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Aspects of renal blood flow and oxygenation in acute kidney injury during severe infections

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Abstract

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Acute kidney injury (AKI) is a common complication in patients treated in the intensive care unit (ICU). Severe infections/sepsis is the most common aetiology, and about half of patients treated in the ICU due to sepsis are affected. Although AKI often resolves over time, the development of AKI decreases the chances of surviving. This thesis aims to investigate mechanisms of AKI development related to renal blood flow and oxygenation, by using both animal experiments and novel techniques available to examine patients.

In Paper I, anaesthetised sheep were randomised to receive a bacterial infusion for 29 hours, or serve as controls. Isolated mitochondria from the animals' renal cortex were investigated in vitro, at the beginning, and at the end of the experiment. Renal function deteriorated significantly in sheep with sepsis compared with the controls, and mitochondrial dysfunction by sepsis, comprised of a reduction of the RCR and an increased CII/CI-relation in State 3, was demonstrated in the sepsis group at the end of the experiment. However, renal cortical mitochondrial efficiency and uncoupling was unaffected by sepsis and cannot explain the discrepancy between oxygen consumption and tubular transport.

Paper II described and characterised AKI during the novel infection COVID-19, in a prospective cohort study of 57 patients in the ICU. Biomarkers for renal damage were measured in urine from 52 of the study participants. The results show that 89% of the study participants developed AKI. Oliguria was common and occurred early after ICU admission. The biomarkers in the urine were generally elevated, however, with only minor differences between patients stratified by severity of AKI.

In Paper III, 19 patients in the ICU due to COVID-19 were examined with magnetic resonance imaging (MRI). Renal blood flows, oxygenation, and water content were measured. The results show that patients with AKI had lower renal blood flows, both in the cortex and medulla, compared with those without. However, no differences regarding oxygenation or water content was demonstrated.

Paper IV investigated whether plasma expansion could affect renal blood flows in 17 of the study participants in Paper III. Ringer's acetate (7.5 ml/kg) was infused and the measurement with the magnetic camera was repeated. The results show that renal blood flows did not increase, either in patients with or without AKI, even though the increase in circulating blood volume resulted in higher blood pressure. However, patients without AKI had a lower perfusion in the renal medulla after plasma expansion.

Keywords: Acute kidney injury, renal blood flow, renal oxygenation, COVID-19, fluids, mitochondria, sepsis, acute respiratory distress syndrome, magnetic resonance imaging

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Luther T, Bülow Anderberg S, Persson P, Franzén S, Skorup P, Wernerson A, Hultenby K, Palm F, Schiffer T, Frithiof R. **Renal mitochondrial dysfunction in ovine experimental Sepsis Associated Acute Kidney Injury** (*Submitted*)

II. Luther T, Bülow-Anderberg S, Larsson A, Rubertsson S, Lipcsey M, Frithiof R, Hultström M. **COVID-19 patients in intensive care develop predominantly oliguric acute kidney injury.** *Acta Anaesthesiol Scand*. 2021 Mar;65(3):364-372

III. Luther T, Eckerbom P, Cox E, Lipcsey M, Bülow-Anderberg S, Hultström M, Martinez Torrente F, Weis J, Palm F, Francis S, Frithiof R, Liss P. **Decreased renal perfusion during acute kidney injury in critical COVID-19 assessed by magnetic resonance imaging: a prospective case control study.** *Critical Care* volume 26, Article number: 262

IV. Luther T, Eckerbom P, Cox E, Lipcsey M, Bülow Anderberg S, Hultström M, Weis J, Palm F, Francis S, Liss P, Frithiof R. **Effect of plasma expansion on renal perfusion assessed by magnetic resonance imaging in COVID-19 ARDS patients with and without AKI: a prospective case control study.** (*Manuscript*)

Contents

Abbreviations

ACEi	Angiotensin converting enzyme inhibitor
ADC	Apparent diffusion coefficient
ADP	Adenosine diphosphate
AKI	Acute kidney injury
ANOVA	Analysis of variance
ARB	Angiotensin II receptor blocking drug
ARDS	Acute respiratory distress syndrome
ASL	Arterial spin labelling
ATN	Acute tubular necrosis
ATP	Adenosine triphosphate
BOLD	Blood oxygen dependent imaging
CEUS	Contrast enhanced ultrasound
CKD	Chronic kidney disease
CO	Cardiac output
COVID-19	Coronavirus disease 2019
COPD	Chronic obstructive pulmonary disease
CPB	Cardio pulmonary by-pass
CRP	C-reactive protein
D	Pure diffusion
D*	Pseudodiffusion
DWI	Diffusion weighted imaging
ETC	Electron transport chain
eGFR	Estimated glomerular filtration rate
Fp	Perfusion fraction
GFR	Glomerular filtration rate
ICU	Intensive care unit
IMV	Invasive mechanical ventilation
IQR	Interquartile range
KDIGO	Kidney disease improving renal outcome
KIM-1	Kidney injury molecule 1
LPS	Lipopolysaccharide endotoxin
MAP	Mean arterial pressure
MRI	Magnetic resonance imaging
NGAL	Neutrophil gelatinase-associated lipocalin
NT-proBNP	N-terminal pro-brain natriuretic peptide.

OXPHOS	Oxidative phosphorylation
P-	Plasma-
PAH	Para-aminohippurate
PC	Phase contrast
PEEP	Positive end expiratory pressure
P/f	PaO2/FiO2
RAAS	Renin-angiotensin-aldosterone system
RBF	Renal blood flow
RCR	Respiratory control ratio
RCT	Randomised controlled trial
RI	Resistive index
ROS	Reactive oxygen species
RSNA	Renal sympathetic nerve activity
RRT	Renal replacement therapy
RVR	Renal vascular resistance
SA-AKI	Sepsis associated acute kidney injury
SAPS 3	Simplified Acute Physiology Score 3
SOFA	Sequential organ failure assessment
T_1	Longitudinal relaxation time
T_2	Transverse relaxation time
TIMP-2	Tissue inhibitor of metalloproteases-2
TKV	Total kidney volume
tpO_2	Tissue partial pressure of O_2
TRUST	T_2 relaxation under spin tagging
UO	Urine output

Introduction

A sudden reduction in urine production followed by symptoms of azotaemia has been known to occur in conjunction with severe illness during most parts of the 20th century. The clinical entity has, for the most part, been labelled acute renal failure in which serious consequences, such as pulmonary oedema, often were fatal. A significant improvement in treatment options occurred in the 1940s, when renal replacement therapies (RRT) were implemented[1]. In Sweden, physicians and researchers in Lund adapted and improved RRT early. During the first decade of its use, it was occasionally used on patients with tracheostomies receiving mechanical ventilation. The importance of fluid balance and the possibilities of renal recovery, even many weeks after the debut of azotaemia and anuria, were emphasised early on[2].

Since then, acute renal failure in patients treated in intensive care units (ICU) has been recognised as a common complication of critical illness and still increase the risk of death despite the possibility of RRT. It has a multitude of different causes; however, sepsis, shock, and as a postoperative complication, especially after cardiac surgery, are major causes in ICU patients. The syndromal nature of acute renal failure in ICU patients, that is not consisting of a single disease but a mix of several, results in difficulties regarding comparisons in frequency, outcome, and disease mechanisms. This has led to the condition being incorporated into what is now referred to as acute kidney injury (AKI) since the beginning of the millennium. AKI is diagnosed by either a new elevation in P-creatinine or a sudden reduction in urine output. The latest definition, the kidney disease improving global outcome (KDIGO) criteria, was published in 2012[3]. It is currently a widely accepted standard but is not considered unproblematic. There are also long-term consequences of AKI development, as every episode increases the risk of developing chronic kidney disease (CKD)[4]. Long-term mortality in AKI survivors is increased, however, mostly by non-renal causes[5].

Today, AKI in the ICU is still widely prevalent and sepsis is the most common etiological factor[6,7]. Sepsis, also a syndrome and one of the leading global causes of death[8], is now defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection"[9]. The diagnosis is made by the combination of the presence of infection and an increase of two points in the

scoring instrument sequential organ failure assessment (SOFA). The more severe form, septic shock, is defined as hyperlactatemia or vasopressor-requiring hypotension despite fluid resuscitation. This is a common indication of admittance to the ICU and further increases the risk of AKI. The pathogenesis of AKI in septic patients has been intensely researched during the last decades, and is considered multifactorial. There is vast heterogeneity within this subgroup of AKI, possibly due to the contribution of different factors such as severity of disease, pathogen, comorbidity, and therapeutic interventions administered. The pathogenesis of AKI in sepsis differs from AKI due to hypovolemia or after cardiopulmonary bypass. The term sepsis associated acute kidney injury (SA-AKI) is used to acknowledge this and has been chosen in this thesis to distinguish this subgroup of AKI.

During the large pandemic of the previous century, the Spanish flu, renal involvement was occasionally reported as common[10]. As of 2020, the new SARS-nCOV-2 pandemic has resulted in a large number of patients needing intensive care due to severe infection. Many are diagnosed with adult respiratory distress syndrome (ARDS), a third syndrome relevant for this report. Defined as "a type of acute diffuse, inflammatory lung injury, leading to increased pulmonary vascular permeability, increased lung weight, and loss of aerated lung tissue"[11]. ARDS is, in practise, diagnosed by criteria fulfilment of bilateral lung opacities on X-ray, hypoxemia requiring positive pressure ventilation, and exclusion of cardiac pulmonary oedema. These three syndromes often overlap, and a substantial number of ICU-treated patients with COVID-19 will simultaneously have all three at some point. For clarity, patients with COVID-19 requiring ICU-treatment will be addressed as such in this thesis and not as patients with sepsis. Further, AKI due to COVID-19 is distinguished from other forms of SA-AKI.

Background

Sepsis associated AKI

As previously stated, AKI is a common complication in the ICU, affecting close to half of admitted patients. [6,12]. A history of diabetes and hypertension are independent risk factors for developing AKI, and patients developing AKI tend to be older and are more often affected by heart failure and previous renal disease. AKI development is independently associated with increased mortality with increased AKI severity further increasing the risk of death. 90-day mortality in ICU patients with stage 3 AKI has been estimated to be close to 40%[12]. SA-AKI accounts for 45–75%[13] of AKI in the ICU with similar associations to increased risk of dying. Survivors of critical illness complicated by AKI, more often have reduced glomerular filtration rate (GFR) at hospital discharge and have an increased risk of CKD-development[4,6]. Treatment options are mainly supportive, in addition to treatment of the underlying disease. Thus, successful interventions promoting AKI-prevention or reversal may lead to substantial benefits for the ICU population.

The multifactorial nature of the pathogenesis of SA-AKI includes a diverse multitude of pathological alterations[14-16]. A summary of some proposed mechanisms relevant to SA-AKI development is presented in **Figure 1,** of which mechanisms relevant to this thesis will be further outlined in the following subheadings.

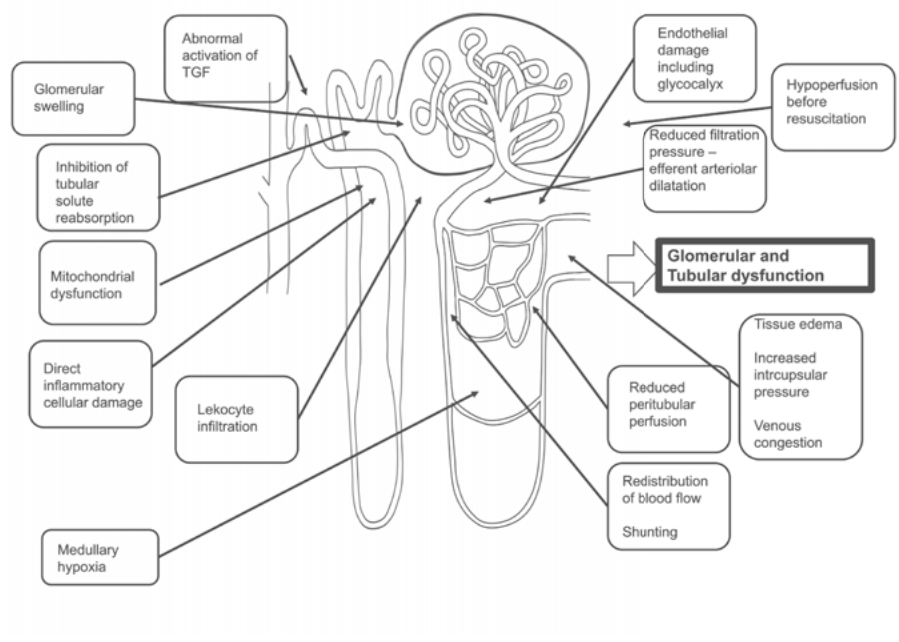

Figure 1. Summary of some mechanisms proposed to contribute to SA-AKI. These hypotheses are based mainly on experimental studies in different animal models and their relative contribution to the clinical entity of SA-AKI is unclear.

Cellular effects of inflammation

The septic dysfunctional host response to infection can be understood as initiated by a danger signal consisting of the different immunogenic debris of bacteria. These molecules are often summarised as pathogen associated molecular patterns (PAMPs), of which lipopolysaccharide endotoxin (LPS) has been frequently used to replicate the sepsis response in laboratories. PAMPs broadly activate different branches of the innate and adaptive immune systems, creating cascades of proinflammatory processes. Activation of the immune system also results in the release of endogenous damage associated molecular patterns (DAMPS), pro-inflammatory molecules associated with an activated host response, with similarities to the immune activation in severe trauma or major burns. The time aspect of the response is relevant because several anti-inflammatory processes are activated simultaneously. This activation later results in a state of compensatory anti-inflammatory response, leaving the patient paradoxically immunosuppressed. This may be a factor in the differences seen between outcomes in clinical trials compared with experiments of anti-inflammatory targeting in sepsis. The initial broad activation of the immune system also negatively affects kidneys. Part of this may be due to direct inflammatory damage. Further, secondary alterations in macrocircula-

tion and microcirculation have the potential to interfere with perfusion pressure and tissue oxygenation. The resulting hypercoagulant state may cause microthrombi formation and contribute to renal dysfunction.

Acute tubular necrosis (ATN) is a common histological phenotype during AKI due to large haemorrhages and hypovolemia, but the phenotype is not commonly prevalent in SA-AKI. Post-mortem biopsies from patients with SA-AKI instead show a pattern of apoptosis and leukocyte infiltration[17]. Animal models also lack histopathological findings of ATN in SA-AKI[18,19]. In ovine (sheep) models, only a few histological changes in tubular cells are initially present[20]. However, signs of glomerular endothelial swelling and leukocyte infiltration in the interstitium are present and can be reduced by targeting the immune response to PAMPs[21]. Direct inflammatory damage to tubular function has also been proposed in studies in rodents[22]. Tubular cells express TLR-4 (a receptor of LPS) and are affected by both circulating and filtrated LPS[23]. This results in downregulated ion transports[24] and altered microcirculatory flows in the peritubular capillaries[25].

There is growing evidence to suggest a prominent role for the inflammatory response to SA-AKI development. Circulating pro-inflammatory factors such as IL-8 are associated with SA-AKI[26] although to a minor degree compared with clinical risk factors. Plasma from septic burn patients with AKI also induces direct cellular damage to renal cells in vitro[27]. Attempts to categorise different molecular patterns to SA-AKI and outcomes have shown that a persisting AKI is associated with increased levels of factors associated with endothelial cell activation, increased pro-coagulant factors, and factors increasing vascular permeability[28]. Genetic factors, such as polymorphisms in the promotor of heme oxygenase 1, a protein with properties protective of oxidative injury, are also more common in patients developing SA-AKI compared to patients not developing AKI[29]. Successful therapeutic interventions beyond experimental settings are still lacking. Randomised controlled trials (RCTs) of TLR-4 inhibitors, which reverse experimental SA-AKI[21], do not reduce mortality[30]. A RCT using human recombinant alkaline phosphatase in SA-AKI could not demonstrate significant improvement in renal function[31]. Absolute improvement in GFR in the later study was clinically relevant, and the study compound may be tried in a better-powered future study.

Mitochondrial dysfunction

Mitochondrial dysfunction has a proposed pivotal role in clinical sepsis[32], as skeletal muscle from septic patients with poor outcomes exhibit lower complex I activity and lower levels of ATP. Similar results were also observed in animal experiments[33]. As a historical note, attempts to elevate oxygen delivery to supranormal levels were previously suggested as favorable[34] based on animal experiments[35] and clinical studies. However, the threshold of "too low" O_2 delivery (critical O_2-delivery level) in humans was soon shown in critical illness and sepsis to be similar to that in healthy adults[36]. Still, septic shock was considered mainly a state of a lack of tissue oxygenation and the proposed mitochondrial dysfunction was highly recognised.

Dysfunction in renal mitochondria by sepsis has been demonstrated in experiments[37] and has also been suggested to be affected in a clinical setting, at least in the most severe cases[38]. In experimental studies in sheep, a difference in lactate/pyruvate ratio can be demonstrated[21] whereas renal ATP levels measured by magnetic resonance imaging (MRI) seem to be unaffected, at least early during the course of disesase[39]. Renal mitochondria are present in cortical and medullary cells where proximal tubular cells are particularly sensitive to electron transport chain (ETC) defects[40]. Mitochondrial density is highest in the proximal tubuli, thick ascending limbs and the distal tubuli[41]. There are also differences in the proximal and distal tubuli. The proximal tubular cells are in a more oxidised state with a membrane potential that collapses quicker in anoxia compared with the tubular epithelial cells in the tall ascending limb[42]. Mitochondria in the cortex are less efficient than in medullary tubular cells[42,43]. Anaerobic metabolism due to glycolysis is mainly a phenomenon in the distal and not proximal tubular cells[44]. Disturbances in the ETC can lead to ATP depletion, increased ROS production and increased oxygen demand due to uncoupling. Disturbances in the ETC have been associated with experimental SA-AKI and mitigated by therapeutic targeting of mitochondrial function and redox scavenging[45]. Other aspects of mitochondrial function, such as removal of defect organelles (mitophagy) and regeneration (biogenesis), have also been investigated in rodent models, and linked to AKI severity and resolution[46-48] as well as the risk of CKD development[49]. The effects of sepsis on renal mitochondria have been proposed to refocus on cell survival in an ATP-depleted environment similar to a state of hibernation[50]. If so, active ion transport would be reduced and less ATP and oxygen would be expected to be consumed[24].

In vitro assessment of ETC may differ from performance in vivo[51,52]. However, renal in vivo assays have mostly been performed in isolated cells or skeletal muscle, and the in vitro assessment of the ETC is still sufficient for clinical use[53]. As both renal cellular response to inflammation and ischemia can

affect renal mitochondria[27], and mitochondria in turn affect the relationship between oxygen consumption and metabolic activity, they are of interest in the study of the interactions of inflammation, circulation and oxygenation in SA-AKI pathogenesis.

