaudry, University of Alabama at Birmingham, Birmingham, Alabama, USA
Prof. Michael Gallimore and Dr DW. Jones, Kent, UK
Prof. Ståle Petter Lyngstadaas, Dept. of Oral Pathology, UiO, Oslo, Norway
Prof. Egil Lingaas and Dr. Reza Assalkhou, Department of Infection Prevention, OUS, Oslo, Norway

Molecular Cardiology

Leader:

Håvard Attramadal Professor, MD, PhD (OUH/UiO)

Scientific staff:

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Vladimir N. Martinov, PhD, Postdoc (NRC)
Thomas G. von Lueder, MD, Postdoc, (OUH)
Geir Florholmen, PhD, Postdoc, (SENRHA)
Jørgen A. Gravning, MD, PhD-Student (OUH)
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Tuyet Anh Pham, MD, PhD-Student (SENRHA)
Eva Maria Rehbinder, MD, PhD-Student (NCCD)
Ole Jørgen Kaasbøll, MD, PhD-Student (NCCD)
Else Marie Valbjørn Hagelin, MSc, Senior Engineer (SENRHA)
Mrinal Das Kumar, MSc-student

Professor Håvard Attramadal

Research Area

Heart failure, the common clinical syndrome characteristic of advanced cardiac disease of diverse etiologies, is a major cause of morbidity and mortality. Indeed, the incidence and prevalence of heart failure in affluent societies are increasing due to demographics with rising proportion of elderly, as well as increased survival of myocardial infarction. Despite implementation of several new treatment modalities during the last 20 years, heart failure is still a progressive and ominous disease indicating that important pathogenic mechanisms remain unmodified by the most current treatment modalities. Thus, there is an impetus for new and more effective pharmacological interventions.

In evolving heart failure multiple compensatory actions are triggered in order to maintain cardiac output, among which is activation of the sympathetic nervous system, the renin-angiotensin system, as well as a number of autocrine/paracrine factors synthesized in myocardial tissue. These compensatory actions also reflect in alterations of cardiac structure, collectively called cardiac remodeling. The most important structural changes are cardiac myocyte hypertrophy and myocardial fibrosis. Although cardiac remodeling may initially balance loss of contractile force, the continuum of these structural alterations often feeds into vicious circles leading to progression of cardiac dysfunction. Despite substantial new insights into the mechanisms of myocardial hypertrophy and fibrosis, many of the nodal points that orchestrate these structural alterations still remain to be identified. Thus, an important focus of our research group is to unravel the signal transduction mechanisms that constitute the dysfunctional signaling responses leading to pathologic remodeling of the heart. Another important conceptual approach is that of delineating mechanisms that either increases or decreases the tolerance of cardiac myocytes to hypoxia or free oxygen radical injury, i.e. potential mediators of cardiac myocyte damage in evolving heart failure. The purpose of these investigations is to provide new knowledge of disease mechanisms enabling development of novel pharmacological interventions for heart failure.

Our research group is a multidisciplinary team of experts in gene technology, molecular and cellular biology, as well as experimental and clinical medicine. The research efforts comprise studies of isolated cardiac myocytes and fibroblasts, integrated physiology in genetically engineered mice, large animal studies, as well as clinical investigations. Our research group is member of Center for Heart Failure Research, University of Oslo (www.heartfailure.no), a thematic research initiative and focus area of research selected by the Faculty of Medicine. Center for Heart Failure Research is also a regional research network sponsored by Helse Sør-Øst Regional Health Authority. The Institute for Surgical Research provides infrastructure with state-of-the-art equipment for high-resolution echocardiography and integrated physiologic assessment of cardiac function in both small and large animals.

Major Aim

Dysfunctional cardiac signaling mechanisms and signals astray are considered major causes of pathologic myocardial hypertrophy and predisposition to heart failure. Increasingly, dysfunctional signaling mechanisms are implicated in increased production of free oxygen radicals, mitochondrial

dysfunction and reduced tolerance to hypoxia and/or free oxygen radicals per se. Thus, the major goal of our research group is to dissect the function of myocardial autocrine/paracrine factors, their cognate receptors, and intracellular pathways in cardiac myocytes and fibroblasts. New knowledge on the function and mechanisms of signaling pathways in the heart may provide basis for development of new and more effective therapeutic intervention in acute coronary syndromes and heart failure.

Current specific aims of the research group:
1) Providing novel insights into the function of myocardial G protein-coupled receptor kinases, i.e. a family of kinases that are important proximal modulators of many receptor-controlled signal transduction pathways involved in regulation of myocardial function in health and disease.
2) Uncovering the function of myocardial autocrine/paracrine factors or cytokines that are activated or induced in heart failure. Current focus is on delineating the functions of secreted CCN matricellular proteins, in particular CCN2/CTGF (connective tissue growth factor) and CCN5/WISP-2 (Wnt-inducible secreted protein-2), secreted regulators of Wnt signaling, as well as the TGF-β superfamily cytokine GDF-15 in heart failure of various etiologies. The CCN proteins (CCN is an acronym for the first three members of this gene family; Cyr61, CTGF, Nov) are cysteine-rich, modular proteins (see Fig. 1) considered to interact with various proteins in the extracellular matrix, including cytokines, growth factors, extracellular matrix proteins, as well as receptors on the cell surface. Yet, the mechanisms of CCN protein action are poorly understood.

Report from 2011:

1) Investigation of substrate specificities and function of cardiac G protein-coupled receptor kinases (GRKs)
We have previously investigated distribution of GRK2, GRK3, and GRK5 in myocardial tissue. These studies revealed that GRK2 was enriched in endothelial cells, whereas GRK3 was confined to cardiac myocytes. GRK5, on the other hand, was ubiquitously expressed among the cellular elements of myocardial tissue. The restricted distribution of GRK3 in cardiac myocytes clearly points to a role for this GRK isoform in regulation of G protein-coupled receptors on cardiac myocytes. However, since both GRK2 and GRK3 could be demonstrated in cardiac myocytes, studies of the substrate specificity of these kinases were an imminent issue. In isolated fully differentiated cardiac myocytes we investigated the substrate specificities of the GRK isoforms GRK2 and GRK3. These studies revealed that GRK2 and GRK3 display striking specificity on G protein-coupled receptors controlling different aspects of cardiac function. Overall, the present data have uncovered the novel findings that GRK3 has substantially higher potency and efficacy than GRK2 at endogenous endothelin receptors and α1-adrenergic receptors. This did not seem to be the case for the β1-adrenergic receptor as GRK3 potency at this receptor appeared much weaker than for the ET-R, and was equipotent with GRK2. Thus, GRK3 emerges as a primary regulator of ET-R- and α1-AR-signaling, which may have important implications in cardiac function. The studies provide biochemical evidence of widely different functional roles of GRK2 and GRK3 in cardiac myocytes. These functional differences are currently subject of investigations in transgenic and gene-targeted mice.
A recent novel finding from our laboratory is that myocardial GRK5 is upregulated in transgenic mice with cardiac-restricted overexpression of CCN2/CTGF causing reduced sensitivity of cardiac β-adrenergic receptors to endogenous agonists. Furthermore, increased GRK5 initiates G protein-independent signaling by recruitment of β-arrestin to the receptor allowing ß-arrestin to act as a scaffolding protein for signaling complexes at the plasma membrane. Indeed, the altered signaling specificity of ERK1/2 initiated by GRK5 elicits cardioprotective actions. These findings have been recapitulated in cardiac myocytes pretreated with recombinant human CTGF. Yet, the signaling pathway(s) implicated in CTGF-induced GRK5 is yet to be characterized. Furthermore, the relative contribution of GRK5 to the cardioprotective actions afforded by CCN2/CTGF remains to be resolved.

2) Role of CCN2 - connective tissue growth factor - in regulation of tolerance towards ischemia-reperfusion injury and in resisting maladaptive cardiac remodeling during chronic pressure overload.
Myocardial CCN2 is highly expressed in the developing heart in fetal life and apparently plays crucial role in cardiac development. However, myocardial expression of CCN2 is repressed in the postnatal heart under physiologic conditions. Interestingly, myocardial expression of CCN2 is reactivated or induced during evolving heart failure. Previous findings from our laboratory demonstrate that induction of myocardial CCN2 appears to be a general response to evolving heart failure, i.e. induction of myocardial CCN2 occurs in heart failure of diverse etiologies. Induction of tissue expression or increased plasma levels of CCN2 is often associated with diseases in which fibrosis is an important morphologic characteristic. However, to what extent CCN2/CTGF actually elicits fibrosis is yet to be demonstrated. Indeed, the physiologic and/or patho-physiologic functions of CCN2 in myocardial tissue have not yet been resolved. Thus, a major focus of our research effort has been to elucidate the function of CCN2 in the heart. Does CCN2 exert salutary actions in heart failure or does CCN2 contribute to progression of heart failure? Does CCN2 cause myocardial fibrosis?

In order to elucidate to the physiologic actions of CCN2 in the heart and to investigate how the actions of CCN2 may contribute in the pathophysiology of heart failure, we are currently investigating various transgenic models with constitutive or conditional overexpression of CCN2 in the heart generated in our laboratory. The transgenic mice with cardiac-restricted, constitutive overexpression of CCN2/CTGF displayed marginal increase of myocardial collagen contents despite 70-fold overexpression of CCN2/CTGF (Ahmed, MS et al. *Am J Physiol Heart Circ Physiol*. 300:H1291-1302, 2011). This finding appears to be consistent with data from transgenic overexpression of CCN2/CTGF in other tissues or organs. Thus, the interpretation of the available data both from our and other research groups is that additional factors are required for CCN2 to induce fibrosis. A surprising, novel finding in our laboratory was that CCN2 exerts striking cardioprotective actions, increasing tolerance towards ischemia-reperfusion injury both *ex vivo* in Langendorff-perfused hearts as well as *in vivo* in mice subjected to transient ligation of the left anterior descending coronary artery *in situ*. These findings have led to filing of patents for protection of the potential commercial development of CCN2/CTGF as a new pharmacologic treatment in acute coronary syndromes with the objective of minimizing myocardial necrosis. Verification of the data in large animal models, commercial development plans, including plans for early clinical testing, are currently being pursued in collaboration with Birkeland Innovation/Inven2 AS, the TTO of University of Oslo and Oslo University Hospital.

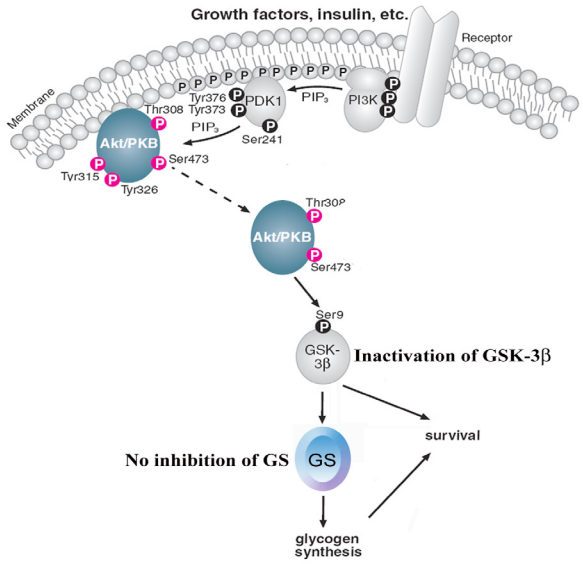

Figure 2. *Schematic illustrating the intracellular signaling pathways of CTGF/CCN2 in cardiac myocytes. A cognate receptor for CTGF upstream of PI3 kinase has not yet been characterized.*

A cognate receptor for CCN2 or any of the other CCN proteins has not yet been characterized. Despite several reported interactions between CCN proteins and extracellular matrix-associated protein, data from our laboratory indicate that CCN2 also acts directly on cells by binding to ligands at the surface of the plasma membrane. Furthermore, analysis of the phosphoproteome of cardiac myocytes following

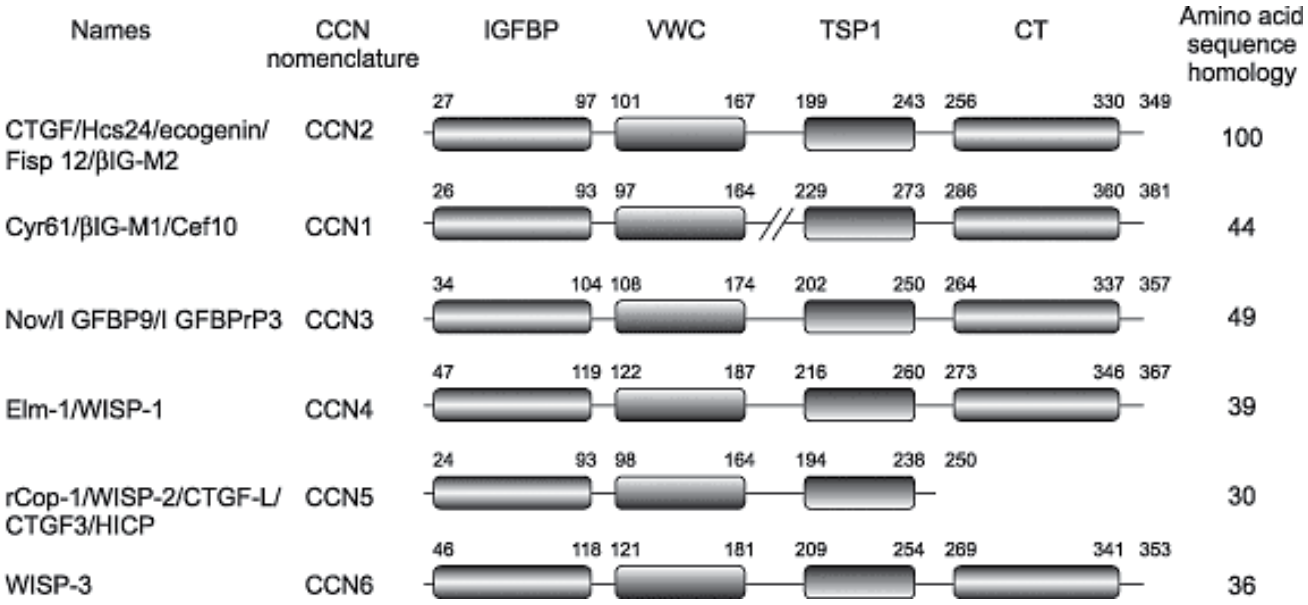

Figure 1. *Schematic demonstrating the modular structure of the CCN family proteins (CCN1-6). IGFBP; insulin-like growth factor binding protein homology domain, VWC; von Willebrand factor homology domain, TSP1; thrombospondin-1 homology domain; CT; cysteine knot homology domain.*

stimulation in the absence or presence of recombinant CCN2 revealed that the PI3 kinase/AKT/GSK-3ß pathway is major intracellular signaling pathway of CCN2 (Fig. 2). Indeed, our data also demonstrate that this pathway is crucial for CCN2-dependent cytoprotection towards hypoxia. The mechanisms of the cytoprotective actions of CCN2 are currently a major endeavor in our research group. To facilitate studies of the mechanisms of CCN protein actions our group has currently established eukaryotic expression systems for large scale production and purification of several of these proteins.

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Integrated Cardiovascular Function

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Center for Cardiological Innovation:

The Center for Cardiological Innovation (CCI) was officially started November 2011. The center was one of seven new centers which received funding through the Norwegian Research Council's program "Centers for research-based innovation". Prof. Thor Edvardsen heads the CCI which is a collaboration with other groups including the Computational Cardiac Modeling group at Simula Research Laboratory and the main industrial partner GE Vingmed Ultrasound. The focus of CCI is improving diagnostic methods for patients with heart failure and patients at risk of sudden cardiac death. The center will constitute a major part of the research of our group the next 8 years.

General objectives:

The Integrated Cardiovascular Function group studies cardiac mechanics in experimental studies and studies in patients. The idea is to develop better diagnostic understanding and solutions into clinical practice.

Specific objectives:

1. To investigate mechanisms of left ventricular (LV) dyssynchrony and develop better methods for selecting patients for cardiac resynchronization therapy (CRT).
2. To investigate hemodynamic effects of CRT in patients with heart failure and narrow QRS.

Professor Otto A. Smiseth

3. To investigate LV mechanical-electrical interactions and improve risk stratification for ventricular arrhythmias.
4. To investigate mechanisms of LV diastolic dysfunction.
5. To develop better diagnostic methods to identify viable myocardium.
6. To develop better diagnostic tools to identify optimal timing of surgery in valvular heart disease.

1. Left ventricular dyssynchrony: Cardiac resynchronization therapy has been documented to be a powerful treatment in patients with severe congestive heart failure, causing reverse LV remodeling, improvement of symptoms and reduction of mortality (Cleland 2005). In left bundle branch block (LBBB) the LV wall contraction is dyssynchronous due to electrical conduction delay to the LV free wall, and CRT resynchronizes the contractions by bi-ventricular pacing and thereby improves the contractile function. Currently, patients are selected for CRT on basis of the presence of wide QRS in the ECG. However, in about 30% of these patients, there is no improvement in symptoms, and in some cases aggravation of symptoms by CRT (Jarcho, 2006). The high number of non-responders represents a major problem with CRT, and better criteria for selection of candidates for this treatment modality are therefore needed. Because ECG has limited ability to identify candidates who will benefit from CRT, several new methods based on the interpretation of myocardial motion as measured by echocardiography have been proposed as more sensitive and specific markers. However, so far echocardiography has no proven clinical value in selection of candidates for bi-ventricular pacing. We suggest that a better understanding of the underlying mechanism of dyssynchrony is important to interpret the echocardiographic findings, and thereby to improve patient-selection for CRT. In LBBB, the pathological motion of the inter-ventricular septum during early systole has been proposed as a good predictor of response to CRT. Previous studies had indicated that early systolic contraction of the

septum into the LV was due to a more rapid rise of right ventricular (RV) pressure than the rise of pressure in the late activated LV. In 2011 we published a new study which opposed the pressure theory and showed that the early-systolic abnormal septal motion is due to active contraction and not merely a passive consequence of pressure differences across the septum. This phase has not previously been considered active when assessing dyssynchrony, and this might explain the failure of most echocardiographic indices of dyssynchrony to predict CRT-response. Our ongoing LBBB research focuses on methods to noninvasively quantify regional myocardial work which is highly dependent on the electrical activation sequence. We have just validated the noninvasive work quantification method and are currently investigating if indexes based on regional work perform better than methods based on shortening/lengthening to identify candidates for CRT.

2. Narrow QRS: Cardiac CRT has been documented to be a powerful treatment in patients with severe congestive heart failure and LBBB. Interestingly, there has also been demonstrated clinical effect of CRT in patients with narrow QRS. As more than two thirds of heart failure patients do not have electrical conduction disturbances, extending the indications for CRT into this patient group is going to have considerate implications. The mechanisms of possible effects of this treatment in heart failure patients with narrow QRS have not been properly investigated. Two possible mechanisms have been suggested: CRT may correct electrical dyssynchrony not seen on ECG or CRT induces changes in the interventricular interaction. The change in ventricular interaction can be obtained by pacing in the left ventricular lateral wall. The LV is then activated earlier than the RV and a concomitant phase shift in the ventricular filling appears. A head start of LV filling relative to RV filling reduces the LV external constraint. External constraint is determined by the RV pressure and the pericardial pressure and constitutes the external resistance to LV filling. The hemodynamical result of reduced external constraint is improved LV filling and increased cardiac output. An experimental dog model is used to explore these electromechanical and hemodynamic consequences of CRT. The study is ongoing, but some preliminary results have already been presented at international congresses.

3. LV mechanical-electrical interactions: Evaluating patients with susceptibility for cardiac arrhythmias and sudden cardiac death is a major challenge in daily cardiology practice. Electrophysiological studies have demonstrated that damaged myocardium (e.g. infarcted or genetically altered) provides the substrate for malignant arrhythmias. Echocardiographic techniques can accurately quantify regional myocardial function. Over the last few years we have studied the correspondence between myocardial mechanical function and risk for ventricular arrhythmias and demonstrated how mechanical dispersion can predict ventricular arrhythmias in patients with long QT syndrome and in myocardial infarction. In a new study we showed that right ventricular mechanical dispersion by strain was related to ventricular arrhythmias in patients with arrhythmogenic right ventricular cardiomyopathy. The study was published in European Heart journal and won Oslo University Hospital excellent publication award fall 2011.

4. Diastolic dysfunction: About half the patients with heart failure seem to have problems related to filling of the heart as the ejection fraction is normal. Main challenges are therefore to understand what causes diastolic dysfunction and how it can be diagnosed. Measurement of the lengthening velocity of the LV during early filling is a standard measurement in the clinic for assessment of diastolic function. We recently studied what factors determined this velocity in an experimental model. The study was published in Circulation 2009 and a mathematical model study that explained the physics of this velocity and generation of early diastolic suction was published in American Journal of Physiology 2011. The Circulation article was announced as one of the most read articles in Circulation in 2011 and it was recognized as among the most important articles in myocardial disease by the editors. The lengthening velocity is one of the Doppler echocardiographic indexes that are used in hemodynamic assessment including prediction of the LV filling pressure. This noninvasive method is highly desired as the invasive gold standard is not without risks. In a prospective multicenter study together with Methodist DeBakey Heart and Vascular Center in Houston we showed that these noninvasive measurements could be used for reliable hemodynamic assessment and determination of filling pressure in patients with acute decompensated heart failure. New echocardiographic technology has recently made assessment of LV twisting and untwisting easily accessible and created an interest in untwisting rate as a potential marker of diastolic function. Similarly to our previous investigation of the determinants of the lengthening velocity we are currently stuying the determinants of untwisting rate.

5. Coronary artery occlusion: Although acute myocardial infarction is treated preferably by early percutaneous coronary intervention (PCI), there is limited access to this treatment, and a large fraction of patients receive intravenous thrombolytics as primary treatment. These patients are referred for "rescue PCI" only when there is no reperfusion after thrombolytic treatment. The main problems with the latter strategy are that all myocardium at risk may have un-

dergone necrosis and therefore PCI is unnecessary. Furthermore, we lack reliable methods to determine if reperfusion has been achieved by the thrombolytic. One of our main objectives is to develop better functional imaging in order to differentiate between viable and necrotic myocardium and to determine when reperfusion has been achieved.

6. Optimal timing of cardiac surgery for chronic valvular regurgitations has been a challenge for years. Development of systolic dysfunction precedes the onset of symptoms in more than one fourth of the patients with this condition. Traditional echocardiographic methods like preoperative left ventricular ejection fraction (LVEF) and cavity dimensions are the most important determinants of survival and LV function after valve replacement for regurgitations. However, volume derived measures of LV function have important limitations in assessing myocardial contractile function where a series of compensatory mechanisms, including an increase in end-diastolic volume and hypertrophy, can mask underlying changes in myocardial force development. Therefore, the purpose of these studies is to investigate whether global systolic strain measured by 2-dimensional speckle tracking echocardiography could detect early onset of myocardial dysfunction in patients with chronic regurgitations and preserved LVEF.

Anders Opdahl

Sebastian Sarvari

Anders Opdahl was the first author of the Circulation paper outlining the determinants of the myocardial lengthening velocity during early diastole. This paper was one of the most read imaging articles in Circulation and was considered by the editors among the most significant research in the area of myocardial disease. Sebastian Sarvari was the first author of the European Heart Journal paper on a new method to detect patients at risk for malignant arrhythmogenic right ventricular cardiomyopathy. This publication received an outstanding research paper price from Oslo University Hospital.

