Anesthesiology and critical care research group- experimental and clinical studies

Group leader: Tor Inge Tønnessen, MD, PhD, Professor

Our research group activities encompass projects of basic laboratory medical and technology sciences, through translational research with animal experiments to bring the results into human studies starting with limited observational studies paving the way into randomized controlled trials. We cover a broad spectrum of topics as described under the different projects.

The research group is an "umbrella organization" for several subgroups with different projects. Currently there are 27 members in the group. We have 12 PhD candidates, 4 post-docs and the rest are senior researchers or professors.

Main research projects:

Experimental and clinical studies on organ ischemia, ischemia/reperfusion, inflammation (Biosensor Research Group; head Tor Inge Tønnessen)

Despite improvements in diagnostic tools and monitoring devices, we still lack good technology that can in real-time monitor the status of vital organs. For instance, after liver transplantation in children, several of the patients get a hepatic artery thrombosis that with current technology is detected too late to save the liver. After coronary artery bypass surgery perioperative infarction occur in 5 – 15 % of the patients and is in many cases not detected in time for effective treatment to occur. Postoperative complications after abdominal surgery like bowel perforation, anastomotic leakage, ischemia, infection and others are also detected too late, and the complications develop into peritonitis and sepsis with high mortality, which could be prevented by early detection. Today, only systemic parameters like blood pressure, heart rate, or with extended monitoring continuous cardiac output are monitored and monitoring technology is improved mainly around these parameters. **The main problem is that there may be extensive organ pathology despite normal values of the systemic parameters. It is therefore a large medical need for monitoring equipment that can give information continuously in real time on the status of individual organs.**

The Biosensor Research Group has main focus on development of intra-organ monitoring systems supporting the hypothesis that pathology can be detected earlier, and treatment be carried out when the damage is still reversible to improve the outcome of the patient. This research is multidisciplinary and translational (physicians in several specialties, physicists, medical technology specialists, immunologists, material specialists and several others participate in our research).

Implantable sensors used in the studies.

We have through several years of research found that **intra-organ PCO_2** is a highly specific and sensitive method for detecting ischemia in the heart, liver, pancreas, intestine, kidney, skeletal muscle and subcutaneous tissue. Because lactic acid generated under ischemic conditions is buffered by bicarbonate to form CO_2, which accumulate in the organ, intra-organ PCO_2 will increase several folds under ischemic conditions. Based on these findings we

reasoned that intra-organ measurement of PCO_2 would have a large clinical potential for detecting ischemia in virtually any organ. There were no available sensors for clinical use available, so **we invented a new conductometric method for measuring pCO_2**. The sensor is 0.7 x 0.7 mm and is constructed in a way enabling insertion in any organ. Data from the sensor is sampled every second, thus we get true real-time measurements and ischemia is detected immediately when it occurs.

Studies carried out with the implantable sensors

Animal studies: We have carried out several studies and only some of them will be mentioned as examples. By using both IscAlert and microdialysis in the same animal for detection of ischemia we can compare the pros and cons of each method. ***Cardiac studies:*** We inserted IscAlert sensor and microdialysis catheters in the myocardium, one in the supply area for LAD, and one in the area supplied by the circumflex artery. We occluded LAD for 1, 3, 5, and 15 minutes with 30 min between each occlusion. IscAlert detected ischemia already after one minute, and PCO_2 rose proportionally with the increasing lengths of occlusion whereas 20 min was required for the microdialysis catheters to detect ischemia. In a second study we carried out CABG with LIMA to LAD. By gradually occluding the bypass graft, hypoperfusion was detected by IscAlert already at 25% decreased blood flow, and decreasing blood flow was highly inversely correlated with increasing PCO_2. **Thus, the ability for immediate detection of cardiac ischemia per- and postoperatively is apparent.**

Studies of intraabdominal organs*:* Based on the clinical important problem that vascular occlusion after liver transplantation frequently occur we wanted to explore if IscAlert was able to detect hepatic artery occlusion and portal vein occlusion. Sensors were inserted in the liver and between loops of small bowel. Flow probes were placed on the hepatic artery and portal vein to monitor blood supply. Vascular occluders were placed around the vessels. Full occlusion of the hepatic artery resulted in immediate increase in PCO_2. With gradual occlusion, significant rise in PCO_2 was found at 40% reduction of blood flow. Occlusion of the portal vein gave rise to immediate increase in intestinal PCO_2 and with gradual reduction of blood flow, significant rise in PCO_2 was found with 40 % flow reduction. IscAlert data correlated well with anaerobic metabolism as detected by the microdialysis catheter, but