Renal Blood Flow in SA-AKI

Dehydration and intravascular volume depletion activates the renin-angiotensin-aldosteron system (RAAS) and the renal sympathetic nervous system. This increases the renal vascular resistance (RVR) and reduces renal blood flow (RBF) and GFR. These systems also increase tubular sodium resorption and conserve water and salt to the extent possible[54], and, thus, protect against more severe dehydration. The reduction in GFR is called pre-renal azotaemia. This was thought to be the main mechanism in several forms of AKI, including SA-AKI[54]. Due to the compensatory nature, pre-renal azotaemia has even been described as a renal success (contrary to failure)[55]. More recent work has described more intricate and diverse pathologies. Even AKI due to reduced RBF by either constriction of circulating volume by dehydration or transient renal ischemia, differs significantly in gene activation[56]. Reductions in RBF have previously been considered crucial to the development of SA-AKI[57] but this has been questioned on the basis of experimental studies conducted mainly in ovine models[58,59]. Whereas rodent models of sepsis more often exhibit a reduced RBF, sheep develop AKI when subjected to bacterial or LPS-infusions without a reduction in RBF. This mimics a common clinical scenario in which AKI develops in severe infection despite the absence of shock[60]. It also falsifies that SA-AKI development necessitates global renal hypoperfusion.

There are systematic reviews of RBF in SA-AKI in animal experiments[61] and of renal blood flow in clinical AKI[62,63]. These studies indicate that in the experimental setting RBF is associated with cardiac output, that is whether the model is hypodynamic or hyperdynamic. This is, in turn, influenced by means of resuscitation or lack thereof. Recent recommendations regarding experimental sepsis studies mandate resuscitative measures in experimental sepsis, except in cases in which lack of resuscitation is explicitly studied[64]. Howeveer, AKI in the clinical setting has repeatedly been associated with reduced RBF and increased intra-renal vascular resistance. A number of clinical studies have demonstrated a higher resistive index (RI) determined by Doppler ultrasound in SA-AKI[65,66]. RI is the difference between systolic and diastolic flow velocities divided by systolic velocity. A RI of 0 would indicate a non-pulsatile flow, and 1 would indicate no flow during diastole. Although RI is commonly used to predict transplant rejection in clinical practice, in SA-AKI, there is poor discrimination at the patient level using RI[66], and it is not easily clinically applicable. Interestingly, RI is not affected by fluid bolus[67], even

when categorising patients in responders and non-responders by systemic hemodynamical variables[68]. RI is also not affected by norepinephrine dose[66], but it can be correlated to persistence (non-recovery) of AKI[65]. Contrast enhanced ultrasound (CEUS) has indicated reduced[69] or highly variable[70] cortical perfusion in SA-AKI, and an unpredictable effect of norepinephrine[70]. In AKI in general, reduced CEUS-perfusion in the cortex and medulla has been linked with the need for RRT and CKD-development respectively[71].

Several studies from the last century used para-ammino huppurate (PAH) clearance to assess RBF, where a change of renal extraction ratio found during SA-AKI may yield less reliable results[72]. More reliable direct measurements have been used to explicitly study RBF in cohorts of patients with sepsis: Brenner *et al.*[73], Prowle *et al.*[74] and Skytte *et al.*[75]. The study by Brenner *et al.* included 8 patients (of whom 7 were in septic shock) examined with a thermodilution renal vein catheter. The first measurements were made within 18 hours of admission; however the mean P-creatinine was 62 µmol/l, and all but one had P-creatinine < 132 µmol/l. There was a quite precise correlation between RBF/CO and GFR ($R = 0.92$, $p = 0.003$), although a poor correlation with thermodilution and PAH clearance indicated a reduced reliability of PAH clearance in this clinical setting. Prowle *et al* reported exams of 10 individuals with SA-AKI, of which 3 had a baseline P-creatinine > 100 µmol/l. They were examined at a median of 3.5 days after AKI-diagnosis, when 9/10 were receiving RRT. RBF was significantly lower than in the healthy controls (median 482ml/min vs 1260ml/min). As in the study by Brenner RBF/CO was lower (median 7.1%) than normal ~20%. In the study by Skytte *et al*, 8 patients with previous normal P-creatinine with norepinephrine-dependent septic shock were examined within 24 hours of admission to intensive care. Three patients had AKI according to the KDIGO creatinine criteria ($KDIGO_{Cr}$) at study inclusion and one additional patient developed AKI during ICU-stay. They were compared with a larger group of 58 patients examined after cardiac surgery without AKI. Septic patients had slightly lower RBF (median 658 ml/min/1.73m^2) than the control group (median 803 mL/min/1.73m^2), but higher than the patients in the previous two studies. There was also a significant increase in renal vascular resistance and a significant reduction in RBF/CO-ratio in concurrence with previous studies.

These findings differ from results in large animals, mainly sheep, in which RBF is usually increased[21] or maintained. Although anaesthesia is associated with lower absolute values in sheep and a RBF/CO well below 20%[76], RBF is not reduced during SA-AKI development. In summary, reduced RBF is not a requirement in experimental SA-AKI, and the role of RBF in the clinical setting remains unclear. Currently, positive effects of therapeutic targeting of RBF cannot be derived by neither the association of reduced RBF in early clinical septic shock nor in manifest SA-AKI. Neither can the opposing fact

that global hypoperfusion is not a relevant factor in certain models of SA-AKI be interpreted as proof of the futility of RBF targeting in the clinical setting.

Oxygenation

The kidney receives approximately 20% of cardiac output and is highly metabolically active. Under normal circumstances, most blood passes through the glomeruli to facilitate glomerular filtration. Deeper medullary areas are sparsely perfused on the brink of hypoxia/hypoperfusion to maintain high extracellular osmolality and enable passive concentration of urine to conserve water. Even under physiological conditions, renal medullary oxygenation is variable. Activation of the renal sympathetic nervous system by a hand grip exercise decreases RBF and regional perfusions but increases medullary oxygenation[77]. During pathological conditions in which RBF is reduced, regional hypoperfusion can occur primarily in the medulla, even when global renal oxygen delivery (DO_2) exceeds oxygen consumption (VO_2). This is the case in haemorrhagic shock, in which neurohormonal activation further reduces RBF and increases oxygen demand by stimulating sodium transport[78]. In ovine experimental SA-AKI, however, global oxygen delivery is intact and often is increased. Instead, disturbed microcirculation in both cortex[18,79] and medulla[80], as seen in porcine models, may cause local hypoxia due to impaired diffusion and redistribution of blood flow[81]. Pathological oxygen shunting has been proposed after short term experiments during endotoxemia in dogs with profound RBF reduction[82]. Oxygen shunting under physiological conditions may protect against hyperoxic damage[83,84], but during sepsis the existence of increased shunting remains to be proven. Whereas global renal oxygen consumption in sheep with SA-AKI remains unchanged[85,86], oxygen delivery seems to redistribute, resulting in reduced medullary tissue oxygenation[87]. Fluid resuscitation transiently increases medullary oxygenation and perfusion, and urine output increases, but the effect wears off quickly[88]. In these models, the reduction of tissue PO_2 precedes the reduction in GFR, which implies a causative effect. In rat experiments, RBF is more often reduced, and tissue hypoxia has been described mainly in the cortex[89,90].

Clinical studies of renal oxygenation in AKI have mostly studied patients after cardiac surgery using renal venous catheters to enable the estimation of oxygen delivery and extraction[91]. Global oxygen consumption is not substantially affected by AKI development. Since the tubular transport of sodium consumes a substantial part of the kidneys' oxygen and ATP[92,93], it is relevant to assess oxygen consumption in relation to the tubular transport of sodium. This reveals a disturbed relationship between oxygen consumption and tubular transport in the clinical postoperative AKI[94]. The study by Skytte *et al.*[75] of septic patients, also demonstrated a similar difference. Note that although only 3 of 8 patients fulfilled AKI definitions at exam, there still was a significant

reduction of GFR in the septic group compared to the control group. A disturbed relationship between (a grossly unaffected) oxygen consumption and sodium resorption has been demonstrated in several studies of clinical AKI, including SA-AKI, and should be considered a feature of AKI. Although catheter-based studies have added substantial understanding regarding renal oxygenation in clinical AKI, a limitation of the method is the inability to examine regional disturbances of the oxygenation, which may be relevant in SA-AKI.

Renal hypoxia, especially in the cortex, is of interest in research regarding CKD development. One hypothesis suggests that hypoxia is a key downstream event that propagates CKD development in many forms of CKD[95]. A few examples of experimental evidence in line with this notion are that treatment with angiotensin II blocking drugs (ARB) increases cortical oxygenation[96], mitochondrial uncoupling induces nephropathy by reducing tissue pO2 without affecting RBF[97], and endothelin A receptor inhibition mitigates experimental diabetic nephropathy by increasing RBF, DO_2, and tissue availability of oxygen[98]. In this regard, RBF and renal oxygenation in SA-AKI can also be researched with the end point of renal recovery or AKI – CKD transition.

Regulation of renal blood flow

Mechanisms to adapt the RBF to maintain an adequate perfusion pressure for the desired GFR are known as the autoregulation of RBF. This is achieved through two principal mechanisms. The myogenic response originates from the muscular layer of afferent arterioles where mechanical stretch (i.e. blood pressure) activates myocytes. A proposed mechanism is the interaction between integrins and the extracellular matrix. This, in turn, activates intracellular phosphoinositol signalling, resulting in an increase in intracellular calcium and membrane polarisation[99]. The tubologlomerular feedback is located in the macula densa. Under physiological circumstances, increased glomerular filtration increases the tubular sodium load in macula densa cells. This leads to an increase in NKCC2 transporter activity, resulting in an increase in intracellular sodium and ATP[99,100]. The exact mechanisms of action on the arterioles are still unclear but possibly mediated by adenosine, which may link mitochondrial dysfunction to an increased activity of tubuloglomerular feedback[40]. There is some evidence of the myogenic response and tubuloglomerular feedback interacting[101]. The presence of other mechanisms has also been suggested[102]. Few studies have been conducted regarding the autoregulation of RBF in sepsis. In one study, there was a plateau of RBF at mean arterial pressure (MAP) >100, indicating present autoregulation during sepsis in mice[25]. Another study demonstrated a reduced dynamic regulation by tubologlomerular feedback using transfer function analysis in LPS-treated mice, which was partly restored by ARB-treatment[103]. In other forms of AKI such as the more studied ischemia/reperfusion model, a disruption of renal autoregulation has

since long been demonstrated[104,105]. Experimental studies in lambs and sheep demonstrated grossly intact autoregulation during early sepsis without AKI[106,107].

Renal RBF is also influenced by systemic factors, such as RAAS-activation and circulating catecholamines, renal sympathetic nerve activity (RSNA), vasopressin, and natriuretic peptides. There are also renal factors, such as intrinsic RAAS effects, nitric oxide (NO), prostaglandins, and endothelin[99,108,109]. The different effects on regional blood flow by endo and exogenous substances have been summarised by Evans *et al.*[110] and are summarized in the following sentences. Vasoconstricting factors angiotensin II and noradrenaline reduce cortical blood flow to a larger extent than medullary blood flow in contrast to vasopressin which mainly reduces medullary blood flow. Endogenous prostaglandins increase medullary blood flow to higher extent than cortical, a mechanism that can be influenced by NSAID. During pathological conditions, NO and bradykinin act as vasodilators in the kidney and increase blood flow proportionally more in the medulla than in the renal cortex.

Clinical therapies

Vasoactive drugs

Norepinephrine is the preferred initial vasoactive agent for the treatment of septic shock. In a small study of cardiothoracic patients in vasodilatory shock with AKI, an increase of norepinephrine to target MAP from 60 to 75 increased RBF and GFR, indicating an increased autoregulatory threshold[111]. A large randomised study regarding MAP goals in septic shock did not demonstrate a benefit of a MAP target of 80 – 85 compared to 65 – 70[112]. A subgroup of patients with previous arterial hypertension exhibited a reduced incidence of AKI with treatment with the higher blood pressure target. This may be due to a premorbid shift in autoregulation that adapts to pressure. A large observational study showed a relationship between time-adjusted hypotension and the risk of SA-AKI development[113] and indicated 73mmHg as a threshold with the best prognostic discriminatory properties. Such observations, however, come with a risk of unadjusted confounding and may differ in different populations with different case mix. Regarding adding vasopressin to norepinephrine to manage hypotension in patients with SA-AKI, vasopressin constricts the efferent arteriole and may increase GFR. In clinical trials, vasopressin tend to decrease need of RRT but do not decrease AKI in general[114,115]. In cardiothoracic patients, vasopressin does increase GFR but also reduces RBF and thus decreases oxygen delivery while increasing demand[116].

Furosemide increases medullary oxygenation in healthy volunteers[117]. It has also been shown to increase urinary pO_2, a proxy for medullary pO_2[86], in humans with SA-AKI[118]. Although furosemide's effects on oxygen demand have been known for decades, studies to support improved clinical outcomes by furosemide treatment, besides effects on fluid balance, are lacking. Low-dose dopamine increases RBF and oxygenation[119] but does not result in a reduced need for RRT or reduced mortality[120]. Fenoldopam, which is a dopamine receptor agonist, has not been able to reduce the RRT need in a larger RCT[121]. Atrial natriuretic peptide (ANP) increases GFR and RBF but also increases oxygen demand by increasing filtration of sodium, an effect that may be overcome by adding furosemide[122,123]. Studies of therapeutic treatment with h-ANP have been small, not targeted at SA-AKI, and the effect on mortality, and no proven benefit regarding mortality[124,125].

Fluid resuscitation

Fluid resuscitation remains a cornerstone in the initial management of sepsis and septic shock, and to reverse hypovolemia in other instances where acute illness or perioperative stress cause cardiovascular instability. From a renal perspective, fluid resuscitation may increase RBF by restoration of MAP and by reducing neuro-humoural factors that decreases renal perfusion. In sepsis, a protocol of early goal-directed therapy in a randomised single centre study substantially decreased mortality[126]. The protocol focuses on optimising oxygen delivery by the administration of vasoactive drugs, fluids, mechanical ventilation and blood transfusion by using physiological triggers such as central venous saturation to indicate inadequate oxygenation. These concepts have been partly incorporated into the standard of care and have been associated with reduced mortality after implementation[127]. The individual components of the protocol, such as the stringent use of mechanical ventilation, have been questioned and three subsequent randomised multicentre trials examining different protocols, including EGDT, were performed almost simultaneously. Neither the ProMISe trial[128] nor ARISE trial[129] could demonstrate any effect on mortality or AKI/ RRT-use by implanting such a protocol in comparison to standard of care. The ProCESS trial which compared two different strategies of protocolised resuscitation in sepsis with the standard of care, did not demonstrate any benefit of protocolised care[130]. A subanalysis specifically investigated SA-AKI incidense[131]. The protocols resulted in 50% more fluids being administrated to the most liberal group during the first 6 hours of care without affecting the risk of AKI-development in patients without AKI. It also did not change AKI progression in patients with AKI at inclusion.

The use of fluid boluses after initial resuscitation is common. There is, however, insufficient knowledge regarding the effects on physiological parameters

or outcome[132]. Fluid overload is also associated with worse outcome[133]. A randomised feasibility trial of a fluid restrictive protocol (CLASSIC-trial) for the management of patients with septic shock after initial resuscitation, did not demonstrate an increased AKI incidence in the fluid restricted group[134]. The frequency of AKI was actually lower, whereas the median use of resuscitative fluids were reduced by 75% during the first 5 days of ICU stay. Nevertheless, in this protocol, oliguria was used as a trigger for fluid administration even in the fluid restrictive group. Thus, there is currently no evidence that resuscitation with a liberal approach to fluid administration can prevent or alleviate SA-AKI.

In the case of ARDS, the FACTT trial of protocolised liberal or restrictive fluid administration demonstrated improved lung function and more ventilator-free days in the restrictive group[135]. There was an increase in AKI development during the first 2 days, although later RRT-use was similar[136]. Also, furosemide use and reducing a positive fluid balance was associated with increased survival. Another sub analysis of AKI incidence, this time after compensation for fluid overload, however indicated better GFR with fluid restriction[137].

The recommended resuscitation fluids is balanced crystalloid solutions. Starch based synthetic colloid use has been demonstrated to increase AKI and mortality in two high quality randomised trials(6S[138] and CHEST[139]). In the SAFE[140] and ALBIOS[141] study, no beneficial effect of albumin use could be demonstrated. The CRISTAL[142] trial of colloids in the ICU indicated that the kinetic appearance of colloids in this setting differs less than expected from crystalloids, resulting in less efficacy in plasma expansion over time than what can motivate routine clinical use. Randomised comparisons of saline 0.9% and balanced crystalloid solutions have been made (SPLIT[143] and SMART[144]), in which the latter larger study indicated a beneficial renal outcome of balanced crystalloid use instead of saline 0.9%. Healthy volunteers have been examined with renal MRI after either saline, balanced crystalloid[145] and colloids[146] indicating reduced perfusion and tissue oxygenation due to the inevitable hyperchloremia due to saline use.

As a final note regarding fluids, a RCT regarding restrictive or liberal perioperative fluid regimens demonstrated a higher incidence of AKI[147] in the restrictive group. A liberal regimen resulted in circulatory effects as a higher cardiac output, implying a role of hypoperfusion and less favourable renal oxygen demand/supply as possible mediators[148]. However, the parameters of fluid responsiveness (breath-to-breath fluctuations in perfusion pressure) did not differ. The presence of perioperative oliguria was associated with an increased risk of AKI, but was a poor predictor[149]. To summarise, fluid admin-

istration can in some instances prevent AKI, but this have not been convincingly demonstrated during severe infections. There is uncertainty regarding to the degree to which renal effects can be predicted using dynamic indices of fluid responsiveness and to the extent to which this effect is mediated by cardiac output.

The data can be used to support the quite opposite hypotheses regarding future strategies in fluid therapy, either that advances in tailoring will likely improve clinical outcomes substantially, or, that differences in fluid therapy within certain limits are unlikely to either benefit or harm. In **Figures 2-3** some simplified stances regarding fluid management is presented.

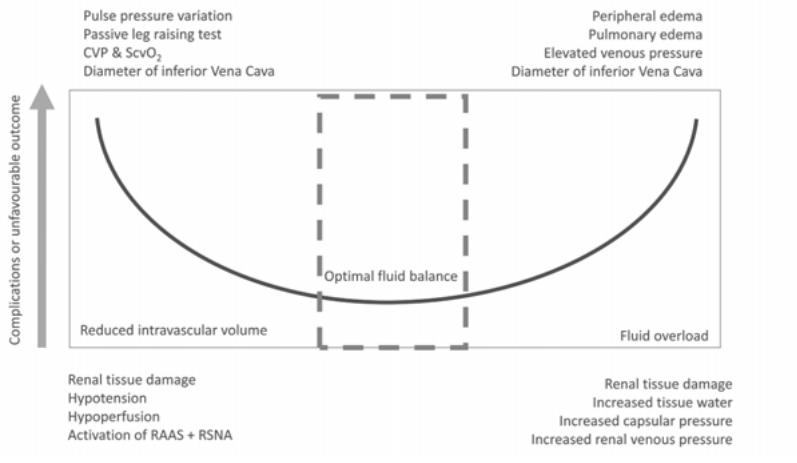

Figure 2. Schematic illustration of the proposed optimal fluid management modified from Bellamy[150] and Ronco *et al*[16]. Some clinical indicators are listed in the upper corners and concomitant pathological mechanisms are listed in the bottom corners. This is a commonly presented concept applied to both AKI and outcome during ICU-care.