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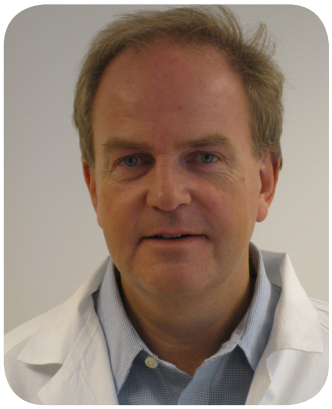

Professor Thor Edvardsen

The Center for Cardiological Innovation (CCI) was formally established 31st October 2011. It is one out of seven new centers that was awarded status as a Centre for Research-Based Innovation (SFI) through the Research Council of Norway SFI program. The CCI has a total budget of roughly 210 MNOK, in which 80 MNOK is to be allocated via the Research Council of Norway. The CCI is hosted by Oslo University Hospital and is a collaborative effort between Oslo University Hospital, Simula Research Laboratory, the University of Oslo, and the industry partners GE Vingmed Ultrasound, CardioSolv and Kalkulo.

The aim of the Center for Cardiological Innovation (CCI) is to develop the next generation of ultrasound systems for cardiology. The proposed tools and technologies will be created through linking currently isolated diagnostic systems with advanced biomedical research, advanced patient-specific computer simulation, and multi-modality visualization techniques. The targeted clinical uses of the proposed innovations are for better triage and treatment of patients at risk of sudden cardiac death or suffering from heart failure, two of the biggest challenges in cardiology today.

The CCI plans to use research results to develop the next generation of cardiac ultrasound equipment, intended to achieve three goals:

1. Record electrical (ECG), mechanical, and anatomical (cU/S) data in a new integrated 3- dimensional cardiac scanner system.
2. Use recordings from the scanner to build patient-specific computer-based heart models
3. Simulate the patient-specific effect of treatment on the model and optimize the treatment.

Professor Thor Edvardsen, MD, Center Director.
Photo: Andreas B. Johansen NFR

Heart failure

Heart disease is the most common cause of death in the West. Heart failure, in particular, is a rapidly growing health problem in the industrialized world. Cardiologists have powerful treatments to combat heart failure, but these can only postpone the progression the disease. Cardiac resynchronization therapy (CRT) is a powerful treatment for severe heart failure patients, and leads to decreased mortality and relief of symptoms. Following current guideline criteria, however, approximately 30% of patients will experience lack of response. Different novel echocardiographic indices have been proposed to improve patient selection criteria for CRT, but have failed. Studies of myocardial mechanics are therefore essential. A new concept for identifying potential CRT responders has been identified by CCI. These methods have, so far, been carried out in experimental studies and in small-scale patient studies. The next step will be to integrate these metrics into ultrasound scanners and to employ these novel principles in larger clinical trials.

Malignant arrhythmias

Approximately 500,000 people in the U.S. will suffer annually from sudden cardiac arrest; a correspondingly smaller, but no less significant, number in Norway is 5,500 deaths. Effective treatments currently exist; the most effective is implantation of an internal cardioverter defibrillator (ICD). However, current guidelines for implantation of ICD following myocardial infarction do not include patients with relatively preserved myocardial function, despite the fact that most episodes of sudden cardiac arrest occur in these patients. CCI has therefore developed a new strategy for the prediction of malignant arrhythmias in patients with preserved myocardial function after myocardial infarction. Echocardiographic assessment of mechanical dispersion has been promising in small studies of patients with post myocardial infarction and other myocardial diseases resulting in scar tissue. A larger multi-center trial testing this method is ongoing, and results will likely be published during 2012. Future plans include testing of this mechanical dispersion index in the context of other cardiac diseases and integration of this index in echocardiographic scanners.

Model-based cardiac simulation

The utilization of patient-specific data acquired via cardiac ultrasound and ECG to build tools that are both clinically useful and commercially viable is a central goal of the CCI. This requires development of a signal and image-based modeling pipeline in order to efficiently extract necessary information from patient scans, the development of geometrical models suitable for computational simulations, and the development of the so-called "inverse" ECG solution that translates ECG recordings from the body surface into information about what's happening within the heart itself. It is additionally necessary to further develop the underlying physical model of electromechanics that will serve as the basis for modelling the individual patient's heart. The signal and image-based modelling pipeline involves:

- Acquisition and recording of cardiac ultrasound and ECG data into a common 3D geometrical framework
- Processing of recorded data to a format suitable for simulation
- Estimation of tissue fiber angles of cardiac muscle and at the organ level for inclusion within models
- Simulation of the heart's mechanical and electrical function in order to provide additional insight to clinicians at OUS and a marketable innovation to GEVU.

As a part of the CCI, GEVU's tool for automatic delineation of left ventricular anatomy and 4D strain was further extended with an export possibility to allow these geometric models to be processed by software external to the ultrasound scanner.

Modelling needs for meshes suitable for simulation have also been defined by SRL. In addition, the investigation of tools for creating hierarchical volumetric meshes of the heart by SRL and KAL, as based on the data exported from GEVU's software, has also played a central role in the CCI since start-up.

CCI has studied the angle of heart muscle fibers and sheets with respect to a frame of reference and to one another as this information is crucial accurately simulate the electromechanics of the heart. As it is not currently possible to extract this information from ultrasound recordings, CCI (via CardioSolv) has developed a reliable rule-based method and an associated tool to estimate fiber directions in any given geometrical model, given certain standard inputs resultant from image-based scans.

Research Ultrasound Sandbox

The software embedded in the GEVU scanner and workstation is subject to strict QA procedures for development, testing and release. In order to allow researchers within the CCI to develop and test methods without the restrictions of corporate procedures, the design of a research software for sandboxing of new methods has been initiated. This software will communicate closely with the EchoPAC workstation or GEVU scanner, to avoid duplication of functionality already present in the product. The "sandbox" will be designed in such a way that it will be possible to port functionality from the sandbox to the commercial product line.

An initial version of this research ultrasound sandbox has been made. At present, it is possible to retrieve a cine-loop of images from the scanner and play it back. The software can also subscribe to events triggered by the scanner, which will allow interactive behavior between the applications.

3D TEE probe

Ultrasound imaging of the heart is limited by the narrow imaging windows between the ribs, as ultrasound cannot penetrate bone. However, more up-close imaging can be achieved by using a trans esophageal echocardiogram (TEE) probe. Such a probe is inserted down the esophagus while the patient is intubated. It can give a non-obstructed, high-resolution view of the heart.

2D TEE has been available for a long time, but to make a 3D TEE probe (with many hundreds more transducer elements) is an engineering challenge. 3D imaging can acquire volumes in real-time, which can be rendered semi-transparently in 3D, or as multiple 2D slices. GEVU is now getting very close to finalizing such a probe, and several prototypes have been made. Recently, an animal experiment was conducted with the latest generation, 3D TEE probe prototype. Earlier generation prototypes have had several problems, but this probe gave good quality images. However, the experiment revealed some unsolved problems with regard to delineation of curtain structures in 2D images. This will be addressed in further developments.

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Research area and aims

Vilhelm Magnus Laboratory for Neurosurgical Research is a research group of the Oslo University Hospital and encompasses the Neurosurgical Departments at Ullevål University Hospital (UUS), Rikshospitalet-Radiumhospitalet Medical Center (RR), and the University of Oslo. The goal of the laboratory is to build a bridge between the basic biological sciences and clinical neurosurgery, to explore the biology underlying neurosurgical conditions, and to facilitate translation of new knowledge from the basic research disciplines into the clinic. Research efforts therefore encompass both normal brain cell development and disease states such as tumours. Investigations aim to understand these processes and develop methods to treat disease as well as promote cell replacement to heal damaged brain tissue.

Stem cells from the adult human brain

A central dogma in neuroscience has been that the mature brain is unable to produce new neurons. Towards the end of the 20th century, studies in birds and rodents came to question this doctrine as new markers for labeling neurons combined with techniques for identifying cells that had been born in adult life, suggested that new neurons sometimes may develop later in life in some species.

At the turn of the century, these findings were to some degree extended to the human brain, as a few research groups had been able to culture immature cells from the human ventricular wall and hippocampus. It was still not known, however, whether it would be possible to differentiate these cells into functional neurons, i.e. cells with typical neuronal action potentials with the ability to communicate via synapses.

The putative existence of an adult human brain stem cell type with the ability to proliferate and differentiate into mature neurons created huge interest as one could now envisage treatment of neurological diseases with either transplantation of stem cells that have been expanded in vitro or by mobilization of endogenous progenitor cells. The work on developing functional neurons from cells from the human ventricular wall was started in Professor Langmoen's laboratory at the Karolinska Institute in Stockholm by Morten Moe and Mercy Varghese. Human tissue was harve-

sted from the wall of the lateral ventricle in temporal lobe specimens resected due to epilepsy. In keeping with earlier results from other groups they were able to expand stem cells from the ventricular wall as cell clusters (neurospheres) in vitro. Following dissociation and exposure to differentiation cues (mainly withdrawal of growth factors and addition of serum) these cells went through characteristic steps of morphological and electrophysiological development and developed into the three principal building blocks of the brain:

1. Astrocytes
2. Oligodendrocytes
3. Neurons

Our group was first to demonstrate that it is possible to transform immature progenitor cells from the adult human ventricular zone into functional neurons, i.e. cells with typical neuronal action potentials and the ability to communicate through synapses (Fig. 1). Essentially, our group was able to develop a small nervous system from a single human stem cell in vitro. This "nervous system", consisting of glial cells as well as a large number of neurons, communicated via synapses. These results were selected from more than 15000 abstracts for presentation at the press conference of the Society for Neuroscience seven years ago. See Westerlund U et al ***Exp Cell Res*** 2003, Moe MC et al ***Brain***, 2005 and ***Neurosurgery*** 2005.

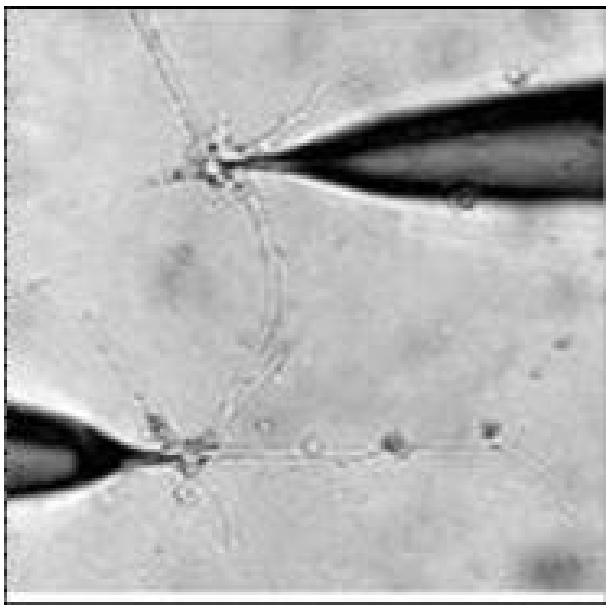

Fig.1
Dual patch-clamp recording from neighboring neuron-like cells, demonstrating synaptic communication between the cells

For stem cells to be useful in the clinical situation, it must be demonstrable that after transplanting them to another adult brain they can survive and integrate into the recipient neuronal circuitry. Using rats with a selective lesion of the hippocampal CA1-region (a small part of cerebral gray matter), Håvard Ølstørn and Morten Moe demonstrated that stem cells from the adult human brain are not only able to survive in the rat brain, but also selectively target and migrate to the area with the lesion (Olstorn H et al ***Neurosurgery***, 2007).

Use of specific antibodies against human nuclei (HuN) demonstrated survival of the transplanted cells and showed that the grafted cells frequently express the immature marker human nestin. Less frequently, the cells expressed the immature neuronal marker doublecortin and the glial marker GFAP. Ølstørn and Moe further showed that by using 'predifferentiation', i.e. "pushing" the cells in a neuronal direction prior to transplant, it was possible to significantly enhance the development of neurons following transplantation. This study for the first time showed that stem cells from the adult human brain are able to survive and differentiate in another adult brain. The first part of the study secured Ølstørn the first prize at the Scandinavian Neurosurgical Society's Annual Congress in 2005. See Olstorn H et al ***Neurosurgery***, 2011.

Innovative scientific techniques

The Vilhelm Magnus lab now has an extensive scientific and student staff dedicated to developing and utilising the best up-to-date molecular cellular biological techniques in fulfilment of its broad aims of understanding brain cell development and disease and thereupon working towards treatment innovations. PhD students get to explore the various aspects of particular techniques and subsequently apply them to experimental questions. Screening methods such as microarrays (Cecilie Sanberg) and proteomics (Linda Paulson) can provide data to be used to delineate differences between normal and cancerous tissue. Statistical and bioinformatic analysis then suggests candidate genes for follow up. These then need to be confirmed using molecular techniques such as quantitative PCR to confirm transcription of mRNA followed by Western blot (Zanina Grieg) to confirm protein involvement. FACs (facilitated cell sorting and cytometry, Einar Vik-Mo, Mrinal Joel) is a very versatile technology that can give specific measurements of cell subgroup behaviour within cell populations and allows for elaborate assessment of experimental parameters. The ability for distinction of many wavelengths of fluorophore as well as the development of sophisticated antibody-fluorophore conjugates means that data relating to cell cycle, division

rate, phenotype and even transitory signalling pathway activation can be assessed within control and experimental cell populations. As well xenotransplantation of cell types to chick embryo (Mrinal Joel) or immune-compromised (SCID) mice (Awais Mughal, Artem Faizullin) provide powerful experimental tools that combine the prowess of surgeon and cell biologist alike.

The ability to knock out or down candidate gene expression is a powerful tool for discerning biological roles as well as involvement in disease pathology. The lab (under the expert guidance of Biljana Stangeland) has developed the expertise to employ lentiviral methodology whereby designer constructs can produce targeted short interfering (Si) and short hairpin (Sh) RNA for transcriptional block as well as express label such as GFP in tandem allowing identification and tracking of transduced cells. Lentiviral constructs integrate permanently into the genome of experimental cell lines giving faithful tracking of cell progeny. As well this most sophisticated molecular cell technology provides scientific rigor and convincing inference of mechanism.

Stem cells and brain cancer

Brain cancers in principle always recur despite apparent complete removal under the operating microscopic and subsequent adjuvant therapy. This is particularly true for the most common intracranial tumour type, the glioblastoma (GBM), where 50 percent of treated patients die within one year from diagnosis. In parallel with results emerging from other research institutions, our group has shown that only a subpopulation of cells in brain cancers have the ability to proliferate and initiate new tumours following transplantation to immunodeficient mice. This cell population infiltrates surrounding brain tissue, appears resistant to both irradiation and chemotherapy, and is the likely explanation for recurrence.

In a leading study, Mercy Varghese and Morten Moe showed that these cells share a number of the properties of normal neural stem cells of the adult human brain. Håvard Ølstørn demonstrated that stem cells isolated from tumours could reproduce those tumours in immunodeficient mice, whereas stem cells from normal human brain did not result in tumours (Fig. 2) (Varghese M et al ***Neurosurgery***, 2008).

Both normal and tumour stem cells showed a high proliferation rate when cultured to do so. Interestingly, the proliferation rate fell dramatically also in tumour stem cells when they were induced to differentiate. Normal and tumour stem cells showed a similar pattern of differentiation, i.e. in neuronal and glial directions, although differentiated cells from the tumour were clearly abnormal morphologically and differentiation in itself progressed much faster. Einar Vik-Mo performed a number of experiments studying the effect that in vitro culture of tumour stem cells has on the cells' ability to form tumours, to differentiate and to undergo genotypic and expressional changes (Vik-Mo et al, ***Neuro***

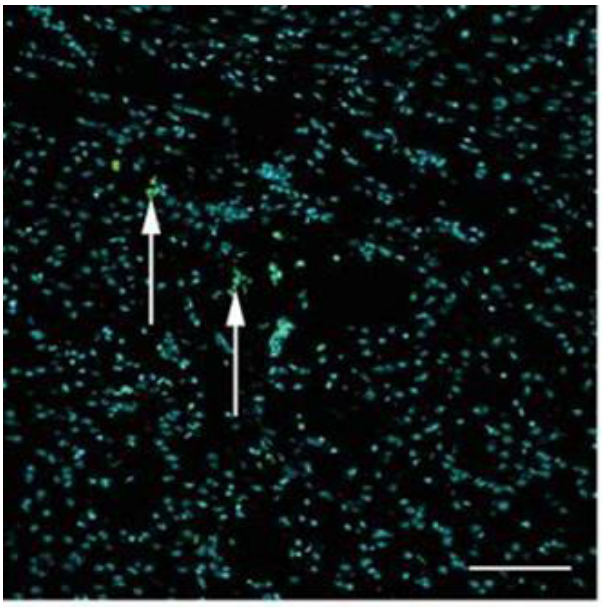

Fig.2a
Transplantation of normal progenitor cells from the adult human brain (top panel, green) did not result in tumor formation, whereas stem cells isolated from brain tumors (green, bottom panel) reproduced a highly invasive tumor in the mouse brain. Blue nuclear staining in both panels.

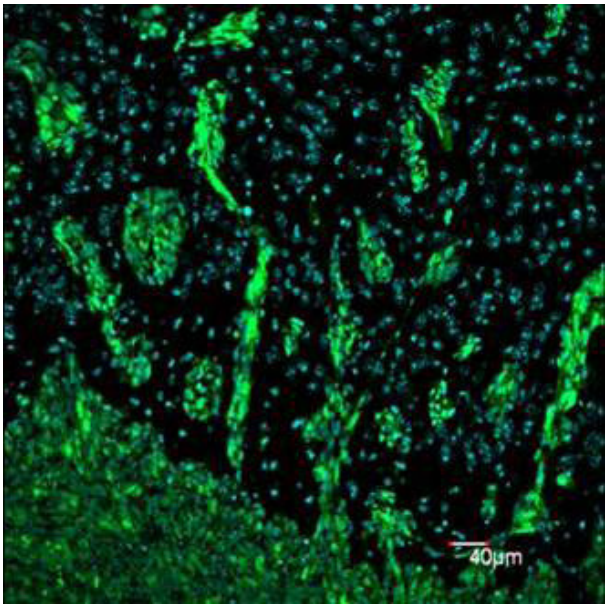

Fig.2b

Oncol. 2010). He has also explored the cellular organization of neuro- and tumourspheres, looking at the cellular heterogeneity of such spheres (Vik-Mo et al, ***Exp Cell Res***. 2011). By sorting tumour cells based on surface antigens, we hope to establish methods for better identification of the progenitor population.

We have also used this technology and experience to establish a clinical protocol (Einar Vik-Mo, Birthe Mikkelsen). This protocol is designed to harness the patients' own immunity. The inclusion of patients into the **"Phase I/II trial of vaccine therapy with hTERT, survivin and tumour stem cell derived mRNA- transfected dendritic cells in patients receiving standard therapy for glioblastoma"** started in February 2009. So far 22 patients have been recruited to the study. This clinical trial is backed up from the collaboration through the Cancer Stem Cell Innovation Center (SFI CAST) and is a collaboration with the Neurosurgical department, Avd. for klinisk kreftforskning, Avd. for celleterapi, and Avd. for immunologi, Institutt for kreftforskning, Radiumhospitalet and the Oncological department at Oslo University Hospital.

Microarray technology

Cecilie Sandberg has used microarray technology to compare the global gene expression in normal stem cells and tumour stem cells, in order to identify possible targets for treatment and to better understand the biology of the cell population that escapes current treatment and causes recurrences. The results of this comparison study show a significant upregulation in tumour stem cells of genes connected to regulation of focal adhesion, actin cytoskeleton, axon guidance as well as the Wnt signalling pathway. Putative target genes have been confirmed at the protein level using immunohistochemistry and Western blot. This work is currently submitted for publication. Currently, the genes' roles in glioma are investigated using shRNA-knockdown based technology and its effect on proliferation, apoptosis and sphere-forming capacity. The roles of the possible targets in the Wnt pathway are investigated by Kirsten Strømme.

Biljana Stangeland, investigated a set of 20 genes that were up-regulated in GBM tumour cultures using C. Sandberg's micro-array data. The study compared gene expression in nine primary GBM stem cell cultures to that in five primary neural stem cell cultures from the adult human brain. Rigorous bioinformatic filtering identified 20 genes whose RNA expression levels were very high in the primary Glioma stem cell cultures but were undetectable in normal adult brain stem cells. The identified genes are involved in cell-cycle/division, epigenetic regulation, signaling and down-regulation of tumour-suppressors. Several of the candidate genes are implicated in cancer (breast, ovary and colon), while others have no known associations to cancer or have unknown functions. A total of nine new GBM primary cultures were further analyzed by real-time polymerase chain reaction (qPCR), Western blot and Immunohistochemistry on tissue sections and cell cultures. qPCR confirmed the increased expression of 17 of the candidate genes. These results were in good accordance with the information provided by public data bases (Rembrant, TCGA). Western blot performed on 17 candidates showed increased protein levels in 9 cases thus demarcating these as best candidates for molecular targeting. The rest of the proteins featured aberrant isoforms, probably due to alternate splicing, modifications, cleavage or degradation. Further bioinformatics analysis identified a subset of 5 candidates that are particularly highly upregulated in GBM when compared to low-grade gliomas. To explore the functional importance of the potential target genes the lab established lentiviral-based shRNA delivery and started to test the potential of gene knock-downs (KDs) to inhibit growth of tumour cells. Preliminary results look very promising with publications in preparation.

Wnt downstream genes are potential targets for a cancer therapy-role of FAM84B

Using a mouse model with conditionally activated beta-catenin, Stangeland and co-workers identified that FAM84B, a member of FAM84 family, is a downstream target of the Wnt pathway. FAM84B was upregulated in the brains of animals that contained conditional gain of function of beta-catenin during embryonic development. FAM84b was also upregulated in neurosphere cultures stimulated with Wnt3A. FAM84B was highly upregulated in all tested glioblastoma cultures at both the RNA and protein levels. Using lentiviral short hairpin technology knock-down of the FAM84B gene was performed.

Decline of expression of the full length FAM84B protein resulted in a very significant reduction in the sphere forming ability of resulting tumours. Further analysis revealed decline of the activated form of beta-catenin in the knockdowncultures when compared to the original tumours and to non-silencing controls. Currently cells are being transplanted to SCID mice to measure tumourigenicity.

Aquaporins or water-channels are targets indicated by Cecilie's work. Former medical student, Guri Fossdal investigated the expression of these in tumour stem cells and their differentiated counterparts. Aquaporin 9 was shown to be associated with glioblastoma by Realtime PCR, immunohistochemistry and Western blot (Fossdal et al, TSWJ Cell Biology, 2011).

Awais Mughal started as a PhD student in the group in 2009. His project is also generated by Cecilie's microarray data focusing on upregulated gene candidates confirmed by Biljana Stangeland. A selection of gene candidates that may serve as clinical targets is being investigated with immunohistochemistry and Western Blots to evaluate protein expression. Again Lentiviral based shRNA-technology is applied to silence genes of interest and establish stable Knock-Down cultures. The gene silencing efficiency is measured on mRNA- and protein level and the cultures are subjected to functional studies. In vitro functional studies investigate effects on proliferation, apoptosis and sphere-formation. The biological importance of these findings is then further investigated using our xenotransplantation model in immunodeficient mice. Mrinal Joel is using this same methodology and has established a role for the PBK gene in glioblastoma.

Mrinal is also studying transplantation of GFP-transduced brain tumour stem cells to an embryonic environment using the chick embryo model. She investigates the behaviour and differentiation potential of tumour cells when placed in this environment. So far, interestingly, tumour cells display a more restricted activity in an embryonic environment compared to adult. Further studies will be based on the analyses of proliferation, cell death and differentiation ability of these cells into other cell types. Co-culture studies of the tumour cells with the cells from the central nervous system of chick embryo are also under investigation to examine these effects. Another of Mrinal's lines of investigation has been the use of phosphoflow cytometry as a method to map phosphoprotein signal transduction networks in GBM.

Proteomic studies

Proteins upregulated in tumour stem cells were investigated by former Post-Doc Linda Paulson. She compared human normal stem cells with tumour stem cells. This may reveal why the two types of cells behave differently in the brain and therefore may help in designing treatments that have a major impact on clinical practice.