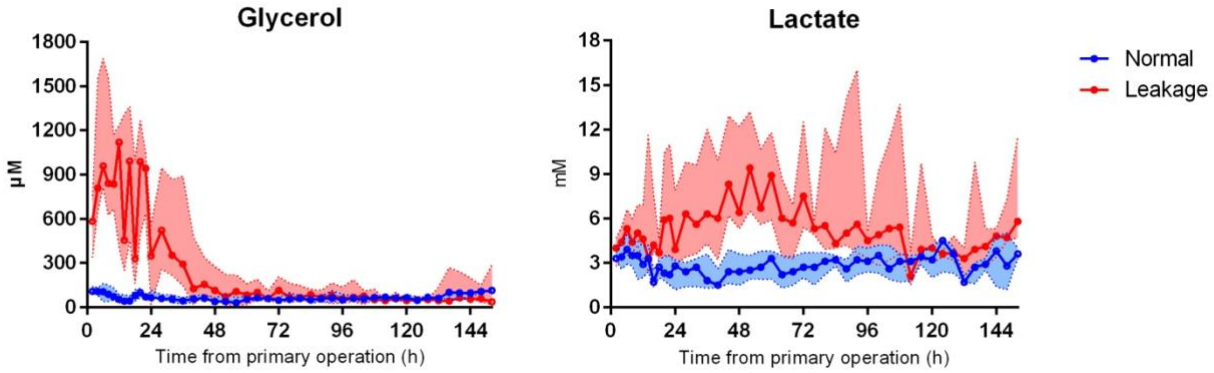
Clinical studies

Studies on liver transplanted patients

Following an in vitro microdialysis catheter study which determined optimal pore size of the catheter membrane, composition of fluid and pump velocity, a pilot study on 20 liver transplanted patients was performed. The results indicated that the method may detect rejection in addition to ischemia, but due to small number of patients the results could not be statistically validated. Accordingly we performed a large study on 73 liver transplanted patients. This is by far the largest study worldwide for real-time detection of postoperative complications after liver transplantation, enabling us to present statistically validated results: Metabolic status of the liver was monitored and the metabolites were analyzed bedside with minimal delay in results, so the patients were immediately taken to the operating theater for removal of thrombus or vascular reconstruction. Bedside differentiation between rejection and ischemia was possible.

Hepatic artery occlusion was detected with 100% sensitivity and specificity with bedside analysis of lactate and pyruvate. Under these ischemic conditions lactate increases, pyruvate decreases and the lactate:pyruvate (LP) ratio increases. Rejection was detected by increase in lactate *and* pyruvate and no increase in LP-ratio. Rejection was detected by microdialysis median 3-7 days before circulating liver enzymes increased. All rejections were verified with biopsy. Rejection was detected with 88% sensitivity and 83% specificity.
In 50% of the episodes of vascular occlusion, metabolic alterations as measured by microdialysis were the only indicator of tissue hypoperfusion. In the remaining cases, pathologic intrahepatic metabolism was observed several hours before current standard of care. **This underscores the great advantage of intra-organ monitoring compared to current methods for detection!**
We have also carried out a study on specifically on the pediatric population. We found the incidence of both vascular catastrophes and rejection was considerably higher than in the adult population. Accordingly, the official post-transplant regimen at Oslo University hospital has been changed and we now insert microdialysis catheters in all pediatric liver transplant patients. This shows that our research has changed the standard of care for these patients.

Whipple´s procedure (pancreaticoduodenectomy)
Pancreatic cancers are among the deadliest cancers with 5 year survival < 10 %. This is due to lack of diagnostics to detect the disease before it has disseminated. However, in patients with cancers detected at a time when they are eligible for surgery, has a better prognosis and is the only treatment modality with cure as a realistic goal. Pancreaticoduodenectomy (Whipple´s procedure) is a surgical procedure where a large part of the pancreas, the duodenum, and the bile ducts are removed in patients with pancreatic or duodenal cancer. Patients undergoing pancreaticoduodenectomy are facing postoperative complications such as fistulas (anastomotic leakage), ischemia, infections and hemorrhage. It has been estimated that 20-65 % of the patients experience at least one complication and many become critically ill. To improve the results early detection of dangerous and potentially reversible complications are of immense importance.



Accordingly, we studied if microdialysis catheters placed at pancreatic and biliary anastomosis would detect anastomotic leakage at an earlier time that current standards of care. 35 patients were investigated. After finishing the surgical procedure, three custom made microdialysis catheters with 300 mm shaft length and a 30 mm long dialysis membrane with a molecular weight cutoff of 100 kDa (CMA 65, M Dialysis AB, Stockholm, Sweden) were inserted percutaneously through a hypodermic needle and fixated to surrounding tissue in near vicinity (<1 cm) of the pancreaticojejunostomy, the hepaticojejunostomy, and the

surface of the cauda pancreaticus. The microvial samples were analyzed at the bedside for glucose, glycerol, lactate, and pyruvate (Iscus, M Dialysis AB, Stockholm, Sweden) hourly during the first 24 hours and thereafter every second hour. The lactate to pyruvate ratio (L/P) was automatically calculated. The catheters were kept in situ until they malfunctioned or the patient was discharged.
Of the 35 patients included, nine patients had very high glycerol values in the microdialysis catheter at the pancreatic anastomosis. All these patients developed pancreatic fistula (anastomotic leakage) verified either by high amylase activity in drained fluid at postoperative day (POD) 3 or later, or it was diagnosed during reoperation. From POD 2 lactate increased to high levels and was increased for many days. A typical example is shown in the figure. Thus, early increase in glycerol means that pancreatic enzymes like phospholipases are released into the peritoneum where it cleaves phospholipids to glycerol and free fatty acids. This elucidates an inflammation/infection due to erosion of tissue by pancreatic enzymes. One to three days after leakage in the pancreatic anastomosis, four patients experienced also a biliary leakage most likely due to erosion caused by leakage of pancreatic fluid. This study clearly shows that in several cases, complications like serious pancreatic leakage can be detected earlier by monitoring by microdialysis catheters than by current standards of care. In this study we found that an elevated glycerol concentration (>400 μmol/L) during the first 12 hours post-surgery, had a 100% sensitivity and 93% specificity for POPF. This was an observational study, and the microdialysis data were blinded to the surgeons. Accordingly, no treatment was initiated based on the microdialysis results.

