Surgical Intensive Care Medicine

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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 and disturbances in the plasma protease cascade systems leading to dysfunctional host defense and 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 and the abnormal plasma proteolysis in patients with infection. A major focus of our work is research into the cellular mechanisms and proteins involved and to facilitate translation of new knowledge from basic research into clinical practice.

Ongoing Projects in 2013

Regulation of CCN1 in a Porcine Model of Intestinal Ischemia-Reperfusion
The extracellular matrix (ECM) has generally received little attention in research. In contrast to the classical ECM proteins, rather being part for the framework for tissue structure, the matricellular CCN family functions to modulate cellular responses to environmental stimulation

Professor Ansgar O. Aasen

with organ-protective properties. Except for CCN5, all CCN proteins contain four conserved domains that include insulin-like growth factor-binding protein (IGFBP), von Willebrand factor type C repeat (vWC), thrombospondin type I repeat (TSR), and a carboxyl-terminal (CT) domain. The CCN1/CYR61 protein plays an important role in tissue regeneration and inflammation by regulating a wide range of cellular processes, including immune cell adhesion, cell survival and endothelial proliferation. The vWC and CT domains of the protein have several integrin-binding sites that may mediate the CCN1s functions.

We have investigated the regulation of CCN1 in the circulation and intestinal tissue in a porcine model subjected to long intestinal ischemia-reperfusion (IIR). A jejunal ischemia-reperfusion model was used in this experiment. Four pigs were fully anesthetized. The abdomen of the animal was opened and a 30-cm long jejunum was subjected to local intestinal ischemia by occluding the arteries and veins for six hours. Intestine biopsies were taken from i) ischemic area (IS) ii) randzone area (RZS) -a segment with sub-normal circulation close to the edge of the ischemic area-, and iii) non-ischemic intestine (NIS) -a segment from an area that was not subjected to ischemia as baseline control segment. Portal and systemic blood samples of the animals were also collected as follows: before induction of ischemia (baseline –BS), 3 hours after induction of ischemia, 6 hours after induction of ischemia or just before reperfusion, and 5, 10 and 30 minutes after reestablishment of intestinal circulation after 6 hours ischemia.

Histological analysis (H&E staining) of these sections showed a little inflammation in NIS while the ischemic, and at lesser level RZS, segments were largely damaged. Messenger RNA of the biopsies measured by real time PCR showed a significant upregulation of CCN1 gene in both IS and RZS segments compared with NIS as it is shown in Figure 1. Western blotting corroborated the upregulation of CCN1 at protein level (Figure 2).

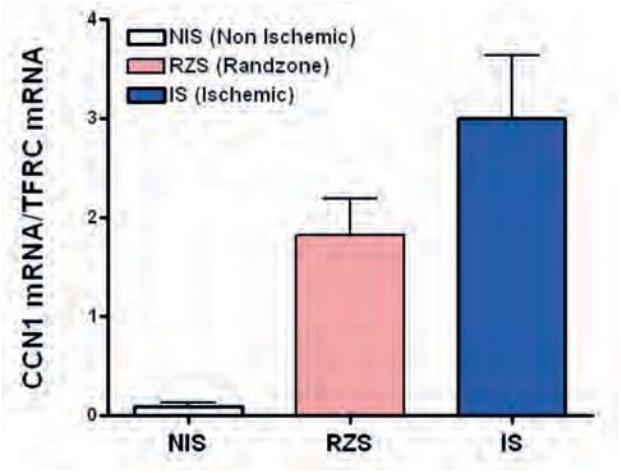

Figure 1. CCN1 gene is strongly expressed in the ischemic and randzone segments in comparison to non-ischemic segment.

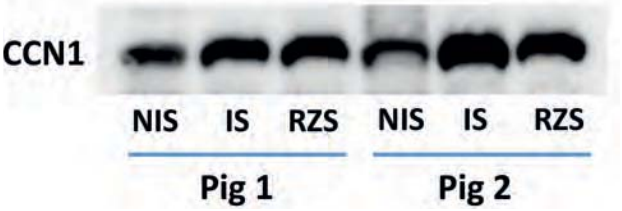

Figure 2. CCN1 protein is strongly upregulated in the ischemic and randzone segments in comparison to non-ischemic segment.