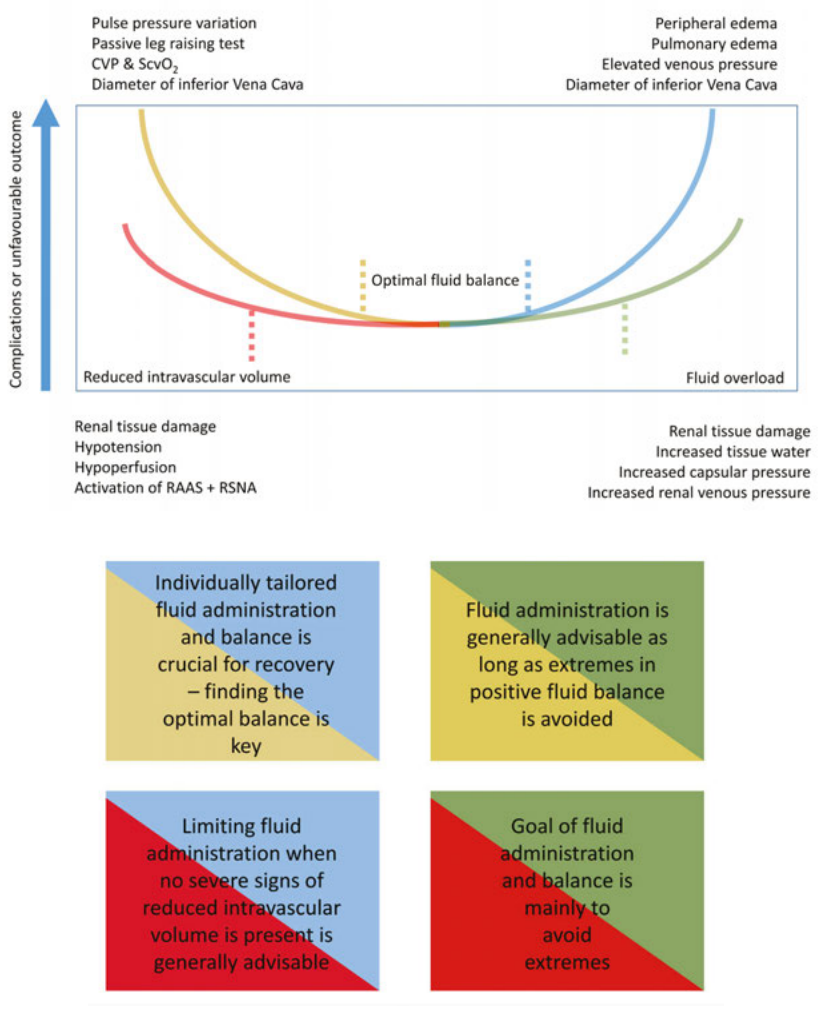

Figure 3a+b. Figure 3a schematically illustrates a limitation to the concept of the optimal fluid balance due to a lack of defined quantitative information. The red and green curves are rescaled to lower *causal* influence on the probability of unfavourable outcomes. This widens the range in which the fluid balance is considered clinically optimal and indicates that some differences in fluid management are clinically irrelevant. Figure 3b illustrates simplified stances based on these assumptions for a specific clinical scenario, depending on which curves are assumed to be true. Both a blue/yellow and red/green perspective can be consistent with results in negative trials regarding different protocols for fluid resuscitation.

Biomarkers of AKI

Urine biomarkers of renal tissue damage have been used to predict the risk of AKI development. Successful attempts to prevent the incidence and progression of AKI in a clinical setting after cardiac or abdominal surgery have been made[151-153]. This is done by identifying high-risk patients as patients with elevated biomarker levels, and directing a care bundle towards these patients. In more detail the bundle include ACEi/ARB discontinuation, monitoring of renal function, avoidance of nephrotoxic agents, blood glucose control and haemodynamic optimisation with advanced hemodynamic monitoring. These interventions are somewhat general in nature, and the effects seen may be explained by other unmeasured factors attributable to increased attention. Biomarkers have been used for the early identification of AKI in sepsis[154,155]. The levels of urine biomarkers increase several hours before AKI detection. However, when other known, more easily accessible risk factors are analysed, the additional value of a biomarker to predict AKI is limited[156]. The clinical use of renal biomarkers is therefore still not widely implemented for most ICU-subpopulations[157]. Reducing AKI simply by 'detection', is not a natural recipe for success; for example the implementation of a large automated alert system of AKI risk did not prevent AKI in a hospital setting while still resulting in more renal consultations[158]. Directed clinical interventions with proven effects are much needed.

The biomarkers used also reflect some degree of tubular dysfunction[16]. Elevated neutrophil gelatinase-associated lipocalin assessed in urine (uNGAL)[154] is associated with distal tubular damage, and kidney injury molecule 1 (KIM-1) is associated with damage in proximal tubules[159]. Tissue inhibitor of metalloproteases-2 (TIMP-2) is a cell cycle arrest marker associated with tubular cellular stress, and may elevate earlier than others. Cystatin C is a 13kD protein filtered in the glomerulus and normally reabsorbed in the proximal tubuli[160]. Although all these molecules rise early during AKI, and may therefore be considered somewhat interchangeable[161], the different properties of the biomarkers may be of interest when examining pathophysiological processes[157] in studies. Whereas filtered NGAL may increase by IL-6 elevation[162] (a prognostic marker in COVID-19), uNGAL could perhaps exhibit a different pattern than other markers of tubular dysfunction. Further, uNGAL is generally elevated by systemic inflammation, as in sepsis[154,156].

Urinary albumin is not used for early AKI identification. Under normal conditions only a very small proportion of albumin passing the glomeruli is filtered. Filtered albumin is reabsorbed in the tubuli by endocytosis[163]. Damages in the filtration barrier, increasing albumin filtration induce albuminuria. Elevated U-albumin is also a marker of nephropathy in hypertension or diabetes, often regarded as a marker of glomerular injury, a perhaps too simplistic view,

as filtered albumin may also induce pathological changes in tubuli[164,165]. Usefulness of U-albumin in COVID-19 AKI may be related to implications of outcome, as increased U-albumin have predicted poorer renal recovery in ICU-patients with SA-AKI[166].

As a final remark, the accuracy of P-creatinine as a biomarker for GFR in the ICU is an area of ongoing research. Compared with GFR estimation by iohexol clearance methods, eGFR by P-creatinine overestimates GFR in ICU-patients while eGFR by Cystatin C often underestimates GFR with neither being supperior[167]. Further, creatinine clearance is variable and non-precise compared to Cr-EDTA clearance in early clinical AKI[168]. There are indications that Cystatin C may be a better marker of long term mortality[169] after care in ICU. The KDIGO definition is, however, widely accepted and no updates have been made since 2012, which is why using P-creatinine for AKI diagnosis and severity classification is still a valid approach.

Initial reports of AKI in COVID-19

As a recapitulation of the information available during the initial phase of the pandemic, there were initial reports of AKI only being of marginal phenomenon in COVID-19[170]. Small retrospective cohorts of ICU patients published in the later part of February/March 2020 indicated an AKI incidence of around 20%[171,172]. In an early larger report of 5700 hospitalised patients in New York, a substantially higher incidence of AKI (22%) were reported[173]. There were more patients with AKI (n=523) than patients admitted to intensive care (n=373), which may imply an even higher incidence in a selected subpopulation with a more severe disease. RRT use in COVID-19 patients treated in the ICU was close to 25% nationwide in the United Kingdom up until April 2020[174], clearly contrasting the initial reports.

Much information on the pathophysiological mechanisms of COVID-19-associated AKI is lacking. Critical COVID-19 is associated with a state of hyperinflammation with increased plasma-levels of proinflammatory cytokines such as TNF-alpha, IL-6 and IL-8[175,176], which are also seen in other severe infections with risk of AKI development, in which some overlap of inflammatory AKI mechanisms are possible. Cytokine levels are however not higher than in sepsis or severe trauma and are not appropriately described as a “cytokine storm”.

A clear relationship in the time between intubation and the initiation of invasive mechanical ventilation (IMV) and an increased occurrence of AKI has been found[177-180]. The time relationship between IMV and AKI may at least partly be explained by the general progression of disease severity. However,

the close time relationship also raises concerns of haemodynamical effects induced by the application of positive pressure ventilation with positive end-expiratory pressure (PEEP) and the administration of sedative drugs, causing a negative influence on renal function.

Despite previous knowledge of fluid management in non-COVID-19 ARDS (and SA-AKI), concerns have been raised that the very common occurrence of AKI in ICU-treated COVID-19 patients may be related to extracellular fluid constriction[181,182], and the importance of individually adjusted fluid management have been stressed[183]. Recommendations for meticulous avoidance of hypovolemia have been widely communicated[184], with the potential to achieve a change of practice of a more liberal fluid administration. The relationship between mechanical ventilation and the perceived link with hypovolemia constitutes a rationale for investigating the renal physiological effects on perfusion and oxygenation and the renal effects of a fluid bolus in critical COVID-19.

Aims

The aims of the studies in this thesis are as follows:

I. To investigate the function of isolated renal mitochondria in comparison to renal oxygen handling and in an ovine model of SA-AKI.

II. To investigate the incidence and characteristics of AKI in COVID-19 patients admitted to intensive care by comparing the two KDIGO criteria over time, as well as their relation to biomarkers for kidney tissue injury.

III. To investigate differences in renal perfusion, oxygenation and water diffusion, using MRI, in COVID-19 patients admitted to intensive care with or without AKI.

IV. To investigate the effects of plasma expansion on renal global and regional perfusion using MRI in COVID-19 patients admitted to intensive care with or without AKI.

Methods

Animal model

Paper I is based on experiments with an ovine model of SA-AKI. Mixed breed sheep were anesthetized, mechanically ventilated, and instrumented with carotid arterial, central and pulmonary venous, and urethral catheters for the purpose of monitoring and sampling. A left paraspinal subcostal incision was made and the left kidney located and freed with mainly blunt dissection. A high-resolution (160Hz) arterial flow-probe, based on ultrasound for determining renal arterial blood flow, was placed around the left renal artery after visual exclusion of ectopic arterial supply. The left renal vein was cannulated with a 4Fr (diameter 1⅓ mm) single lumen vascular catheter enabling blood sampling to determine oxygen content. Sepsis was induced with infusion of live *Escherichia coli*. The bacteria were cultured from a specific frozen stock and stimulated to growth and division, log-phase, by instillation in lysogeny broth (LB-medium) prior to infusion. This induces a systemic inflammatory response, on the cellular and molecular levels different from an infusion of LPS alone. The response is characterised by hyperthermia, increased pulmonary arterial pressure, hypotension, reduced systemic vascular resistance, consumption of platelets, and SA-AKI after approximately 6-18 hours. Creatinine clearance was used as a surrogate marker of GFR and assessed every 6 h. Anaesthesia was maintained throughout the experiment. Crystalloid fluids were administered as a 30ml/kg initial resuscitation, and a maintenance of 2ml/kg/h Ringer's acetate and 2ml/kg/h buffered glucose 10%. Additional fluid boluses were administered at a predetermined fall in cardiac output. Noradrenaline was used to treat hypotension. After completion of the protocol, tissue from the left kidney was harvested and fixated by immersion. The planning and initiation of experiments were conducted prior to the minimum quality threshold in pre-clinical sepsis studies (MQTiPSS)[64] and the latest version of ARRIVE 2.0 guidelines for reporting[185] were published. However, the protocol adhered to the major recommendations as the necessity of fluid resuscitation, recommendation of a microorganism commonly found in human sepsis (and against LPS), monitoring the well-being of animals, and definition of organ dysfunction as a life-threatening deviation from normal based on objective evidence. Volatile anaesthetics reduce RBF[76] in sheep and are associated with increased RSNA[186]. Propofol anaesthesia and instrumentation also reduce

RBF but less than volatile anaesthetics, and propofol anaesthesia is associated with less RSNA than volatile anaesthesia[187]. Neither reduces tissue pO2[187].

In vitro respirometry of isolated renal mitochondria

Isolated cortical mitochondria sampled by a 5 mm skin punch at the beginning and end of the experiment were evaluated. The tissue was immediately cooled in ice-cold isolation consisting of 250 mM sucrose, 10 mM HEPES, 1 mM EGTA, and 1 g/l BSA, pH 7.4 compensated with KOH. Mitochondrial properties are well preserved at this temperature[188]. The tissue is homogenised in a glass homogeniser. Isolated mitochondria were prepared by repeated differential centrifugation in which cellular nuclei and unlysed cells were centrifuged and removed at first, and repeated centrifugation at higher speed with removal of supernatant and buffy coat[43] resulted in a mitochondria rich pellet. A suspension was made with a preservation medium which was then be added to the respirometer with a respiration medium.

A gold and silver based Clark type electrode measured the concentration of oxygen in a micro chamber of 2 cm^2 and respiration rates could be calculated as the negative time derivative of oxygen concentration. Flux values were corrected for back diffusion and leakage from materials and any consumption by the electrode. By successional adding substrates and Complex inhibitors, mitochondrial respiration could be determined as the steady O_2 flux values at different conditions. Flux values then were corrected for protein concentration in each sample, in turn determined spectrophotometrically. The initial respiration with substrates but without stimulants is called State 2 respiration. The addition of ADP is called State 3 respiration, which mimics lack of cellular energy and stimulates maximal OXPHOS capacity. Succinate is needed for Complex II activation and subsequent addition of Complex I inhibitor Rotenone resulted in only Complex II mediated OXPHOS respiration at maximum capacity. Respiratory control ratio (RCR) and Complex I / Complex II relationship was derived by division of the different respiratory rates. LEAK respiration in turn was determined during the presence of oligomycin, an ATP-synthase inhibitor, which inhibits the normal electron transport. Proton leak, spontaneous or by uncoupling proteins (such as UCP family or ANT) results in continuous oxygen consumption which was measured as an O_2 flux. Phosphate/oxygen (P/O) ratio was determined in the respirometer with the addition of an infusion pump module, creating a steady state situation, with continuous addition of ADP at a concentration mimicking half maximal State 3 respiration. Since the continuous addition (consumption) of ADP was known, and oxygen consumption (determined as O_2 flux) is dependent on mitochondrial efficiency, the latter could be determined as the P/O ratio. Schematic illustrations of the ETC and the substrates/inhibitors are presented in **Figures 4-5**.

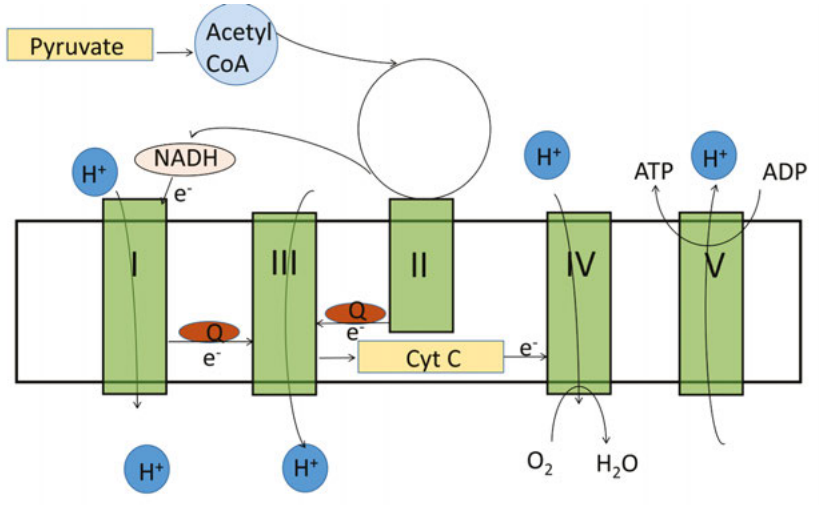

Figure 4. Schematic illustration of the electron transport chain consisting of complexes I-V and the net flux of hydrogen ions (H^+). Reduced nicotinamide adenine dinucleotide (NADH), acetyl coenzyme A (Acetyl CoA), coenzyme Q (Q) and cytochrome C (Cyt C) are indicated at their sites of action.

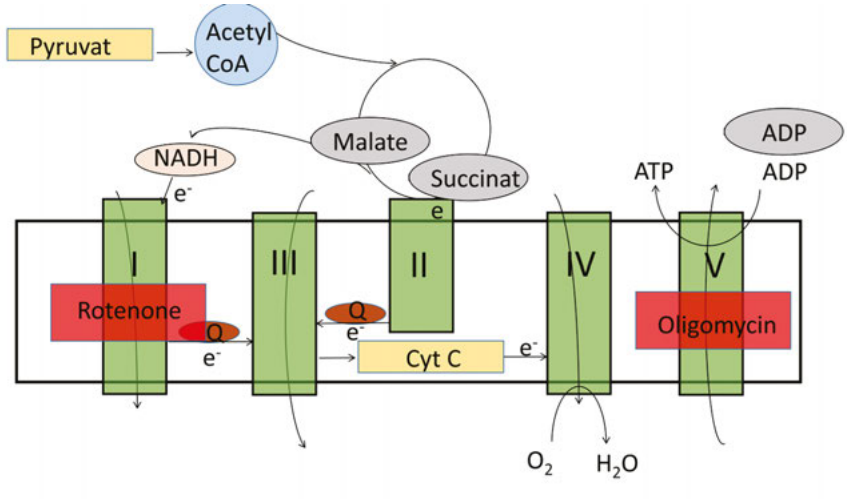

Figure 5. Schematic illustration of the electron transport chain with site of action of substrates in grey and inhibitors in red. Substrates and inhibitors are added sequentially according to the description in the text were O_2-flux was determined in between.

Results were analysed with repeated measures analysis of variance (ANOVA) in which a significant interaction effect was followed by post-hoc testing with planned comparisons using Statistica 13.5.0.17 (TIBCO Software, Palo Alto California). Missing values before the end of the experiment were imputated as group means at baseline, or derived from related intra-individual measurements when possible. Missing values of descriptive variables were excluded.

KDIGO criteria characterisation of ICU cohort

Paper II used data from a sub-cohort consisting of the first 57 consecutive adult patients with confirmed or suspected COVID-19 admitted to the ICU and included in a prospective cohort study at Uppsala University. Exclusion criteria were pregnancy, breastfeeding, pre-existing end-stage renal failure, or dialysis. The data and medical history were collected from the electronic medical record and analysed with end-points consisting of fulfilment of the KDIGO criteria over time. Time was standardised as ICU days, in which a 24-hour period between 7 AM–7 AM with admission date assigned as ICU day 0 in concurrence with the electronic medical record and routine care to enable time comparison of laboratory data and clinical parameters. Clinical characteristics at admission and premorbid conditions were summarised. Fluid balance was defined as all fluids given either intravenously or orally, with quantified losses from catheters and drains, including urine output and faecal losses subtracted, with no estimation of insensible losses. Fluid balances were calculated for each ICU day and expressed as ml/kg in conjunction with the total volume of administered fluid. Baseline p-creatinine was defined as the lowest p-creatinine value within 7 days of hospital admission, or, when lacking, in the year before hospitalisation if available. Hourly urine outputs were recorded during the ICU stay and analysed using actual body weight for the occurrence of oliguria. Recorded outputs were manually checked for errors. Any period fulfilling the KDIGO urine output criteria was registered with the time to each stage of the AKI. Time periods without complete registration were disregarded. Statistical inference testing with continuous variables was made by groupwise comparison by maximum AKI stage using Kruskal–Wallis nonparametric ANOVA. Dichotomous variables were tested using Fisher's exact test or the chi-square test. R version 3.6.3 was used for the calculations. Missing data were excluded from the statistical analysis. Continuous variables are expressed as means and standard deviations (SD) or percentages.