Monitoring of patients with microdialysis following pancreaticoduodenectomy—the MINIMUM study: randomized controlled trial
Trial registration: Clinicaltrials.gov (NCT03631173).

With close monitoring of this high-risk anastomosis, we aim to detect complications at an early stage and intervene before the effects of leakage become severe. Therefore, based on the result from the observational study, we have designed a randomized study to test the hypothesis that microdialysis monitoring will improve patient care after pancreaticoduodenectomy.

Background: Postoperative pancreatic fistula after pancreatoduodenectomy is a much-feared complication associated with substantial mortality and morbidity. The current standard for diagnosing postoperative pancreatic fistula, besides routine clinical examination, include radiological examinations, analysis of pancreatic drain amylase activity, and routine blood samples. Another promising method is by intraperitoneal microdialysis to monitor intraperitoneal metabolites measured at the pancreaticojejunostomy, thereby detecting what occurs locally, before chemical events can be reflected as measurable changes in systemic blood levels.
Methods: The MINIMUM study is a prospective, randomized, controlled, single center enrolling 200 patients scheduled for open pancreatoduodenectomy comparing the microdialysis method to the “standard of care.” Half of the included patients will be randomized to receive an intraperitoneal microdialysis catheter implanted at the end of surgery and will be monitored by microdialysis as an additional monitoring tool. The other half of the patients will not receive a microdialysis catheter and will be monitored according to the current standard of care. The primary objective is to evaluate if the microdialysis

method can reduce the total length of stay at the hospital. Secondary endpoints are the frequency of complications, length of stay at the hospital at our institution, catheter malfunction, number of infections and bleeding episodes caused by the microdialysis catheter, patient-reported quality of life and pain, and cost per patient undergoing pancreatoduodenectomy. The patients will be randomized in a 1:1 ratio.
Discussion: Intraabdominal microdialysis could potentially reduce morbidity and mortality after pancreatoduodenectomy. Furthermore, there is a great potential for shortening the in-hospital length of stay and reducing the financial aspect considerably. This study may potentially open the possibility for using microdialysis as standard monitoring in patients undergoing pancreatoduodenectomy. The hypothesis is that the microdialysis method compared to "standard care" will reduce the total length of hospital stay. All patients are included, database closed and we are analyzing the data.

New method for real-time detection of tissue ischemia; IscAlert study

- REK sør-øst C : (REK) – 66553
- Clinicaltrials.gov id: NCT04879875

Eudamed CIV ID CIV-NO-20-07-034084

Design: Open, prospective, descriptive, single-center observational study in patients undergoing orthopedic limb surgery with planned use of a tourniquet.
Background: There are currently no good monitoring systems on the market to detect insufficient blood supply in real time, so in this study we wanted to investigate whether a Norwegian-developed medical sensor (IscAlert) would be able to do this.
Carbon dioxide, pCO2, measured directly in the tissue/organ is an important marker for whether there is sufficient blood supply. The sensor IscAlert has been developed to be able to measure pCO2.
Method: This is the first human study designed to investigate the feasibility and safety of the IscAlert™ system. Patients included were patients scheduled for elective extremity surgery with the use of a perioperative tourniquet >30 minutes. Three sensors (two intramuscular, one subcutaneous) were placed in the intervention arm/leg (ischemia), and two sensors (one intramuscular, one subcutaneous) in the control arm/leg (no ischemia). The sensors in the control limb were removed before the end of anaesthesia, while 40% of the patients kept the sensors in the operated limb for up to 72 hours to investigate whether the sensor continued to show stable readings. The patients were followed throughout the hospital stay, and a telephone consultation was carried out 7 and 30 days after discharge from hospital.
Result:
First patient included on 25 May 2021. Last patient at 30-day follow-up was 24 July 2022. Fifty patients (32 men) >18 years (mean age 53.5 years) were included, five were excluded after inclusion. Recordings of pCO2 three minutes before tourniquet release showed a statistically significant difference of 3.9 kPa ±0.24 ($p<0.0001$) in mean pCO2 levels between extremities. No pain, inflammation/infection or bleeding more than 5 ml from the insertion site was recorded, neither during the operation nor during the follow-up period. During the study period, the patients did not register any pain from the sensor (NRS 0). Five adverse events were noted, none related to study procedure or examiner.
Conclusion: The IscAlert system is a safe and reliable monitor of tissue ischemia with continuous and real-time measurements.

Detecting tissue Ischemia in reconstruction flaps by a novel CO2 biosensor; The DIMENSION-study.