We also wanted to study the cell types that produce CCN1 protein in response to ischemia. Immunofluorescence staining indicated that endothelial and epithelial cells may have a major role in production and secretion of CCN1 in response to hypoxia (Figure 3). This suggestion will be further studied by double immunofluorescence staining. Furthermore, animal blood samples taken during ischemia and after reperfusion showed no enhancement of CCN1 and TNF-level, indicating that CCN1 is locally regulated (not shown).

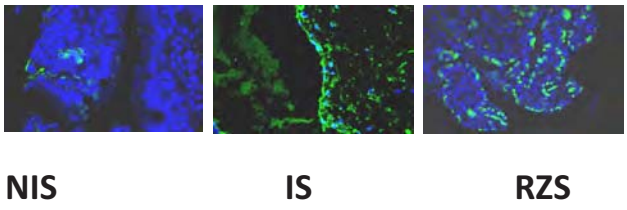

Figure 3. Immunofluorescence staining of the intestine biopsies shows CCN1-positive cells (green) co-stained with DAPI (blue) in the non-ischemic segment (NIS) ischemic segment (IS) and randzone segment (RZS).

Altogether, these results indicate that CCN1 is upregulated in intestine subjected to ischemia. These observations raise the interesting possibility that CCN1 may act as a local danger or survivable signal that may lead to repair and elimination of ischemic damage. Our studies now focus on identifying the source of CCN1-protein cells after ischemia as well as identifying a potential role of local increased levels of CCN1 in intestinal ischemic injury.

CYR61 in an orthopaedic wound after major orthopaedic surgery

Cyr61/CCN1 is a multifunctional matricellular protein that has recently emerged as a potential player in the regulation of inflammatory responses. Experimental research has uncovered pro-inflammatory effects and chemotactic capacities of this protein. Clinical investigations found it to be elevated in patients with chronic inflammatory conditions. However, its regulation in acute non-infectious inflammation in humans has not been investigated previously.

Ten otherwise healthy patients (13 - 18 years old) undergoing major orthopaedic surgery for idiopathic thoracic scoliosis were recruited to the study. Fluid from the surgical drain and systemic blood was collected 0, 1, 2, 4, and 6 hours after wound closure and analyzed for CCN1 contents, neutrophil counts, selected cytokines, and markers of primary haemostasis activation.

CCN1 levels increased rapidly in the drained fluid, reaching significance at 1 hour after wound closure, whereas its levels remained unaltered in the systemic circulation throughout the observation period. The platelet activation marker soluble (s) P-selectin increased in the drained fluid but not in systemic blood during the early post-operative phase, resulting in a strong correlation between CCN1 and sP-selectin in the drained fluid (0.649, p=0.042).

	CCN1
Platelets	0.591 (p=0.09)
sP-selectin	0.649 (p=0.042)*
Neutrophils	-0.171 (p=0.638)
TNF-	0.515 (p=0.232)
IL-1	0.519 (p=0.232)
IL-6	0.401 (p=0.250)
IL-8	0.684 (p=0.02)*
IL-10	-0.043 (p=0.905)
G-CSF	0.493 (p=0.148)

*Table 1. Pearson's correlation of subject means. sP-selectin: soluble P-selectin, TNF-: tumor necrosis factor alpha, IL: interleukin, G-CSF: Granulocyte colony stimulating factor. *p<0.05*

Levels of interleukin (IL)-6 and granulocyte-colony stimulating factor (G-CSF) were elevated in the drained fluid only from two hours after wound closure and neutrophil counts, tumor necrosis factor – alpha, IL-8 and IL-10 were elevated from 4 hours after wound closure.

In conclusion, the present study demonstrates that CCN1 accumulates in fluid drained from a surgical wound but not in the systemic blood after major surgery. It uncovers that CCN1 levels increase before classical cytokine mediators at sites of tissue-injury and suggests a connection between platelet activation and CCN1 accumulation. Altogether, these data may suggest that CCN1 is an early actor in acute inflammation in humans.

The matricellular "cysteine-rich protein 61" is released from activated platelets and increased in the circulation during experimentally-induced sepsis

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. Interestingly, the magnitude of CCN1 regulations demonstrated strong correlations with the inflammatory activity in the organs during development of dysfunction. Furthermore, exposing hepatocytes to TNF-alpha resulted in a dosage-dependent CCN1 mRNA regulation.

In the present study we have provided a comprehensive description of CCN1-regulation in the circulation and vital organs during experimentally induced sepsis with developing organ dysfunction.