Urinary biomarkers

Urine spot check samples collected within a day of study inclusion were used to assess biomarkers of renal tissue injury in 52 patients from the ICU cohort

in Paper II. Urine Cystatin C was measured on the Mindray™ with reagents from Gentian (Moss, Norway). TIMP-2, KIM-1, and NGAL levels were analysed using a commercial sandwich ELISA kit (DY1757, DY971, and DY1750B, R&D Systems, Minneapolis, MN). The results were evaluated as measured concentration, normalised to U-creatinine, and by dichotomising the samples into positive/negative using cut-offs from the literature. The cohort was divided into groups by the highest stage of AKI according to the different KDIGO criteria during the period two weeks after admission or until discharge. Group wise comparisons for continuous variables was made using Kruskal–Wallis ANOVA and for post-hoc comparison pairwise Wilcoxon Rank Sum Tests with Bonferroni adjustment of p-values. Binary variables were evaluated with Fisher's exact test. R version 3.6.3 was used. Samples were stored cold and frozen the same day as collected and preserved at -80. Freezing/thawing and storage at -80 only have minor effects[189,190].

MERSEP-COVID study

Papers III and IV were part of the MERSEP-COVID study which are illustrated in **Figure 6**. Adult patients with confirmed COVID-19, with, or considered at risk of AKI admitted to the ICU were screened for inclusion, and informed consent was obtained from the patient, or next of kin if the patient was unable to give consent. Exclusion criteria were pregnancy, pre-existing end-stage renal failure or dialysis, contraindications for MRI scanning (e.g. Pacemaker). Patients with instability in vital parameters to a degree where MRI was not feasible (e.g. prone-positioning) were also excluded from scanning. The included patients were transported to MRI with dedicated ICU staff. Mechanically ventilated patients are ventilated with an MRI-compatible Maquet Servo-i MR-Conditional ventilator (Getinge AB, Solna, Sweden) with the same PEEP as before transport and FiO2, respiratory frequency, and inspiratory pressures adjusted to maintain target SpO_2 and minute ventilation. The sedation regimen and vasoactive treatment, when present, were continued throughout the exam. Saturation with pulse oximetry and invasive arterial pressure was monitored continuously and recorded manually every 5 minutes. Breath hold was induced with an expiratory pause when invasive mechanical ventilation was present. The remaining medical data and histories were collected from the electronic medical records. The AKI was determined using the KDIGO creatinine criteria. Patients fulfilling the KDIGO creatinine criteria on the day of the exam or within 12 hours were allocated to the AKI group, while the others were allocated to the No AKI group.

Patients were examined with MRI sequences described in more detail below, assessing RBF, oxygenation, and water content. Due to a lack of established

normal values in this population, existing measurements from 12 healthy volunteers of a similar age previously published were added to the analysis in Paper III *post hoc*. However, the main preplanned comparison was group-wise between patients in the AKI and No AKI groups.

Paper IV consists of a subcohort of the patient population in Paper III. After the initial scan, plasma expansion was achieved with a rapid infusion of 7.5 ml/kg Ringer's acetate, followed by a new multimodal MRI scan with a reduced number of sequences only assessing perfusion and oxygenation. The protocol is registered at Clinical Trials NCT02765191.

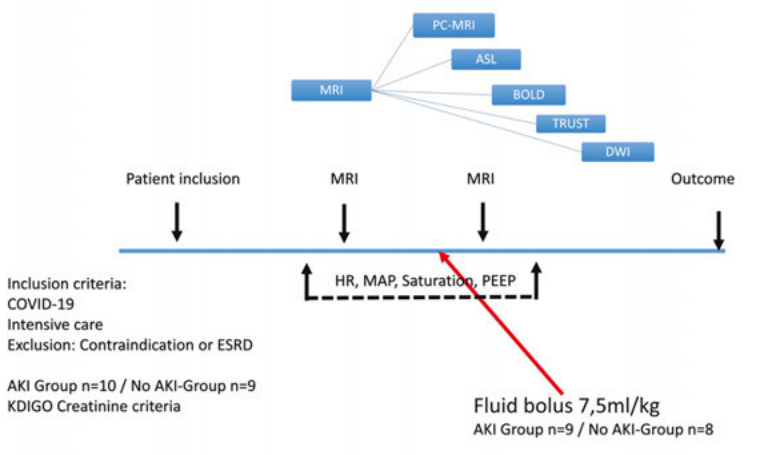

Figure 6. Overview of the study protocol in MERSEP-COVID.

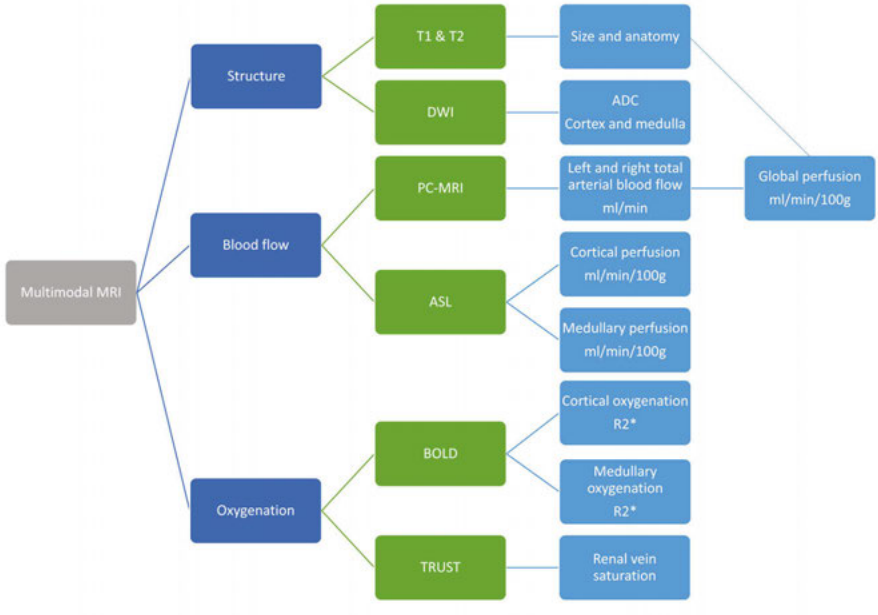

Figure 7. Branched overview of the multimodal renal MRI. Physiological properties are in dark blue, names of the protocols are in green, and output variables are in light blue.

The MRI modalities

The scanning protocol used in the MERSEP-COVID study is a multimodal renal MRI protocol described in more detail in a previous publication[191]. These are outlined in **Figure 7**. T_1 and T_2 weight imaging are the two most common sequences used in clinical MRI and are therefore not described further.

Non-invasive magnetic resonance imaging (MRI) enables the assessment of parameters reflecting RBF, tissue oxygenation and tissue composition[192]. These modalities emerged at the end of the last millennium and were initially developed for imaging of the brain often referred to as functional MRI. Experiments in pigs have validated blood oxygen level dependent (BOLD) MRI as a signal of renal oxygenation by comparing it with invasively measured tpO_2[193]. Reductions in BOLD due to controlled hypoperfusion in animals have also been shown[194]. Experimental studies in humans have resulted in normal values of renal perfusion and oxygenation[192] and a demonstration of the circadian influence on RBF[195]. Further, an increase in medullary oxygenation by furosemide injection in healthy volunteers can be demonstrated[117,196]. Perfusion studies have demonstrated a decrease in cortical perfusion assessed by

arterial spin labelling (ASL) by saline but not balanced crystalloid infusion in healthy volounteers[145]. Although previous studies are consistent with these measurements reflecting perfusion and oxygenation, the measurementss acquired by MRI are also influenced by other factors that do not affect the invasive techniques used in experimental research. A description of the MRI modalities follows below.'

Diffusion weighted imaging (DWI)
DWI uses the diffusion of water (known as Brownian motion) as a contrast in images. The random movement of water molecules is normally constrained by semipermeable membranes surrounding organelles and cells and is influenced by the composition of non-water tissue. Differences in intracellular and extracellular compartments such as oedema influence the signal. Output can be expressed as a mean of attenuation of apparent diffusion coefficient (ADC)[191] from an anatomically defined region (e.g. cortex or medulla). Small voxel ADCs make up the image contrast (analogous to Hounsfield units in computerised tomography) and are expressed as an image in clinical use. A difference in mean ADCs have been demonstrated in CKD[197] compared to non-CKD patients. The ADC values attained by DWI for anatomical studies are influenced by a setting called the b-values, which amplifies diffusion effects. At low b-values, water motion in capillaries and tubules has a proportionally larger contribution to ADC. When measuring ADC using multiple ascending b values, curve fitting using the intravoxel incoherent motion (IVIM) mathematical model allowed us to separate the theoretical contribution from each principally contributing factor. These are labelled true diffusion (D), pseudodiffusion (D*) and perfusion fraction (fp).

Phase Contrast (PC-MRI)
The clinical use of phase contrast is to measure blood flow in larger vessels. Spins (originating from water molecules) moving in the same direction as a magnetic field with a gradient are affected differently than stationary spins, and the difference is proportional to velocity. By using a bipolar gradient with a neutral net effect on stationary spins, the intensity of the signal from moving is proportional to speed. In practice, the renal artery is visualised using regular morphological T_1 mapping and a grid is placed at a suitable part[198]. Flow velocity is measured several times each cardiac cycle, creating a pulsatile flow curve similar to a flow probe. From these, flow can be measured by including the known diameter of the vessel. Vascular anomalies with turbulent flow and/or double arteries may cause difficulties in flow estimation. Further, deviances in the measured angle from the true flow direction affected the results. The examination is made during a < 20 s expiratory breath hold depending on heart rate. The output measure primarily used was RBF in each kidney expressed as ml/min.

Arterial spin labelling (ASL)
In this modality, water is magnetically labelled intra-arterially and used as a contrast medium. By subtracting images with labelled water from non-labelled images, regional images reflecting perfusion of the labelled water are created[199]. Image acquisition is made using respiratory triggering due to its sensitivity to motion artefacts. An initial scan examines the arrival time of the labelled blood by differing post-labelling delay times. In our protocol, 40 pairs of labelling and measurements are made during approximately 8 minutes depending on the respiratory rate[191]. The measurement in MERSEP includes flows in the renal medulla. Output is mean perfusion expressed as ml/min/100g based on values from anatomically defined grids (e.g. cortex and medulla).

Blood oxygen level dependent (BOLD)
This modality is based on the different paramagnetic properties of deoxyhaemoglobin and oxyhaemoglobin, where the presence of deoxyhaemoglobin shortens T2* signals. This scan is sensitive to motion artefacts. Other factors besides tissue oxygenation that affect deoxyhaemoglobin content, such as regional blood volume and flow, acidosis, hypercarbia, and haemodilution, may affect the BOLD signal. The measurement is made during a 16-second expiratory breath hold. Output can be expressed as mean R2* (which is the inverse of T2*) from manually defined regions of interest (ROIs). It can also be expressed as gradients of a "twelve layer concentric objects" (onion peel division of kidney) or as a histogram or map of fractional tissue hypoxia with a compartmental model[191] . For primary analysis, the mean R2* from the ROI maps was chosen. Differences in R2* can be considered to express relative differences in oxygenation but are not reliably converted to pO2 or without context-specific calibration.

T_2 Relaxation Under Spin Tagging (TRUST)
TRUST is based on T_2 relaxation times as is BOLD but uses radio frequency labelling of blood to procure a measurement from renal venous blood. Venous saturations can be calibrated to provide saturations within a few percent[200]. It has been used intracranially and has just recently been adapted for the examination of abdominal organs[201]. It is applied to a grid in the left renal vein and the measurement takes up to 5 minutes. From the measured R2 of venous blood, saturation in percent can be calculated and is dependent on haematocrit.

Statistical analysis

In Paper III, the mean of the measured variable from both kidneys (when available) was used as the end point for comparison, with the exception of total RBF, which was defined as mean times 2. If a measurement in a single kidney was missing or unreliable, the value from the other kidney was used instead.

Missing data were excluded. When continuous variables were compared between the two groups, a Mann–Whitney U test was used. A Kruskal–Wallis one-way ANOVA was used to compare the two study groups with healthy volunteers. Post hoc correlations presented in the summary were made using the product-moment correlation. Descriptive data were calculated using Excel 2016 (Microsoft, Santa Rosa, California), and other statistical calculations were made using Statistica 13.5.0.17 (TIBCO Software, Palo Alto, California).

In Paper IV, the scientific question was analysed using paired before–after measurements and an interaction effect. Both kidney measurements were used in a nested mixed model repeated measures ANOVA in which each kidney was compared with its own previous measure, taking into account which individual the kidney belonged to. First, a mixed linear model was created in which fluid bolus status, AKI status, and an interaction were used as fixed effects, whereas individual and kidney were used as random effects. Model fit was assessed using visual evaluation of residuals/fitted and QQ plots. Where heteroscedasticity was considered prevalent, logarithmic transformation was used, and the new model was similarly re-evaluated. Residuals were thereafter tested for normality using Shapiro-Wilk's method, and the model was accepted if normality could not be refuted. The accepted model was analysed using ANOVA type III with Kenward Roger's method for determination of degrees of freedom. A significant effect of either fluid bolus status or interaction refuted the null hypothesis of no effect of fluid bolus in either the AKI or No AKI groups and was evaluated further using pairwise comparisons for the effect of plasma expansion within each group, with p-values adjusted using Tukey's method. Estimated marginal means were used to determine the means and confidence intervals of the measurements presented in the summary and manuscript. These were derived from the final model and reflect the effects and precision evaluated in the formal analysis. R studio 22.07.01 using R 4.1.2 was used for statistical analysis with packages lme4 (1.1-27.1), lmerTest (3.1-3), and emmeans (1.7.0) using functions lmer, anova, and emmeans. Graphs were created using the ggplot2 (3.3.5) package. Continuous data are presented as the mean (95% confidence interval) in the text, if not stated otherwise.

Ethical considerations

In Paper I, an animal model was used to perform the experiments. The animals were not purpose-bred but were acquired from the same carer, who was responsible for treating the animals in accordance with Swedish regulations of animal welfare before arrival at the laboratory. The experiments were conducted in a state of terminal anaesthesia and in accordance with the principles

of the 3 Rs (Replacement, Refinement and Reduction) first proposed by William Russell and Rex Burch. The animals were not considered to be subjected to severe pain or suffering. Approval for the study was obtained in advance from the Regional Ethics Committee for Experiments in Animals, Uppsala, Sweden (C60/16 with amendments 5.8.18-02074/2017 and 5.8.18-14384/2017) and all animals were handled in accordance with the directives from the European Commission for animal research. In Papers II, III, and IV, we included patients during their current treatment in intensive care. Informed consent was given by either the patient or next of kin if the patient was not able to give consent at the time of inclusion. When informed consent was given by the next of kin, surviving patients were given verbal and written information about the studies and approved participation. They were given the opportunity to ask questions and were informed of their rights regarding withdrawal and handling of sensitive personal information and that declining participation and withdrawing from the study would not affect the care provided. Acquiring informed consent in an intensive care setting without breaching patients' autonomy is delicate, as patients are vulnerable and affected by disease. This is an inherent problem for some research in healthcare in cases such as when patients are either acutely ill or recently given a severe diagnosis. However, informed consent is generally not waived for this reason, and there is an ethical imperative in aiming to improve care for patients through research. In Papers III and IV, care to not affect the standard of care outside the examination was made, and rigorous safety precautions regarding transportation were undertaken. Information from the exam could also be shared with clinicians treating patients. No medical intervention aimed at correcting hypovolemia was withheld as part of the study before the exam. The study from which Paper II is a part was approved by the National Ethical Review Agency (EPM; No. 2020-01623). The study from which Papers III and IV were made was approved by the Uppsala Regional Ethical Review Agency (No. 2014/381 with amendment No. 2020-01996 and No. 2021-04798). The Uppsala University Hospital directory for clinical research, innovations, and education also approved all patient-related research.

Results

Paper I

A total of 21 animals were included in the final analysis. Of 13 sheep in the sepsis group, 5 were terminated prematurely after a mean 22 ± 2.5 hours due to irreversible cardiopulmonary deterioration. 8 animals in the control group and 8 sepsis group completed the full pre-defined course of the experiment. The anaesthesia-regiment was comparable between groups (propofol + sevoflurane, n = 4 + 4 in the control group and n = 7 + 8 in the sepsis group).

Sepsis was not associated with renal hypoperfusion. Mean left renal blood flows at the end of experiment were similar in the Sepsis group, 261 ± 194 ml/min, as in the control group, 240 ± 84 ml/min, (n.s.). Renal VO_2 was unaffected by sepsis, with a mean left renal VO_2 of 3.5 ± 1.5 ml/min in controls and 3.6 ± 1.6 ml/min in septic animals at the end of the experiment (n.s.). Renal sodium resorption was substantially reduced in sheep with sepsis, where mean sodium resorption at the end of the experiment was 0.8 ± 0.7 mmol/min compared to 9 ± 5 mmol/min in the control group ($p < 0.001$). Septic sheep also developed a disturbed relation between sodium resorption in relation to oxygen consumption, with a markedly lower mean sodium resorption per ml consumed O_2 at the end of the experiment (septic sheep 0.1 ± 0.1 mmol/ml compared with controls 1.4 ± 0.8 mmol/ml, $p < 0.001$).

Respirometry in isolated mitochondria was affected by sepsis. The respiratory control ratio was reduced at the end of the experiment in the sepsis group compared to the control group, indicating a disturbance in renal mitochondrial coupling (6 ± 1.5 vs. 8.2 ± 1.6, p = 0.006) (Fig. 8A). A reduced complex I mediated state 3 capacity (Fig. 8C) at the end of the experiment compared to the baseline in septic animals (1.8 ± 0.4 vs. 1.5 ± 0.6 $pmol \cdot s^{-1} \cdot \mu g^{-1}$, p = 0.002) was also present, whereas no significant difference in State 2 (Fig. 8B) could be demonstrated. The ratio complex II /complex I of state 3 was increased at the end of the experiment in the Sepsis group (1.6 ± 0.2 vs. 1.3 ± 0.1, p = 0.002), but not in the control group (Fig. 8D). This was mainly caused by a reduction in complex I activity in the sepsis group at the end of the experiment. No differences in mitochondrial efficiency (P/O ratio with complex I substrates) or mitochondrial uncoupling (LEAK respiration) were demonstrated (Figs. 8E and 8F). Mitochondrial morphology was examined in podocytes in

3 randomly selected glomeruli in all included ewes cortical biopsies. No pathological ultrastructural changes were observed in the podocytes in any of the animals. Further, mitochondrial morphology was examined in five randomly selected tubuli in three animals from the sepsis group and three in the control group without differences between the groups. However, some morphological artefacts in tubular cells in general, were noted, due to the insufficiency of the fixation by immersion.