She adapted a method called SILAC proteomics. SILAC is a straightforward approach for in vivo incorporation of a label into proteins for mass spectrometry-based quantitation. SILAC relies on metabolic incorporation of a given 'light' or 'heavy' form of an amino acid into the proteins; amino acids with substituted stable isotopic nuclei (e.g. containing deuterium, 13C, or 15N). Thus in an experiment, two cell populations are grown in culture media that are identical except that one of them contains a 'light' and the other a 'heavy' form of a particular amino acid. When the labeled analogue of an amino acid is supplied to cells in culture instead of the natural amino acid, it is incorporated into all newly synthesized proteins. The process is efficient and reproducible as the incorporation of the isotope label is 100% and does not affect protein behaviour. By adding stable, non-radioactive isotopic forms of amino acids to media when growing cells, it is possible to get a mass difference of 6 kDa in the same protein from different samples. Thus we can quantify relative protein abundance as cellular and metabolic processes occur. Experimental results achieve high fidelity with minimal bias, allowing relative quantitation of even small changes in specific protein abundance. Data obtained by Linda are currently being incorporated in manuscripts in preparation.

Håvard K. Skjellegrind started as a PhD student in 2010. He is doing live imaging of single cells, aiming to identify the "true stem cells" in heterogeneous normal stem cell and tumour stem cell cultures. He has already published on mitochondrial membrane potential in brain cells and at present is also examining this behaviour in single cells as a potential marker for "stemness".

Towards tissue repair

An avenue where adult human neural stem cells offer tremendous promise is in the treatment of degenerative diseases such as Parkinson's disease. Parkinson's disease is characterized by loss of pigmented dopamine-secreting cells in the substantia nigra. Though transplantation of embryonic stem cell- or fetal stem cell-derived dopaminergic cells has shown promising results, the results have not been consistent. Furthermore, the ethical issues regarding the use of the aforementioned cell types, limit their clinical use. Dopaminergic cells derived from adult human brain stem cells have obvious benefits: firstly, they pose no ethical challenges and secondly, they make autologous transplantation possible. We are investigating the potential of adult human brain stem cells to develop into dopaminergic neurons. PhD graduate Mercy Varghese obtained preliminary results that in vitro, adult human brain stem cells can develop into neurons expressing tyrosine hydroxylase, a rate-limiting enzyme in dopamine synthesis. This has been confirmed by Wayne Murrell and Emily Telmo.

Stem cells isolated from regions external to the brain.

Mercy Varghese and coworkers isolated neural progenitors from the adult human filum terminale. This terminal end of the spinal cord has been referred to as a fibrovascular tag without neurogenic potential and of no clinical significance. Similar to brain stem cells mentioned earlier these cells from

the filum terminale generated functional neurons capable of firing action potentials. When transplanted into the adult central nervous system, the filum terminale-derived neural progenitors survived, differentiated and showed targeted migration to a site of injury. See Varghese M et al ***Stem Cells and Development***, 2008.

Wayne Murrell joined the Vilhelm Magnus lab as Senior Scientist at the end of 2007. Wayne is from Australia and for the previous seven years led laboratory research at Griffith University, Brisbane, on neural stem cells derived from a region of the peripheral nervous system, the olfactory mucosa. Wayne is first author of a paper Multipotent stem cells from adult olfactory mucosa (Murrell et al, ***Dev Dyn*** 2005) which demonstrates the potential to generate neurospheres, direct their differentiation towards neuronal and non-neuronal lineages and suggests autologous tissue repair is a real possibility. This paper was pivotal in gaining support from the Australian Federal Government for the establishment of an Australian National Adult Stem Cell Centre in Brisbane working on human-derived neural stem cells for potential to treat human disease. Wayne has broad experience in the fields of molecular, developmental and cell biology. In recent times he has investigated stem cell transplant in various animal models of disease including Parkinson's, heart attack, disc degeneration and motor neuron diseases (Murrell et al***, Stem Cells*** 2008, ***Spine J*** 2009, ***Dis Model Mech*** 2010, ***N Engl J Med*** 2010). Now he helps guide the efforts of the researchers of the Vilhelm Magnus lab. Any effective tissue replacement therapy will require capability to produce authentic stem/progenitor cells in bulk quantities whilst maintaining cell integrity.

We have now developed culture methods for the rapid isolation, maintenance and proliferation of undifferentiated adult stem cells. Many suggestions for improvement made by former Post-Doc John Bianco have been quantitated systematically by Wayne Murrell and Emily Telmo. Today, a robust technique for the culture of these cells in billions is now established. At present, studies are being performed to assess the fate of these stem cells and their differentiation potential. Directed differentiation of these cells towards a specific fate such as down the dopaminergic pathway to obtain dopaminergic neurons is being examined. This work is currently in preparation for publication.

What are the cellular processes of healing? What is involved in tissue repair? Surgical ablation of vital brain tissue or the damage accidents cause to the spine and subsequent paraplegia are both relevant scenarios for cell replacement therapy. As well treatments of neurological disorders such as Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's are desirable. We have shown that stem cells exist throughout the adult human nervous system. Human neural stem cells can be manipulated to differentiate to defined phenotypes in vitro. Thus we believe that human neural stem cells can repair damaged neural tissue. Are neural stem cells from different regions equivalent? Or can they be induced to be interchangeable? These are fundamental questions pertaining to any attempt at human neural tissue repair. For instance could cells from the filum terminale or the olfactory mucosa be used to repair the human brain?

Olfactory mucosa has been shown a potential source for autologous stem cell therapy for Parkinson's disease (Murrell et al, ***Stem Cells***, 2008). Parkinson's disease is a complex disorder characterised by degeneration of dopaminergic neurons in the substantia nigra in the brain. Stem cell transplantation is aimed at replacing dopaminergic neurons because the most successful drug therapies affect these neurons and their synaptic targets. Murrell et al. have shown that neural progenitors can be grown from the olfactory organ of persons even with Parkinson's disease. These neural progenitors proliferated and generated dopaminergic cells in vitro. They also generated dopaminergic cells when transplanted into the brain and reduced the behavioural asymmetry induced by ablation of the dopaminergic neurons in the rat model of Parkinson's disease. These results indicate that Parkinson's patients could provide their own source of neuronal progenitors for cell transplantation therapies.

"The transplantation of adult human neural stem cells in rat model of Parkinson's disease" is an ongoing aim of the lab (Wayne Murrell, Emily Telmo, Artem Faizullin). The specific aims of this study are: 1.To investigate the ability of transplanted neural stem cells to reduce the signs of disease in Parkinsonian rats. 2. To estimate and compare the therapeutical efficacy of neural stem cells transplants from different sources (olfactory mucosa, subventricular zone and filum terminale). 3. To test the hypothesis that neural stem cell transplants will produce dopaminergic neurons in the striatum and/or will promote increased innervation of grafted striatum by surviving dopaminergic afferents.

Artem Fayzullin joined the Vilhelm Magnus Lab in 2010. As well as working on transplantation of normal human stem cells he is acquiring expertise in cell filming from Håvard Skjellegrind and will record cell movements of brain cancer stem cells as a contribution to the aims of CAST.

This report therefore summarizes the Vilhelm Magnus lab's current work on the cell biology of the human brain and nervous system. We seek to broaden knowledge of cell development encompassing the biological concepts of

stem cells, cancer stem cells and the development of tumours, cell physiology of anesthesia, and as well the future development of cell replacement therapy.

Awards and publications

Einar Vik-Mo was awarded his well deserved PhD during early 2011. Our group achieved seven refereed publications during 2011.

International collaborators of the Vilhelm Magnus lab:

David Tirrell, California Institute of Technology, Los Angeles, USA
Charles Liu, University of Southern California, Los Angeles, USA
Monica Nistér, Karolinska Institutet, Stockholm, Sweden
Anders Björklund, Wallenberg Center/University of Lund, Sweden
Ernest Arenas, Karolinska Institutet, Stockholm, Sweden
Alan Mackay-Sim, Australian National Adult Stem Cell Centre, Brisbane, Australia
Yasuhiro Watanabe, Tottori University, Japan
Winston Hide, Harvard University, MA
Dolph Hatfield, NIH, Washington, USA
Ludesi, Malmö, Sweden
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Experimental Orthopaedic Research

Leader:

Lars Nordsletten, Professor, MD, PhD, Leader (OC/UiO)

Professor Lars Nordsletten

Scientific staff:

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Olav Reikerås Prof MD, PhD, (UiO/OUH)
Jan Erik Madsen Prof MD, PhD, (UIO/OC)
Asbjørn Årøen MD, PhD (OC)
Stig Heir MD, PhD (Martina Hansens Hospital)
Harald Steen MD, PhD (OUH)
Per Ludvigsen MSc, Engineer (UiO/ OUH)
Sverre Løken MD, PhD (OC)
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Finn Reinholt Prof MD, PhD (UiO/OUH)
Jan Brinchman Prof MD, PhD (UiO/ OUH)
Stein Erik Utvåg, MD, PhD (AHUS)
Geir Hjorthaug, MD, PhD-student (OUH)

Research area

Joint injuries, diseases and fractures are main reasons for disability in the community and are often subjected in younger age groups. It involved large costs for the society and improved health care in this area would be a significant improvement both for the individual and the society. The Experimental Orthopaedic Research group applies experimental methods on different aspects of orthopaedics, including research on clinical material (biopsies, joint fluid, and retrievals), animal experiments and cell culture. Mechanical testing of structures, including live anaesthetized animals, and materials has been one of the main parts of our research methods. The experimental work in the laboratory is close connected to ongoing or clinical studies under planning for improvement of orthopaedic care of these patients in the community and the involvments of the clinicians is one of the strength in the group.

Aims

Develop a novel treatment of focal cartilage defects
Reduce the numbers of deficient fracture healing
Improve healing of tendon grafts in orthopaedic surgery
Delineate the best biomaterial surface for prothesis surgery

CARTILAGE RESEARCH

Malfunction of the knee joint is often associated with cartilage injury. Whether healing or restoration of lost or wounded portions of articular cartilage with newly formed fully functional cartilage is possible remains one of the unsolved problems in orthopaedic practice. These knee patients have more problems than patients with rupture of anterior cruciate ligament rupture and experience severe limitations in their daily life. Despite this the knowledge about the best treatment and if surgical treatment do offer a better outcome than the natural history is still not documented. A better understanding of articular cartilage biology, pathophysiology and biomechanics are definitely warranted. Focused research questions in the group, have been to improve the understanding of the cartilage repair process and to figure out if mesenchymal stem cells should be used instead of chondrocytes for cartilage repair. The ongoing work of the cartilage research group can be divided in three main areas

Experimental cell-culturing cartilage research

The group has worked intensively providing the best cell source for cartilage repair. Mesenchymal stem cells harvested from the bone marrow implanted in hyaluronan-based scaffold have been intensively studied in the laboratory for production for production of articular hyaline cartilage specific markers. Theoretical these cells have a promising potential for repair of normal hyaline cartilage. The collaboration with the Ex Vivo Laboratory (RH) headed by Jan Brinchmann has improved our ability to be supplied with improved cells for cell based cartilage repair and to test and develop new scaffolds.

Experimental cartilage research in an animal model

An important issue in cartilage treatment is the location of defect in the knee and the consequence this might have for the treatment of defect and risk of degenerative changes. One experimental study has been performed to specifically investigate this issue and the location is essential as shown in the current study. The difference in the outcome between microfracture and mosaicplasty has been difficult to evaluate clinically and an experimental study has been conducted to evaluate these clinically techniques. Changes are observed in the subchondral bone that is of concern for the long-term risk of degenerative changes in the joint. Mesenchymal stem cells implanted under a commercially available scaffold for repair of articular defect have been evaluated experimentally to look at feasibility of this technique and the amount of cartilage obtained in the defect.

Clinical projects

The experimental work is close connected to the clinical studies of the group and there is an ongoing study on stem cell treatment versus chondrocyte implantation. Patients with cartilage defects of the knees treated with chondrocyte implantation have been evaluated with electron microscopy biopsies to look at ultra organization of the tissue and deaths of chondrocytes in a recent publication.

Deficient fracture healing

Delayed fracture healing or non-union may occur in patients with systemic diseases, with hormonal or nutritional deficiencies, or in patients taking specific medications like non-steroidal anti-inflammatory drugs (NSAIDs and COXIBS). NSAIDs have been reported in general to affect bone metabolism, and indometacin has been shown to reduce fracture healing experimentally, and to inhibit ectopic bone formation clinically.

In these studies we have investigate fracture healing in rats on a short time medication with an injectible COX-2-inhibitors, and in rats made severely osteoporotic by estrogen and vitaminD depletion. Parecoxib is a new and selective NSAID targeting cyclooxygenase-2 (COX-2). Through their selective action these drugs are supposed to lack many of the main side effects of other NSAIDs and thereby, expected to be drugs of choice in near future for several patient groups. The effects on bone metabolism and healing have, however, not been elucidated. Furthermore, it is demonstrated that COX-2 is required for both intramembranous and endochondral bone formation. Thus there are reasons to concern regarding the potential negative effects of these drugs on bone metabolism and bone repair. Concern has been expressed about its potential negative effects on bone. The present study was designed to investigate the effects of short-term administration of parecoxib on bone healing. Based on the current knowledge our hypothesis is that the drug will delay the healing process and this is verified and published.

Another project on intramedullary nailing and external fixation in lower leg fractures evaluates bone healing by quantitative micro computer tomography (qmCT), in addition to mechanical testing and densitometric measurements. This includes development of a segmentation technique applied to qmCT images and an investigation of correlation between the segmented qmCT data and mechanical properties. The group documents the efforts and results made in these novel projects in a recent publication. Allograft is important both in bony defects as well as in arthroscopic surgery and experimental work on the biological events of allograft is recently published.

Tendon healing to bone surfaces/tunnels Healing of tendon material to bony surfaces is of major importance in both shoulder and knee surgery where understanding of the biology and biomechanics in this process is a key element. A recent experimental paper from the group demonstrates that this is significantly affected by COX-2-inhibitors.

PhD-dissertations

There were two PhD-dissertations in 2011. Stig Heir defended his PhD on cartilage injuries and treatment. Ulf W. Sigurdsens thesis was on experimental fracture healing.

Cell Transplantation and Tissue engineering

Leader:

Aksel Foss, Prof, MD, PhD, MHA, FEBS (OUH)

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Hanne Scholz, MSc, PhD, Senior Scientist (OUH)
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Tim Scholz, MD, PhD, FEBS (OUH)
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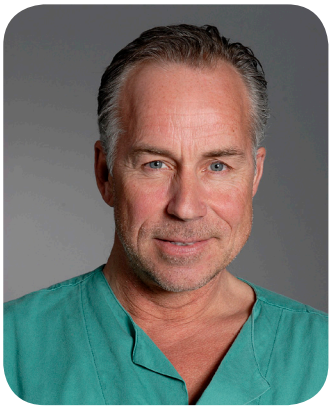
Karsten Midtvedt, MD, PhD (OUH)
Geir Hafsahl, MD, PhD (OUH)
Hilde Hestvåg, Study nurse (OUH)



Professor Aksel Foss

Research areas

Cellular therapies are evolving as alternatives to tissue and whole organ therapies in reconstructive medicine and cell transplantation is a fast growing field in medicine. In front of a broad area of therapeutic options is islet cell transplantation, as treatment for unstable type 1 diabetes (see below).

The research group was established at Institute for Surgical research in 2005 and has been focuses on investigate new strategies to counteract detrimental effects on islet prior to engraftment and to improve islet growth and function after transplantation. In 2009 the first doctoral thesis from the work of the research group was published (Tormod Lund). A master degree was published in 2008 (Ingrid Aursnes Stølen).

The Research Group for Cell transplantation is part of the Nordic Network for Clinical Islet Transplantation (NNCIT) and works in close collaboration with Uppsala University. During the last years extensive work has been carried out to establish facilities for isolation and culture of human islets as well as other cell types such as hepatocytes, and mesenchymal stem cells, with the aim to create a 'Center for Cell Transplantation' at Oslo University Hospital. A working group of key individuals from Oslo University Hospital, University of Uppsala and Karolinska Institute have joined to bring forward a robust inter-Scandinavian collaboration to address new potential cell therapies and a defined research program has been established. The strength in this organization is that the people involved are a mix of full time researchers and active clinicians who quickly can transfer new knowledge from the laboratory into the clinic, which is mandatory in modern medical science.

For further advance in the field of cell transplantation, knowledge obtained from islet transplantation research is important. Inflammatory reactions are key elements in islet isolation, culture, and transplantation as it is in many other biological processes, as well as the implantation site is important determinants for the outcome of cell transplantation. Therefore, islet transplantation represents a unique functional model for further knowledge within other types of cellular transplantation such as hepatocyte transplantation.

General Objectives Pancreatic Islet Transplantation

- To utilize the present knowledge on clinical islet transplantation and to induce C-peptide production in unstable type 1 diabetics, thus improving quality of life and reducing long term complications of type 1 diabetes.
- To develop strategies to suppress inflammatory reactions elicited by islet transplantation and to establish non-toxic immunosuppressive protocols in islet transplantation, able to induce immune tolerance.
- To explore and develop striated musculature as an alternative site for islet transplantation.
- To obtain methods for in vivo surveillance of islet cell engraftment, distribution and function by bioluminescent imaging.

Background

Diabetes is lifelong chronic disease affecting hundreds millions of individuals, and the incidence is rapidly increasing worldwide, particularly in the western society with a prediction that 366 mill people will be affected by diabetes in 2030.

There is currently no cure for diabetes, and exogenous insulin administration, either as injection or via a subcutaneous pump, is the selected treatment for most patients suffering from the disease. However, it is apparent from numerous clinical studies that insulin does not offer the effective glucose homeostasis required to prevent the deadly long-term complications of the disease, and better treatment options are highly warranted. Moreover, there are also new findings supporting that patients suffering from type 2 diabetes has also reduced beta cell mass leading to insufficient endogenous insulin production.

Clinical islet transplantation is an important and a promising minimally invasive cell transplantation therapy for restoring insulin secretion in a selected group of type 1 diabetes (TD1) patients by replacing destroyed insulin producing beta cells with donor islets containing functioning beta cells (Fig.1A). Therefore replacement of beta cell mass with islet cell transplantation has the potential to be a cure for diabetes.

However, non-immune-mediated islet graft loss poses a significant barrier to sustainable long-term insulin independence. Obstacles to be overcome are beta-cell injury during islet isolation, slow or inhibited vascularization of the islets after transplantation, ischemic injury to the graft, and the toxicity upon the islets of immunosuppressive drugs (Fig.1B). In addition, the islets are transplanted via the portal vein, which leads to the formation of emboli in the portal vein branches of the liver and induces local hypoxia in the surrounding liver tissue resulting in inflammation and graft failure.

The survival of the transplanted islets is judge by measurements of insulin levels, and glucose homeostasis. These measurements give little or no information on the health of the transplanted islets and the continued loss of islets from the transplanted tissues. Importantly, non-invasive imaging technology has advanced rapidly in recent years, and a variety of methods including magnetic resonance (MRI), positron emission tomography (PET) and optical imaging (bioluminescence) have now been applied to image pancreatic islets. This is of great importance to understand the sequence of events following cell transplantation.

The common denominator causing loss of beta cells seems to be inflammation and the single most important inflammatory reaction may occur in the recipient upon transplantation: IBMIR, referred to as the "instant blood-mediated inflammatory reaction". Anti-inflammatory strategies, applicable to the donor, to islets in pre-transplant culture and to the recipient, are one way to limit the loss of islets caused by IBMIR. From others and our work it seems that the detrimental effects of IBMIR provide an explanation for the need for

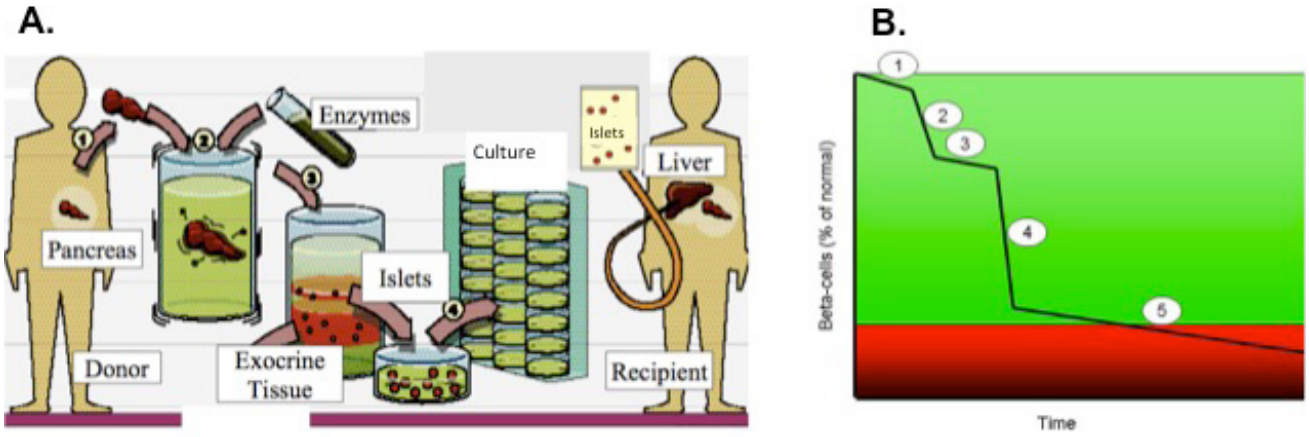


Fig.1 A: *Pancreas procurement (1), islet isolation (2-4), and islet cell transplantation into recipient (TD1 patient). B: Schematic presentation of loss of islets from the time of organ retrieval to revascularization in the recipient. Loss of islets during brain death and organ harvest (1), during islet isolation (2), during pre-transplant culture (3), during the islet transplant procedure (4), and during post-transplant period prior to revascularization (5). Green area represents insulin-independence, red area insulin dependence for the patient. From Olle Korsgren.*

islets from 2-4 donors in order to obtain normoglycemia in the transplantation series. Another approach to avoid direct islet-blood interactions is to explore alternative sites for islet transplantation. A readily available transplantation site might be striated musculature, which has been employed in auto transplantation of parathyroid tissue for years.

Mesenchymal stem cells (MSCs) are promising multipotent stromal cells for cellular therapy. It is established that MSCs can exert immunosuppressive activity on T cells responses and islet activation. Most of the clinical studies have used autologous MSCs. However, also allogenic MSCs from third party donors have proven to be effective. If MSCs can be useful in our studies will be tested and remain to be seen.

1. Ongoing clinical trails

Open, prospective, controlled study to evaluate the effect of islet transplantation on quality of life in selected patients with unstable type 1 diabetes.

It is well established that islet transplantation regularly induces sustained C-peptide production in type 1 diabetics, resulting in reduced insulin need and improved metabolic control. Further, C-peptide production corrects insulin sensitivity and fatty acid profile and improves Quality of Life and recently it has been shown that C-peptide positive type 1 diabetics have reduced late complications of the disease. Despite optimal insulin therapy, more than 1000 young T1D patients in Norway suffer from unawareness of hypoglycemia with recurrent episodes of unconsciousness. The Islet Cell Research Group is conducting an ethically approved study where the goal is to induce C-peptide production, not to seek insulin independence in such patients. 15 patients will be recruited in the study with eight patient enrolled up to now. Primary end point is QOL at 12 months.

Open randomized multi-center study to evaluate safety and efficacy of low molecular weight sulfated dextran in islet transplantation.

Low molecular weight dextran sulfate (LMW-DS) is a strong candidate to prevent early islet graft destruction caused by the instant blood-mediated inflammatory reaction. In addition, LMW-DS has shown to increase endogenous release of islet protective hepatocyte growth factor, which could be an additional beneficial effect of LMW-DS during the first critical hours after transplantation. 72 patients will be recruited in the study. The first patient in Oslo was enrolled in the study in May 2010, 6 patients have been transplanted and 4 patients are on the waiting list.

Clinical Islet Transplantation as established treatment option for patients with brittle diabetes.

The department of transplant medicine at OUH has recently included islet transplantation as a treatment option for patients with brittle diabetes.

2. Ongoing experimental projects

Establishing improved non-diabetogenic immunosuppressive protocols in islet transplantation, able to induce immune tolerance.