- REK KULMU A 589762
- Clinicaltrials.gov id: NCT05487820
- Eudamed CIV ID CIV-22-06-039727

Design: Open, prospective, single-center intervention study in patients requiring reconstructive surgery.
Background: One of the main causes of serious illness and death is ischaemia. If the ischemia is detected in time, it will be possible to save the organ. The study will use the biosensor IscAlert, which in real time (continuously) can measure carbon dioxide (PCO2) in the tissue, and which is an indicator of ischaemia.
Method: Sensor(s) are inserted into musculature and subcutaneous tissue in a patch of tissue in patients undergoing elective reconstructive surgery in the ENT area. The purpose of the study is to detect ischemia in real time in the patch by the fact that the CO2 level in a tissue with a reduced/suspended blood supply will have higher CO2 values than in a tissue patch where the blood supply is good. If impaired blood circulation is detected, the closed blood supply can be opened at such an early stage that the patch still survives. 1 sensor is also placed in a healthy tissue in the neighboring area of the operated patch. The PCO2 and temperature levels are compared between the patch and control tissue and between the patches receiving ischemia vs. those who do not get ischemia. The sensors are removed after 10 days, possibly before depending on reoperation or other events.
Progression: Inclusion is ongoing. First patient included 10 October 2023. 7 included per January-24. Inclusion period assumed to be 17 months, but can be extended further. 56 patients will be included prospectively. Interim analysis planned.
Preliminary result: 8 patients included so far, 7 with a good outcome. In one patient ischemia was detected by IscAlert and the patient was taken to the OR for reoperation.

Early Discovery of Ischemia after Replantation Surgery of the extremities; EDIR study

- REK SØR-ØST KULMU B 340841
- Clinicaltrials.gov id: NCT05297266
- Eudamed CIV ID CIV-NO-21-11-038006

Design: Open, prospective, single-center intervention study in patients requiring replantation surgery.
Background: There are currently no good instruments on the market to detect insufficient blood supply in real time, so in this study we wanted to investigate whether a Norwegian-developed medical sensor (IscAlert) would be able to do this.
Carbon dioxide, pCO_2, measured directly in the tissue/organ is an important marker for whether there is sufficient blood supply. The sensor IscAlert has been developed to be able to measure pCO_2.
The study is designed to compare pCO_2 levels in replanted extremity that develop thrombosis/ischemia versus those patients where no ischemia occurs.
Method: IscAlert sensors are inserted into the replanted extremity (finger) at the end of the primary operation. One sensor is also placed in an undamaged control finger. Monitoring period is 10 days. The PCO_2 and temperature levels are compared between the replant finger

and the control finger and between the fingers that get ischemia vs. those who do not get ischemia. If impaired blood circulation is detected, the closed blood supply can be opened so early that the finger(s) still survives. The sensors are removed after 10 days.
Progression: Inclusion is ongoing. First patient included on 12 January 2023. Inclusion period assumed to be 17 months. One will include 80 replanted extremities (approximately 60 patients) patients prospectively. Interim analysis planned.
Preliminary results: Data so far confirm that circulatory disturbances in replanted fingers are detected by measuring an increase in CO_2 and a drop in temperature. IscAlert detect ischemia earlier that current standard of care.

Detecting tissue Ischemia in reconstruction flaps by a novel CO_2 biosensor; The DIMENSION-study.
Design: Open, prospective, single-center intervention study in patients requiring free flap reconstructive surgery.
Background: One of the main causes of serious illness and death is ischaemia. If the ischemia is detected in time, it will be possible to save the organ. The study will use the biosensor IscAlert, which in real time (continuously) can measure carbon dioxide (PCO_2) in the tissue, and which is an indicator of ischemia.
Method: Sensor(s) are inserted into muscle and/or subcutaneous tissue in a free plap of tissue in patients undergoing elective reconstructive surgery in the ENT area. The purpose of the study is to detect ischemia in real time in the reconstructed flap by the fact that the CO_2 level in a tissue with a reduced/suspended blood supply will have higher CO_2 values than in a tissue patch where the blood supply is good. If impaired blood circulation is detected, the closed blood supply can be opened at such an early stage that the reconstructed flap still survives. One control sensor is also placed in a healthy tissue in the neighboring area of the operated flap. The PCO_2 and temperature levels are compared between the flap and control tissue and between the patches receiving ischemia vs. those who do not get ischemia. The sensors are removed after 10 days, possibly before depending on reoperation or other events.
Progression: Inclusion is ongoing. First patient included 10 October 2023. 12 included per February-24. Inclusion period assumed to be 17 months, but can be extended further. 56 patients will be included prospectively. Interim analysis planned.
Preliminary result: Of the included patients two had postoperative ischemia detected by IscAlert and underwent reoperation.

Developing novel methods for detection of ischemia/reperfusion tissue harm in abdominal organs (Strand-Amundsen, Hou, Tønnessen)
We have used sensors to investigate the passive electrical properties, active electrical properties, imaging and wavelength spectroscopical properties of intestine and liver. These tissues were investigated in vivo and ex vivo with large animal models, and ex vivo with human tissues, to assess how sensor parameters change with the state of the tissue (perfusion, ischemia, structural and cellular injury levels, transplantation).
Examples of projects related to the small intestine:
Investigation of the feasibility of VIS-NIR spectroscopy to differentiate between degrees of ischemia-reperfusion injury in porcine small intestine. The study found that the VIS-NIR spectroscopy method together with a PLS-DA model showed promising results and can be