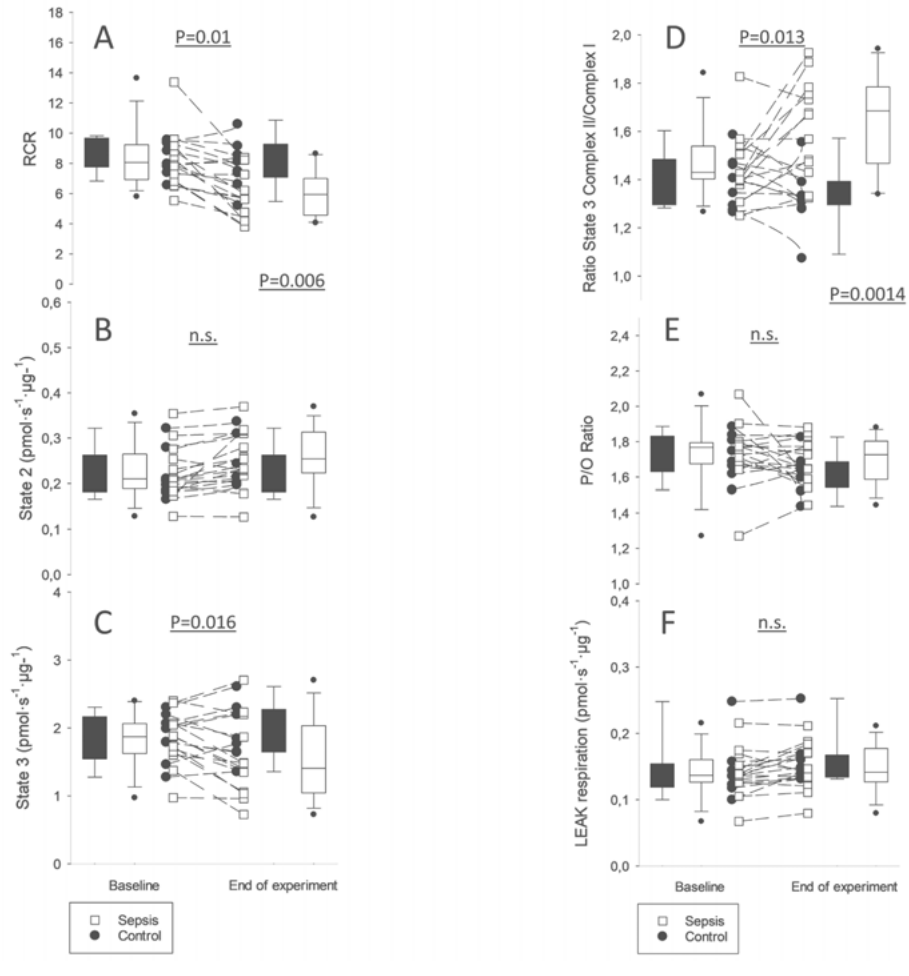

Figure 8. Results of mitochondrial parameters RCR, state 2, state 3, C II/C I, P/O ratio and leak at baseline and end of experiment in control group (n = 8) and sepsis group (n = 13). Results are presented as boxplots (ends) and individual data points (between), where dashed lines indicate changes within each individual. The header indicates the results of the interaction effect in the ANOVA. P-values from post hoc tests between groups are added below box-plot when $p < 0.05$.

Paper II

Acute kidney injury incidence according to different criteria and times

A majority of patients (n = 51/57, 89%) developed AKI according to the KDIGO definition. Of these, 29/57 (51%) patients were classified as KDIGO stage 2, and 13/57 (23%) stage 3 AKI. By analysing only one criterion at a time, 36/57 (63%) patients developed AKI according to creatinine criteria ($KDIGO_{Cr}$) and 44/57 (77%) patients developed AKI according to urine output criteria ($KDIGO_{uo}$). Both criteria were fulfilled in 29/57 (51%) patients. AKI severity according to the two KDIGO criteria was, however, not well-matched. A total of 27/57 (47%) patients had predominantly oliguric AKI and developed the highest AKI stage due to oliguria. Oliguria without fulfilment $KDIGO_{Cr}$ at any time during the study occurred in 15/57 (26%) patients with AKI. Of these 12/15 (80%) progressed to KDIGO stage 2 AKI according to urine output criteria. AKI due to creatinine criterion without any episodes of oliguria occurred less frequently (n = 6, 9%). AKI according to the creatinine criterion occurred in 15/57 (26%) patients before ICU admission, and later development (on ICU day 3 or beyond) of AKI according to creatinine criteria was common (12/36, 33%), especially for stage 2 and stage 3 AKI (12/18, 67%). Patients who developed oliguria severe enough to be classified as AKI tended to do so before the end of ICU day 2 (37/44, 84%). Oliguria preceded creatinine rise in 18 out of 20 cases (90%) ($p < 0.001$) in patients without AKI at admission that later fulfilled the KDIGO AKI definition by both criteria. The development of the different criteria over time can be viewed in **Figure 9**, and the matching of criteria is illustrated in **Figure 10**.

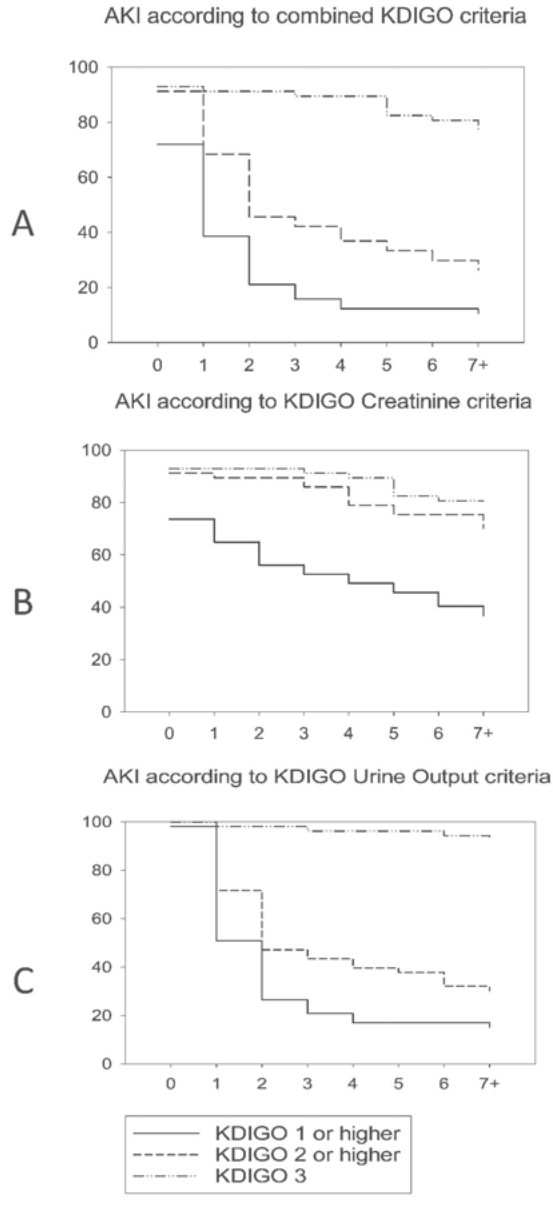

Figure 9. Cumulative incidence of AKI as the proportion of patients without AKI according to different criteria in graphs A-C. The different stages are illustrated by different lines in relation to ICU days in the x-axis. The y-axis is the percentage of patients without AKI to the stage according to the line.

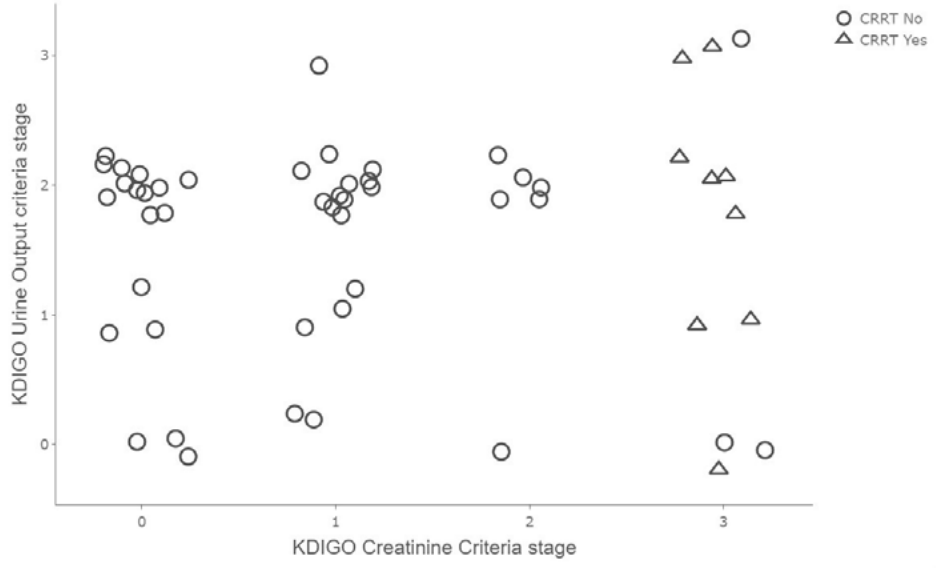

Figure 10. Plot of the relationship between AKI stages by the two different KDIGO criteria. Individual participants are illustrated by symbols, highlighting with patients receiving continuous renal replacement therapy (CRRT). Patients not classified by both criteria are excluded from this plot.

Patient characteristics, comorbidities, and clinical outcome

The mean age of the study population was 59 (± 14) years, and women comprised 23% of the study population. The most common comorbidities were hypertension (54%), diabetes mellitus (28%), and chronic pulmonary disease (25%). Forty percent of the patients were treated with angiotensin converting enzyme inhibitors or angiotensin II receptor blocking drugs (ACEi/ARB) (Table 1). Baseline P-creatinine was available for all patients with a mean of 73 ± 20 µmol/L and only 6 (11%) patients had a baseline P-creatinine above the reference interval (women 90 µmol/l, men 105 µmol/l). Symptoms of COVID-19 started on average 11 ± 3 days before ICU admission. Invasive ventilation was given to 68% of patients during their ICU stay and was associated with AKI ($p = 0.01$). Most patients were circulatory stabile upon arrival, with a mean arterial pressure (MAP) of 93 ± 16 mmHg. Although four patients received vasopressor drugs at admission, no patients were hypotensive (MAP <65 mmHg). Patients who developed stage 3 AKI tended to be older, have a higher body weight, and have higher P-creatinine at admission. Continuous renal replacement therapy (CRRT) was initiated in 9/57 (16%) patients at a mean of 152 ± 113 hours after admission. The indications for CRRT initiation were the consequences of AKI in all patients. Mortality within 30 days of ICU admission was 28%. The clinical parameters are summarised in **Table 1**.

Table 1. Clinical data regarding comorbidity, disease severity at admission and outcome by grouping of maximum KDIGO AKI stage. Variables are expressed as means and SD or percentages.

	No AKI	KDIGO 1	KDIGO 2	KDIGO 3
N	6	9	29	13
Age (years)	53±22	63±9	56±15	67±6
Sex (% female)	33	44	24	0
Body weight (kg)	78±14	77±16	89±14	93±27
BMI	25±3	26±8	30±5	32±7
Pulmonary disease (%)	17	33	31	8
Hypertension (%)	33	56	52	69
Heart failure (%)	0	11	3	0
Ischemic heart disease (%)	0	22	7	15
Malignant disease (%)	0	11	7	8
Diabetes (%)	0	44	28	31
ACEi/ARB treatment (%)	17	56	41	38
Baseline P-Creatinine (µmol/l)	69±14	69±25	73±21	80±13
Elevated baseline P-Creatinine (%)	0	11	14	8
At ICU admission:				
SAPS 3 score	47±5	58±9	51±10	55±9
Glasgow Coma Scale 15 (%)	80	67	79	92
Heart rate	81±9	96±26	91±13	86±15
Mean arterial pressure	92±14	99±20	92±17	92±15
COVID day	11±4	11±4	11±3	10±3
P-Creatinine at arrival (µmol/l)	79±18	81±34	83±38	244±443
Invasive ventilation (%)	0	33	17	8
Vasopressor treatment (%)	0	22	3	8
During ICU stay:				
Highest P-Creatinine (µmol/l)	89±20	95±28	116±54	441±410
Invasive ventilation (%)	17	67	69	92
30 day mortality (%)	17	33	17	54

Fluid balance

Patients in general had a positive cumulative fluid balance at the end of the first whole day in the ICU (ICU day 1) with a mean positive balance of 18 ± 19 ml/kg and a mean total volume of administered fluids of 57 ± 27 ml/kg. There were no significant differences between groups in absolute values or normalised to actual body weight, or the proportions of patients with negative fluid balance at the end of ICU day 1. No significant differences in fluid balance at the end of ICU day 1 were found in the subgroup of patients without

AKI at the time of admission. There were also no significant differences in cumulative fluid balance at the time of KDIGO AKI stage criteria fulfilment between stages 1, 2 and 3. In absolute numbers the mean volume of administered fluids were 4.6 ± 1.7l at the end of ICU day 1 resulting in a mean fluid balance of +1.4 ± 1.5l. Data stratified by KDIGO stage is presented in **table 2**.

Table 2. Administered fluid volumes and fluid balances at the end of ICU day 1 presented groupwise as the maximum KDIGO stage during the 14 day study period. Variables are expressed as means and SD or percentages.

During ICU stay:	No AKI	KDIGO 1	KDIGO 2	KDIGO 3
Total volume of fluids at end of ICU day 1 (ml/kg)	72±57	59±22	54±23	50±15
Cumulative fluid balance at end of ICU day 1 (ml/kg)	27±21	13±19	19±20	16±19
Proportion of patients with positive cumulative fluid balance at end of ICU day 1 (%)	83	89	76	69

Renal injury biomarkers

There were differences in creatinine-corrected U-cystatin C levels (p = 0.004), with the highest levels in stage 3 AKI (0.4 ± 0.4 mg/mmol) and the lowest in stage 2 (0.1 ± 0.1 mg/mmol). In patients classified using urine output only with disregard of P-creatinine, stage 2 AKI was associated with lower levels of corrected U-cystatin C (0.1 ± 0.1 mg/mmol) compared to stage 1 (0.3 ± 0.2 mg/mmol) and no oliguria (0.3 ± 0.3 mg/mmol) (p = 0.001 and 0.001, respectively). There were no significant differences in the levels of uNGAL between the groups. U-cystatin C was elevated above the cut-off value in 39/52 (75%) patients, whereas U-NGAL was higher than 100 ng/ml in 13/52 (25%) patients. KIM-1 and TIMP-2 were elevated above the cut-off value in 35/52 (67%) and 26/52 (50%) patients, respectively, with no significant differences between the groups in either absolute or corrected means or proportions above the cut-off.

Table 3. Levels (mean ± SD) and proportions of urinary biomarkers at ICU admission in different AKI stages, defined by the combined KDIGO criteria.

	No AKI	KDIGO 1	KDIGO 2	KDIGO 3
N	6	9	27	10
U-Cystatin-C$_{Corr}$ (mg/mmol) ††	0.3±0.3	0.2±0.1**	0.1±0.1**	0.4±0.4
U-Cystatin C (mg/l)	0.9±0.7	1.2±0.8	0.8±0.6	1.6±0.9
U-Cystatin-C > 0.414 mg/L (%)	83	89	63	90
U-NGAL (ng/ml)	169±221	95±79	73±140	239±362
U-NGAL$_{Corr}$ (ng/mmol)	45±82	27±37	10±24	60±99
U-NGAL > 100 ng/ml (%)	33	33	11	50
U-KIM-1 (ng/ml)	2.3±3.3	3.2±2.8	4.5±3.8	3.5±2.5
U-KIM-1$_{Corr}$ (ng/mg)	2.5±2.3	5.1±3.8	5.3±6.2	4.1±2.6
U-KIM-1 > 2.37 ng/mg (%)	33	67	70	80
U-TIMP-2 (ng/ml)	6±3	16±29	9±6	12±9
U-TIMP-2$_{Corr}$ (ng/mg)	10±5	45±99	10±6	14±9
U-TIMP-2 >7.58 ng/ml (%)	20	20	64	56

† = p <0.05, †† = p <0.01, ††† = p <0.001 indicates difference between all groups.
* = p <0.05, ** = p <0.01, *** = p <0.001 indicates difference between groups.

Albuminuria at ICU admission was common, and 38/52 patients (73%) had at least microalbuminuria while 9/52 (17%) presented with severe albuminuria (**Figure 11**). There were no significant differences in U-albumin between AKI stages.

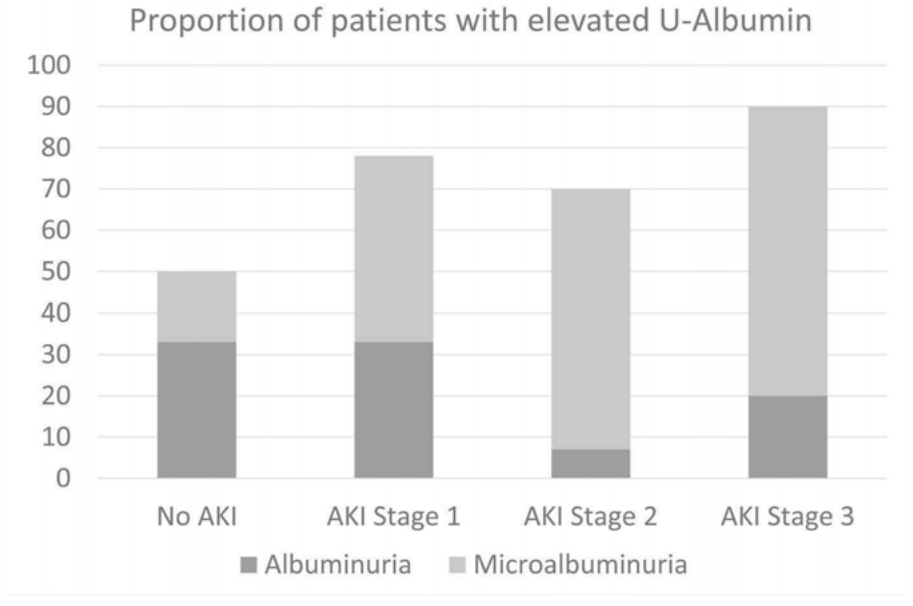

Figure 11. Proportion of patients (in percentages in y-axis) with elevated U-albumin presented group wise according to highest KDIGO AKI stage.

Paper III

The analysis included exams of nineteen patients in the two study groups, 10 in the AKI group and 9 in the No AKI group. A third healthy volunteer group consisting of 12 individuals from a previously published study were added post hoc[197]. The median ages were similar in the three study groups (Table 4). There was a common occurrence of comorbidities in patients with COVID-19, most commonly a history of hypertension (63% of patients). During the current hospitalisation, all patients had at least one P-creatinine measurement within the normal range prior to the MRI scan. The AKI and No AKI groups were well separated in regard to renal outcomes. During the whole course of the hospitalization 80% of patients in the AKI group had at least one episode of severe AKI (≥ Stage 2 according to KDIGO creatinine criteria) compared with none of the patients in the No AKI group. At the day of the scan patients were generally circulatory stable. Vasoactive drugs was used for 63% of patients in low doses. The current episode of AKI for patients in the AKI group

had debuted in median 1 day [IQR 0–2] prior to the exam, and 2 (20%) patients had already developed severe AKI (≥ stage 2) at the time of the exam. Patient characteristics are further described in **Table 4**.

Patients in the AKI group had lower total RBF, cortical and medullary perfusions compared with patients in the No AKI group (**Figure 12**). Compared with the healthy volunteers, RBF in the AKI group but not in the No AKI group was lower. Cortical perfusion was higher in healthy volunteers than in both groups of patients. There were no differences in BOLD relaxation rates assessing renal regional oxygenation between the groups, in neither renal cortex nor medulla. The lowest relaxation rates (consistent with low concentrations of deoxyhaemoglobin) were found in either of the two groups of patients with COVID-19. Measures of renal venous oxygenation assessed with TRUST had several non-valid measurements without any demonstrable group differences. The measurements of perfusion and oxygenation are further presented in **Figure 12**. DWI could not demonstrate any significant difference between the patients with and without AKI (**Table 6**). Cortical T_1 was longer in the AKI group (1560 ms [1524–1638]) compared with healthy volunteers (1459 ms [1400–1525], $p = 0.009$).