A key factor for success in islet transplantation is said to be avoidance of steroids as shown by the Edmonton group in 2000. The IS regimen in the Edmonton protocol consists of tacrolimus, rapamycin and IL-2 antagonist induction therapy. Following the publication of the Edmonton group almost all clinical trials have utilized this type of IS after the transplantation (although it is well documented that tacrolimus induces de novo diabetes in up to 25 % of allotransplanted patients). The aim of this project is to identify an immunosuppressive maintenance regimen that inhibits IBMIR, has minimal islet toxicity and the ability to induce tolerance. In a step-wise methodological approach different immunosuppressive compounds are screened for diabetogenic properties and effect on proinflammatory cytokines (IL-8, MCP-1).

Intracellular regulation of immunosuppressive drugs in human islets

Together with the i2mc research group, headed by Prof. Stein Bergan we are investigate the pharmacokinetics, pharmacodynamics and pharmacogenomics of the immunosuppressive drugs in human islets in vitro.

Experimental islet transplantation to striated muscle

Islet transplantation through the portal vein is current clinical practice. However, it has been recognized that this implantation site has several characteristics that can hamper islets engraftment survival, such as low oxygen tension, activation of the innate immune system, and the provocation of the inflammatory response, IBMIR. Thus, alternative sites for islet transplantation are demanded. The ideal site to our opinion, must be well vascularized to offer a suitable environment for implantation as well as being easily accessible. The intramuscular site has attracted recent interest due to positive long-term outcome of auto-transplantation of parathyroid glands to the muscle, and recently success of islet auto-transplantation to muscle. We have previously shown that islet transplantation into striated muscle is feasible site in the rat, but less efficient compare to the liver. To investigate whether the mesenchymal stem cells have

the potential to support the islets in the intramuscular site we have established a model where we transplant the islets with or without MSC into abdominal muscle of diabetic rodent (Fig.2).

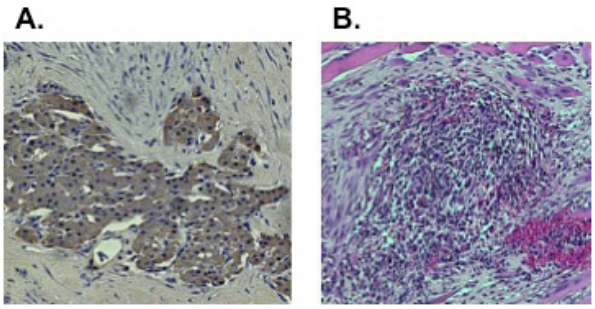


Figure 2 *Islet-MSC graft transplanted to the intramuscular site in the rat. A: Islets are identified by insulin staining (brown) and B: infiltration of inflammatory cells by HE staining.*

In vivo surveillance of transplanted islets

Transplanted islet cell mass is not static but changes in response to the environment, e.g. inflammation at the transplant site, toxicity of immunosuppressants and influence by the immune system. Non-invasively monitoring of changes in islet mass following islet transplantation is important to provide new insight into factors regulating post transplant function. Bioluminescence imaging has the impact to detect molecular and cellular processes in vivo.
In collaboration with the Transplantation Research Laboratory, UCSF, we have established a mice model utilize luciferase-expressed beta cells to visualized insulin producing islets in vivo by bioluminescent imaging. We have shown that the imaging signal correlates with beta cell mass (Fig. 3). Thus, this non-invasive model can be utilized to monitor changes in beta-cell mass over time in individual animals. The bioluminescent imaging technique is an important tool for the research in islet transplantation, and is implicated in many of the ongoing studies (Fig.4).

Anti-inflammatory treatment during islet transplantation, study the molecular mechanisms and clinical significance

Biological therapy has emerged as a cornerstone in the treatment of chronic inflammatory diseases. These agents target specific pathological processes without the broad immunoregulatory effect their predecessors had. Anakinra (IL-1 receptor antagonist) and Tocilizumab (anti IL-6 receptor antibody) are both novel biological clinically approved agents targeting specific receptors and thereby reducing

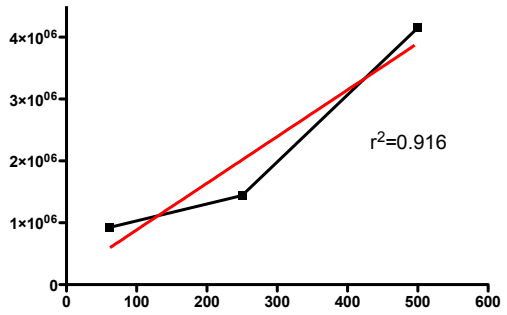


Figure 3. *Linear relationship between the amount of transplanted islets and the intensity of the luminescent signal. Three different doses (100, 250 or 500) of MIP-Luc islets were transplanted under the kidney capsule. Bioluminescence images were taken 8 min after injection of D-luciferin (200mg/kg) and signal intensity was measured and expressed as photons/sec.*

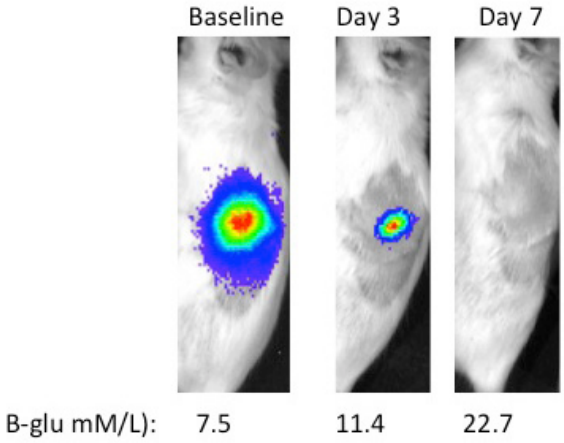


Figure 4. *Bioluminescence in B6 Albino mice transplanted with synergistically MIP-luc islets under kidney capsule. A. Representative image of transplanted MIP-luc islets before (baseline) and day 3 and 7 after induction of diabetes with alloxan treatment (correspondent blood glucose levels shown below).*

inflammatory responses in rheumatoid arthritis. Whether these two agents also can modulate the inflammation cascade and improve viability in human islets are unknown and has been investigated. Results in this study shows that in vitro treatment of human islets with anti-inflammatory agents significantly improves islets survival and function (Fig.5).

Study effects of new compounds on human beta cell mass and function by using a double islet transplantation model in mouse.

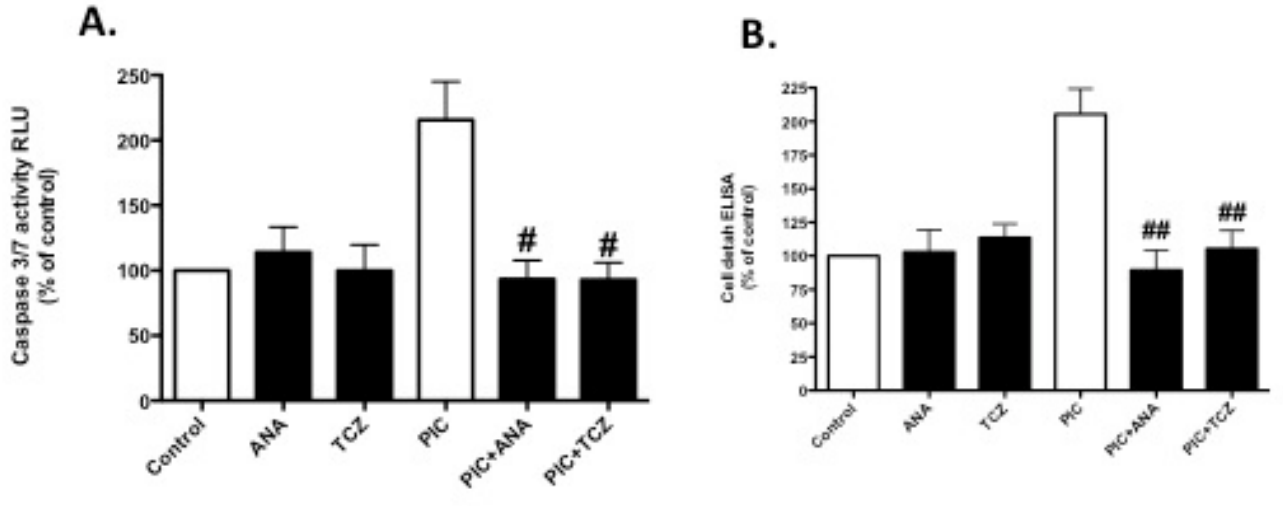


Figure 5 *Human islets treated with Anakinra or Tocilizumab for 48 hours after induction of cytokine-induced apoptosis showing significantly decreased A: caspase activity, and B: cell death.*

Patients with type 2 diabetes are often treated with intensive lifestyle interventions involving a combination of diet and physical activity, which can delay or prevent the disease. However, the disease usually gets worse over time, and diet and exercise may not be enough to control blood glucose levels resulting in needs for medications that include injections of insulin. New therapeutic options for treatment of type 2 diabetes are needed, and some new ideas have also become available such as the incretin-based therapies. In collaboration with AstraZeneca AB research team and Uppsala University, we have established a double islet transplantation model to be able to investigate the impact of new compounds on the expansion of beta cell mass and improvement of function (Fig.6). Briefly, two islet transplantations are performed in the same animal. First, mice islets are transplanted under the left kidney capsule to restore hyperglycemia, the second transplantation are performed after a recovery time of 2-4 weeks. A suboptimal dose of human islets are transplanted under the right kidney capsule and the grafts is allowed for a recovery period for up to 3 weeks, the first graft is removed by left sided nephrectomy which allows for followed islet function and possible proliferation in the second graft in response to different interventions in vivo.

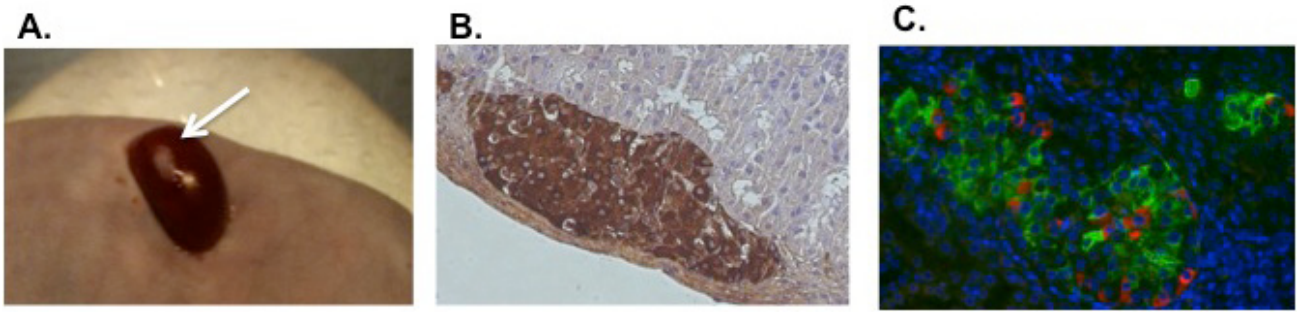


Figure 6. *Transplantation of human islets under the kidney capsule into diabetic nude mice. A: Human islets (white arrow) at the subcapsular site. B: Section of the human islet graft showing insulin-producing cells in vivo, indicated by insulin staining (brown) of islet in the graft from cured nude mice, and C: immunofluorescence section of the islet graft showing functional islets producing insulin (green) in a proliferations state, Ki67 proliferation marker (red), with nuclear stained blue.*

General Objectives Hepatocyte Transplantation

Scientific work aiming at implementation of hepatocyte transplantation into the Clinical Transplant Program at OUH

- Development of effective primary Hepatocyte Culture Techniques
- Development of Hepatocyte Transplant Techniques at various sites in Animal models
- Development of Experimentally and Clinically applicable Imaging Techniques for Evaluation of Hepatocyte Engraftment.
- Approaches to overcome the Instant Blood-Mediated Inflammatory Reaction (IBMIR) to hepatocytes at the time of transplantation.
- Enhancement of hepatocyte engraftment by optimizing immunosuppression combinations.

Background Hepatocyte Transplantation

Liver transplantation (LTX) is the only clinically proven and therefore standard treatment for patients with acute and chronic liver disease, including selected patients with primary and secondary liver malignancies. However, due to organ shortage a significant number of patients are dying on the waiting list. Several initiatives such as split-liver (one liver for two recipients) and live donor transplantation have been introduced to defy organ shortage. However, additional approaches are emerging as a possible supplement or even a substitute to LTX.

Traditionally, hepatocyte cell therapy has been given through extracorporeal bioartificial liver devices. The success with these devices has been limited and attention has now increasingly turned towards clinical hepatocyte transplantation. Experimental studies and some clinical trials have shown that hepatocyte transplantation may be useful in patients as a bridge to LTX or while waiting for the liver to regenerate (acute liver failure, acute-on-chronic liver failure and acute decompensation after liver resection). In selected patients with single enzymatic defects in the hepatocytes (tyrosinemia, primary hyperoxaluria, α-1-antitrypsin deficiency, hemophilia), transplantation of hepatocytes without this enzyme defect may even heal the disease. More than 200 patients with acute and chronic liver failure have been treated with hepatocyte transplantation.

In principle, our knowledge within the field of islet isolation, culture and transplantation may also be applied for hepatocyte transplantation. Furthermore, islet isolation facilities can be used also for hepatocyte isolation.

Experimental projects to be started

Routes of hepatocyte transplantation

As in islet transplantation, hepatocytes may be transplanted into the liver via the portal vein and it has been demonstrated that hepatocytes then translocate from the portal pedicle into the space of Disse by disrupting the sinusoidal endothelium and then join with adjacent host hepatocytes. Hepatocytes are regularly well engrafted in the liver (better than islets). Other potential implantation sites are striated musculature, peritoneal cavity, mesenteric leaves, the spleen, lung parenchyma, under the kidney capsule, and in the subcutaneous space.

Therapeutic hepatocyte mass.

Hepatocytes occupy almost 80% of the total liver volume. The cell mass required to restore adequate liver function in a patient is dependent on the underlying disease. The cell mass needed to correct a single enzymatic defect is significantly less than for treatment of acute liver failure. Thus, it has been suggested that 1- 5% of normal liver mass may be sufficient to restore adequate function of a single enzymatic liver defect, while a considerably larger hepatocyte mass is required for the treatment end stage liver failure. 10% of the liver mass (150 g) can be transplanted safely as hepatocytes via the intraportal route into the diseased liver. Since both fulminant and chronic liver failure require replacement of more than 10% of functional hepatocytes into the liver, additional ectopic sites such as the peritoneal cavity or musculature, may provide alternative sites for hepatocyte implantation.

An important distinction from islets is that there exists a wide range of endogenous hepatic growth stimulators that increase the mitotic activity of transplanted hepatocytes and thus increases total hepatic mass. Several growth factors such as insulin and hepatocyte growth factor (HGF) can be injected and thereby possibly speed up the mitotic activity further. Interaction between foreign material and blood is almost universal following infusion and research into diminishing these blood-mediated reactions are warranted.

Sources of hepatocytes

A limiting factor of clinical hepatocyte transplantation has been the shortage of mature functioning human hepatocytes. Traditionally, hepatocytes have been obtained from livers discarded for LTX, commonly due to excessive steatosis. Other potential sources of hepatocytes are remnant parts of livers after reduced sized LTX, fetal livers that are

too small for LTX and livers from patients transplanted for other causes than diseased hepatocytes such as polycystic livers and livers explanted due to hemangiomas. Another important source of hepatocytes could be livers from PSC patients (without cirrhosis) transplanted due to the finding of malignant cells in the extra-hepatic biliary system. PSC is the most common cause of LTX in Scandinavia, comprising more than 20% of all LTXs. The suitability of such livers for hepatocyte transplantation should be explored, and if suitable, a significant number of livers will be available for hepatocyte transplantation in the Nordic countries. Hepatocytes can be derived from various sources such as cell lines (tumor-derived and immortalized normal hepatocytes), progenitor cells (embryonic, fetal and adult stem cells), and other hepatocyte-like sources (matured in vitro or in vivo).

Cryopreservation and banking of hepatocytes

The most important indications for hepatocyte transplantation beyond enzymatic defects in children are acute fulminant hepatic failure and liver decompensation following extensive liver resections in patients with liver malignancies. In these acute situations, the need for readily available stored hepatocytes is imminent. To achieve this goal, a functional freezing protocol needs to be developed for cryopreservation and banking of viable metabolically active hepatocytes on a permanent basis. Several cryopreservation techniques are available but need further refinement.

TISSUE ENGINEERING

Tissue Engineering (TE) combines research within cellular biology and cellular transplantation with material- and engineering science to develop biologic substitutes. The goal is to restore and maintain normal organ function that has been damaged due to disease, trauma, cancer therapy or by other causes.

TE is a relatively new interdisciplinary and multi disciplinary field of research that has seen intense developments in recent years. It involves the in vitro seeding and attachment of cells into a scaffold. After proliferation, migration, the cells differentiate into the specific tissue. Current scaffolds are mainly made up of synthetic polymers.

TE utilizes living cells as engineering materials. Recently there has been a great trend towards the use of adult mesenchymal stem cells from bone marrow or fat. These cells can differentiate into a variety of tissue types also including endocrine and nerves.

The first trachea transplantation

For the first time in history, a patient in 2011 was given a new trachea made by TE technique using a synthetic scaffold seeded with his own stem cells.

The operation was performed on June 9th at Karolinska University Hospital in Huddinge, Stockholm by Professor Paolo Macchiarini. Professor Macchiarini led an international team including colleagues from UK and USA who provided the tracheal scaffold and a specifically designed bioreactor used to seed the scaffold with the patient's own stem cells. The patient is still in good health 9 months after the operation.

Research plan

At Oslo University Hospital the three clinics F, G/E and C in 2011 decided to start a joint project in Tissue Engineering with the aim to provide our patients with treatment solution based on tissue engineering techniques. Professor Aksel Foss has accepted to be the leader of the project.

Initial phase of the project is estimated to last for 2-3 years and includes:

- Establishment of the research group on Tissue Engineering
- Establishment of an international network of cooperation
- Develop a tracheal transplant for clinical use based on TE techniques
- Develop research and clinical testing on vascular grafts based on TE technique

Members of the TE-project group: Aksel Foss (leader), Ansgar O. Aasen, Hanne Scholz, Gunnar Kvalheim, Tormod Lund, Morten Moe, Pål-Dag Line, and Otto A. Smiseth

Conferences Attended 2011

IPITA Congress, June 1-4 2011 Prague, Czech Republic
CTS-IXA 2011 Joint Congress, October 22-26, Miami, Florida, USA
AASLD Liver meeting 2011, November 4 - 8, San Francisco, California, USA

Collaborators:

Professor Peter Stock and Ass. Professor Qizhi Tang, University of California, San Francisco (UCSF), USA
Professor Peetra Magnusson, Uppsala University Hospital, Sweden
Professor Camillo Ricordi, University of Miami, Miami, FL, USA
Prof. Tomas Totterman, Uppsala University Hospital, Sweden
CMO Per Gjorstrup, Resolvyx Pharmaceuticals, Inc. Bedford, MA, USA
AstraZeneca R&D Mølndal, Sweden
Professor Pål Aukrust, Institute for Internal medicine, Oslo University Hospital
Senior scientist Thor Ueland, Institute for Internal medicine, Oslo University Hospital
Professor Stein Bergan, Department of Pharmaceutical Biosciences, Oslo University Hospital
Professor Gunnar Kvalheim, Department of Cellular Therapy, Oslo University Hospital
Professor Bo Gøran Ericzon Karolinska University Hospital, Huddinge, Sweden

Experimental microsurgery and Transplantation

Leader:

Pål-Dag Line, Director, MD, PhD, Department of transplantation, Division of Cancer Medicine, Surgery and Transplantation, (OUH)

Group members:

Jihua Shi, MD, Section for Transplantation Surgery, PhD Student , (OUH)
Bjarte Fosby,MD, Section for Transplantation Surgery, PhD Student, (OUH)
Helge Scott, Professor, MD, PhD, Division of Pathology, (OUH)
Henrik Huitfeldt MD PhD Division of Pathology, (OUH)
Vivi Bull Stubberud, RN, Division of Surgery, (OUH)
Aksel Foss MD PhD, Professor, (OUH)
Svein Dueland MD PhD, (OUH)

Head of Department Pål-Dag Line

Introduction

The research group has been a collaborative project between the Transplant Unit at the Department of transplantation, other research groups at Rikshospitalet, in particular at the Institute of Pathology. After the foundation of the Dicision of Cancer medicine, surgery and transplantation and new research-group organisastion, we have joined the research group for Transplantation and malignancy headed by Svein Dueland MD PhD.

Our group has for many years established various experimental transplantation models and other surgical models in order to form a solid platform that allow us to explore our main fields of interest: transplantation immunology and hepatic regeneration and cancer.

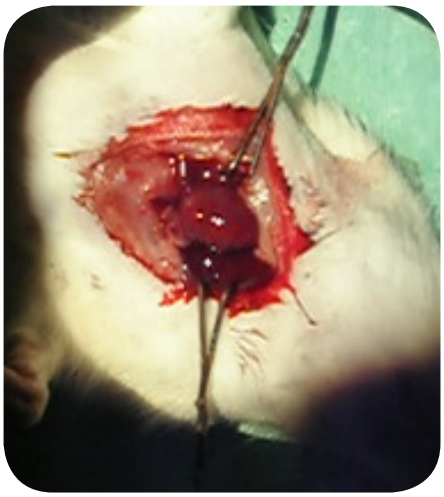


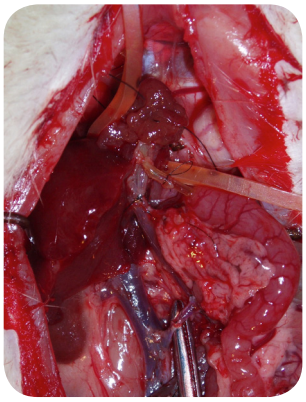
***Figure 1**. Heart transplanted to the neck of the recipient animal.*

The transplantation models

In the cuff-based cervical heterotopic transplantation technique we transplant the donor heart to the neck of the recipient. Anastomoses between the graft aorta and the carotid artery, and between the pulmonary artery and the external jugular vein are cuffed as reported by Heron. A transplanted heart is shown in picture 1.

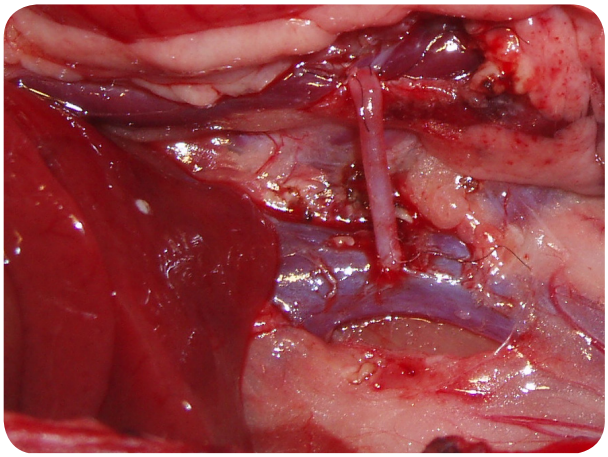
Compared to conventional techniques of anastomosis the cuff method reduces graft cold ischemia and bleeding from the anastomoses. Another advantage with the method is the superficial localization of the graft in the recipient's neck which simplifies registration of graft function and enables exact judgement of endpoint for the rejection process. Graft ischemia is typically 5-10 min, and the technical success rate above 90%.

We have also established orthotopic liver transplantation models in the rat. This is a non arterialized, model, where the portal vein and the infrahepatic cava is anastomosed by cuff technique whereas the proximal, suprahepatic cava is sutured. The model is performed partly as a whole graft transplant, but when indicated partial liver transplantation of various size is achieved by performing lobar resections on the graft.. The model can also be combined with porto-caval shunting, utilizing a carotid artery graft as a shunt.