well-suited as a real-time intraoperative method for assessing intestinal ischemia-reperfusion injury, due to its easy-to-use and non-invasive nature.
We studied the characteristics of various degrees of ischemic-reperfusion injury of the small intestine using dielectric relaxation spectroscopy. The dielectric constant and conductivity showed clear differences between healthy, ischemic and reperfused intestine segments. Machine learning models were employed to classify viable and non-viable segments based on the frequency dependent dielectric properties of the intestinal tissue, providing a method for fast and accurate intraoperative surgical decision-making.
Investigation of changes in the passive electrical properties of ex vivo porcine and human intestine during ischemia. We compared microdialysis measurements and histological findings from biopsies with the time development of impedance parameters, to assess how the changes in a pig model compare to changes in human tissue.
We investigated the feasibility of using microscopic images taken from the surface of the small intestine in-vivo in a pig model, combined with deep learning, to assess ischemia-reperfusion injuries. Different deep learning models were evaluated and compared, ResNet50 outperformed the Bayesian CNN, CNN with decision-level fusion and traditional CNN. The uncertainty estimation provided by the Bayesian CNN model can be important in clinical settings. In combination with the explainable AI - SHAP method, not only can we assess the viability state of the small intestine, at the same time, we can explain what regions of the intestine the model used as a basis for decision making.
Examples of projects related to the liver:
We monitored the microwave dielectric properties of porcine livers to investigate how these properties associate with ischemia-reperfusion injury. The livers in this study were divided into three groups: control with no injury (CON), biliary injury by hepatic artery occlusion (AHEP), and overall hepatic injury by static cold storage (SCS). We found that the changes in dielectric conductivity can be used to differentiate different injury conditions after normothermic machine perfusion.

Sterile inflammation in acute critical illness and organ transplantatation (Pischke et al)

Sterile inflammation is dependent on the innate immune system. We aim to attenuate this inflammation to enhance organ and systemic protection and regeneration.
Ischemia/reperfusion injury (IRI) is a key challenge in several widespread diseases like heart infarction, circulatory arrest, traumatic injury, and organ transplantation. These conditions have different etiologies, but the underlying mechanism is the same, i.e. cell death due to the stop of blood supply during ischemia and aggravation of organ damage after the restoration of blood supply during reperfusion.
The projects within this topic aim to resolve this challenge by significantly reducing tissue injury in the course of IRI with both preclinical and clinical studies.

Projects are listed here with links to short project descriptions:

1. Mechanisms of sterile inflammation
    a. Mitochondrial DNA
    b. Misfolded proteins (fibrils)
    c. Stem cell survival and interplay with the innate immune system
2. acute critical illnesses
    a. Cardiac arrest and traumatic brain injury

    b. Liver transplantation (SALT study)
    c. Point-of-Care assessment of Complement Activation in critically ill patients
3. Ex situ machine perfusion of donor organs before organ transplantation
    a. Liver transplantation
    b. Kidney transplantation

1a. Mitochondrial DNA elicits innate immune and specifically complement system activation

This project aims to develop a new therapeutic approach targeting the interaction between mitochondrial DNA (mtDNA) and the innate immune system, namely complement and Toll-like receptors (TLR). mtDNA is released from injured cells and induces sterile inflammation in diseases like e.g. cardiac arrest, trauma and transplantation. We aim to test the hypothesis that ***mtDNA and the complement system augment each other's effects and thus sterile inflammation in a vicious circle; mtDNA activates complement and complement activation releases mtDNA from cells.*** The major aim of the project is to (i) reveal the mechanism behind mtDNA-complement system/TLR interaction and (ii) break this vicious circle. This is an in vitro pre-clinical project in collaboration with the Department of Immunology.

1.b Misfolded proteins activate the innate immune and specifically complement system activation

This project aims to investigate how misfolded proteins (aka. Fibrils and amyloids) activate the innate immune system. Although the precise function of amyloids in our bodies remains unknown, accumulating evidence suggests that they play a significant role in immune system dysregulation, paving the way for the development and progression of several illnesses. Amyloids are implicated in a range of disorders spanning different organ systems and include systemic amyloidosis, Alzheimer's disease, type 2 diabetes, and many more. Each of these illnesses exhibits unique clinical manifestations, highlighting the diverse impact amyloids can have on various organs and biological processes. Our primary objective is to decipher how amyloids modulate innate immune responses, particularly the complement system. We aim to describe this novel interaction and investigate if targeted innate immune system inhibition can alter these detrimental processes. This is an in vitro pre-clinical project in collaboration with the Department of Immunology, OUS and the Department of hematology, Mayo Clinic, Rochester, USA.

1c. Stem cell survival and interplay with the innate immune system

Stromal/stem cell therapy emerges as a promising avenue, particularly in inflammatory scenarios like organ grafts for transplantation. Stem cells are recognized for their remarkable ability to elicit pro-angiogenic, anti-inflammatory, and antioxidant effects through the secretion of diverse molecules such as mRNAs, miRNAs, and proteins. Decidua stem cells (DSCs) exhibit these desired traits and have in addition low tumorigenicity and thrombogenicity, the ability to regulate immune responses and differentiate into organ-specific cells. Our research group has access to clinical-grade DSCs. However, DSCs are short-lived after infusion and the mechanism is unknown. Here, we investigate if DSCs are affected by the innate immune system in an in vitro approach and clinical study investigating the effect of DSCs on graft-versus-host disease in patients after allogenic bone-marrow transplantation. This is an in vitro pre-clinical and clinical project in collaboration with the

Department of Immunology, Department of Surgical Research, and the Department of hematology, all at OUS.