Post hoc simple linear regressions were conducted to explore physiological factors that may affect renal perfusion and to facilitate the interpretation of regional oxygenation data. A negative linear relationship between the renal arterial resistive index and renal perfusion was demonstrated. No relationship was found between the measures of renal perfusion and PEEP or MAP.

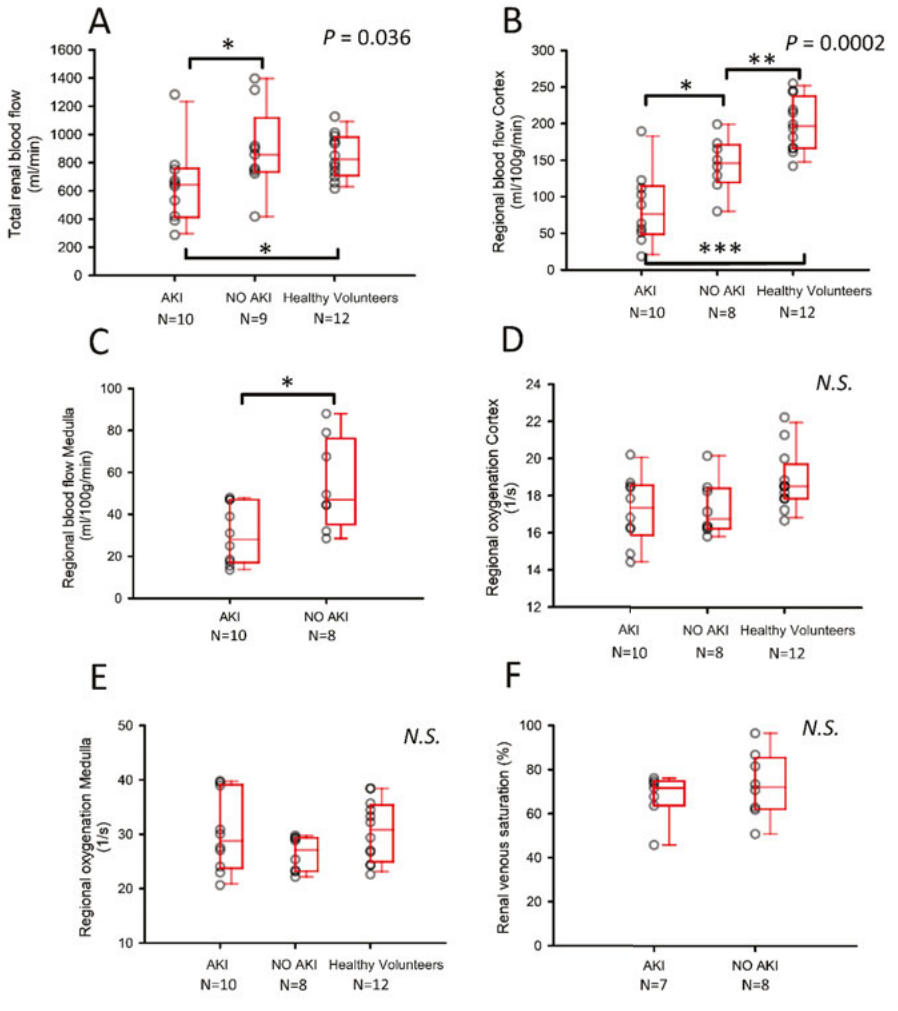

Figure 12. Box and scatterplots of the MRI results of the AKI and No AKI groups, along with 12 healthy volunteers of similar age. Valid numbers of MRI examinations are specified for each parameter and group. p values from Kruskal–Wallis ANOVA. *, **, *** signifies p <0.05, 0.01, and 0.001, respectively, in the Mann–Whitney U test.

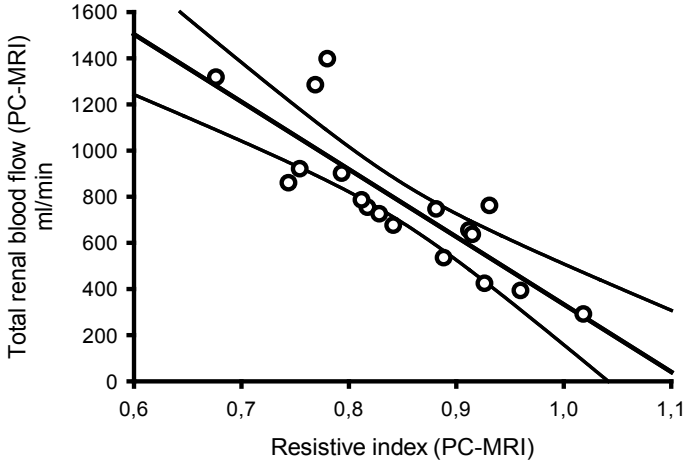

Figure 13. Scatterplot demonstrating the relationship between the resistive index and total RBF in 18 patients in the ICU-treated study population with COVID-19. Added to the plot is a line indicating the estimated correlation with a 95% confidence region. The correlation is significant with a $p < 0.001$ and $r = -0.76$.

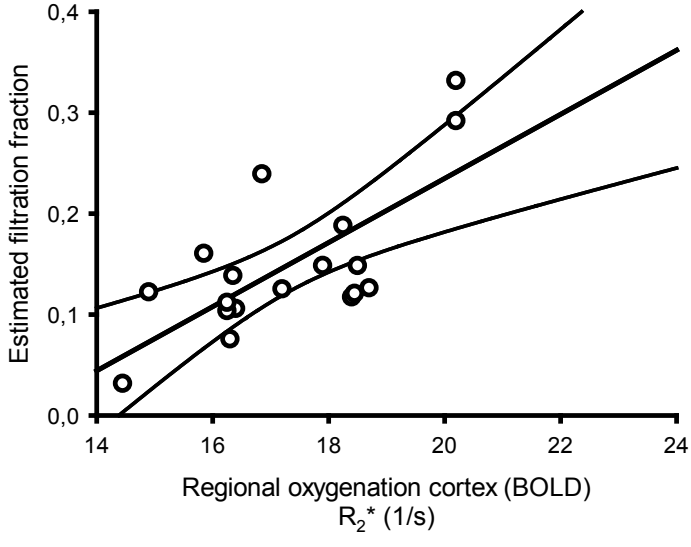

Figure 14. Scatterplot demonstrating the relationship between the cortical BOLD and the estimated filtration fraction in 18 patients in the ICU-treated study population with COVID-19. Added to the plot is a line indicating the estimated correlation with a 95% confidence region. The correlation is significant with a $p = 0.001$ and $r = 0.70$. Filtration fraction was estimated as: eGFRCreatinine/(total RBF×(1−haematocrit)).

Table 4. Patient characteristics, comorbidities and outcome in the 19 patients treated in ICU for respiratory failure due to COVID-19 included in study.

	AKI (N=10)	NO AKI (N=9)
Age, years [IQR]	66[64–72]	65 [53–70]
Male, n (%)	8 (80%)	8 (89%)
Height, cm [IQR]	173 [169–177]	180 [170–187]
Weight, kg [IQR]	94 [82–102]	86 [80–102]
Body Mass Index, [IQR]	32 [27–35]	27 [26–36]
Hypertension, n (%)	7 (70%)	5 (56%)
History of treatment with ARB/ACEi, n (%)	7 (70%)	6 (67%)
Diabetes, n (%)	4 (40%)	2 (22%)
Ischemic heart disease or congestive heart failure, n (%)	3 (30%)	2 (22%)
Ischemic heart disease, n (%)	3 (30%)	1 (11%)
Congestive heart failure, n (%)	0 (0%)	1 (11%)
Asthma or COPD, n (%)	2 (20%)	0 (0%)
History of CKD, n (%)	0 (0%)	0 (0%)
Baseline Plasma-Creatinine, µmol/l [IQR]	68 [65–77]	66 [58–73]
Most severe AKI-stage during hospital stay, [IQR]	2[2–3]	0[0–1]
AKI stage 2 or 3 any time during hospital stay, n (%)	8 (80%)	0 (0%)
RRT at any time in ICU, n (%)	2 (20%)	0 (0%)
SAPS 3, [IQR]	54[52–56]	53[50–55]
Days of symptomatic COVID-19 at ICU-admission, [IQR]	9[8–11]	9[9–10]
Treatment with dexametasone, n (%)	8 (80%)	7 (78%)
IMV at any time during ICU stay, n (%)	10 (100%)	7 (78%)
Days with IMV, [IQR]	18[15–22]	14[6–18]
Vasoactive treatment at any time in ICU, n (%)	10 (100%)	7 (78%)
Moderate or severe ARDS, n (%)	10 (100%)	8 (89%)
Severe ARDS, n (%)	8 (80%)	5 (56%)
90-day survival, n (%)	6 (60%)	6 (67%)

Table 5. Patient characteristics of physiologic parameters on the day of the MRI exam in the 19 patients included in the study who were treated in ICU for respiratory failure due to COVID-19.

	AKI (N=10)	NO AKI (N=9)
Current Plasma-Creatinine, µmol/l [IQR]	104[101–114]	67 [64–77]
Current eGFR (Creatinine), ml/min [IQR]	56[49–59]	87 [78–90]
Current KDIGO Creatinine AKI Stage	1[1–1]	
Last recorded hourly urine output, ml/kg [IQR]	0.9[0.3–1.7]	1 [0.8–1.7]
Last recorded hourly urine output, ml [IQR]	65[35–131]	110 [80–150]
Furosemide within 12 hours before MRI scan, mg [IQR]	8[1–10]	0 [0–0]
Time since latest furosemide, h [IQR]	7[5–20]	28 [17–36]
Any furosemide within 3 hours before MRI scan, n (%)	2 (20 %)	1 (11 %)
Net fluid intake at exam day, ml [IQR]	318[-15–629]	184 [-16–429]
SOFA score, points [IQR]	7[7–8]	6 [4–6]
Days of symptomatic COVID-19, n [IQR]	17 [14–19]	12 [11–14]
Days in ICU at exam, n [IQR]	8 [4–8]	3 [2–5]
Plasma-CRP, mg/l [IQR]	98[71–140]	115 [64–186]
Days since start of invasive ventilation, n [IQR]	4 [2–5]	2 [2–3]
Arterial oxygen saturation	96 % [93–97]	95 % [95–98]
Arterial pO_2, kPa [IQR]	10 [10–11]	10 [10–12]
Arterial pCO_2, kPa [IQR]	6.3 [5.7–6.6]	5.5 [5.2–6]
Arterial pH, [IQR]	7.39 [7.37–7.41]	7.42 [7.4–7.44]
P/f-ratio, kPa [IQR]	21 [20–27]	26 [25–33]
PEEP, cmH_2O [IQR]	14 [13–15]	10 [9–12]
Mean arterial pressure, mmHg [IQR]	80 [79–85]	81 [80–98]
Sinus rhythm, n (%)	10 (100 %)	8 (89 %)
Blood haemoglobin, g/dl [IQR]	11.5 [11.3–12.3]	12.8 [11.4–12.9]
Central venous saturation, [IQR]	73 % [72–77]	67 % [65–74]
Vasoactive drug, n (%)	8 (80 %)	4 (44 %)
Noradrenaline dose, µg/kg/min [IQR]	0.05 [0.01–0.07]	0 [0–0.03]
Plasma-lactate, mmol/l [IQR]	1.4 [1–1.9]	1.5 [1.3–1.6]
Plasma-NT-proBNP, ng/l [IQR]	201 [131–633]	373 [236–491]

Table 6. Additional results of renal multiparametric MRI. The data are presented as medians [quartile range]. Significant differences from Mann-Whitney U-Test between AKI and No AKI groups are indicated by ‡ if $P < 0.05$. Significant differences from the one way Kruskal-Wallis ANOVA between all groups are indicated by † if $P < 0.05$. Valid numbers of MRI exams are specified for each measure and group.

	AKI	***n***	**NO AKI**	***n***	**HEALTHY VOLUNTEERS**	***n***
Perfusion						
Global perfusion (ml/100g/min)	168 [142–216]	10	202 [174–235]	9	220 [174–242]	12
Regional Perfusion Ratio (Cortex/Medulla)	2.4 [2.2–3.3]	10	2.2 [1.7–3.4]	7		
Resistance Index ‡	0.90 [0.82–0.93]	10	0.79 [0.75–0.86]	8		
Renal vascular resistance (mmHg/ml/ min)	0.11[0.098–0.15]	10	0.095[0.092–0.098]	9		
Structure and regional water diffusion						
Total kidney volume (ml)	356 [331–437]	10	390 [359–447]	9	403 [345–430]	12
T_1 cortex (ms) †	1560 [1524–1638]	8	1522 [1497–1638]	8	1459 [1400–1525]	12
T_1 medulla (ms)	1838 [1732–1872]	8	1792 [1695–1870]	8	1732 [1661–1850]	12
T_2 cortex (ms)	120 [113–134]	8	124 [119–141]	8		
DWI Cortex ADC ($x10^{-3}$ mm^2/s)	1.9 [1.9–2.1]	8	2.1 [1.9–2.1]	8		
DWI Medulla ADC ($x10^{-3}$ mm^2/s)	1.9 [1.9–2.0]	8	2.1 [1.8–2.1]	8		
DWI Cortex D ($x10^{-3}$ mm^2/s)	1.8 [1.7–1.9]	8	1.9 [1.7–2]	8		
DWI Cortex D* ($x10^{-3}$ mm^2/s)	26 [23–29]	8	27 [24–28]	8		
DWI Cortex F_P	0.13 [0.11–0.15]	8	0.13 [0.11–0.14]	8		
DWI Medulla D ($x10^{-3}$ mm^2/s)	1.8 [1.7–1.9]	8	1.9 [1.7–2]	8		
DWI Medulla D* ($x10^{-3}$ mm^2/s)	26 [23–17]	8	27 [26–28]	8		
DWI Medulla F_P	0.14 [0.11–0.17]	8	0.15 [0.12–0.16]	8		

Paper IV

This paper included a subcohort consisting of 17 patients from Paper III, with ARDS due to COVID-19. Patients who received a standardised fluid bolus and were included in the analysis if they had at least one complete pair of perfusion measurements from at least one kidney. Nine patients had AKI and 8 did not, totalling 33 pairs of perfusion measurements with 17 in the AKI group and 16 in the No AKI group. The haemodynamic response to plasma expansion was distinct, with a maximal increase in MAP by 19 (12–27) mmHg. The response was similar in both study groups and decreased during the exam (**Table 7)**. 7 of 9 patients in the AKI group and 7/8 patients in the No AKI group increased the ratio MAP/HR by >10%. In both groups, a reduced cortical relaxation rate assessed by BOLD was measured during the last part of the scan, indicating that haemodilution due to plasma expansion was still present (**Table 7**).

Total RBF was similar before and after plasma expansion in both groups. The ratio of blood flow after/before plasma expansion was 1.03 (0.94–1.13) in patients with AKI and 0.99 (0.90–1.08) in patients without AKI. There were also no significant effects on cortical perfusion, with ratios after/before of 1.07 (0.91–1.25) and 0.88 (0.75–1.04) in the AKI and No AKI groups, respectively. Medullary perfusion was reduced in patients without AKI after plasma expansion with a significant decrease to a ratio of 0.61 (0.44–0.83) ($p = 0.0027$), unlike the patients with AKI, who had similar values before and after, with a ratio of 1.05 (0.77–1.4). The data are further presented in **Figure 15.**

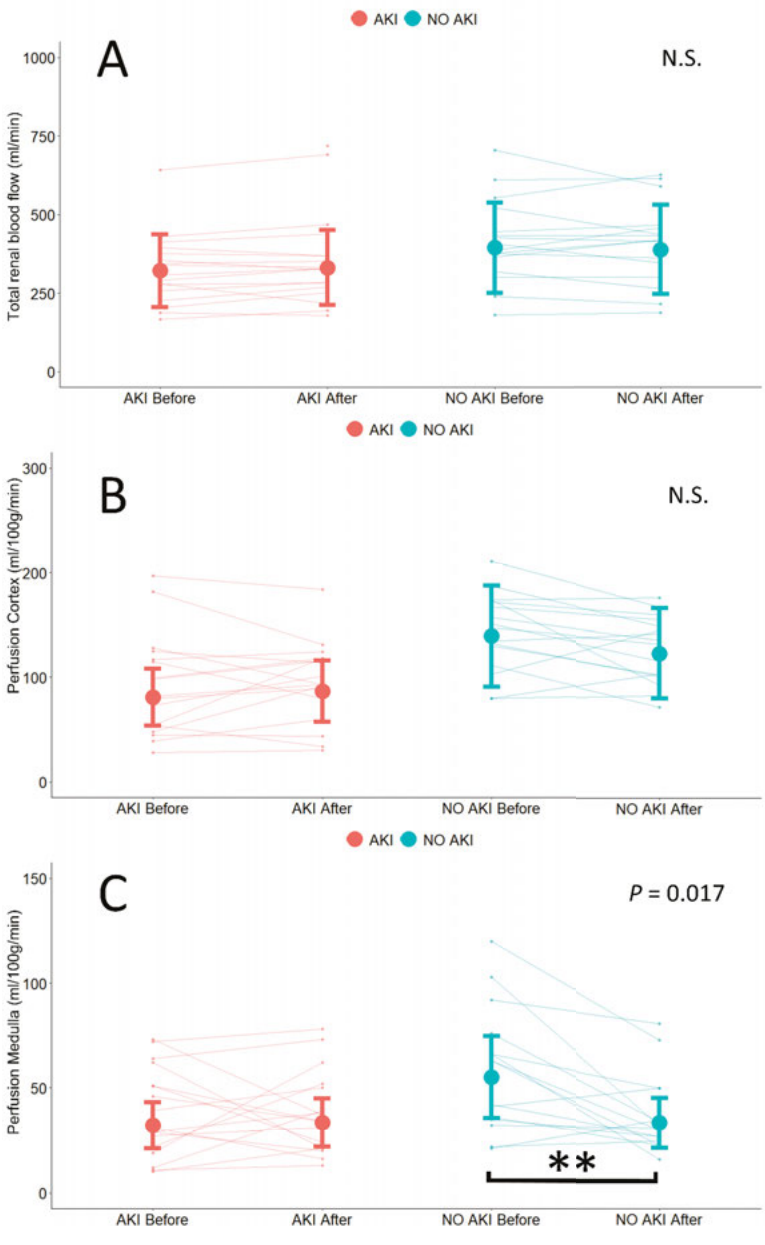

Figure 15. The main results are presented for each measurement of perfusion using MRI with phase contrast estimation in A, ASL cortex in B, and ASL medulla presented in C. Individual measurements are presented as dots colourised by AKI status. The estimated marginal means and confidence intervals are presented as an overlaying bar plot. P-values in ANOVA for the interaction effect between group and plasma expansion are presented in the upper right corner of each graph. N.S. denotes non-significant. ** denotes p <0.01.

Table 7. Additional results are presented by group and in relation to plasma expansion status below. If not stated otherwise, the data consist of estimated marginal means with confidence intervals in parenthesis derived from the mixed linear model used. Interaction effects were modelled in all analyses without any significant effect in the analyses presented in this table. Haemodynamic parameters before plasma expansion are presented compared with maximum/minimum (max/min), and last MAP recorded at the end of the scan (end).