Picture 2 (left) shows a transplanted rat Liver

Picture 3 shows a portocaval shunt where a carotid artery graft is sutured end-to-side to the vena porta and cava respectively.



Ongoing research topics

Our main research topic for 2011 has been related to growth and differentiation of hepatocellular carcinoma. Hepatocellular carcinoma (HCC) ranks fifth in frequency worldwide among all malignancies and is the third most common cause of cancer mortality, with about 600000 cancer related deaths annually. Liver resection or transplantation represents the only potential curative treatment modalities for most HCC patients. Despite successful surgery achieving R0 resection, tumour recurrence is a major problem and might be as high as 75 % at five years of observation.

Growth of hepatocellular carcinoma in the regenerating liver

Recurrence of HCC after resection is presumably caused by undetected, disseminated micrometastasis or de novo cancer in the liver remnant. The cellular and molecular changes resulting from hepatectomy, and the subsequent liver regeneration process may influence the kinetics of tumor growth and contribute to recurrence. Accumulating clinical and experimental evidence suggests that factors involved in liver regeneration may also stimulate the growth of occult tumours and the reactivation of dormant micrometastasis.

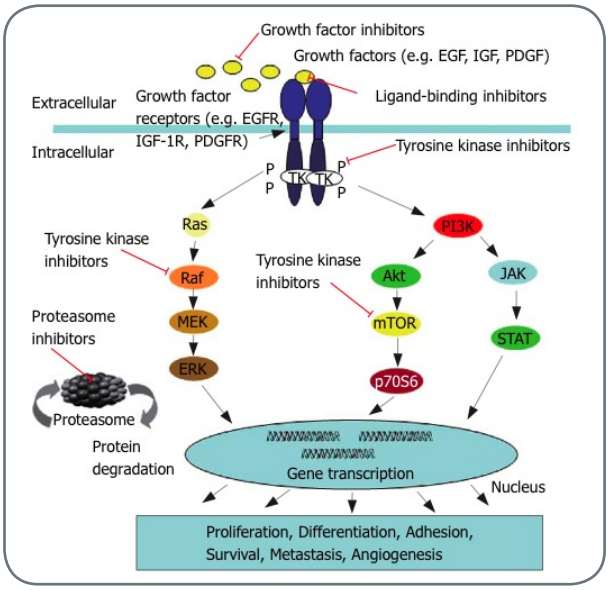


Figure 1. *Major growth factor receptor signallling pathways in HCC:*

Some of the most prominent growth factors are oulined in Figure 1 (adapted from Höpfner M et al. World J Gastroenterol 2008 January 7; 14(1): 1-14)

It is an important clinical question how one can control the equilibrium between suppressing tumor recurrence and promoting liver regeneration after liver resection or reduced-size liver transplantation. Kinoshita and Tanaka reported that the angiogenesis inhibitor TNP-470 prevents liver metastases of VX2 carcinoma after hepatectomy.

In one of the concluded projects of 2011, we tested the hypothesis that microscopic HCC tumours developed after extended hepatectomy display a greater growth rate compared to tumors in unresected livers and that tumor proliferation is related to the size of the partial hepatectomy. Furthermore, it was postulated, that the tumors developed in the resected liver show sign of greater invasiveness compared with tumors in unresected livers. Male inbred Buffalo rats underwent laparotomy and were divided into groups according to the size of partial hepatectomy. A micro hepatoma was established in the liver remnant through injection of 1x106 Morris Hepatoma Cells (McA-RH7777) into the parenchyma. Varying grades of hepatectomy (group T0= 0%, Group T30=30%, Group T70= 70%, Group T80=80%, Group C= Control) was performed concomitantly. After three weeks of observation the animals were sacrificed, and tumor growth and morphology, metastasis, Intracellular Expression VEGF, VEGFR, EGF, and Ki-67, Microvessel Density (CD34) an Calpain subunit 3 (CASPN) were registered.

TABLE 2. Tumor Growth and Invasiveness in the HCC Micrometastasis Model

	Group T0	Group T30	Group T70	Group T80
Macro-examination				
Tumor volume (mm^3)	61.74 ± 17.92	95.37 ± 8.92	507.94 ± 38.73*	489.08 ± 27.39*
Tumors per animal (n)	1.0 ± 0.0	1.0 ± 0.0	2.33 ± 0.21*	2.0 ± 0.0*
Fluorescence microscopy of the lungs†				
Lung invasion (n/N)	3/6	3/6	6/6*	6/6*
AFP-positive cells per view field	7.33 ± 2.58	10.33 ± 3.71	25.67 ± 3.9*	35.67 ± 4.18*

*$P < 0.05$ versus groups T0 and T30.
†See Fig. 2B.

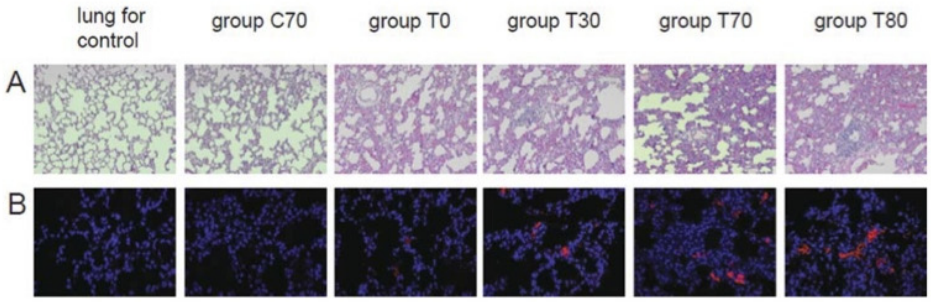


Figure 2. Histological examination of lungs 21 days after hepatectomy and tumor cell implantation. (A) Histological examination of lung tissues with hematoxylin and eosin staining (×100). Groups T70 and T80 showed more severe inflammatory lung injury. (B) Histological examination of lung tissues with paraffin fixation and immunofluorescence staining (×400). Nucleus staining with Hoechst 33258 (blue) and cytoplasm staining with the tumor-specific marker AFP (red) showed that tumor cell clusters and tumor thrombi were positive in the alveolar atrium. The number of AFP-positive cells in the lungs increased with the size of the partial hepatectomy.

The magnitute of hepatectomy, clearly stimulated posto-perative tunor growth (Table 2), and the expression of VEGF, CASPN, and CD 34 was enhanced in the high resection groups. Resection size was also related to malignant cell in-filtration in the lungs, as an expression of metastatic disease (figure 2).

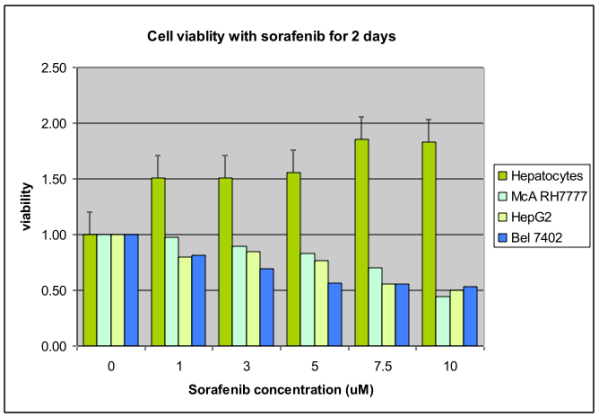
The reults from the current study were published in Liver Transplantation in 2011:

Shi, J.-H., Huitfeldt, H. S., Suo, Z.-H., & Line, P.-D. (2011). Growth of hepatocellular carcinoma in the regenerating liver. Liver transplantation : doi:10.1002/lt.22325

The effect of RAF targeted therapy on HCC in the regenerating liver

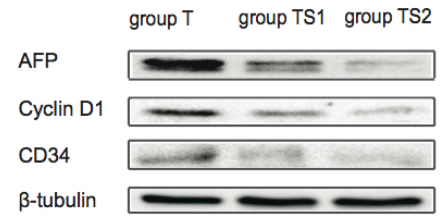
Since liver regeneration following hepatectomy appears to stimulate HCC growth, adjunct therapies that targets hepatoma cells but that not inhibits liver regeneration is of clinical interest. This study investigates the effect of the RAS/RAF pathway inhibitor sorafenib on rat liver cells as well as rat and human hepatoma cells in vitro (McA RH7777, HepG2, Bel 7402).We also tested the effect of two dosage regimens of sorafenib affected liver regeneration and tumor growth in an in vivo model of liver regeneration following hepatectomy. Animals were divided into groups ase indicated in table 3.

In th in vitro experiments, hepatocytes showed higher cell viability compared with hepatoma cell lines after being cultured for 2 days in the medium with Sorafenib induction and EGF stimulation (Figure 3)

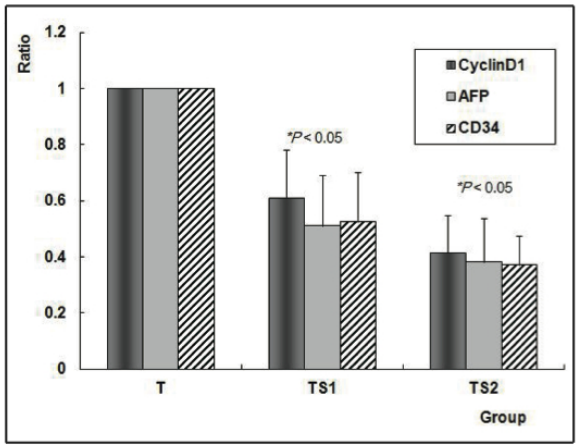


In the in vivo experiments, sorafenib did not significantly inhibit liver regeeration in normal liver tissue as evaluated by Cyclin D1 expression, and liver function was comparable between group C and croup HS. There was a distict and significant effect of sorafenib on tumor growth, and expression of AFP, Cyclin D1, and CD34 in the tumour tissue. The inhibitory effect of sorafenib was aslo evident in reduction of pulmonary micrometastasis in the sorafenib treated groups.

These findings could support the use of this class of drugsas an adjunct to surgical therapy for HCC. The results from these studies have been submitted to the Journal of Surgical Oncology.



A



B

Table 3. Investigated groups of the in-vivo experiment

	group C (n=6)	group HS (n=6)	group T (n=6)	group TS1 (n=6)	group TS2 (n=6)
Hepatectomy	70%	70%	70%	70%	70%
Implantation of McA-RH 7777	-	-	+	+	+
Sorafenib	-	2.5 mg/kg	-	2.5 mg/kg	10 mg/kg

Does hepatic progenitor cells play a role in HCC growth and biological behaviour following hepatectomy? .

Liver regeneration following surgery may stimulate the growth of occult HCC tumors. Large liver resections can in itself mobilize peripheral hematopoietic stem cells into the liver that differentiate into hepatic progenitor cells (HPC). It has been speculated whether progenitor cells might participate in hepatic tumorgenesis or contribute to tumor growth and metastasis after surgery. The aim of this project was to explore how activation of HPCs affects tumor growth and malignant development in vivo following partial hepatectomy and to study the effect of HPCs on hepatoma cells in vitro. Varying grades of hepatectomy (0%, 30% and 70%) with concomitant implantation of hepatoma cells in the liver remnant were performed in Buffalo rats. HPC activation was verified by double immunofluorescence staining for cytokeratin 19 and αFP/CD133.

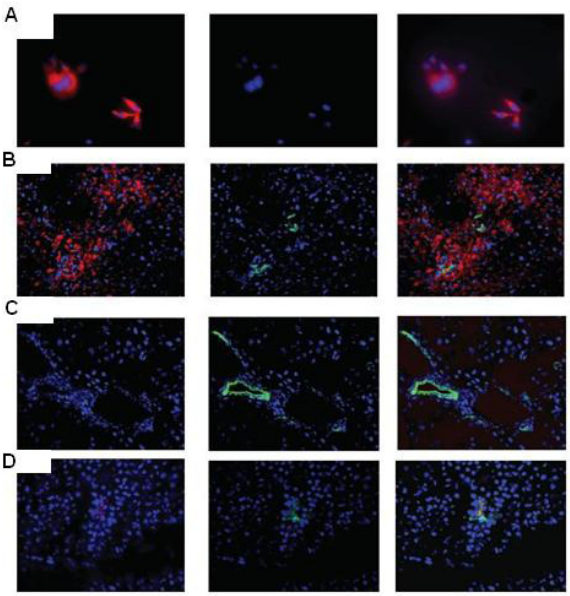
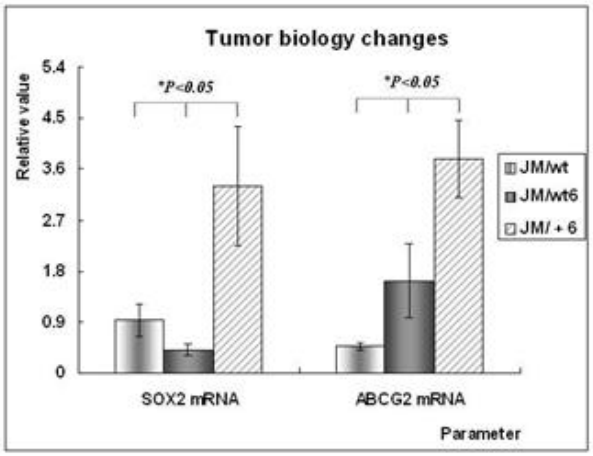
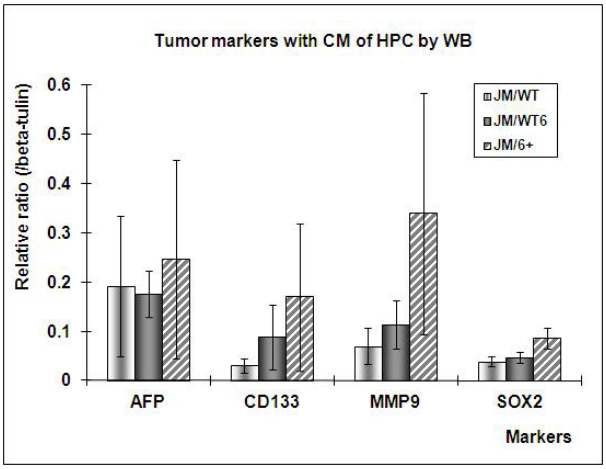
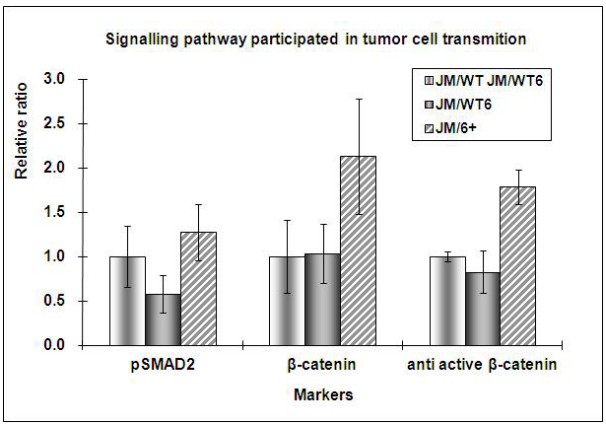


Figure 5. *Double immunofluorescence staining with αFP (red), CK19 (green) and Hoechst 33258 (blue) (400).*
A McA-RH7777 cells.
C Regenerating liver after 70% hepatectomy.
D Positive expression of HPC in liver tissue after 70% hepatectomy with tumor

The effects of HPCs (WB-F344) on wild type hepatoma cells (JM1/WT) in vitro were investigated by co-culturing technique whereby the HCC cells (JM1/WT) were cultured in the supernatant of the HPC cells (WB-F344) for 6 passages, thus creating a distict subgroup of hepatoma cells (JM1/6+), and proliferation rate, sensitivity to Adriamycin and expression of invasive factors and factors linked to «stemness» of the tumor (AFP, CD133, MMP9, SOX2) were assessed. JM1 cell co-cultured with conditioned medium from WB-F344







	Group TJ70 (n=7)	Group TJW70 (n=6)	Group TJ/+70 (n=5)
Hepatectomy	70%	70%	70%
Hepatoma cells implantation	JM1/WT 1.0×10^6	JM1/WT 1.0×10^6	JM1/6+ 1.0×10^6
WB-F344 injection	-	+ 0.2×10^6	-
Volume (mm3)	1006.7±555.9	1613.1±520.1*	1645.8±497.9*
Local metastasis	2	4	5*
Distand metastasis	0	1	3*

*P<0.05 (compared with Group TJ70).

Table 4. *Tumor development in vivo with the effect of hepatic progenitor cells.*

(JM1/6+) showed more aggressive characters marked with the increased drug resistance (IC50) to Adriamycin and enhanced expression of AFP, β-catenin, MMP9, CD133 and ABCG2 in vitro with RT-PCR (Figure 6) and WB (Figure 7).

TGF-β and WNT pathways were activated in JM/+6 compared with JM/WT1 and JM/WT6 (Figure 8).

The in vivo effect of HPCs on tumor growth was tested in 3 groups of Fischer rats undergoing 70% hepatectomy and implantation of JM1 hepatoma cells: wild type (group TJ70), wild type co-cultured with HPC (Group TJ/+70) and wild type + HPC transplantation (group TJW70). Vigorous tumor development after WB-F344 injection in rats was evidenced as the increased morphological tumor size, metastasis (Table 4) and an enhanced expressions of tumor biological markers including Cyclin D1 and MMP9.

The results from the current study indicates that progenitor cells could play a critical role in growth and biological behaviour of HCC development after surgery. This could also give new insights to the role of liver cancer stem cells. This study will be sent for publication in 2012.

Transplantation and Malignancy

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Introduction

Organ transplantation requires lifelong immunosuppression. A side effect is increased post-transplant de novo malignancy. During the last decades, the effectiveness of standard immunosuppressants in allograft transplantation has improved and so has the incidence of de novo cancer. A recent study of 905 recipients of transplanted hearts, lungs, or both has shown a 7.1 times increase in de novo cancers compared to the general population. Cancer-related death following transplantation is increasing and accounts for 13% of post transplant mortality.

Regulatory T cells (T regs) maintain self-tolerance to autoantigens and are involved in the pathogenesis of various clinical conditions such as autoimmune diseases, chronic viral infections and cancer. T regs appear more frequently in peripheral blood lymphocytes of cancer patients than healthy controls and interestingly, it seems that high levels of T-regs are a prerequisite for allograft tolerance following transplantation. By manipulation of the interaction between CD4+ CD25+ T regs and dendritic cells, it may become possible to influence host offence and defense in cancer and organ transplantation. In these aspects it is of particular interest that immunosuppressive drugs used in transplantation have both an anti-rejection and anti-neoplastic activity. Rapamycin (Sirolimus, Rapamune®) is an established drug for prevention allograft rejection by blocking the intracellular pathway complex mTOR. It also appears that a Sirolimus based immunosuppression protocol has beneficial effects on tumor recurrence and survival with an acceptable rate of rejection and toxicity in liver transplanted HCC patients. Rapamycin is a potent VEGF antagonist showing significant anti angiogeneic effects in addition to a direct inhibitory effect on tumor growth and proliferation. The drug has shown clinical effect and objective x-ray responses and stabilization of disease in different types of cancer, such as advanced breast and renal cancer that has previously progressed on other treatments. Accordingly, rapamycin is an effective anticancer drug in addition to its immunosuppressive effects. This supports the use of the drug for patients transplanted for cancer and in patients with de novo post transplant malignancy.

The SECA study

In 2006 we acquired an ethical approval (S-05409 Regional Ethics Committee, Helse Sor-Ost) for a clinical pilot study (SECA-study) to investigate liver transplantation as treatment option for selected patients with non-resectable liver metastases after colorectal carcinoma (CRC), using the mTOR inhibitor Rapamycin as standard immunosuppression from postoperative day 1.

By November 2011, 21 patients have been transplanted in the study. The Kaplan-Meier estimates of overall survival at one, three, and five years post Lt were 95%, 68% and 60%, respectively. The 95% confidence interval for 5-years survival was 33% to 85%. Non-resectable colorectal liver metastases is a devastating disease with a 5-year overall survival of less than 10 % after start of first line chemotherapy. Sixteen of the 21 patients in the SECA study had progressed on first or later lines of chemotherapy at the time of inclusion in the study. Such patients have a median expected OS of 12 months or less.

The results are also comparable to those of studies for liver resection for colorectal metastases (However, it should be

kept in mind that the SECA patients were not eligible for resection). The overall 5-year survival in these studies is generally between 30-40%. For larger hepatic tumor load, the results are even lower. The patients in the SECA study had generally a large tumor burden (the median number of liver tumors was 8 (Range 4-40) and the median size of largest metastase was 4.5 cm (Range 2.8- 13)). They were all deemed non-resectable even when available treatment options such as liver expansion techniques, repeat resections and ex situ liver resections after chemotherapy were taken into account. If resectable, expected 5 year OS of the study population would have been only 25 % when each patient were scored according to Fong clinical risk score for resectable colorectal liver metastases.

Metastatic recurrence of disease appeared in 18 of 21 patients. Most of these were pulmonary and these progressed slowly. Also, a significant proportion of the recurrences were accessible for surgery and at follow up seven patients (33%) had no evidence of disease. Hepatic tumor load prior to liver transplantation, time from primary surgery to Lt, and progressive disease on chemotherapy at time of liver transplantation were identified as significant prognostic factors. A combination of prognostic factors can likely to act as substrate for patient selection strategies that could further improve the outcome.

The SECA II study.

The SECA II trial was initiated on January 2011as a randomized trial where Ltx was compared to best available oncological treatment. Because of the large difference in survival in the SECA study compared to all previously reported chemotherapy studies, an amendement in protocol was approved by the Regional Ethics Committee in November 2011. This changed the objective of SECA II to a randomized controlled trial between Ltx and hepatic resection for patients with large but resectable colorectal metastases with a non-randomized Ltx study for patients with favorable prognostic factors as an aside.

Study Objectives of SECA II

- In a randomized controlled trial to explore whether liver transplantation in selected patients with liver metastases from CRC can obtain significant life extension and better health related quality of life compared to patients receiving chemotherapy or surgical resection.
- To explore if patient selection according to nomo-grams for outcome of colorectal cancer can define a subgroup of patients with a 5 year survival of at least 50% or even cure from the disease

Study endpoints

Primary endpoint

- Overall survival

Secondary endpoints

- Survival related to Memorial Sloan-Kettering nomograms for recurrence after liver resection of metastatic CRC.
- Disease free survival (RECIST criteria and time to lung lesions of ≥10 mm)
- Time to start of new treatment (change in strategy)
- Quality of life (EORTC QLQ-C30), time to decrease in physical function and global health score
- Registration of symptoms (NCI-CTCAE version 4.0)
- Diagnosis of other malignancies
- Determine nomo-gram scores of patients who survive for 5 years or more
- Survival in relation to biological markers (plasma TIMP-1 and TPA , circulating tumour cells and micro-metastatic disease in bone marrow biopsies at time of Ltx, different micro-arrays of liver biopsies, regulatory T-cells, infiltrating T-lymphocytes in liver biopsies and KRAS and BRAF mutations.
- Correlation between recurrence/metastases and other biological parameters (proteomics, micro array, micro metastases in bone marrow and lymph nodes and circulating tumour cells)

Study population

In part A of the study patients with 6 or more liver lesions will be randomized between liver resection and liver transplantation since surgical resection in this group of patients have a 5 years survival of only 0-30%.

In part B, CRC patients with non-resectable liver only metastases with at least 10% response according to RECIST criteria after 8 weeks (first evaluation) of first line chemotherapy will be randomized between continuing best available chemotherapy and liver transplantation. Patients randomized to chemotherapy will continue the ongoing regimen and will receive further lines of chemotherapy at the discretion of the treating physician. Patients randomized to Ltx will continue chemotherapy until Ltx. Within 10 weeks after Ltx the patients will receive capacitabine (1000mg/m2 two times a day, 2 out of 3 weeks for 6 months). Patients randomized to Ltx who have not received a Ltx within 3 months after randomization due to donor deficiency, will be taken off the transplantation list and included in the chemotherapy arm of the study.