2a. Cardiac arrest activates the innate immune system with implications for long-term prognosis

Acute brain injury and cardiac arrest are the number one causes of death and disabilities in the world. Over half of the patients develop long-term complications and have lower survival rates over a long period compared to healthy controls. The common hallmark for both conditions is Ischemia/Reperfusion Injury (IRI). IRI is known to impact cellular metabolism and may lead to the activation of inflammatory mechanisms. Here, we aim to describe the impact of innate immunity in general, in particular complement activation on short- and long-term outcomes compared to healthy controls. Blood samples and clinical data from two observational studies are assessed and compared to matched healthy control populations. We have already found that mild traumatic brain injury led to a significant systemic increase of cytokines compared to matched healthy controls, which persisted for up to one year. These findings are remarkable as previous studies had reported prolonged systemic inflammation in severe traumatic brain injury only and had been associated with worse clinical outcomes. While the clinical outcome was not assessed in this study, clear associations with demographic and clinical variables were found. Resuscitated cardiac arrest patients with poor outcomes (defined as coma or death) demonstrated higher levels of the complement activation products C3bc and sC5b-9. sC5b-9 at admission was independently associated with poor outcomes and sub-sequent endothelial activation. In acute situations like cardiac arrest and circulatory shock due to heart failure, we demonstrated that the activation product sC5b-9 is the most sensitive and accurate assay for measuring the level of complement activation compared to C3bc and the sC5b-9/C5 and C3bc/C3 ratio.

2.b Ischemia Reperfusion Injury in Liver transplantation - Study of Oxidized Albumin in Liver Transplant patients (SALT)

In this study, we aim to show whether liver failure patients receiving liver transplantation have increased oxidized albumin, decreased albumin function, and increased oxidative stress at seven different time points pre-, per- and postoperatively indicative of the pre-transplant state and ischemia/reperfusion injury upon liver transplantation. In addition, we will assess activation of the innate immune system and the association thereof to pre-transplantation liver state, and oxidized albumin at the same time-points and outcome. This is a clinical pilot study with the inclusion of liver transplant patients at OUS and Karolinska Sykehuset, Stockholm, Sweden.

2c. Point-of-Care assessment of Complement Activation in critically ill patients

This project focuses on evaluating the feasibility of a Point of Care (PoC) method for measuring complement activation in critically ill patients. The complement system, traditionally known for combating infections, also plays a crucial role in immune surveillance and housekeeping activities. However, when the balance between complement activation and regulation is disturbed, it can contribute to adverse processes and various clinical conditions.

Currently, identifying patients for whom complement activation is a significant factor in their disease process poses a challenge. Existing diagnostic methods require time-consuming laboratory assessments, leading to delays in timely and appropriate treatments. To address

this issue, HycultBiotech has developed a CE-marked PoC platform for measuring complement proteins C3, C3d, and complement activation product sC5b-9. This PoC method can provide rapid results within an hour, making it suitable for use in critical care settings. The aim of this project is to compare the results of the PoC complement activation assessment with standard laboratory measurements.
HycultBiotech will provide the PoC complement assessment machine and consumables, while the control measurements will be conducted at the complement laboratory at the Department of Immunology, Rikshospitalet, Oslo University Hospital.
Ultimately, this project aims to validate and establish the accuracy and reliability of the PoC method for measuring complement activation. If successful, this PoC approach could revolutionize complement activation diagnostics, enabling faster and more accurate treatment decisions for critically ill patients. This project is a collaboration with HycultBiotech, The Netherlands and the Department of Immunology, OUS.

3a. Ex situ machine perfusion of liver donor organs
Ex situ liver machine perfusion is an innovative procedure designed to preserve and assess donor livers before transplantation. Traditionally, donor livers are stored on ice, which provides a limited window of time for transplantation. However, with ex situ liver machine perfusion, the organ is connected to a specialized perfusion machine that mimics the conditions of the human body. This allows for continuous perfusion, oxygenation, and assessment of the liver's function and viability. By keeping the organ in a more physiological state, ex situ liver machine perfusion extends the preservation time, enabling thorough evaluation and potential treatment of the liver before transplantation.

The current research status regarding the clinical use of ex situ liver machine perfusion is promising. Numerous studies have demonstrated its potential benefits, including improved organ quality assessment, reduction in primary graft dysfunction, and the possibility of using suboptimal livers that would otherwise be discarded. Additionally, ex situ machine perfusion allows for the assessment and treatment of the liver's innate immune system activation, which plays a vital role in transplant outcomes.

The innate immune system is the body's first line of defense against pathogens and tissue damage. However, during the organ transplantation process, activation of the innate immune system can lead to inflammation, tissue injury, and graft rejection. Ex situ liver machine perfusion provides a unique platform to assess and manipulate the liver's innate immune response. By modulating the immune system activation during perfusion, researchers aim to mitigate inflammation and reduce the risk of organ rejection.

Though significant progress has been made, the translation of ex situ liver machine perfusion from research to clinical practice is still ongoing. Further studies are needed to optimize protocols, evaluate long-term outcomes, and establish the safety and efficacy of this technique in large-scale clinical settings. Additionally, comprehensive investigations regarding the interaction between ex situ perfusion and the liver's innate immune system activation are crucial for refining the procedure and unlocking its full potential.

The future holds great promise for ex situ liver machine perfusion, as it represents a significant advancement in liver transplantation. By prolonging organ viability, assessing liver

function in real-time, and modulating the innate immune system activation, this technique has the potential to revolutionize transplant medicine and improve patient outcomes. It might even get implemented for acute liver failure patients with the aim to restore liver function and thus prevent transplantation.