Group	AKI		NO AKI	
Plasma expansion status	Before	After	Before	After
Haemodynamic parameter				
MAP max (mmHg) †††	80 (72–89)	98 (90–107)**	90(81–99)	110(101–119)**
MAP end (mmHg) †	80 (72–89)	84(76–93)	90(81–99)	99(90–108)*
Heart rate min (bpm) †††	79(68–91)	75(64–86)*	65(53–77)	56 (45–68)**
Renal measurements				
Resistive Index	0.87 (0.78–0.97)	0.89(0.79–0.98)	0.79(0.70–0.89)	0.80(0.71–0.89)
BOLD Cortex (1/s) †††	16.9 (15.7–18.0)	16.3(15.2–17.4) ***	17.4(16.2–18.8)	16.9(15.7–18.3) ***
TRUST Valid exams	N=6	N=6	N=7	N=7
TRUST Yv (%)	71.5(62.2–80.8)	70.7(61.4–80.0)	76.3(67.7–84.9)	75.0(66.4–83.6)
TRUST R2 (1/s)	10.0(7.40–12.7)	10.6(7.99–13.2)	10.0(7.59–12.5)	10.4(7.98–12.8)

*, **, *** indicates p-value < 0.05, 0.01, 0.001 respectively, in pairwise comparison within group after Tukey's adjustment

†, ††, ††† indicates p-value < 0.05, 0.01, 0.001 respectively, of before-after effect of plasma expansion in ANOVA

Discussion

Main findings

In Paper I, renal cortical mitochondrial dysfunction comprised of a reduction in the RCR, and a disturbance in the relation between complex I and complex II activity in state 3, was demonstrated in an ovine model of SA-AKI. Further, we demonstrated a disturbed relationship between renal oxygen consumption and renal sodium resorption that cannot be explained by a change in renal mitochondrial efficiency or by renal mitochondrial uncoupling.

The main result of Paper II is that 89% of critically ill COVID-19 patients developed AKI in a single centre cohort during the first wave of the SARS-COV-2 pandemic. Early development of oliguria was predominant, and preserved GFR as estimated using plasma creatinine was not uncommon. Increased levels of urinary biomarkers of epithelial kidney injury, as well as increased urinary albumin excretion, may indicate tissue damage at both the glomerular and tubular levels. However, we could not demonstrate a clear relationship between levels at admission and the final AKI stage.

In Paper III, our main findings are that total RBF and regional perfusion were lower in critically ill COVID-19 patients with AKI compared with similar patients without AKI, while no demonstrable effects in regional oxygenation could be found.

From the results of Paper IV, we conclude that renal perfusions were not increased by plasma expansion by a standardised balanced crystalloid bolus of 7.5 ml/kg, either when AKI was present or absent, despite generating significant increases in MAP. Further, renal medullary perfusions were lower after plasma expansion in patients without AKI.

Renal mitochondrial function in SA-AKI

In Paper I we demonstrated an effect of the electron transport chain (ETC) by sepsis comprising a reduction of the RCR and a disturbed relation between complex I- and complex II-mediated state 3 respiratory capacities. This was mainly caused by a reduced complex I dependent state 3 respiration induced

by sepsis. RCR is suggested to be a valid parameter of mitochondrial dysfunction in isolated mitochondria[202], and as such it is sensitive although not specific. Our findings are in contrast to two previous large animal studies in septic pigs, in which prolonged endotoxemia failed to demonstrate any in vitro changes in renal mitochondrial function[203], whereas pigs with peritoneal sepsis developed a State 2 reduction[37]. However, the animals in the later study did not develop a decrease in creatinine clearance; thus, the implication of this finding is unclear. Reduced complex I activity in skeletal muscle has been associated with decreased survival in ICU-treated patients with septic shock[32] and has also been demonstrated in animal models of sepsis[33,203]. This is considered a result of prolonged NO exposure which induce complex I inhibition, both reversibly and irreversibly[204]. Wheras reduced mitochondrial function is also ameliorated by inhibition of inducible nitric oxide synthase (iNOS)[205] it is noteworthy is that inhibition of iNOS does not have renal protective effects in large animal models of sepsis[206]. Besides modulation of cytochrome C oxidase[207] there are effects of NO that may also be beneficial in the context of SA-AKI. NO promotes mitochondrial stability, and iNOS depletion results in the accumulation of dysfunctional mitochondria[208]. As an increase in all cortical NOS-isoforms has been demonstrated in a similar sheep SA-AKI model[20], this is likely the cause of the altered complex I respiratory capacity in this experiment. Although a maintained intrarenal ATP-level was demonstrated early in septic sheep[39], the effect on the ETC may still affect renal function if metabolic conditions change[42].

We also demonstrated a disturbed relationship between oxygen consumption and tubular sodium transport. This is consistent with previous studies in sheep[85], studies in patients with SA-AKI[75] and post-cardio-pulmonary by-pass associated AKI[94] and can be considered a feature of AKI in the clinical context. Tubular sodium transport is a major source of ATP demand where a reduction of tubular transport by furosemide administration results in a reduction in oxygen consumption by approximately a third[209]. The marked reduction in sodium transport due to SA-AKI in our study would thus be expected to affect global renal oxygenation if no other oxygen-demanding process had increased. Renal oxygen consumption was invariable throughout the experiment in our study, as described by others[210].

Mitochondrial uncoupling has been proposed to counteract increased oxidative stress[211]. Morphological changes in renal mitochondria that could indicate damage and an increased risk of unspecific proton leak have also been described in post-mortem biopsies from patients with SA-AKI[38]. Our results indicate that it is likely that the maintained renal oxygen consumption is not caused by a reduced P/O-ratio, or increased proton leak, and that renal mitochondria can utilise oxygen for ATP-production with the same efficiency as in non-septic animals. Other contributing possible contributing mechanisms

include, a disrupted barrier between tubular cells with back-leak of sodium, higher ATP demand for cell repair, or infiltration of metabolically active leukocytes[212].

COVID-19 and AKI

The finding in Paper II that nine out of ten intensive care patients with COVID-19 develop AKI is in agreement with a previous study[177] but substantially higher than the proportion seen in general ICU populations without COVID-19[12]. More studies have been published demonstrating similar results. In New York, the occurrence of AKI during the first wave of COVID-19 were 87,2%[180], 89,7%[177]and 76%[213] in more than 1500 patients treated in the ICU or with mechanical ventilation. Similar results have been demonstrated in a smaller single centre study in france[214] and a study of 71 patients from 4 French ICUs showed an incidence of 80%[215]. In this sense, our prospective cohort is coherent with international experiences. In studies reporting lower rates of AKI during intensive care, nearly two thirds of all reported cases of AKI was stage 3[216] which may indicate underreporting of less severe cases. All were retrospective cohorts from medical records. There are likely regional differences and differences over time. Regarding these outcomes, a recent meta-analysis with 8086 patients in ICUs from several continents found an incidence of 46% with 19% receiving RRT[217]. These patients were, however, not limited to the first wave. During a longer collection of data more variants of the virus are included which adds to differences in disease severities. As the results of the RECOVERY-trial indicate treatment with dexamethasone almost halves the need for RRT besides reducing mortality[218], new treatment options may also decrease the occurrence of AKI during COVID-19.

A majority of patients with stage 3 AKI by creatinine in this cohort received CRRT, and biomarker levels in this group were high. It is common for AKI studies to focus on AKI development within the first 48-72 hours[156,219] after ICU admission. This is partly because the older Acute Kidney Injury Network (AKIN) criteria required an abrupt (within 48 hours) reduction in kidney function, but also to focus on AKI associated with the condition requiring intensive care rather than the effects of treatment. In this cohort however, more severe AKI with a reduced glomerular filtration rate tended to occur later. This is consistent with the long duration of stay seen in critically ill COVID-19 patients, as well as the gradual progression of the illness during intensive care[220]. This is in contrast to other early reports in which the median time of RRT initiation from hospital admission was merely 2 hours, and 0 hours after AKI diagnosis was made[177]. Another multicentre study report a median 3 days between ICU-admission and RRT-initiation[221]. The reasons for this may include

differences in hospital practices, population characteristics, and the prospective observational design of this study in which the AKI diagnosis was made independently from the treating clinician. Further, early versus late initiation of RRT in the ICU is an area of ongoing research in which a benefit of early initiation has not been demonstrated[222].

A common occurrence of albuminuria in ICU-treated COVID-19 patients was demonstrated in our study. Albuminuria has recently been reported in COVID-19 patients but not previously considered in association with AKI[223]. In septic ICU patients, microalbuminuria is associated with AKI[224]. Further, albuminuria is a risk factor for poor outcomes in hospitalised patients[225] and is a known risk factor for the development of chronic kidney disease after AKI[226]. Follow up data of patients with critical COVID-19 indicate an increased risk of reduced GFR in survivors with stage 3 AKI compared with those without[227]. Further investigation of the influence of albuminuria on long-term prognosis should be encouraged, aiming at targeted intervention for high-risk individuals.

Measurement of biomarkers of tubular injury at inclusion was not easily applicable for AKI risk assessment in this cohort. Biomarkers generally tend to correlate well with the AKI stage and with each other in intensive care populations[16]. One potential explanation for this discrepancy is the varying testing time points in relation to the onset of the disease. During AKI after cardiopulmonary bypass or ischemia/reperfusion injury, the time and duration of the insult are generally known and can be tied to an early rise in biomarkers that may remain high for a time after the injury[16,228]. In the case of COVID-19-induced AKI the process is ongoing, which may indicate that biomarker development over time could be more informative, as has been shown in sepsis[154].

Similarities and differences between SA-AKI and COVID-19-AKI

When a new infectious disease emerged during the work with this thesis several of the same scientific questions as in AKI associated with bacterial sepsis were raised. Similarities include the systemic inflammation. In COVID-19, increased levels of plasma and urinary cytokines are associated with AKI development[175,229]. Although not accurately described as a cytokine storm, the elevated levels of some cytokines are in parity with what is seen during sepsis[176]. Further, treatment of COVID-19 pneumonia with systemic corticosteroids reduces the risk of receiving RRT by approximately 40%, a relative risk

reduction greater than the need for IMV[218]. As previously described, experimental SA-AKI can also be attenuated by modulation of the immune response[21]. A procoagulant state and activation of the complement system have been reported in COVID-19 and activation of procoagulant factors have also been linked to persistent AKI in bacterial sepsis[28,183,230]. Some histological findings from patients with COVID-19-associated AKI differ from those of SA-AKI and show signs of acute tubular injury[231,232], although not to the extent expected by severity of AKI. Evidence of the presence of virii in tubular cells[233] have been reported but not been replicated to an expected extent[183]. Collapsing glomerulonephritis has been described in several cases of COVID-19-associated AKI, as well as thrombotic micorangiopathy, the latter however not in a majority of cases and the role of thrombotic micorangiopathy in AKI-development still remains unclear[183,234]. These are post-mortem findings which in some instances may include a longer period of profound hypoxemia before the tissue was examined. Further, the report summarised histological findings from 243 patients with COVID-19, of whom not all had AKI[183]. Biopsies from experimental SA-AKI and post-mortem biopsies from patients instead show a pattern of apoptosis and leukocyte infiltration with a notable lack of histopathological pathology in relation to the degree of AKI[17,19]. This indicates the possibility of diversions in pathological mechanisms between bacterial SA-AKI and AKI due to COVID-19. The lack of available animal models of COVID-19 associated AKI currently limits the possibility of clarifying the relevant mechanisms.

Risk factors for AKI in COVID-19 include older age, a history of hypertension, diabetes, cardiovascular disease and increased disease severity, which is similar to bacterial sepsis[6,177,180]. Male sex as a risk factor for mortality, AKI development, and RRT need has been reported in COVID-19, and has not been reported, at least to the same degree, in SA-AKI due to other causes[6,177,180,235]. In Paper III we assessed tissue composition and DWI parameters between patients in the AKI and the No AKI groups. We could not demonstrate differences between the two patient groups, but T_1 values in the COVID-19 cohort and especially in the AKI group differed from those of the healthy controls. Changes in tissue composition may be due to either COVID-19 or comorbidities. Comorbidities influence the risk of AKI development in a manner similar to SA-AKI. Supportive of differences in tissue composition reflecting the underlying risk of AKI is that patients with CKD have both a lower ADC and a longer T_1 compared with with healthy controls[197]. Thus, the previous unnoticed renal effects of comorbidities could influence these findings. An argument against this stand is a previous study demonstrating longer T_1 in the acute phase of AKI[236]. In that study the T_1 was reduced partly after 3 months and completely to a healthy population's mean after a year of recovery and was accompanied by a similar reduction from increased to normal in total kidney volume. Thus, higher T_1 values may reflect higher water

content in inflammatory, oedematous tissue. Increased intracapsular pressure due to oedema has been proposed as a contributing mechanism in both COVID-19-AKI and SA-AKI, but the lack of difference between the AKI and No AKI groups cannot support this claim in our study.

In previous studies of SA-AKI the timing of AKI development in relation to the septic insult can be divided into early (within 24-48 hours) and late onset AKI[13]. Late development of SA-AKI has been associated with worse outcome[237]. In Paper II, the late development of AKI was not uncommon; however, the clinical consequences may not be transferrable between these two entities. In the case of bacterial sepsis, antimicrobial therapy is usually initiated soon after disease onset, and complications occurring after a therapeutic effect should be noticed, and can therefore be a more aggravating sign of more severe disease. During the period in which Paper II was conducted, therapy was mainly supportive and thus rather reflects the natural course in the more severe cases, and later occurring complications may not be linked to therapeutic failure to the same degree.

Finally, a common concern when managing patients in the ICU with risk of, or manifest AKI is the possibility of iatrogenesis as a result of the multiple interventions undertaken. Some aspects related to intensive care have been examined in Papers III and IV and further discussed below. Although some findings regarding AKI in COVID-19 will not be generalisable outside its context, it is possible that aspects mainly reflecting factors related to the execution of intensive care will be.

Oliguria

In Paper II, a difference in the time trajectory was observed regarding the two KDIGO criteria for AKI. Categorising the groups by the different criteria generated differences in biomarker levels. In patients with oliguria classified as AKI stage 2, isolated oliguria was common and lower levels of corrected U-Cystatin C were found at admission. The urine output criterion was criticised soon after publication because they did not predict mortality in parity with the creatinine criteria in general ICU-patients[238]. Even when the KDIGO criteria were created, the imprecision regarding body weight and the influence of drugs on the estimation of urine output were acknowledged but left unadressed[3]. This was part of the reason why only the creatinine criteria were used for AKI diagnosis in paper III and IV. We also expected a delay between inclusion in the study and the MRI exam, in which interventions addressing current oliguria would likely have been made. Later studies of oliguria in the ICU have demonstrated an increased risk of end-stage renal disease and death in patients with periods of oliguria and also found that the risk increases with

both duration and severity of oliguria[239-241], in which the most severe prolonged oliguria (<0.1ml/kg/h) is highly associated with initiation of RRT or death[239]. A large retrospective study published after the completion of data collection for Papers II, III and IV in non-COVID mixed ICU patients found an association of oliguria for more than 12 hours with adjusted mortality[242].In that study, oliguria without concomitant creatinine elevation consisted of approximately half of the patients with AKI, which is consistent with our findings. Although less is known about the clinical relevance of oliguric renal failure in COVID-19, severe oliguria (<100ml/d) before initiation of RRT in critically ill patients with COVID-19 is associated with increased mortality[221].

Fluid balance and plasma expansion

In Paper II, fluid balance in general was positive after initial care, although it was not liberal. The cumulative fluid balance in the ICU cohort in our study of 1.4 l after ICU Day 1 can be related to the liberal and restrictive group in the FACTT trial where the fluid balances were +2.5 l and +1.2 l, respectively, after the first 24 hours after randomisation[135]. During the same time period the patients in our COVID-19-cohort received a mean 4.6 l of fluids, whereas the liberal group in the FACTT trial received 5 l and the restrictive group 4.2 l. Our cohort had a larger proportion of men, which may have influenced the relative need. Further, the patients in the FACTT-trial were randomised almost 48 hours after ICU-admission, and in general had a positive fluid balance and was more often treated with vasopressors. Taken together, the data in our study do not indicate that the patients in our study were exposed to extremes in fluid balance management, or that the fluid management is non-representative by international standard of care. This is confounded by the fact that fluid balance prior to intensive care was not known, and insensible losses aggravated by fever may be larger during COVID-19.

Hypovolemia and dehydration have been proposed to contribute to COVID-19 associated AKI[184] and instances of high renin values in combination with high levels of plasma sodium[181]have been reported. In Paper IV, we therefore tested the hypothesis that plasma expansion could increase renal perfusion in patients with COVID-19 in patients with or without AKI. However, neither measurements with phase contrast nor ASL could demonstrate an increase in renal perfusion in any of the groups consisting of normotensive critically ill patients. A previous randomised study comparing plasma expansion by either crystalloid or colloid fluid bolus in ICU patients after cardiac surgery demonstrated a transient increase in RBF[243]. Larger fluid boluses were used in that study: 20 ml/kg of crystalloid or 10 ml/kg colloid. Plasma expansion by crystalloid fluid infusion is often transient, with prolonged half-lives during hypovolemia or hypotension[244]. We therefore assessed the renal perfusion

shortly after completion of the fluid bolus when the plasma expansion is still likely present and used the BOLD sequence at the end of the exam to verify this[245]. Larger bolus sizes than those used in our study are not commonly used in clinical practice in the ICU[246,247]. The bolus size chosen in the present study thus emulates the plasma expansion reasonably achievable at the bedside during similar clinical conditions. The smaller volumes used in the present study resulted in a greater difference in MAP and heart rate than those reported in the post-operative patients. This is likely because the administration time was shorter in our study intended to maximise the plasma volume expansion while limiting increases in fluid balance. As both phase contrast and ASL are valid non-invasive measurements of renal perfusion[191], plasma expansion is unlikely to facilitate a relevant increase in renal perfusion in our study population.

The renal resistive index was not affected by plasma expansion in concordance with previous studies of mechanically ventilated patients. A 500 ml fluid challenge did not affect RI regardless of fluid responsiveness or AKI status[67], similar to the results in another study specifically investigating patients with SA-AKI[68]. One previous study aimed at critically ill patients in general, however, found a small decrease in RI after fluid challenge, which correlated with increased UO and MAP[248].

In our study, renal medullary perfusion was reduced by plasma expansion in the No AKI group but not in the AKI group, which was not an expected finding. An important caveat of medullary ASL is that the relative precision is lower, since flows and signals are smaller than in cortical measurements. With some variation between species, there are differences in the autoregulation of medullary perfusion compared to total and cortical blood flows[110,249,250]. In rats, medullary autoregulation is reduced during normovolemia[250] compared with a fluid depleted state. Although we cannot present a probable mechanism of these findings that can explain the phenomenon in the No AKI group solely, we note that a paradoxical decrease in renal medullary perfusion has been noted during a rapid increase in blood pressure during experimental conditions[251]. When interpreting the reduction of medullary perfusion in patients without AKI, one should acknowledge the possible biases for which the before–after design of the evaluation of the intervention is susceptible, including regression to the mean bias.