Patients fulfilling inclusion criteria in part B and have metachronous liver metastases, having a primary pN0 disease

and CEA<100 ng/ml at time of primary diagnosis as well as at time of metastatic disease will not be randomized but will all receive liver transplantation (part C). This selected group of patients is expected to obtain a 5 year survival of 50-70 %.

At time of progressive disease all patients will be considered for the best treatment options, including further chemotherapy as well as surgical resections/radiation therapy/radiofrequency ablation (RFA).

Characterization of the primary tumor, liver metastases and temporal T-cell immunity in the SECA-patients

1.1 Recent studies have addressed the importance of regulatory T-cells (T regs) in suppressing T-cell immunity to tumor-associated antigens. There is evidence that T regs may be a main obstacle of successful tumor immunotherapy and that patients who exhibit allograft tolerance have increased levels of T regs. Temporal levels of T regs are measured at both preoperatively and at fixed intervals postoperatively. Lymphoid infiltration in tumors will be assessed, as this has shown to be an independent prognostic factor in CRC.

1.2 Cytokine profiles will be assessed at Ltx and at defined time points at follow up. The cytokine profiles will be evaluated in regard to immunosuppressive treatment, to the cancer disease and in recognition of the liver as an active immunological organ participating in local and systemic host defense.

1.3 Samples of tissue from the metastases as well as normal liver tissue are being collected and frozen (-70ºC) for later investigations. The properties of this tissue and the possible relation to the clinical course of the patients will be explored. The exact modalities of analysis are not yet fully established as de-freezing is a nonreversible event that needs meticulous planning. The analyses will be performed after all patients have been included in the trial. There are several possible paths to follow:

1.4 Tumor gene expression profiles will be determined in metastases and in normal liver tissue (oligonucleotide micro array analysis)

1.5 The role of disseminated tumor cells ((DTC, micro metastases). The prognostic value of disseminated tumor cells in bone marrow (BM) has been investigated in several studies comprising patients with epithelial cancers such as breast-, colorectal- and gastric cancer. The presence of DTC in BM seems to be of predictive value for the development of metastases not only in bones but also in other distant organs even in tumors that rarely develop skeletal metastases such CRC. Concerning breast cancer it has been shown that the detection of disseminated tumors cells in bone marrow by immunocythochemical methods is a strong and independent prognostic factor. Although not completely unequivocal, possibly because of a certain degree of methodological concern and lack of standardization, identification of DTC in BM also seems to predict disease free survival for patients with CRC. The incidence of DTC in BM in patients with isolated liver metastases from CRC is low when compared to patients with primary CRC or disseminated CRC disease, but their presence is associated with a poor prognosis. There is strong evidence, at least from breast cancer that DTC in the peripheral blood from patients with N0 disease is correlated with a high incidence of systemic relapse and mortality. In CRC the inconsistency of detection is larger and a more standardized methodology and sets of markers are called for. Nevertheless, it is probable that such a correlation will be found using a combination of markers. Little is known about the molecular characteristics of isolated DTC cells. Recent technological advances involving enrichment of the relevant cell population, amplification procedures for DNA and RNA as well as high-throughput molecular screening tools now render such characterization feasible. Samples containing from DTCs in BM, systemic- and portal blood give the possibility to compare molecular characteristics of cells from these compartments with each other and with the hepatic metastatic tissue. A novel aspect is that peripheral blood is collected for DTCs at Ltx, both at induction of anaesthesia and after manipulation of the liver in order to assess the potential effect of manipulation of the liver. Samples from BM, portal- and systemic blood will be processed at the Department of Tumor Biology immediately after sampling, using standard procedures for mononuclear cell isolation i.e. immunobead rosetting and preparation of cytospins for immunocythochemical analysis. Tumor cells isolated by immunobead rosetting will be further enriched using the CellPick system followed by RNA/DNA isolation and molecular profiling.

Mechanisms of regulatory T cell immune suppression and characterization of the regulatory T cell – effector T cell interaction in vitro, with special emphasis on the potential of cytostatic drugs as immunosuppressants in SECA-patients and in transplanted patients with de novo malignancy.

The concept of transplantation of cancer patients raises several immunologic considerations. 1) Immunosuppressive treatment to prevent rejection may promote cancer recurrence. 2) High tumorload in the liver may lead to a relative immune suppression that possibly involves regulatory T cells (TR cells), thus the patients may need less intense im-

munosuppressive treatment than liver transplant recipients treated on other indications. 3) Cytostatic drugs with immunosuppressive effects may be an alternative to standard immunosuppressive treatment for the SECA patients.

1.1 The role of regulatory T cells in cancer immunity in SECA patients. Regulatory T cells have been shown to inhibit anti-tumor immune responses in various studies in patients with malignant diseases such as colorectal cancer, ovarian cancer, breast cancer and melanoma. In this project we aim to investigate the suppressive role of regulatory T cells in anti-tumor immune function in the SECA patients. This cohort of patients will be compared to patients with lower tumor burden and who are eligible for local liver resection (the Ullevål patient cohort). Anti-tumor immune function will be evaluated i) prior to transplantation; ii) four months after; iii) one year after transplantation and; iv) at the time of recurrence.

1.2 Identification of down-stream cell-signaling pathways involved in TR cell function. As a part of a broader strategy to test known anti-cancer drugs with immunosuppressive effects to prevent rejection and reduce risk of recurrence in the SECA-patients, we wish to assess the effects of known anti-cancer drugs, immunosuppressive drugs and small molecular compounds (Src-, PI3-, MEK and p38-kinase inhibitors) on TR cell function and effector T cell function. Compounds with a preferential, different or selective effect on either T cell subset, may point to important physiological differences in the intracellular signal transduction machinery with potential clinical value. Cell proliferation and intracellular cytokine production will be assessed by flow cytometry in CD4+ and CD8+ T cells.

1.3 The physiological role of regulatory T cells and the mechanisms of action in interaction with effector T cells. In this in vitro project, we will aim to elucidate the physiologic role of regulatory T cells and the mechanisms of action in interaction with effector T cells. Regulatory T cells have been extensively studied for the last ten years. However, several fundamental questions remain unanswered. The molecular interactions underlying the immunosuppressive activity of regulatory T cells are not known. Furthermore, the temporal and cellular requirements of the immunosuppressive activity of the regulatory T cells during the cell-cell interaction with the effector T cells during an ongoing immune response, have not been thoroughly dissected.

Naturally Occurring Regulatory T cells

We have been addressing several basal l properties of Treg function by series of experiments studying the activation Requirements and Kinetic Properties of Human

Regulatory T cells :

Naturally occurring regulatory T (TR) cells are crucial in maintaining self tolerance by dominant suppression of potentially self-reactive T cells in peripheral tissues. The activation requirements, the temporal aspects of the suppressive activity and mode of action of human naturally occurring TR cells are subjects of great controversy. Ex vivo, TR cells display great variability in the suppressive activity dependent on donor and experimental conditions. Here we show that by ex-vivo anti-CD3/anti-CD28 stimulation and subsequent fixation in paraformaldehyde, TR cells acquire full suppressive activity after 6 hours of stimulation. However, the activation requirements are heterogenous as 60% of healthy blood donors have fully suppressive TR cells without the need of ex-vivo stimulation. The suppressive activity of TR cells operates in a contact-dependent manner that is not dependent on dendrittic cells or other antigen-presenting cells. The suppressive activity of paraformaldehyde fixated TR cells can be fully obliterated by trypsin treatment indicating that cytokine secretion is not required for suppressive activity, but that a cell-surface protein is directly involved. By using inhibitors for downstream signaling pathways involved in T cell activation, we were not able to inhibit the upregulation of TR cell mediated suppressive activity, indicating that this mechanism may be dependent on several pathways that operate in concert.

These findings establishes a method for further studying a core suppressive mechanism in TR cells wich in turn could lead to therapeutically exploitable targets.

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Plastic and reconstructive surgery

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Introduction

Plastic and reconstructive surgery is performed to restore normal anatomy and function in patients with congenital and acquired disorders, and in patients with tissue defects after trauma or cancer surgery. During the last decades research in plastic and reconstructive surgery has led to development of a large number of treatment options for patients with different kinds of disorders and defects. These methods are often based on experimental research which has been refined through clinical procedures. The main outcome is improved quality of life and patient satisfaction based on restoration of anomalies and dysfunction.

Research areas

Free tissue transfer is a relatively new technique which has revolutionized the field of reconstructive surgery over the past three decades. During the 1970s, reconstructive surgeons started to use the microscope to perform anastomosis of small vessels (±1mm). Tissue, based on these small vessels, could be transposed from a distant part of the body (donor site) to the location where reconstruction was needed and the vessels anastomosed to a recipient artery and vein. In 1989 a new area of free flap surgery was initiated with the introduction of flaps based on perforator vessels. This technique improved reconstruction by reducing donor site morbidity and by allowing new alternative flap designs. There is a constant need for optimising the reconstruction techniques to give the best possible result with minimal disadvantages at the donor site. Another new area in almost all surgical fields is the introduction of regenerative medicine. With this method new cells and tissue structures can be cultured to reconstruct various kinds of defects. Our research group has focused on the following areas:

1. Microcirculation, wound healing and microsurgery

a. Microcirculation in random flaps on rats and the effect of prostaglandin E1

In order to investigate the distribution of blood and microcirculation in random flaps we have designed a rat model that enables us to perform multiple measurements with laser Doppler perfusion imaging (LDPI)*. A random flap is raised with width-length proportions of 1:5. The flap is monitored

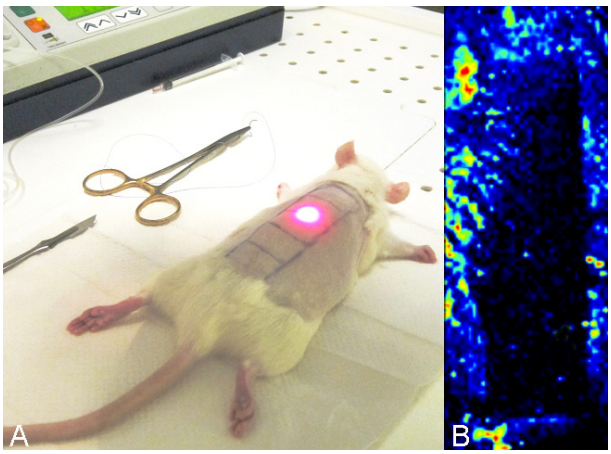

Figure1. *(A) Preoperative measurement with LDPI of a random flap on the dorsum of the rat. PGE1 is planned given iv. in the tail. (B) A LDPI scan after raising the random flap based cranially. Perfusion is measured in the five zones.*

in 5 equally sized squares on which a LDPI measurement is performed every hour for 6 hours (fig 1). The circulation is evaluated for every square with regards to the blood distribution within the flap.

Several studies suggest a positive effect of prostaglandin E1 (PGE1) on the circulation of flaps. In the same rat model as described above we compare the circulation of random flaps with i.v. infusion of PGE1 alternative saline and perform LDPI measurement.The circulation is evaluated and comparison between the control and intervention groups is performed and verified statistically.

b. Microcirculation and wound healing

To resemble a clinical situation, we are using animals with skin structure and function similar to the human skin. Pig skin has many similarities to human skin, including histological appearance and wound healing ability. We are using Norwegian pigs (Norsk landsvin) with weight between 25 and 30 kg in our studies. Microcirculation and histological measurements are performed to evaluate the effect of different reconstructive procedures or other interventions on wound healing. To investigate microcirculation and wound healing in an isolated setting, we use rat models as described below.

c. Experimental perforator flaps and other rat models

Dissection of the perforator flaps preserves the muscle and minimizes the donor site morbidity. Nevertheless, the method may have undesirable effects on the muscle because of damage of its innervation, blood supply or by direct injury when dissecting the perforator. We are performing studies to evaluate the surgically technique to reduce this damage to a minimum using Wistar rats where two symmetrical abdominal lipocutaneous flaps are raised around the midline (fig 2). One side is used for intervention which is compared to the other side. After dissection, the flaps are fixed to the original position by a continuous suture. Microcirculation, flap viability, wound strength and histological changes are measured preoperatively and during the first week after the operation.

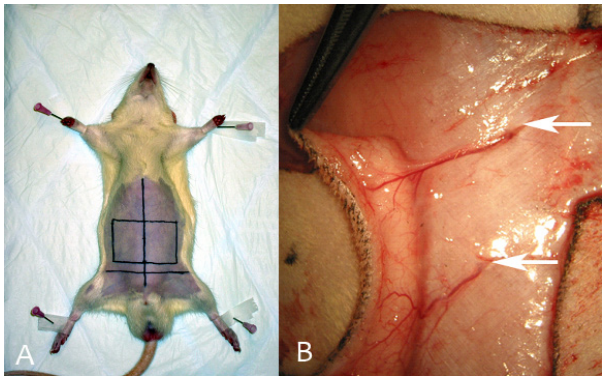

Figure 2. Perfusion in the Location of the perforator flap on the abdomen of the rat (left). Two perforators piercing the rectus abdominis muscle are seen trough the microscope (right)

To continue improvements in both a clinical and scientific setting research using animal models is important. The groin flap based on the superficial inferior epigastric artery (SIEA) is well described. We have established a new model where the SIEA flap is transposed to the back of the rat with good conditions for flap monitoring, without danger of flap autocannibalisation. This model is used when performing studies on microcirculation and histological changes where we want to compare different interventions on the flap or the animal over a longer period of time.

d. Changes in microcirculation of the skin during sepsis and cardiogenic shock

Sepsis and cardiogenic shock are diseases with high mortality. New equipment for monitoring central hemodynamics has not improved survival as expected. In 1922 Freedlander studied skin microcirculation with a microscope in patients with sepsis and found decreased capillary density and increased heterogeneity of capillary density. New studies with advanced microscopes on patients with sepsis and cardiogenic shock show microcirculatory alterations in tongue mucosa in the way Freedlander described. In addition, alterations in erythrocyte flow velocity appear without changes in central hemodynamics. These characteristic changes may be valuable in diagnosing sepsis at an earlier phase, and may also have a potential in treatment guiding. Still microcirculatory monitoring is not used as a routine in any clinical field to examine patients with sepsis or cardiogenic shock.

Our hypothesis is that systemic diseases will induce microcirculatory changes everywhere in the organism. We also believe that we will see changes with our microscope before we see them in central hemodynamics and bloodtests. In our last study we have induced fecal sepsis in eight pigs (Norwegeian Landrace pigs, 27-33 kg) and three controls. Pigs are used of the same reason as described in 1b. In the model they have been measured regularly (every 90 minutes) from before sepsis induction to immediately before death by four different non-invasive techniques in four areas of interest (two skin sites, eye and tongue,). The techniques are Laser Doppler techniques (LDPM, LDPI), spectroscope (for microvascular tissue oxygenation) and an "in vivo" microscope. The analysis of microcirculatory data are done by two blinded observers and microcirculatory data will be compared to central hemodynamics and bloodchemistry and immunology. We have previously done clinical studies with microscope and LDPM on patients on extra-corporeal membrane oxygenation (ECMO) for cardiogenic shock.

e. Microcirculation and reinnervation in human perforator flaps.

The deep inferior epigastric artery perforator (DIEAP) flap from the abdomen is one of the most suitable perforator flaps used for breast reconstruction (fig 3). This procedure has had a significant impact on the field of plastic and reconstructive surgery, because of the high number of women requiring breast reconstruction after cancer surgery. Based on the experimental research and clinical experience, our group is performing investigations to optimize the reconstruction technique and to minimize the donor morbidity.

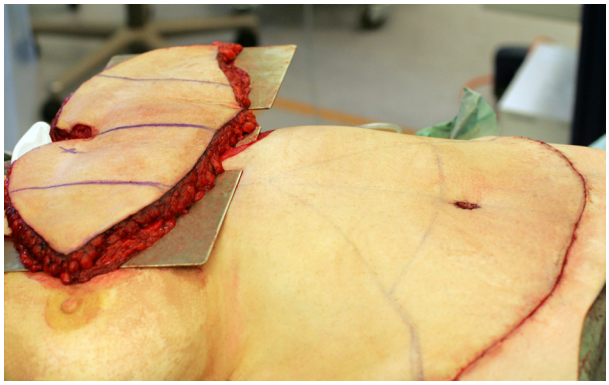

Figure 3. *The abdominal flap is transposed to the thorax and is ready for revascularization.*

Until now little attention has been paid to reinnervation of the flap. We have investigated the spontaneous reinnervation of the DIEAP flap after breast reconstruction and at the donor site at the abdomen (fig. 3). Pressure thresholds have been analysed on the skin using Semmes-Weinstein monofilaments. Histological studies to evaluate the reinnervation in skin are planned both for the perforator flaps and for the donor site.

Through better understanding of flap anatomy, physiology and better surgical technique the complication rate has decreased and the cosmetic outcome has improved. However, partial flap necrosis is still a recurrent complication that can affect the final cosmetic result and the patient satisfaction. In most cases this can be avoided by discarding parts with unreliable capillary refilling after transferring the flap to the recipient site. We are performing quantitative evaluation of the perfusion zones and skin areas with LDPI* in order to get a more exact picture of the microcirculatory differences in the DIEAP flap (fig 4) and other skin flaps.

Laser induced fluorescens of (ICG) is a new sensitive method for evaluation of tissue perfusion. In another project ICG videoangiografy is used to evaluate tissue perfusion of

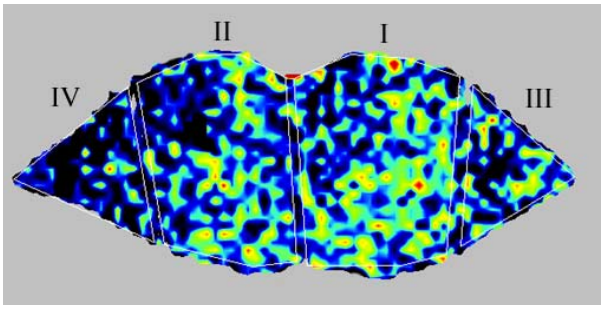

Figure 4. *Colour map of blood flow for the DIEAP flap processed by laser Doppler perfusion imaging. The flap has been divided into perfusion zones. The colour scale red-yellow-green-blue-black represents perfusion values where red is the highest and black the lowest value.*

the DIEAP flap during conventional abdominoplasty. The perforators are isolated to investigate their effect on the microcirculation of the flap.

These studies will have major clinical impact on all surgical procedures involving flap surgery in order to improve surgical outcome of the reconstructed part and to reduce the donormorbidity.

*Measurements of microcirculation with laser Doppler perfusion imaging (LDPI)

Measurements of microcirculation are a central part of all our animal and human experiments. It is performed with a PIM 3.0 LDPI from Perimed, Stocholm, Sweden. The LDPI generates, processes and displays colour-coded images of tissue perfusion. An optical scanner guides a low power laser beam stepwise to the tissue surface. The LDPI measures microcirculation to a depth of a few hundred micrometers. When the laser beam hit moving erytrocytes in the subepidermal plexus the light is backscattered and detected by a photodetector, this convert the light intensity to electrical signals and colour-coded images

Microcirculation endringer I hud ved ssytemsykodom

2. Treatment of facial palsy

a. 3-dimentional evaluation of outcome after surgical reanimation of facial palsy.

Patients with persistent facial palsy are evaluated for surgical treatment. One of the treatment options is dynamic reconstruction with cross-facial nerve grafting and subsequent gracilis muscle transfer to the face. In cooperation with the department of plastic surgery in Vienna, Austria, we are analysing the 3-dimentional outcome of these surgical procedures.

b. Medical treatment of Bell`s palsy

Bell`s palsy is an acute, idiopathic, unilateral peripheral facial palsy of unknown cause with an incidence of 30 per 100,000 inhabitants per year. Treatment of Bell`s palsy has been a matter of debate for decades, and treatment with corticosteroids and antivirals have been the most commonly described treatments. To evaluate the treatment effect of these two drugs, the Scandinavian Bell`s palsy study was performed from 2002 to 2007 at 17 different clinics in Scandinavia (Fig. 5) . This randomized, double-blind, placebocontrolled, multicenter trial included 839 patients with Bell`s palsy with a 12-month follow-up. Patients were randomized to treatment with prednisolone plus placebo, valacyclovir plus placebo, prednisolone plus placebo, and placebo plus placebo. The primary endpoint and some secondary endpoints have already been published showing significantly higher recovery among prednisolone treated patients. We are still analyzing some secondary endpoints that will be published in 2012 and 2013.

3. Regenerative medicine

Regenerative medicine is of great interest in plastic surgery due to the possibility to reconstruct defects which has been difficult or impossible to handle with traditional surgery. Still, there are many aspects of the techniques which have to be improved before they can replace the methods used to day. Our group has focused on the following areas:

a. Fat transplantation

Transplantation of autologous fat has been performed for many decades. Improvements in harvesting techniques and adventages such as availability and biocompatibility have led to its widespread application. In addition, potential positive effects of regeneration on the surrounding cells has been described. We are using fat transplantation in a number of different clinically conditions. However, there are still areas where the use of fat transplantation not has been sufficient described, and where the longterm outcome of the procedure is unknown. We have investigated the use of fat transplantation to the velum and pharynx in patients with velopharyngeal insufficiency (VPI). In these patients there is an incomplete velopharyngeal closure during speech producing hypernasality. Fat transplantation can possible improve this closure and perceptual speech assessments and MRI evaluation (fig 5) is performed to investigate this effect.

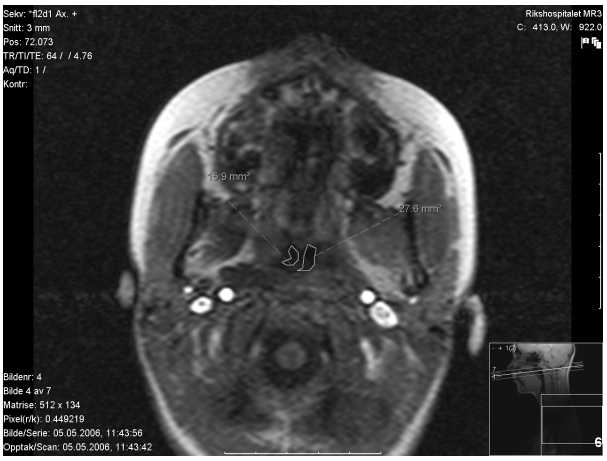

Figure 5.
Axial image preoperatively during phonation. The velopharyngeal gap area is measured.

The survival of autologous fat transplantation cells is described to be about 50%. In cooperation with the Norwegian Centre of Stem Cell Research studies are performed to investigate how this survival can be improved and to evaluate the effect of the transplanted fat on tumorigenesis in the recipient tissue.

b. Cultured urothelial cells

In reconstructive surgery within the genitourinary tract, autologous urothelial cells cultured in vitro could be of considerable value. To acquire urothelial cells for in vitro engineering of urothelium, bladder washings from adult patients as well as children can be performed. These samples will contain enough proliferative and colony-forming uroepithelial cells to regenerate urethral mucosa in vitro. The cultures could be expanded to confluent, stratified sheets, which can be used for reconstruction of the urethra in urogenital anomalies or in patients with other needs (transsexuals, reconstruction after trauma or cancer surgery). The laboratory work will give a large improvement in the clinical treatment of these patients.

c. Cultured epidermal cells

This project is performed in cooperation with the department of Ophthalmology (OUS), and focus on how cultured epidermal epithelial cells can be (1) successfully cultured on electrospun scaffolds, (2) optimally stored within a small temperature interval, (3) successfully stored in a tailor-made medium, and (4) reliably transported under specific conditions. These methods might ultimately be applicable in the treatment of a number of diseases as for example burns, chronic wounds, stem call deficiency in the cornea, and more.

Projects guided by external project leaders

Hypoxia and reoxygenation in newborn piglets and human infants
A study of oxidative stress after hypoxia and reoxygenation.