This project is the most extensive project of the research group and involves preclinical studies on the effect of machine perfusion with different perfusate solutions on injured livers, the innate immune system, and the metabolism.

The preclinical pig study has been concluded (and reports are in the writing process), while the clinical studies are started or about to start.

Further information can be found here: [link to EVINCE og EVOLVE studiene.docx]

This project is a collaboration with the Department of Immunology, Clinic of technical innovation, Department of Transplant Surgery, all at OUS and the University Medical Center Groningen, Netherlands.

3b. Ex situ machine perfusion of kidney donor organs

In this project, we strive to understand and enhance this groundbreaking technique for kidney preservation and transplantation.
Ex situ kidney machine perfusion involves the use of a specialized device to perfuse and maintain a donated kidney outside of the body. This technique enables the assessment and optimization of the organ's viability and function before transplantation. During ex situ perfusion, the kidney is connected to a machine that delivers a controlled flow of oxygenated blood or preservation solution, providing the necessary nutrients to sustain the organ.

One critical aspect of ex situ kidney machine perfusion is the interaction between the perfused kidney and the innate immune system. Activation of the innate immune system is a natural response to injury or transplantation, but it can also have both positive and negative effects on the perfused kidney.While some level of inflammation is necessary for tissue repair and regeneration, excessive or prolonged innate immune system activation can have detrimental effects on the perfused kidney.
Our research focuses on deciphering the intricate relationship between ex situ kidney perfusion and innate immune system activation. By investigating the underlying mechanisms and developing targeted interventions, we aim to optimize the preservation and transplantation of kidneys. Ultimately, our goal is to enhance transplant outcomes and increase the number of viable organs available for transplantation.
Currently, we investigate pre-clinically pig and discarded human kidneys in collaboration with the Department of Immunology, OUS, the Department of nephrology, Aarhus University Hospital, Denmark, the Department of Surgical Research, University Medical Center Groningen, The Netherlands and the non-profit organization 34LIVES, West Lafayette, USA.

VAPOR-2 (Tønnessen, Hausken)

Rationale: Volatile anaesthetics (VA) like sevoflurane and isoflurane have the ability to reduce ischemia and reperfusion injury (IRI). This phenomenon is called anaesthetic conditioning (AC). Depending on the timing of administration of the anaesthetic it is defined as pre- (before ischemia), per- (during ischemia) or post- (directly upon reperfusion) conditioning. Protective effects of AC on the heart have been demonstrated in vitro, in various animal species and in randomized controlled clinical trials. In contrast evidence for AC in the kidney is restricted to in vitro and animal work.
Since IRI is inevitable in organ transplantation and AC could be an effective way to reduce IRI, we designed the VAPOR trial.
After a follow up period of 2 years there was a significant difference in acute rejection in favour of the sevoflurane groups. In conclusion, sevoflurane based anaesthesia was associated with higher urinary KIM-1 and NAG levels in LDKT however this was not reflected in inferior graft outcome. Remarkably a lower acute rejection rate was seen in the sevoflurane groups. We therefore proceeded with the VAPOR-2 trial, a multicenter trial comparing these 2 anaesthetic agents in renal transplantation with kidneys of DBD and
Objective: To compare the effect of a sevoflurane based anaesthesia versus a propofol based anaesthesia on the incidence of delayed graft function in recipients of DCD and DBD donor kidneys.
Study design: Prospective randomized controlled European multicenter clinical trial with two parallel groups
Study population: Patients ≥18 years scheduled for kidney transplantation with a kidney from a DBD or DCD donor
Intervention (if applicable):
Patients will be included and randomised to one of the following groups:
Group 1 PROP (control): propofol: a propofol-remifentanil based general anaesthesia.
Group 2 SEVO (intervention): Sevoflurane: a sevoflurane-remifentanil based general anaesthesia.
Main study parameters/endpoints:
DGF defined as need of dialysis the first week after transplantation excluding one time dialysis for hyperkalemiaAcute rejection episodes within the first year after transplantation
Graft and patient survival
GFR at , 3, , 12 months PNF defined as a permanent lack of function of the allograft
Length of hospital stay
Postoperative complications of all kind
kidney biomarkers
Mechanism of immunomodulation/protection
The study recently reached it´s goal of including 488 patients from Groningen, Aarhus, Barcelona and Rikshospitalet. Rikshospitalet included 122 patients. Follow up is one year.

Treatment of postoperative pain after abdominal surgery (Hausken, Tønnessen, Haugaa)

After open abdominal surgery (laparotomy) there is a current controversy about which treatment modalities are the best. After open liver resection thoracic epidural analgesia (TEA) with opioids and local anaesthetics is considered the standard of care. As this procedure labour intensive, may have serious complications like epidural bleeding or abscess and has an analgesic failure rate of about 20% we decided to compare this with intravenous