Female CD-1 mice served as baseline controls or were subjected to coecal ligation and puncture (CLP) for 18 - 96 h and CCN1 regulation was analyzed in selected organs and in the circulation. A 5-, 5-, and 3-fold increase in circulating CCN1-protein were respectively observed at 18, 48, and 96 h post-CLP.

Hepatic and pulmonary CCN1 mRNA expression was down-regulated by 80, 60, and 55% and 85, 80, and 65% at 18, 48, and 96 h post-CLP, and undetectable in circulating white blood cells. To identify a potential source for the circulating protein, mouse and human platelets were explored and revealed to contain CCN1. Human platelets were stimulated in vitro by thrombin and a specific PAR1 agonist (SFLLRN). Both agonists induced an instant CCN1 release and the effect of SFLLRN was blocked by the specific antagonist RWJ56110.Taken together, the current study provides novel evidence that platelets comprise a source for CCN1 release to the circulation in sepsis. It demonstrates that the increase in circulating CCN1-protein is opposed by a profound CCN1 mRNA repression in vital organs and suggests that the restoration of pulmonary protein levels is mediated by the sequestering of platelets and innate immune cells in the microvascular network. The functional impact of these observations awaits further investigations but encourages more research into the role of this protein in lifethreatening infections.

Thrombin Generation is a sensitive tool to predict haemostatic changes in sepsis in an animal model

Septicemia is almost inevitably associated with changes in the haemostatic equilibrium ranging from a discrete drop in platelet count to full-blown disseminated intravascular coagulation (DIC). Routine monitoring of haemostasis in septic patients is largely based on evaluation of prothrombin time (PT), International normalized ratio (INR), activated partial thromboplastin time (aPTT), platelet count, fibrinogen levels, D-Dimer, Thrombo-Elasto-Gram (TEG) measurements and, eventually, bleeding time. Abnormal results often indicate poor prognosis and outcome.
In septicemia time and early detection of disturbances leading to life threatening situations may be of vital importance. The tests mentioned are generally insensitive in early septicemia.

There has been increasing interest in so-called "global assays" where the final formation of fibrin and fibrinolysis is evaluated to reveal the early changes. The most promising of these tests is the fluorogenic Thrombin Generation (TG) assay, which measures active thrombin in a plasma sample.

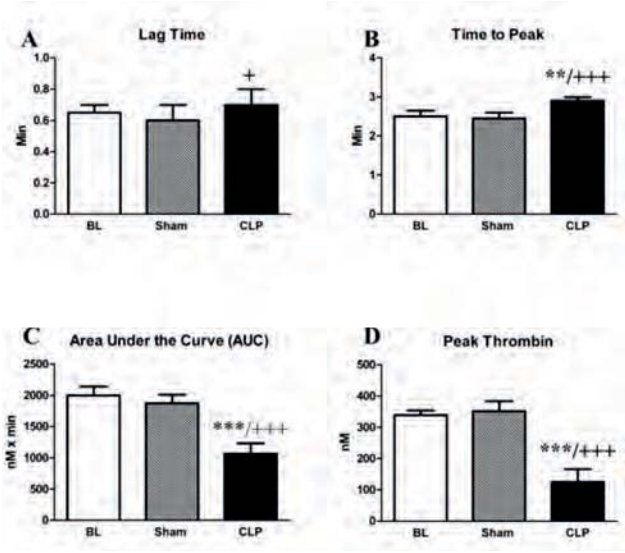

Figure 4. Thrombin Generation in early-stage experimental sepsis.

We have compared this method to TEG, PT, INR, aPTT and evaluated AntiThrombin (AT) and Thrombin/ Antithrombin (TAT) in a rodent model where septicemia was induced by coecal ligation.

Our conclusion is, as expected, that thrombin formation precedes fibrin formation. This measurement is a very sensitive way to detect haemostatic disturbances early in septic animals. The TG assay should be further developed to a “bedside” (easy to use) test for clinicians.

Assessment of the viability of Ischemic Small Intestine using Bioimpedance Measurements

In gastrointestinal surgery related to intestinal ischemia, the surgeon has to assess the viability of intestines that have been exposed to ischemic injury. There are at present limited means by which tissue viability can be assessed. The standard clinical method is still visual inspection and palpation. This method is non-specific and unreliable, and requires a high level of clinical experience. Bioimpedance has been utilized to measure changes in electrical parameters during ischemia in tissues like the liver, skeletal muscle, and the heart. The physical changes on the cellular and structural levels after the onset of ischemia result in time-variant changes in the electrical properties of the tissue. We have investigated the possibility of utilizing bioimpedance measurements to support intraoperative assessment of the viability of ischemic small intestinal tissue.