Principal Investigator
Rønnaug Solberg, MD, PhD, Researcher Dept. of Paediatric Research, OUS-RH

Research group
Professor Ola Didrik Saugstad, MD, PhD, FRCPE
Leonid Pancratov, MD, PhD-student
Tor Einar Kalish, MD OUS
Ewa Henkel, MD, PhD-student Karolinska Sjukhuset, Stockholm

Background and Aims
This research project will continue to study adverse effects of perinatal hypoxia-ischemia (HI) and reoxygenation. We use our newborn piglet model to simulate the situation newborn infants in need of resuscitation might be in. We have a special focus on oxidative stress and possible early intervention strategies that could reduce cell damage and long term consequences. The endogenous antioxidant capacity can be overwhelmed after HI insults, leading to increased concentrations of reactive oxygen species like the hydroxyl radical and peroxynitrite, - for which there are no known detoxification systems. The use of antioxidants may attenuate oxidative stress induced damage.

Projects 2011
During 2011 we have had two experimental studies where we have tried out new gas mixtures for immediate resuscitation/reoxygenation and an intravenously given antioxidant. Preliminary results indicate less apoptosis. We study gene regulation, lipid peroxydation, DNA damage, metabolic pathways and histopathological changes.

Development of a kit for non-sutured anastomosis

Principal Investigator
Sumit Roy, Stavanger Universitetssjukehus, Stavanger

Project manager:
Gunnar Kleppe, Prekubator AS, Stavanger

Project Staff:
Aurora Pamplona, Sera Sebastian, Vivi Bull Stubberud, Oslo Universitetssykehus-Rikshospitalet, Oslo

I. Background
A structurally sound, geometrically optimal anastomosis is the key to therapeutic success in vascular surgery. A simple technique for achieving this goal without suturing has for long been the focus of research. The few products that are currently commercially available for the purpose, cannot be incorporated in synthetic blood vessel substitutes ("vascular grafts") commonly used in peripheral vascular surgery, or are designed for very specific applications. To satisfy the unmet need for a versatile method for anastomosing tubular organs such as blood vessels without sutures, a simple, inexpensive implant has been developed. Using the implant and a deployment tool, two tubular organs can be rapidly anastomosed, with a simple, technique that even surgical novices will be able to master in a short time. A kit comprising a set of implants and deployment tools could hence be a valuable addition to the surgical armamentarium. To determine whether the envisaged kit had the propects of becoming a clinical reality, funding was secured from Norges Forskningsråd with two goals in mind:
(a) Research & development: to complete the development and preclinical testing of the kit for carrying our non-sutured anastomosis
(b) Commercialization: to explore the possibility of licensing or selling the intellectual property underlying the kit.

II. Research & Development:
Preliminary testing of the anastomosis kit was carried out on the heart in an animal model, because the coronary arteries offer probably the most challenging environment for evaluating new methods for creating anastomoses. However it remained unknown whether the results obtained with tests on coronary arteries faithfully reflected the behaviour of the implants when scaled up for larger structures such as the aorta or arteries supplying the brain, or the upper and lower limbs.

To achieve the goals of the R&D component of the project the following tasks were set:
- Modification of the design to allow its use for a wide range of vascular operations (phase 1)
- Establishment (in an animal model) the feasibility of performing vascular anatomoses with prototypes based on the final iteration of the design (phase 2)
- Determination of the natural history vascular anastomoses created with the implant (phase 3)

The first study from the first phase of the project, which was performed at the Institute for kirurgisk forskning, Oslo Universitetssykehus-Rikshospitalet in 2011, is the subject of this report.

A. Aim:
The primary goal was to modify the design of the implant to allow the creation of angled ("Y-shaped") end-to-side anastomoses.

B. Activities:
(a) Design and fabrication of prototypes:
Based on the preliminary experience with the use of the implant in an animal model the anatomical demands animal model to be used for testing purposes, design specifications were established for the implant and the deployment tool. Prototypes of the implant were fabricated in two sizes: "large" for anastomosing vascular grafts with lumen 7 mm and and "small" for anastomosing vascular grafts with lumen 4 mm in diameter. Appropriately sized prototypes of the deployment tool were also fabricated.

(b) Preclinical evaluation:
The prototypes were tested in juvenile pigs A commercially available thin walled, expanded polytetrafluoroethylene vascular graft (CarboFlo®, Bard Peripheral Vascular Inc) was anastomosed to a common iliac artery or the aorta. Up to 4 anastomoses were performed in each animal. Time required for creating the anastomosis was recorded. The anastomosis was visually monitored and any blood loss qualitatively graded. Interventions necessary to stop bleeding were recorded.

(c) Results:
Evaluation of the implant was carried out on five animals. All survived the experimental procedure. Nineteen anastomoses were attempted. After three modifications to the design, creation of an end-to-side anastomosis with an angled configuration with the implant proved to be a realistic proposition. Six of the nine anastomoses performed with the final iteration of the prototype were water tight, while leaks from the anastomsoses that could be controlled within 3 minutes with light manual compression occurred from the remaining three. A serendipitous observation from testing the first iterations of the design was that inadequate coaptation of the edges of the graft and the artery could be easily rectified by the application of glutaraldehyde-activated bovine serum albumin, a commercially available tissue adhesive (BioGlue®, CryoLife Inc). Deployment of the implant however was not as technically simple as anticipated. The median time for the anastomosing procedure was 5 minutes. Only one graft could be anastomosed in less than 3 minutes.

C. Implications:

For the non-sutured anastomosis kit to achieve its full potential the following refinements of the implant and the deployment tool could to advantage be explored:

1. Implant:
(a) Design:
Relatively minor modifications that should be easy to incorporate would make the implant appreciably easier to deploy
(b) Material:
Fabricating the outer component of the implant with a metal or alloy with a higher elastic modulus than titanium would not only simplify deployment of the implant, but also facilitate its removal when deemed necessary.

2. Deployment tool:
The ergonomics of the deployment tool could be appreciably improved by making it much lighter and scaling it down in size.

III. Commercialization:

Only preliminary exploration of the commercialization prospects of the envisaged kit was performed during 2011. The results of the efforts are awaited.

Cyclic AMP signalling in inflammation and sepsis

Principal Investigator
Petter Kirkeby Risøe, Cand.med. (UiO)
(Currently studying at the University of Yale)

Supervisor
Maria K. Dahle, MSc, PhD (UiO)
Guro Valen, Professor, MD, PhD (UiO)
Håvard Attramadal, Professor, MD, PhD (OUS/UiO)

Aim
Investigate the role and regulation of the intracellular cyclic AMP signalling system in innate immune cells, with a particular focus on cAMP-producing adenylyl cyclases.

Background
As a former member of the Group for Surgical Intensive Care Medicine, these projects are rooted in the field of innate immune responses and sepsis. The intracellular second messenger cyclic AMP (cAMP) acts as a negative regulator of proinflammatory cytokine release from macrophages, leukocyte adhesion, T-cell signaling, cytotoxic responses, endothelial disruption and cytokine-mediated signaling events. By utilizing ten different isoforms of cAMP synthesizing adenylyl cyclases (ACs) and an even larger family of cAMP hydrolyzing phosphodiesterases (PDEs) organized in subcellular microdomains, the cAMP signaling system can mediate a multitude of cellular effects. Circulating levels of cAMP have been found to be diminished in septic patients, and in animal models of systemic inflammation and endotoxic shock, elevating cAMP by inhibition of PDE4-isoforms has been reported to protect organ function and reduce fatalities.

Projects 2011
In 2011, Risøe et al. published that the micro RNA miR142-3p is elevated in rat cecal ligation and puncture sepsis, and attenuate AC expression in liver macrophages (Risøe et al., Cecal ligation and puncture sepsis is associated with attenuated expression of adenylyl cyclase 9 and increased miR142-3p. Shock. 2011;36:390-395). In parallel, a manuscript on lipopolysaccharide-mediated AC regulation in human blood, revealing differential regulation of the isozymes in a gender-specific manner, is now under final preparations, along with his PhD thesis.
Since June 2011, Risøe has been a student at The University of Yale, making his last preparations for his dissertation from there.

External support
These projects are greatly indebted to the financial support of Norske Kvinners Sanitetsforening, as well as the benefaction of the Institute for Basic Medical Sciences by the provision of laboratory facilities and first-rate scientific assistance.

Role of the Liver X receptors (LXRs) in inflammatory modulation

Principal investigator:
Joanna Ågren, MD, PhD student (OUS/UiO)

Supervisors
Maria K. Dahle, PhD (UiO)
Michael R. Daws, PhD (UiO)
Håvard Attramadal, Professor, MD, PhD (OUS/UiO)

Research area
Sepsis is a clinical syndrome where an infection (e.g. pneumonia or urinary tract infection) causes systemic illness. Delayed initiation of treatment may lead to organ failure (severe sepsis) and/or septic shock. Severe sepsis has an overall incidence of 2.8/1000 population and a mortality rate of 30%. It is believed that sepsis, and the following organ injury, develops when the body's own regulation of the inflammatory responses to an infection is out of control and defense mechanisms overshoot, rather than from the infectious agent alone. Thus, the complex pathogenesis of sepsis involves several factors, including dysregulation of the immune and endocrine systems, disseminated intravascular coagulation (DIC), genetic susceptibility, and derangement of energy metabolism.

Aims
Our major aim is to study factors that may modulate the overshooting inflammatory responses in sepsis and promote a better balanced immune defense. We believe that the liver X receptor (LXR) may have such properties and intend to elucidate the potential of LXR as an inflammatory modulator in sepsis.

Liver X Receptor and Inflammation
Liver X receptor (LXR) is a ligand binding transcription factor and belong to the family of nuclear hormone receptors. The two subtypes of LXR, LXRα and LXRβ, sense intracellular metabolites of cholesterol and are important regulators of cholesterol, fatty acid and glucose metabolism. Studies have also indicated that the LXRs are involved in regulation of both acute and chronic inflammation. We have previously demonstrated that activation of LXR by a synthetic agonist (GW3965) reduces liver injury and modulates systemic inflammation in rats subjected to acute endotoxemia or polymicrobial sepsis, and that liver injury is increased in LXR-deficient mice in the same models. Our in vitro studies have also shown that the release of pro-inflammatory mediators from white blood cells (Kupffer cells (liver macrophages) and human monocytes) is reduced when the cells are pretreated with the synthetic LXR agonist before stimulation with the gram negative cell wall component lipopolysaccharide (LPS).
To study the impact of LXR on inflammation, we use models that closely resemble the endogenous responses during an infection. For example, we use a model of human whole blood as well as cultures of freshly isolated human white blood cells (monocytes, macrophages, neutrophils and T cells). We also use murine models to study the systemic effect of LXR on inflammation. The cecal ligation and puncture model resembles acute polymicrobial sepsis, and the oil induced arthritis model is used to study immune responses in chronic inflammatory disease.
During 2011 Joanna Ågren has finished her medical internship at Drammen hospital. The experimental studies for her thesis are completed, and her focus is now on finishing her Ph.D. thesis.

Modulation of monocyte inflammatory responses: Effects of 9cisRA and the impact of surgical trauma

Principal Investigator:
Ingrid B. Moss Kolseth, MD (UiO)
(PhD student at the Institute of Basic Medical Sciences since July 2011)

Supervisor:
Maria K. Dahle PhD (UiO) until July 2011.

Aims
Elucidate the influence of 9cisRA on the inflammatory responses of human monocytes
Evaluate the state of inflammatory responses and inflammatory modulation mechanisms in whole blood from colon cancer patients before and after laparoscopic surgery.

Background
Vitamin A or retinol is crucial for the body homeostasis. Two nuclear receptors mainly convey the activity of retinoic acid (RA). The retinoid acid receptors (RARs) bind all-trans-(atRA) and 9-cis retinoic acid (9cisRA). The retinoid X receptors (RXRs) bind 9cisRA only. In previous studies by Kolseth, 9cisRA was shown to modulate inflammatory responses in human adherent monocytes.
Surgery has a profound effect on innate immune responses. The whole blood model allows ex vivo monitoring of human inflammatory responses and inflammatory modulation pathways in circulating immune cells. In a collaborative study with Professor Egil Johnson, MD and PhD-student Dag T. Førland and co-workers at Oslo University Hospital Ullevaal we investigate the status of immune modulatory mechanisms in patients having elective colon resections performed with minimal invasive techniques. 20 patients with resectable colon cancer (left and right) (T1-3 N0-1) have been included.

Projects 2011
The practical work on this project was finalized, and two manuscrips were finished in July/August 2011, before Ingrid B. M. Kolseth started her PhD studies. One paperhas already been accepted and was published online, and the othermanuscript was submitted in December 2011.

Kolseth IBM, Ågren J, Lyngstadaas SP, Wang JE, Aasen AO, Dahle MK. 9-cis retinoic acid inhibits inflammatory responses of adherent monocytes, and increases their ability to induce classical monocyte migration. Journal of Innate Immunity. 2011 Dec 29 (Epub).
Kolseth IBM, Førland D, Risøe PK, Kjeldsen SF, Ågren J, Reseland JE, Lyngstadaas SP, Johnson E, Dahle MK. Attenuated inflammatory responses and modulation effects of 9-cis retinoic acid after laparoscopic surgery for colon cancer. Submitted, SCLI

Training Courses and Seminars (Norwegian)

Introduksjon til nyfødtmedisinske teknikker og prosedyrer

07.03.2011 – 11.03.2011
Kurs O-25272

10.10.2011 – 14.10.2011
Kurs O-25273

Kurskomité:
Thor Willy Ruud Hansen (kursleder), Morten Grønn, Arild Rønnestad, Terje Rootwelt, Inger Elisabeth Silberg, Per Arne Tølløfsrud, alle Nyfødtseksjonen, Rikshospitalet. Gro Furset Flatekval, Avdeling for komparativ medisin, Rikshospitalet. Vivi Bull Stubberud, Insitutt for kirurgisk forskning, Rikshospitalet. Thoams Rajka og Vigdis Skaug, Barneklinikken og Undervisningssenteret ved Ullevål Universitetssykehus.

Målgruppe:
Leger under utdanning i barnesykdommer med særlig fokus på de helt ferske og uerfarne. Kurset kan også ha interesse for andre kolleger i det barnemedisinske faget som arbeider ved avdelinger der visse typer nyfødtmedisinske prosedyrer utføres svært sjelden. Videre vil kurset være relevant for anestesileger som arbeider ved avdelinger der akuttbehandling av nyfødte ivaretas av anestesiolog.

Læringsmål:
Deltakerne skal i løpet av kurset få demonstrert teknikker og selv få utføre praktiske øvelser. Målsettingen er å gi deltakerne grunnleggende kunnskaper og ferdigheter slik at de med større trygghet kan utføre prosedyrer på syke nyfødte.

Temaoversikt:
Følgende teknikker/prosedyrer vil bli undervist:
- Innleggelse av perifer venenål
- Innleggelse av perifer arterienål/-"kran", inkludert etablering av trykkmåling
- Kapillær blodprøvetagning
- Innleggelse av navlevenekateter
- Innleggelse av navlearteriekateter
- Innleggelse av perkutant sentralvenekateter
    1. "Peel away" teknikk
    2. Seldinger teknikk
- Innleggelse av thoraxdren
    1. Vanlig rett dren med mandreng
    2. "Pig-tail" dren med Seldingers teknikk
    3. Bruk av drenasjekammeret
- Spinalpunksjon
- Endotrakeal intubering
- Blærepunksjon (suprapubisk)
- Lokalbehandling av ekstravasering
- Innleggelse av intraossøs nål for infusjon

Introduksjon til nyfødtmedisinske teknikker og prosedyrer

Kursleder Thor Willy Ruud Hansen instruerer kursdeltakere.

Program

Mandag	Tirsdag	Onsdag	Torsdag
09:00 Teknikk: Perifer venenål. Perifer arterie + etablering av trykkmåling Modell:Rotte (føtter/hale)	09:00 Teknikk: Perkutan CVK. Modell: Rotte.	09:00 Teknikk: Intubering. Modell: 1) Dukke. 2) Gris.	09.00 Teknikk: Spinalpunksjon. Intraossøs infusjon. Modell:Gris.
12:00 Pause	12:00 Pause	12:00 Pause	12:00 Pause
13:00 Teknikk: Venøs bl.prøve Art. bl.prøve Kap. bl.prøve. Modell:Rotte	13:00 Teknikk: Navlekateter. Modell: Human navlesnor.	13:00 Teknikk:Thoraxdren. Modell:Gris.	13:00 Teknikk:Blærepunksjon. Blærekateterisering. Modell:Gris. Kursevaluering.
16:00 Slutt	16:00 Slutt	16:00 Slutt	16:00 Slutt

Basalkurs i laparoskopisk kirurgi

25.08. -26.8.2011
Kurs O-25802

Kurskomité:
Trond Buanes og Arne R. Rosseland (kursledere), Anders Debes, Fredrik Halvorsen, Ronald Mårvik, Erik Trondsen, Bjørn Edwin, Thomas Moger, Marianne Berg, Vivi Bull Stubberud.

Målgruppe:
Leger under utdanning i generell kirurgi og gastroenterologisk kirurgi.

Læringsmål:
Indikasjoner, operasjonsmetoder, resultater og kvalitetssikring innen laparoskopisk kirurgi. Kurset vil omhandle teoretisk undervisning og praktiske øvelser på simulatorer samt D-boks og pop-trainere.

Temaoversikt:
Laparoskopets oppbygging og vedlikehold, fysiologiske effekter av pneumoperitoneum, anestesi ved laparoskopi, laparoskopi ved akutt abdomen og galle. Praktiske øvelser under supervisjon på modeller og simulatorer vil være en vesentlig del av kurset.

Basalkurs i laparoskopisk kirurgi

Kursleder Arne Rosseland instruerer kursdeltakere.

Program

Torsdag	**Fredag**
8.30 Registrering/kaffe & rundstykker	9.00 Akutt abdomen Rolf Riis
9.00 Introduksjon av kurset (Arne R. Rosseland)	9.15 Diagnostisk laparoskopi Anders Debes
9.10 Kirurgisk trening(Fredrik H. Halvorsen)	9.55 Laparoskopisk appendectomi(Fredrik H. Halvorsen)
9.25 Basal laparoskopisk teknikkArne R. Rosseland	10.30 Pause
9.45 Etablering av pneumoperitoneum Thomas Moger	10.45 Laparoskopisk cholecystectomi-Trond Buanes
10.00 PAUSE. Kaffe og frukt	11.15 Diskusjon
10.15 Laparoskopiske rack(Olympus)	11.30 Lunsj
10.30 Diatermi(Covidien)	12.15 Praktiske øvelser
10.45 Ultralydsaks(Johnson&Johnson)	14.55 Praktisk kurs prøve
11.00 Laparoskopisk suturering Ole Christian Olsen	15.00 Kursprøve
11.20 Lunsj	16.00 Evaluering av kurset.Slutt
12.15 Praktiske øvelser, eget program	
16.00 Slutt for idag.	

Thorako-/laparoskopisk kirurgi

30.03. – 01.04.2011
Kurs nr. O-25595

Kurskomité:
Trond Buanes og Arne R. Rosseland (kursledere), Erik Trondsen, Bjørn Edwin, Marianne Berg, Vivi Bull Stubberud, og Stefan Kutzsche

Målgruppe:
Leger under utdanning i generell kirurgi og gastroenterologisk kirurgi.

Læringsmål:
Indikasjoner, operasjonsmetoder, resultater og kvalitetssikring innen laparoskopisk kirurgi. Kurset vil omhandle teoretisk undervisning og praktiske øvelser på simulatorer samt demonstrasjonsoperasjoner.

Temaoversikt:
Laparoskopets oppbygging og vedlikeholdFysiologiske effekter av pneumoperitoneumAnestesi ved laparoskopiLaparoskopi ved akutt abdomenCholecystecomiAntirefluxkirurgiLaparoskopisk cancerkirurgiLaparoskopi i urologienThoracoskopisk kirurgiPraktiske øvelser under supervisjon, på modeller og anestesert gris vil være en vesentlig del av kurset.

Operasjonsstue klargjort for kurs

Thorako-/laparoskopisk kirurgi

Onsdag	Torsdag	Fredag
Møteledere: **Bjørn Edwin og Erik Trondsen**	**Møteledere:** **Ole Christian Olsen & Ronald Mårvik**	**Møteleder:** **Arne Rosseland**
8.30 Registrering/kaffe & rundstykker 9.00 Åpning av kurset. Arne R. Rosseland, Kirurgisk avdeling, OUS – Rikshospitalet 9.05 Hvordan trene laparoskopi? Anders Debes, Sykehuset Østfold 9.20 Simulatortrening. Fredrik Halvorsen. OUS - Rikshospitalet 9.30 Anestesiteknikker. Hva er den ideelle anestesiform ved laparoskopiske prosedyrer. Kirsti Myre, Anestesiavdelingen, OUS Ullevål 9.45 Hvordan etablere pneumoperitoneum (åpen/lukket tilgang - videoillustrert). Buanes 10.00 Thoracoskopisk kirurgi. Steinar Solberg, Thoraxkirurgisk avd./RR 10.15 Pause 10.30 Urologi. Bjørn Brennhovd, Kirurgisk avdeling, OUS - Radiumhospitalet 10.45 Gynekologisk akutt abdomen. Anton Langbrekke, Gynekologisk avdeling, OUS - Ullevål 11.05 Teoretisk gjennomgang av diatermi ved representant fra Covidien 11.20 Teoretisk gjennomgang av ultralydsaks (vibrasjon) ved representant fra Ethicon 11.35 Lunsj 13.00 forts. Anders Debes, Trond Buanes, Bjørn Edwin, Bård Røsok, Olaug Villanger, Arne R. Rosseland, Vivi Bull Stubberud, Erik Trondsen, Stefan Kutzsche og representanter for firmaene. 13.00 Praktiske øvelser ved Rikshospitalet, Institutt for Kirurgisk ForskningInstruktører: Ole Christian Olsen, Ronald Mårvik, Fredrik Halvorsen, 15.00 Pause 19.30 Kursmiddag	7.45 Kaffe og rundstykker 8.00 Utredning og klargjøring før laparoskopisk cholecystectomi, Choledoccussteiner Erik Trondsen 8.20 Laparoscopi ved akutt abdomen/ akutt appendicitt. Ole Christian Olsen, Kirurgisk avdeling, Drammen 9.05 Laparoskopisk brokk-kirurgi: TEP. Elisabeth Hegstad 9.25 Ventralhernier. Røsok 9.40 Pause 10.00 Galleveiskirurgi Laparoskopisk kolecystectomi How to do it - prinsipper. Buanes 10.15 The easy gallbladder/intraoperativ cholangiografi & difficult gallbladder (video). Buanes 10.30 Gallegangsskader	7.45 Kaffe og rundstykker 8.00 Avansert laparoskopisk kirurgi (milt, pancreas, binyre, lever). Edvin 9.00 Praktiske øvelser ved Rikshospitalet, Institutt for Kirurgisk Forskning, Intervensjonssenteret og Ullevål 12.00 Lunsj 14.45 Kursprøve med evaluering 15.30 Slutt

Bakvaktkurs i akuttpediatri

31.10 – 01.11.2011
Kus O-25426

Kurskomité:
Kursledere: Overlege, professor dr.med. Thor Willy Ruud Hansen, Kvinne- & Barneklinikken, Oslo Universitetssykehus Sognsvannsveien og Overlege Thomas Rajka, Kvinne- & Barneklinikken og Utdanningssenteret, Oslo Universitetssykehus Kirkeveien. Kurskomité: Seksjonsoverlege Terje Alsaker, Nyfødtavdelingen, Haukeland Universitetssykehus.ledende spesialsykepleier/ Kari R Hovde, Kvinne- & Barneklinikken og Utdanningssenteret, Oslo Universitetssykehus Kirkeveien. Veterinær/leder PhD Gro Flatekval Furset, Avdeling for komparativ medisin, Operasjonssykepleier Vivi Bull Stubberud, Institutt for Kirurgisk Forskning, Oslo Universitetssykehus Sognsvannsveien.