Patient Controlled Analgesia (IV-PCA) where the patient pushes a button on a “pain pump” to get a bolus dose of opioid. PCA is easier to manage, cheaper but there are doubt if the analgesic effect is sufficient for this patient group. We therefore conducted a randomized, controlled, noninferiority trial to investigate if intravenous, patient-controlled opioid analgesia (IV-PCA) and nonsteroidal analgesia (ketorolac or diclofenac) could be as effective as thoracic epidural analgesia (TEA; local anesthetic/opioid/epinephrine) in patients undergoing open liver surgery. Dexamethasone single dose and acetaminophen were also given to both groups. Patients were randomly assigned to receive either IV-PCA (IV-PCA, n = 66) or TEA (n = 77). The primary endpoint, mean NRS pain score was 1.7 in the IV-PCA group and 1.6 in the TEA group, establishing noninferiority. Pain scores were lower in the TEA group on PODs 0 and 1, but higher or equal on PODs 2 and 5. Postoperative hospital stay was significantly shorter for patients in the IV- PCA group (74 vs 104 h, $P < 0.001$). The total opioid consumption during the first 3 days was significantly lower in the IV-PCA group. In accordance with our findings the Enhanced Recovery After Surgery (ERAS) Society Guidelines do not recommend TEA in open liver surgery.
Thoracic epidural analgesia (TEA) is not widely used for postoperative pain management in liver transplantation due to hepatic coagulopathy-related increased risk of inducing an epidural hematoma. However, an increasing number of patients are transplanted for other indications than the end-stage liver disease and without coagulopathy allowing insertion of an epidural catheter. This study is a retrospective observational single-center study of all adult patients undergoing first-time liver transplantation at Oslo University Hospital between January 1, 2008, and December 31, 2017. Out of 685 first-time liver transplantations in a 10-year period, 327 received TEA, and 358 did not. There were no serious complications related to insertion or removal of the TEA catheters. Patients in the TEA group had less pain with a mean numeric rating scale at postoperative days 0–5 of 1.4 versus 1.8 ($P = 0.008$).
Standard of care for postoperative analgesia after pancreas transplant has been thoracic epidural analgesia (TEA). A high incidence of venous graft thrombosis necessitated a change to a more aggressive anticoagulation protocol. To minimize the risk of epidural hemorrhages, carried out a study comparing TEA with rectus sheath block (RSB) Compared with TEA, RSB was equally effective and safe for postoperative analgesia in heavily anticoagulated pancreas transplant patients.

Detection of deep infection after surgery for locally advanced rectal cancer with microdialysis catheters (Asvall, Haugaa, Tønnessen, Thorgersen)
Locally advanced rectal cancer (LARC) in need of neoadjuvant chemo-radiotherapy ((C)RT) before surgery with abdominoperineal resection (APR) develop a high rate of deep pelvic surgical site infections (SSI). Vague symptoms delay diagnosis. Ischemia is suggested to be an important risk factor in the pathogenesis. We hypothesize that typical ischemic changes as elevated lactate and lactate/pyruvate ratio (L/P ratio) measured with microdialysis evolve in patients who develop deep pelvic SSI.
We included 50 patients undergoing open APR after neoadjuvant (C)RT for LARC. At the end of surgery microdialysis catheters were placed in remnant tissue of the pelvic floor. Metabolic parameters including lactate, pyruvate, glucose and glycerol were measured, and the L/P-ratio was calculated for 10 days.
11 patients in need of additional reconstructive surgery and one with intraoperative complications were excluded. Of the remaining 38 patients 12 developed deep pelvic SSI

(abscess group) and 26 didn´t (no-abscess group). Diagnosis of deep pelvic SSI 9(6-17) days after surgery was based on clinical and radiological criteria.
Mann-Whitney U test was used in descriptive statistics to compare groups. Linear mixed model analyses was used managing the microdialysis data.
The day of surgery mean lactate in the abscess group was 6.0 mmol/L whereas 3.6 in the no-abscess group. Overall mean lactate was 1.9 mmol/L higher in the abscess group (P=0.002). L/P-ratio at the day of surgery was higher in the abscess group, 38, versus 21 in the no-abscess group. Overall mean L/P-ratio was 34 units higher in the abscess group (P=0.001).
Deep pelvic SSI after open APR for LARC increased lactate and L/P-ratio measured with microdialysis already from the day of surgery.
Comprehensive surgery, bacterial contamination, ischemic and immunological changes after (C)RT may contribute to this. Microdialysis can be of diagnostic value.

Effect on circulation of Intermittent negative pressure on the lower extremity Høiseth et al)

We have participated in a project on the effect of intermittent negative pressure on the lower extremity on circulation and wound healing. We have first carried out preclinical studies on healthy volunteers and patients with peripheral vascular disease, and then a clinical intervention study. The project is a collaboration with the company Otivio, which supplies the product FlowOx® (https://flowox.com/). In the last 5 years, this has led to co-authorship of 6 articles and co-supervision of PhD for Øyvind Heiberg Sundby (disputed 2018) and Henrik Hoel (disputed 2022). The project is now being continued by PhD candidate Nigel Callender, where I am co-supervisor.

Methoxyflurane

Methoxyflurane is an analgesic/sedative that has relatively recently been approved for use in Europe. The undersigned has taken part in a project which first looked at the analgesic effect and then at the hemodynamic effect of the drug in experimental hypovolemia, published in two articles.

Hypovolemia and hemodynamic monitoring

The undersigned participates in projects that focus on hemodynamic effects of experimental hypovolemia and experimental and clinical monitoring of volume status and fluid response with co-authorship of 7 articles and co-supervision for Ingrid Elise Hoff (disputed 2019). I am currently main supervisor for two PhD candidates in this subject; Sole Lindvåg Lie and Håvard Djupedal as well as one research student; Aura Koistinaho.
Swimming in cold water, hypothermia