We have accomplished 5 initial pilot studies in a porcine model of small intestinal ischemia, and 7 further studies on porcine models based on the initial findings. After induction of anesthesia, a warm ischemic model with full mesenteric occlusion for 6 hrs was implemented in a 30 cm part of the jejunum. Measurements were conducted placing electrodes on the serosa of the jejunum, applying a constant voltage, and measuring the resulting admittance. Several electrode setups were tested. We used the recognized and commonly used Solartron 1260/1294 impedance analyzer setup. As a control we used measurements on parts of the jejunum with normal perfusion in the same porcine model.

The 2-electrode Silver-Silver chloride setup appeared the best of the tested setups for measuring small intestinal ischemia interoperably. The collected data from the porcine models show significant changes in electrical parameters of

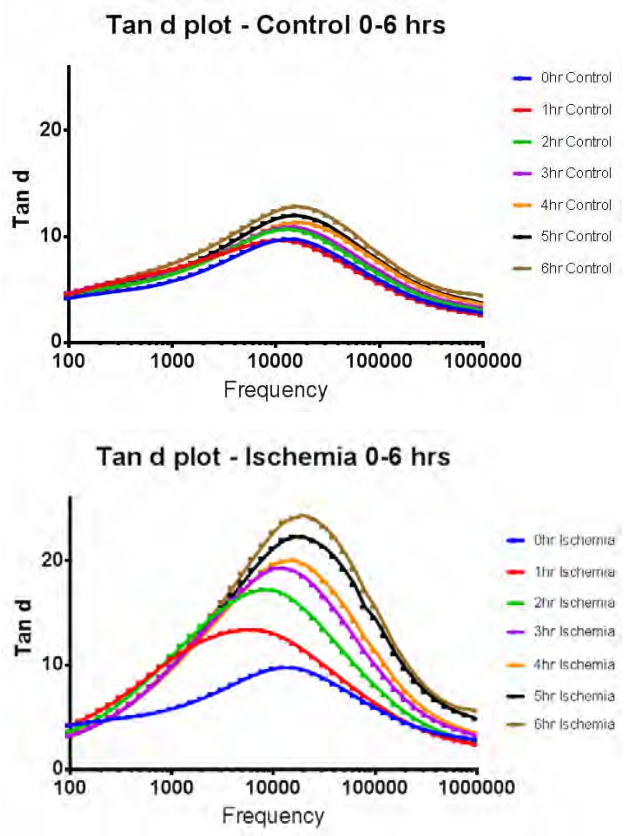

Figure 5 Left: Control - Right: Ischemic small intestine (Jejunum, porcine model)- The figures show: Tan d (loss tangent) as a function of frequency. The data show measurements with the two-electrode setup on a porcine model. Each point on the graph show the mean from 8 measurements on separate porcine models at the same frequency, within a +/-5 minute window of ischemic time development.

modulus, phase, and tan d as a function of ischemic time, compared to the control (Figure 5). Tan d is the loss tangent, a parameter that compares the loss of electromagnetic energy to the energy that passes through a medium.

A Matlab program with pilot algorithms has been developed showing 78,2% sensitivity, 94,2% specificity, 92,7% positive predictive value, and 82,0% negative predictive value in assessing if the porcine small intestine is ischemic or not, based upon analysis of the collected data. The program also shows promising results in discerning the time duration of ischemic small intestine within the tested 6 hour time frame. Histological samples show ischemic changes to the small intestinal tissue that correlate with the measured time variable changes (Figure 6).