Målgruppe:
Barneleger som går bakvakt på barneavdelinger med særlig fokus på de som har behov for oppfrisking av ferdigheter i akuttpediatriske prosedyrer.

Læringsmål:
Deltagerne skal i løpet av kurset få demonstrert teknikker og selv få utføre praktiske øvelser og trene i teamarbeid. Målsettingen er å styrke deltagernes kunnskaper og praktiske ferdigheter slik at de med større trygghet kan ivareta sine oppgaver i akuttpediatri som bakvakt.

Temaoversikt:
Følgende teknikker/prosedyrer vil bli undervist:
- Innleggelse av perifer arterienål/-Akran@,
- Innleggelse av navlekateter
- Innleggelse av navlearteriekateter
- Innleggelse av thoraxdren
- Endotrakeal intubering
- Innleggelse av intraossøs nål
- Arbeidsgrupper innen avanserte medisinske problemstillinger
- Scenariotrening med fokus på lederrollen i akuttsituasjoner

Program

Mandag	**Tirsdag**
Institutt for kirurgisk forskning, OUS Rikshospitalet 0800 Perifer arterienål, Navlekateter Intubering Thoraxdren Intraossøs infusjon MODELL Spedgris Human navlesnor 1200 Lunsj	**Utdanningssenteret, OUS-Ullevål** 0800 Workshop – astma - anafylaksi – sepsis - arrytmier – traumer - avslutning Scenario trening - lederrollen Dukkemodeller 1600 Slutt
Utdanningssenteret, OUS- Ullevål 1300 BHLRAHLR Frie luftveierIntraossøs drill Dukkemodell 1700 Slutt	

Seminars

Date	Name	Title	Department
2/17/2011	Fahri Saatcioglu	Wellness through evidence based practices - Example of the yogic science of breath	Inst for molekylær biovitenskap, Det matematisk-naturvitenskapelige fakultet
3/3/2011	Guttorm Haraldsen	Interleukin 33 - ulv i fåreklær?	Klinikk for diagnostikk og intervensjon, OUS
3/10/2011	Hilde Nilsen	The nematode C. elegans to study molecular mechanisms of the anti-cancer drugs 5-Fluorouracil and valprioc acid	Bioteknologisenteret, UiO
3/17/2011	Bjørg Mikalsen	Genome-wide endothelial cell activation profiles and matricellular protein expression after solid organ transplantation	Adv. for patologi, Oslo universitetssykehus
3/24/2011	Srdjan Djurovic	Dissecting the phenotype in genome-wide association studies of psychiatric illness	Professor, Senter for psykoseforskning, OUS, Ullevål
4/28/2011	Ola D. Saugstad	Resuscitation of newborn. From oxygen to room air.	Dept. of Pediatric Research, OUS
5/12/2011	Knut Erik Berge	The role of PCSK9 in cholesterol metabolism	Enhet for hjertegenetikk, Avd. for medisinsk genetikk
9/15/2011	Joel Glover	In vivo tracking av humane stamceller ved bruk av magnetiske mikrokuler samt litt om Nasjonalt senter for stamcelleforskning	Professor, Leder for Stamcellesenteret
10/20/2011	Geir Florholmen	The hypertrophic response in isolated cardiomyocytes	Forsker, PhD, Institutt for kirurgisk forskning
11/3/2011	Lise Roman Moltzau	NPR-A and NPR-B signal in different functional compartments in failing rat left ventricle	PhD-student, Farmakologisk institutt, RH
11/10/2011	Monica Suarez Korsnes	Paraptosis	PhD, Norwegian School of veterinary
11/24/2011	Einar Vik-Mo	Tumor initiating cells in CNS tumors	MD, PhD, OUS Ullevål
12/1/2011	Shan Zienolddiny	The role of genetic variation in predisposition, prognosis and therapy of lung cancer	Senior researcher, Group for toxicology and biological working environment, National Institute of Occupational Health, Oslo
12/8/2011	Kristina Gervin	Why are identical twins not identical? A role for epigenetic factors in development of disease	PhD-student, Department of Medical Genetics, Oslo University hospital.
12/15/2011	Odd-Arne Olsen	The calpain proteinase- developmental regulator in all walks of (eucaryotic) life	Professor, Universitetet for Biovitenskap, Ås & Høgskolen i Hedmark, Hamar

Publications and PhD-Theses

Publications 2007 – 2011

2011

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9. Myhre AE, Gram-positive endotoxemia and modulation of the innate immune response. Thesis, University of Oslo, 2007

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17. Smiseth OA and Edvardsen T. Tissue Doppler and speckle tracking echocardiography. Editor: Catherine M Otto: Practice of clinical echocardiography, 3rd edition Saunders ISBN: 1416036407 [Book chapter], 2007

18. Vinge LE, Andresen KW, Attramadal T, Andersen GO, Ahmed MS, Peppel K, Koch WJ, Freedman NJ, Levy FO, Skomedal T, Osnes JB, Attramadal H. Substrate specificities of g protein-coupled receptor kinase-2 and -3 at cardiac myocyte receptors provide basis for distinct roles in
regulation of myocardial function. Mol Pharmacol 72 (3):582-591, 2007

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22. Øverland G, Kupffer cell activation in systemic inflammation. Thesis, University of Oslo, 2007

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PhD-Theses

Ulf Sigurdsen

Rønnaug Solberg

Stig Heir

Einar Vik-Mo

Marit Dalen

2011

SIGURDSEN, Ulf Eirik Wangsvik: Tibial bone healing: Experiments with external fixation and intramedullary nailing

SOLBERG, Rønnaug: Resuscitation of the newborn. An experimental study of toxic effects of supplementary oxygen in newborn piglets

HEIR, Stig: Focal Cartilage Defects in the Knee

VIK-MO, Einar Osland: Propagation and characterization of stem like cells from brain tumors - establishment of a clinical protocol for immunotherapeutic targeting of tumor stem cells in glioblastomas

DALEN, Marit Lunde: Hypothermia and room air resuscitation in NT2-N neurons, immature rats and newborn pigs.

2010

DIMMEN, Sigbjørn: Effects of cox inhibitors on bone and tendon healing.

HAUGAA, Kristina Hermann: Prediction of cardiac ventricular arrhythmias by echocardiography in patients at risk.

2009

VON LUEDER, Thomas G: Molecular Mechanisms of Myocardial Hypertrophy and Heart Failure
Experimental Studies on Cardiac G Protein-Coupled Receptor Signaling with Emphasis on Endothelin-1.

LUND, Tormod: Strategies to counteract β-cell loss in pancreatic islet transplantation.

BAINS, Ravi: Isoflurane and sevoflurane: Effects on mitochondrial function in the rat and human brain.

ØLSTØRN, Håvard: Neural stem, cells from the adult human central nervous system and brain tumors. Properties in vivo following xenotransplantation.

2008

VARGHESE, Mercy: Isolation and Characterization of Stem Cells from the Adult Human Central Nervous System and Brain Tumors.

ANDRESEN, Jannicke: Neuroprotective effects of intravenous nicotine administration. An experimental study in newborn piglets.

WANG, Yun Young: Novel targets in modulation of inflammatory responses in experimental endotoxemia and sepsis: focus on the liver X receptor.

2007

ØVERLAND, Gunhild: Kupffer cell activation in systemic inflammation.

MYHRE, Anders E: Gram-positive endotoxemia and modulation of the innate immune response.

PhD-Theses

2006

BØRKE, Wenche Bakken: Myocardial injury and performance in hypoxaemic neonates: Effects of oxygen and carbon dioxide during reoxygenation. An experimental study in newborn pigs.

LARSEN, Geir Arne: Early mechanisms of brain ischemia. An experimental study in isolated rat neurons.

LYSEGGEN, Erik: Assessment of myocardial viability and reperfusion by tissue Doppler echocardiography.

LINDENSKOV, Paal HH: Inflammation in experimental meconium aspiration syndrome.

VINGE, Leif Erik: Myocardial G protein-coupled receptor kinases – Distinct isoform specificities at receptors as basis for diverse roles in regulation of cardiac function.

PAASCHE, Joachim Dericq: Molecular Mechanisms of Regulation and Intracellular Trafficking of the Endothelin Receptors.

AHMED, Muhammad Shakil: Autocrine / Paracrine Regulators of Myocardial and Pulmonary Remodeling in Heart Failure.

SKULSTAD, Helge: New insights into the function of normal and ischaemic myocardium.

LYNG, Kristin: Experimental induction of fetal brain damage in pigs as a model of infection-induced brain damage in humans.

2005

CASTELLHEIM, Albert: Meconium aspiration syndrome, systemic inflammatory response, and the complement system.

MUNKEBY, Berit Holthe: Resuscitation of the newborn with 100% O2 - detrimental effects on the brain, lungs and heart.

SOLÅS, Anne-Beate: Resusciation of the newborn – with or without supplemental oxygen?

URHEIM, Stig: Quantification of myocardial function by Doppler echocardiography.

ÅRØEN, Asbjørn: Cartilage Injuries and The Repair Process.

2004

TØLLØFSRUD, Per Arne: Meconium Aspiration Syndrome – An experimental study in newborn piglets.

MOE, Morten Carstens: Isoflurane and Sevoflurane – Mechanisms of action in the presynaptic terminal – An experimental study in the rat and human brain.

2002

EDVARDSEN, Thor: Assessment of regional myocardial dysfunction by tissue Doppler imaging and strain Doppler echocardiography.

KUTZSCHE, Stefan P: Effects of hypoxemia and reoxygenation on cerebral microcirculation, nitric oxide, hydrogen peroxide and excitatory amino acid concentrations. An experimental study in newborn pigs.

SCHOLZ, Tim: Studies on activation of the humoral defence system and inflammatory cells in liver transplantation.

JØRGENSEN, Pål Foyn: Immunomodulation and innate immune responses in experimental sepsis. A study with emphasis on the monocytemacrophage system.

QI, Wei: A-and B-type cardiac natriuretic peptides-markers of atrial pressure and left ventricular hypertrophy.

FRØEN, Fredrik: Effects on inflammation and nicotine on apnea, respiratory response and hypoxic-ischemic brain damage in the newborn piglet.

2001

WANG, Jacob: Cellular responses involved in sepsis caused by gram-positive bacteria and fungal pathogens.

BORTHNE, Kjell: The role of 1- og -adenoceptors in sympathetic inotropic myocardial control.

MEDBØ, Sverre: Pulmonary hemodynamics during neonatal hypoxemia and reoxygenation. Role of oxygen, endothelin-1 and nitric oxide in piglets.

HYSTAD, Marit: Origins of increased plasma concentrations of Atrial –and Brain natriuretic peptid in heart failure.

PhD-Theses

2000

ROY, Sumit: Interventional radiologic management of deep vein thrombosis.

ØIE, Erik: Autocrine/Paracrine Regulatory mechanisms and myocardial remodeling during ischemic heart failure. An experimental study in rats with special emphasis on the role of myocardial endothelin and adrenomedulin.

KJEKSHUS, Harald: Vasoactive mediators and vascular function.

KLINGE, Randi: Cardiac natriuretic peptides as endocrine markers of cardiac function. A study of changes in plasma concentrations during development and treatment of heart failure and angina pectoris

1999

BJØRNLAND, Kristin : Importance of proteolytic enzymes in tumor cell invasion.

MADSEN, Jan Erik: Neural regulation of bone repair. Experimental studies of fracture healing, posttraumatic osteopenia and bone graft incorporation in the rat.

GUNDERSEN, Yngvar: Modulation of nitric oxide values in endotoxaemia. An experimental study with special emphasis on hepatic circulation and function.

HOSAKA, Junro: Preclinical evaluation of the temporary venous "spring filter" for prevention of pulmonary embolism.

ODDEN, Jan Petter: Cerebral and Ocular Blood Flow responses in the posthypoxemic newborn piglet.

FIANE, Arnt: Pig-to-human xenotransplantation. Inhibition of hyperacute rejection.

LEHNE, Gustav : P-glycoprotein- a target for circumvention of multidrug resistance in cancer.

HVAAL, Kjetil: Attenuation of ischaemic skeletal muscle necrosis.

KAASTAD, Trine Sand: Osteoporotic fractures relative to strength of bone and muscle. Experimental rat models for studying means of prevention.

ÅSBERG, Anders: Effects of cyclosporine A on microvascular function and endothelin-1 in renal transplant recipients.

YU, Xiang-Qing: Hemodynamic and metabolic effects of nitric oxide. An experimental study in newborn piglets with acute lung injury.

SATAS, Saulias: Pharmacokinetics, posthypoxic hypothermia and cerebral microdialysis in the newborn pig.

KROHN, Claus D: Pathophysiological aspects on postoperatively, drained, untreated blood for autologous transfusion.

STEINE, Kjetil: Mechanisms of early diastolic left ventricular intracavitary flow: a study of flow patterns by colour M-mode doppler echocardiography and measurements of intraventricular driving pressures.

ANFINSEN, Ole-Gunnar: Radiofrequency catheter ablation: a study concerning electrode configuration, lesion size and potential complications.

HAALAND, Kirsti: Hypothermia as treatment of asphyxia in a newborn piglet model.

RISE, Ingunn: Cerebral blood flow during high intracranial pressure and blood loss.

1998

FEET, Bjørn: Resucitation of hypoxic newborn piglets with different oxygen concentration

MOEN, Atle: Circulatory effects of surfactant replacement. An experiental study in depleted newborn piglets.

GRØNDAHL, Tor Ø.: Ion fluxes during cerebral ischemia with special emphasis on measurements of intracellular calcium. An experimental study in vitro.

1997

KARLSRUD, Tove Sigstad: Human kininogens as multifunctional proteins. Studies focusing on their function as cysteine protease inhibitors.

TRONDSEN, Erik: Treatment of gallstone disease. Aspects of Surgical Treatment in the early laparoscopic era.

LARSEN, Morten: Isoflurane: Effects on synaptic release and uptake of glutamate and GABA.

PhD-Theses

1996

BENTDAL, Øystein H.: Immune responses in protein-energy-malnutrition. A study in patients with anorexia nervosa, carcinoma of cardia or oesophagus.

STORM, Hanne: Beta-endorphin in victims of sudden infant death syndrome.

KONGSGÅRD, Erik: Charateristics of radiofrequency current catheter ablation. An experimental study *in vitro* and *in vivo.*

BUØ, Laila: Proteolytic mechanisms in tumor spread. Studies on the role of serine proteinases in human gastrointestinal cancer.

HAUGSTAD, Tor: Mechanisms of amino acid release during cerebral ischemia.

AUNE, Arne Kristian: The muscle stabilized knee. Significance for anterior cruciate ligament injury and reconstruction.

LINE, Pål-Dag: Laser Doppler assessment of limb perfusion. Physiological and methodological aspects.

1995

STUGAARD, Marie: Intraventricular filling pattern and diastolic function. Clinical and experimental studies by color M-mode doppler echocardiography.

STEEN, Torkel: Noninvasive evaluation of left ventricular diastolic function. A critical investigation of two proposed methods.

SANDERUD, Jon: Oxygen radical induced pulmonary hypertension.

ROOTWELT, Terje: Resuscitation with 21% or 100% oxygen.

HEGSTAD, Elisabeth: Amino acids in the human brain. A study of possible roles in synaptic transmission and ischemia with special reference to glutamate and GABA.

1994

VEDDENG, Odd J.: Cardiac mechanical effects of selective positive end-expiratory pressure ventilation.

SKJELDAL, Sigmund: Acute Skeletal Muscle Ischemia. Microcirculation and histological changes in rat hindlimbs.

NORDSLETTEN, Lars: Muscle protection against fracture. A biomechanical study in the rat tibia.

SAMDAL, Frode: Suction-assisted lipectomy. A clinical and experimental study.

RISØE, Cecilie: Changes in splanchnic vascular capacitance during experimental and clinical heart failure.

STOLTENBERG, Lauritz: Biochemical, immunological and experimental studies of sudden infant death syndrome (SIDS).

1993

SOLHEIM, Erik: Effects of Bioerodible Polyorthoester on Heterotopic and Orthopic Bone Induction in rats.

KIRKEBY, Ole Jørgen: Incorporation of Bone Grafts. An experimental study in rats.

POULSEN, Jan Petter: Extracelluar oxypurines during hypoxia and reoxygenation.

KROHG-SØRENSEN, Kirsten: Laser Doppler Flowmentry in Evaluation of Colonic Perfusion.

QIAO, Zhi-Gui: Measurement of electroderman and micro-circulatory activities and responses in the human palm.

1992

KONGSGAARD, Ulf E: Changes in the plasma protease systems during open heart surgery.

PINHOLT, Else Marie: Experimental alveolar ridge augmentation studies.

STIRIS, Tom Arne: Ocular blood flow in the newborn. Effects of lights and blood gas variations on retinal and choroidal blood flow in the new born piglet.

1991

RIDDERVOLD, Fridtjov: Atrial mechanical and endocrine function during changes in heart rate and loading. Clinical and experimental studies.

PhD-Theses

BØRSUM, Tone: Investigations on cultured human endothelial cells. Cytotoxic effects of low density lipoprotein. Protein composition and surface structure.

KARLSEN, Steinar Johan: Acute effects of extracorporeal shock waves on kidney function and morphology.

1990

EMBLEM, Ragnhild: Straight ileonal anastomosis in patients with familial poliposis and ulcerative colitis.

MYRENG, Yngvar: Relaxation and filling of the left ventricle assessed by doppler echocardiography. A clinical and experimental investigation.

1989

HALL, Christian: Experimentally induced acute ischemic heart failure. A study in dogs of some endocrine and tissue blood flow responses, with special reference to effects of angiotensin converting enzyme inhibition.

STORDAHL, Arvid: Water-soluble contrast media in obstructed and in ischemic small intestine. A clinical and experimental study.

1988

ROSSELAND, Arne R: Endoscopic papillotomy. A clinical and experimental study.

1987

BRØNDBO, Kjell: Reinnervation of laryngeal muscles. An experimental study in dogs.

SOLHEIM, Ludvig Fjeld: Influence of nonsteroidal anti-inflammatory drugs on bone. A mechanical and biochemical study with acetylsalicylic acid and naproxen in rats.

MATHISEN, Svein R: Healing of synthetic and biological protheses in the cardiovascular system. An experimental study.

1986

RØNNINGEN, Helge: Bone ingrowth in porous fiber titanium Experimental investigation of ingrowth for the purpose of fixation of weight-bearing skeletal implants.

GLENNÅS, Anne: Experimental studies on resistance to certain cytotoxic effects of three gold-compounds, with special reference to metallothionein.

BARTH, Elin: Bioactive, non-porous and bioinert, porous implant materials: comparison of two principles for cementless prosthesis fixation.

1985

ENDRESEN, Liv: Metallothionein. Experimental studies on its protective function against certain anticancer agents.

ENDRESEN, Gerhard KM: Immunological studies on human platelet proteins. Findings in relation to auto-immune diseases.

SMITH-ERICHSEN, Nils: Septicemia evaluated by means of chromogenic peptide substrate assays. A retrospective study in man.

SCHRADER, Harald: Dynamics of intracranial expanding masses, An experimental study with particular reference to the Cushing response.

1983

LÆRUM, Frode: In vivo and in vitro studies of contrast media effects in lower limb phlebography.

BAKKA, Arne: Studies on possible functions of metallothionein.

1982

BREKKE, Inge B: Transplantation of the duct-occluded rat pancreas. Long-term endocrine function and metabolic effects.

1981

AMLIE, Jan P: Mode of actions of digitoxin and digoxin on cardiac electrophysiology and inotropy.

PhD-Theses

GULDVOG, Ivar: Gastric acid and pepsin secretion in dogs. The role of vagal innervation.

FRENG, Atle: Transversal growth of the maxillary base following resections of the mid-palatial suture - a biometrical and morphological study in the man and the cat.

EKELAND, Arne: Influence of calcitonin on healing and intact bone and skin. A biomechanical and biochemical study in rats.

1980

WIE, Henrik: Effects of cyclophosphamide on connective tissues. An experimental study in rats.

HJORT, Erling F: An experimental study on hematogenous pyelonephritis in the rat and its possible bearing on human pathology.

1979

HENRIKSEN, Tore: Interactions of serum lipoproteins with human endothelial cells in culture.

HOLEN, Jarle: The quantification of flow obstructions in the mitral flow channel with doppler echo-cardiography.

ENGESÆTER, Lars B: Biomechanical and biochemical effects of antibiotics on bone and skin.

LILLEAASEN, Per: Hemodilution in open-heart surgery.

WILLE, Sven Øivind: Numerical models of arterial blood flow.

1978

AASEN, Ansgar O: Activities and inhibition of proteases found in plasma during endotoxin shock. An experimental study in dogs.

BERG, Knut Joachim: Effects of acetylsalicylic acid on renal function.

SLAATTELID, Olav: Studies in hypothermic kidney perfusion.

1977

SAUGSTAD, Ola Didrik: Hypoxanthine as an indicator of tissue hypoxia. A study of plasma, cerebro-spinal fluid and brain tissue concentrations.

KVEIM, Morten: The acetate ion as a source of base. Experimental and clinical studies.

ENGE, Ivar: Angiography in regeneration of the liver. An experimental and clinical study.

1976

BERGAN, Anstein: Aspects of bilirubin metabolism in normal and cholestatic dogs.

1975

BLIX, Arnoldus Schytte: The elicitation and regulation of the cardiovascular responses to diving.

SUDMANN, Einar: Vital microscopy of bone remodelling in rabbit ear chambers.

HØIE, Johan: Hemodynamic changes following acute thrombin-induced intravascular coagulation. An experimental study in dogs.

LANGELAND, Norvald: Effects of oestradiol on bone collagen metabolism. An experimental study in female rats.

1974

NUSTAD, Kjell: The relationship between urinary and kidney kallikrein in the rat.

BENUM, Pål: Autogenous transplantation of apophyseal cartilage to osteochondral defects of joints. An experimental study in dogs.

1973

JAKOBSEN, Arnt: Rabbit anti-rat lymphocyte serum. A study on the influence of the antigen dose and immunization schedule on some in vitro characteristics and in vivo immunosuppressive potency.

NESBAKKEN, Ragnar: Aspects of free fatty acid metabolism during induced hypothermia.

1972

SEMB, Bjarne KH: Cardiac transplantation. An experimental study in dogs.

1970

OFSTAD, Egil: Formation and destruction of plasma kinins during experimental acute hemorrhagic pancreatitis in dogs.

TVETER, Kjell: Studies on selective uptake and metabolism of testerone-3H in the prostate and the seminal vesicles of the rat.

TRIPPESTAD, Arne: Aspects of host defence reactions during experimental intestinal strangulation obstruction in rats.

UNHJEM, Olav: Studies on the interaction between androgen and macromolecular components of the rat ventral prostate.

1969

SANDER, Sten: The uptake of oestradiol in normal breast tissue and in induced breast cancer of the rat.

2009:

RISØE, Petter: Cyclic AMP - A promising approach for immunomodulation in sepsis?

RYGH, Una: The role of liver X receptors and adenylyl cylcases in experimental sepsis.

2007

FOSSDAL; Guri: The presence of aquaporins in brain tumours and brain tumour stem cells.

SKJELLEGRIND, Håvard: The role of Ca2+ stores and mitochondria in neuronal ischemia.

ÅGREN, Joanna: Modulation of endotoxemia by CpG DNA and the liver X receptor.

2006

MYHRE, Anders E.: Pathophysiology of endotoxemia

STUESTØL, Jon Fredrik: Systemic inflammation caused by emerging pathogens

Egil Amundsen Lecture

22.09.06	**Professor Iver A. Langmoen** "Stem cells in the Adult Human Brain"
27.04.09	**Professor Olle Korsgren** " Clinical Islet Transplantation: an emerging therapy for patients with type I diabetes"
18.10.10	**Professor Henrik Kehlet** "Fast-track surgery – what is in it and why should we do it?"
11.04.11	**Professor Wolfgang G. Junger** "Purinergic control of immune cell function"

Professor Wolfgang G. Junger was awarded the Amundsen medal and diploma by professor Ansgar O. Aasen.