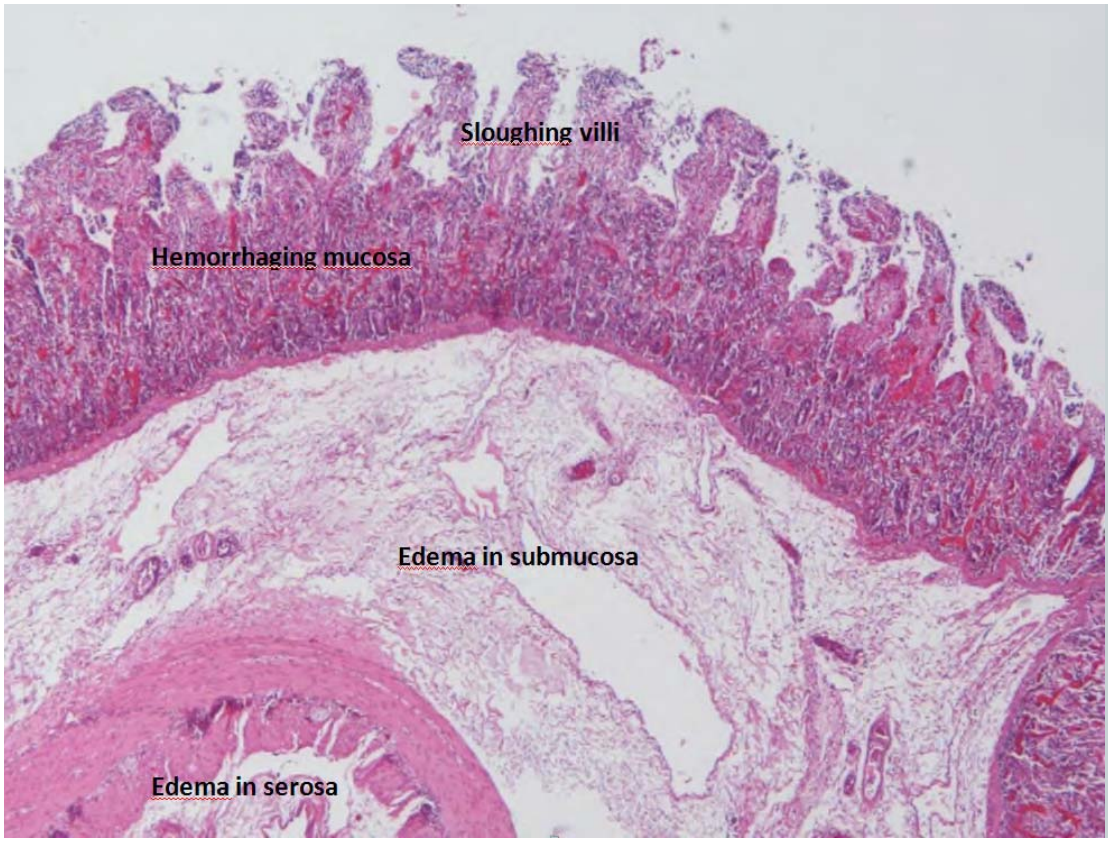

Figure 6. Cross section of porcine jejunum wall with damaged and affected areas after 6 hours of warm full mesenteric occlusion ischemia.

The time development of the electrical properties measured within a 6 hour period of ischemia is statistically significant, and can be correlated with the onset and duration of ischemia (Figure 7).

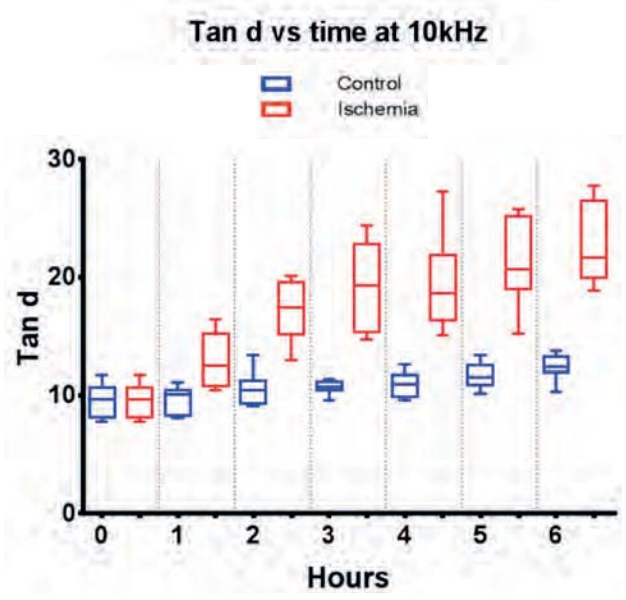

Figure 7. Tan d - Control vs. Ischemic small intestine at 10kHz. Each box plot is based on 8 measurements from 8 separate porcine models, at the same frequency in the same time window (+/- 5 min of the selected hour). They are presented as mean, 1 and 3 quartile and range.

Based on the results from the porcine models, it is not yet possible to assess the viability of the small intestine to the point of irreversible ischemic damage, as the 6 hour ischemic period of porcine model 2 (PM2) did not create a 100% certain ischemically irreversible damaged porcine small intestine. The animal model developed through these experiments seems suitable for further studies related to the determination of the viability of ischemic small intestine.

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