Fluid bolus administration and active management of fluid balance are a common interventions in the management of ICU patients in general. Elevated or increasing P-creatinine as well as mild oliguria are still used as signs of hypovolemia[247,252], and fluid bolus administration is suggested with the expected beneficial effect mediated by changing renal perfusion. Nevertheless, clinicians' expectations of the haemodynamic and renal effects of fluid bolus

administration often differ from the actual effects[253]. Evaluation of the effects of fluid boluses is usually made by measuring haemodynamic changes. However, a previous study has demonstrated that in non-COVID-19 ARDS, increases in cardiac index after fluid bolus administration do not correlate with changes in urine output[254]. A liberal fluid regimen in the treatment of non-COVID-19 ARDS in general has not been associated with a reduced rate of severe AKI but is associated with reduced pulmonary function[136]. An excessive fluid balance is also associated with AKI[255]. During the completion of this thesis, a restrictive approach to fluid management has now been recommended also in COVID-19[246], although it is based mainly on previous findings in non COVID-19 ARDS[256]. Two recent multicentre RCTs (CLASSIC and CLOVERS) focused on initial management of sepsis have not demonstrated a beneficial effect on AKI development with with liberal or restrictive fluid protocols beyond initial resuscitation[257,258]. Further, in a recent randomised feasibility trial of a restrictive or liberal fluid regiment after fluid resuscitation in AKI-patients, a restrictive regimen was favourable in maintaining close-to-neutral fluid balance and was associated with less initiation of RRT[259]. The apparent lack of an increase in renal perfusion by plasma expansion in the patients included in our study is consistent with the references above.

Renal blood flow in COVID-19 and intensive care

To my knowledge Paper III is the first study to reliably measure RBF in patients with AKI due to COVID-19 and we can demonstrate that lower RBFs were more common in patients with AKI at the time of the exam. Reduced total RBF in patients with AKI as found in our study have been described during AKI due to other causes, including bacterial sepsis[73-75,94]. Multiparametric MRI has demonstrated reduced renal perfusion in a previous study of nine patients with severe AKI of different aetiologies at a median of six days after peak P-creatinine[236]. In critical COVID-19, a previous investigation implied reduced renal cortical perfusion using contrast enhanced ultrasound[260]. An increased renal resistive index has also been demonstrated[261] which is associated with AKI progression in general[262]. When we measured RI in the renal artery instead of the arcuate arteries, we also found increased RI during AKI and demonstrated a negative correlation between RI and total RBF.

A reduction in RBF during normotension is accompanied by increased RVR. The mechanisms behind this can include the activation of RSNA and intrarenal release of adenosine and endothelin[263,264]. Activation of RSNA may aggravate AKI and increase RVR under experimental conditions. In a mice model, LPS induced reduced creatinine clearance and reduced RBF without affecting MAP and both RBF and GFR increased when renal denervation was undertaken before LPS injection[265]. By contrast, ovine models of septic AKI as used

in Paper I develop low RVR, in which AKI was not affected by renal venous denervation[266] despite increases in RSNA is found during ovine SA-AKI[267]. Increased RVR has also been demonstrated in patients with AKI due to cardiopulmonary bypass and sepsis[75,94]. In our material, we could not demonstrate a significant difference in a crude estimation of RVR using MAP-PEEP as driving pressure; however, differences in group medians were of similar magnitude as previously reported in clinical studies.

Patients in the AKI and No AKI groups differed besides renal function, as patients in the AKI group were treated longer in the ICU, with higher proportion of IMV and with higher PEEP. We could not find a significant correlation between PEEP during the MRI exam and total or regional renal perfusions, but cannot draw conclusions regarding the influence of respiratory therapies. PEEP has been proposed to contribute to AKI in COVID-19[183]. There is conflicting experimental evidence regarding the renal effects of PEEP, where some experiments demonstrate reduced RBF shortly after the initiation of PEEP, while RBF is unaffected in others even though there is a significant reduction in cardiac index[268-270]. The initiation of PEEP is commonly reported to initiate a reduction in GFR and salt excretion[271], possibly by a reduction of secreted ANP[272], increased venous pressure, reduction of renal perfusion pressure, reduced circulating blood volume, and activation of RSNA[273-275]. Despite our findings, the present studies cannot rule out that the initiation of invasive mechanical ventilation with the application of PEEP may still cause a transient reduction in RBF with a negative impact on renal oxygen delivery.

A limitation of some above-mentioned studies using thermodilutional catheters[75,94] or phase contrast MRI[74] to determine total RBF is that regional hypoperfusion cannot be investigated. Using ASL in Paper III, we demonstrated lower regional perfusion in both the renal cortex and medulla. We also identified discrepancies in the degree of difference between the groups for cortical perfusion compared to total RBF, where the relative difference was greater in regional blood flow. As only a small proportion of RBF pass through the medulla, and after passing the glomeruli, total renal blood flow is expected to be closely linked to cortical perfusion. Although both measurements correlated well with each other, a similar discrepancy was noticed in the previous longitudinal study of nine AKI patients using the same methods of assessing renal perfusion and oxygenation[236]. A possible explanation could be that the two measures PC and ASL have different approaches to measuring blood flow. Renal perfusion is estimated from ASL images by a two-compartment mathematical model, which also assumes similar blood-tissue partition coefficients, that is that the proportion of labelled water that diffuses to the tissue is similar in all patients. A more recent publication also described a similar discrepancy during bacterial septic shock, in which a reduced renal cortical perfusion was demonstrated in patients with severe AKI despite an unaffected

renal arterial flow[276]. Patients were investigated using contrast enhanced ultrasound and renal arterial Doppler, and patients with KDIGO stage 3 were compared with other patients with septic shock with no or lower grade AKI. Absolute RBF estimation by ultrasound is less reliable than phase contrast measurement, but the underlying properties of the measurements are analogous to our paper. The authors interpreted their results as a disturbance in the kidneys' microcirculation which affects tissue wash-in-curves differently than perfusion measurements of flow. The same authors also compared the same measurements in 10 patients with COVID-19 and 13 patients with septic shock, all with stage 3 AKI, with healthy controls[260]. In severe AKI due to COVID-19, reduced renal cortical perfusion similar to septic shock was described and may be a feature of AKI due to severe infections in humans. These investigations support our findings and imply that this phenomenon may be similar in both SA-AKI and AKI due to COVID-19.

Renal oxygenation in COVID-19 associated AKI

In Paper III we could not demonstrate any differences in renal oxygenation in patients in the AKI group compared with the No AKI group, using either BOLD or TRUST sequences. This although regional perfusions in both cortex and medulla were significantly lower in patients with AKI. BOLD imaging instead shows the same level of relaxation rates in our study population as in healthy individuals of similar ages. This is similar to the findings in a previous study in which patients were investigated six days after peak P-creatinine[236]. In our study, the patients were examined earlier after the onset of AKI. In addition, we examined renal intravenous saturation using renal TRUST, which is a novel sequence. A strength of TRUST is its insensitivity to oedema. We could not demonstrate any group level differences; however, unfortunately some exams could not yield sufficient data to be interpreted. The remaining individual levels were more dispersed than expected. Measurements in healthy volunteers yielded estimations of 89 ± 2%[201], and previous studies in the intensive care setting[75] demonstrated less dispersed venous saturations than in the patients in our study. This may be due to limitations in the TRUST sequence. Besides deoxyhaemoglobin, TRUST is affected by MetHb and ferritin, which may be more variable in the study cohort. However, previous comorbidities may also have increased variability, as studies using renal venous catheters in patients with hypertension have exhibited ranges more similar to those in our study[277,278].

In accordance with the results in Paper I, clinical studies imply that renal oxygen demand is not negatively affected by the reduced sodium transport accompanied by the GFR-reduction to the same degree when AKI is present. This is in contrast our results in paper III, which do not support hypoperfusion-

induced regional renal hypoxia as a specific feature of early AKI in COVID-19. A key issue in interpreting the results is whether the results of the BOLD scan truly reflect tpO2, or, if offsets in the relation may occur during COVID-19 associated AKI. BOLD is generated by the occurrence of deoxyhaemoglobin with a linear relationship between intrarenal deoxyhaemoglobin content and R_2*[193]. Absolute measurements may be influenced by changes in tissue water, as increased water content strongly decreases both R_2 and R_2*. Differences therein may therefore attenuate differences in deoxyhaemoglobin content. The influence of water content in our BOLD data is supported by the correlations between R_2*, R_2, T_1, and ADC in the patient group (see Paper III); however, we could not demonstrate group level differences in water content. The possibility of intrarenal microthrombotisation has been previously discussed. If present this may contribute to increased renal resistance and offset tpO2 and BOLD, as thrombotised vessels only transitorily contain deoxyhaemoglobin. Furthermore, a decreased intrarenal blood volume due to vasoconstriction or changes in oxygen transit in tissue could also offset the relation between tPO_2 and renal oxygenation measured using BOLD[279]. We cannot conclude if these mechanisms contribute to our findings or to what extent. Lastly, a *post hoc* analysis in Paper III investigated the correlation between the estimated filtration fraction and cortical BOLD. As decreased GFR reduces the oxygen demand, and the filtration fraction (FF) reflects the supply/demand relation, a relationship between FF and cortical oxygenation is expected. This has been previously demonstrated using BOLD in a human study and is also implied by our results[280].

Strengths and Limitations

There are both strengths and limitations to the studies in this thesis. Starting with Paper I, the duration of the study is relatively long, the model is relevant to the pathogenesis of SA-AKI mediated by inflammation, live bacteria from a microorganism relevant to human disease were used and the large animal model enabled simultaneous measurement of more physiological parameters and baseline sampling of mitochondria. Although we assessed global renal oxygen, the conclusions are limited due to only analysing mitochondria from a cortical biopsy. Thus, conclusions regarding more regional mitochondrial function cannot be drawn. Cortical mitochondria consist of a relevant proportion of all renal mitochondria and the differences in P/O ratios between the cortex and medulla in rats is of magnitude of 5 – 10%[43]. In that regard, a larger effect would be expected in the cortical mitochondria to fully explain the level of oxygen consumed in relation to sodium absorption. Further, as proximal tubular cells may also be more prone to injury due to respiratory chain defects than more distal tubular cells, these findings may still be of relevance to AKI

progression[40,42]. Effects of a reduced mitochondrial density would not be visible in the analysis of isolated mitochondria and are beyond the scope of this study. Morphology was examined in the cortex and cannot exclude morphological alterations in medullary or tubular cells. As the cells in the tubuli are more sensitive to degradation after fixation by immersion, the available data comes with limitations that prevents firm conclusions regarding mitochondrial ultrastructural changes in tubular cells.

The animals were anaesthetised throughout the experiment, which influenced parameters as RBF and may have aggravated SA-AKI[76]; however, the drugs used are common in intensive care and our results demonstrated that the effects were not caused by instrumentation and anaesthesia alone. As five animals were terminated before the end of the protocol, representing a premorbid state, and we did not subject the animals to any intervention specifically designed to preserve renal mitochondrial function in experimental sepsis, we cannot draw conclusions regarding the causal role of the observed mitochondrial dysfunction in the pathogenesis of SA-AKI. However, as the focus of the manuscript was on which role the observed mitochondrial function plays in oxygen handling, we still consider our conclusions valid. Other experimental studies have also demonstrated that targeting mitochondrial ROS generation in sepsis ameliorates AKI[281]. As targeting mitochondrial dysfunction has yet to be proven beneficial in a clinical setting where clinical studies of high dose supplements of nutrients affecting lactate clearance and redox-states have not proven useful[282,283], more research in both clinical and experimental settings is needed.

Paper II comprises an early description of a new disease in the form of a prospective cohort study with a high inclusion rate of eligible patients. Further, attempts were made to further characterise the novel disease by including an analysis of biomarkers early after admission to the ICU. Limitations related to the study cohort include the fact that screening may have missed short stays, which causes a bias towards longer stays, excluding very early deaths and discharges. Despite this risk of bias, where we only examined patients where consent was available, our results concur with larger retrospective studies at various locations regarding the incidence of AKI. During some phases of the pandemic, high dependency units were used to a greater extent to care for patients using non-invasive respiratory support. This may have increased the likelihood of a generally more severely diseased study population than under other circumstances. Another major limitation is the relatively small number of patients, and multiple comparisons are made, which, combined with the low proportion of patients without AKI, make a more detailed analysis of the clinical usefulness of biomarkers hard to interpret. As a single centre study local practices may influence outcomes, however, ICU care at the department in

question generally adheres to international standards and guidelines and is comparable to similar units in the region.

It is also probable that using lean or predicted body weight would reduce the incidence of AKI defined by urine output alone, which is a general limitation of the KDIGO criteria. Urine output, as assessed in Paper II, has been shown to be associated with mortality and can therefore be considered of interest in this population as well[242]. There are data missing regarding urine output both before ICU admission, and for some patients during intensive care, which may delay the time to urine output criteria fulfilment. However, it is likely that this limitation underestimate the true incidence of AKI and should not affect the overall conclusion. Baseline P-creatinine before disease onset, but within a short time of admission, was not generally available, as is generally the case in AKI studies. Finally, urinary biomarkers were collected at inclusion and may not have the temporal resolution to be accurate for AKI prediction. A theoretical window of clinical usefulness may occur both before admission and later during the course of the disease. General conclusions regarding a non-existent relationship between fluid balance/fluid administration and AKI-development should not be made based on Paper II alone. The presented comparison is limited to a single time point, and was chosen to be close to when oliguria in general occurred in this cohort. There were large differences within each group regarding fluid balance at the specific time points analysed, and there were only a few observations in some groups.

The strenghts in Paper III and IV include an early investigation in relation to the AKI and the inclusion of a comparator group of COVID-19 patients treated in the ICU without current AKI at the time of the exam. A novel technique was used, enabling the possibility of acquiring new insights in the pathogenesis of COVID-19-associated AKI and the effects of one of the most common interventions in the ICU. Still, relatively few patients were included in both groups, all from a single centre. Although the study cohort represented a relevant part of the patients in the ICU, it did not include the most severely deteriorated patients where MRI was not feasible. Additional limitations related to the MRI sequences include the fact that TRUST is a more novel sequence in renal MRI, and the pitfalls in renal application are less explored. We observed more missing values due to technical problems using this sequence than expected, and a larger variation in the range of estimates was present in the TRUST measurements compared with BOLD. In Paper III, differences in total renal blood flow between the groups were partly attenuated after adjusting for total kidney volume (TKV). Reduced TKV (because of the loss of functional mass) predisposes AKI development, while AKI development in itself increases TKV[236]. Although we could not demonstrate significant differences in TKV between the groups, adjustment may have introduced more uncertainty to the data by these mechanisms.

Neither of the clinical studies in the papers can resolve the causal contribution of a reduced RBF seen during established AKI. We must also consider a reverse causal relationship in which factors associated with AKI development also predispose patients to have reduced RBF. Supporting this possibility is the fact that RBF assessed with PC and ASL is strongly correlated with eGFR in CKD[197,284]. By contrast, our patients all had at least one measurement of P-creatinine within the normal range during the current hospitalisation before the exam, and possible pre-morbid kidney disease would likely be limited. Although the COVID-19 cohort and the healthy volunteers' imaging data were acquired on different scanners, the main comparison between patients with AKI and No AKI with COVID-19 was not affected by this. In the same sequences and we have previously reported similar measurements between the two scanners in young healthy volunteers[191,192].

In Paper IV, severely hypovolemic patients with a resulting circulatory instability were not included in the study. As such, conclusions cannot be made about the effects of plasma expansion on renal perfusion during initial resuscitation and stabilisation. This is a common problem in studies where RBF estimation is not readily available at the bedside. The selection of study participants based on indices indicating fluid responsiveness could have generated a more robust haemodynamical response and increased the generalisability of our results in relation to current practice. Further, a more detailed measurement of the haemodynamic response, including CO, could have facilitated the interpretation of our results. This because CO may not have been increased despite MAP elevation, and an increase in central venous pressure by plasma expansion may have attenuated the increase in renal perfusion pressure[254]. In our study, simultaneous CO assessment was not feasable due to technical limitations. However, 14 of 17 participants showed an increased ratio of MAP/HR > 10%, which is in parity with the number of patients expected to be true fluid responders after a dynamic evaluation of fluid responsiveness. Improvements in haemodynamic variables in general (reduced HR and increased MAP) are common end points when evaluating the effect of a fluid bolus in clinical practice[253]. Finally, the blood pressure transducer during the study was fixed to the same relative level as the patient's heart. The MRI milieu, however, limits the possibilities, and allowing movement with the patient was prioritised. Thus, the reported absolute blood pressure levels may be less reliable than the differences within individuals.

Conclusions

I. SA-AKI in sheep is associated with alterations in the mitochondrial electron transport chain in the renal cortex, consisting of a reduction in RCR and an increase in the ratio Complex II/Complex I. However, no reduction in cortical mitochondrial efficiency explaining the disturbed relation between sodium transport and oxygen consumption could be demonstrated.

II. AKI occurred in a majority of patients admitted to the ICU before the introduction of targeted therapies in a single center cohort. Oliguria was common, and levels of urinary biomarkers at admission were generally increased without a clear relation to the final AKI stage.

III. Decreased renal perfusion assessed by MRI was more common in patients with critical COVID-19 and AKI compared to similar patients without AKI. However, no effects on renal oxygenation nor water diffusion could be demonstrated.

IV. Plasma expansion by standardised fluid bolus did not increase renal perfusions assessed by MRI, neither when AKI was present nor in the absence of AKI in normotensive critically ill patients with ARDS due to COVID-19.

Future perspectives

As presented in this thesis, AKI is a common complication during severe infections requiring ICU care. Disturbances in some aspects of RBF and oxygenation have been demonstrated in experiments and in clinical observational studies. A future challenge, in the clinical setting, is to determine the circumstances in which a reduced RBF aggravates renal tissue damage, or mainly is a result of it. Thus, in the event of the introduction of bedside methods measuring similar renal parameters as described in this thesis, further work is needed to determine the causal role of differences in RBF and oxygenation. Although a reduced RBF is not a prerequisite for AKI development, the additional harm of periods with a reduced RBF due to hypotension, may be further investigated in experimental studies. Using the combination of clinical data and biomarkers, subtypes of clinical SA-AKI can also be statistically created[26,28]. A future challenge is to link such subdivisions to mechanisms demonstrated in experimental research, to be able to increase the chances of clinical benefit with targeted interventions. Observational imaging studies of patients, such as presented in this thesis, which explores pathological mechanisms, may provide support for such links.

Interventions that target RBF and oxygenation face several challenges. Non-renal effects of the compounds used to target mitochondrial function may limit the clinical utility. Thus, finding methods to limit the intervention to the kidney may increase the likelihood of success. The novel family of drugs inhibiting SGLT2 have the advantage of effects limited to kidney. More data from safety trials is also available than experimental compounds. SGLT2 inhibition have is beneficial in several instances of CKD, and increases tissue oxygenation in the renal cortex. Thus, investigating whether this mechanism may reduce the severity of AKI, or reduce the risk of CKD-development after an episode of AKI, will likely be attempted.

Increases in GFR increases oxygen demand, as have been discussed, and renoprotective therapies can be associated with limited decreases in GFR. This may limit the usefulness of changes in GFR and urinary output as a surrogate for clinical outcome. Whereas there is in general a clear relation between the AKI phenotype and poor clinical outcome, well supported by a large quantity of studies, a discrepancy may occur when introducing new therapies, or when

attempts to alter renal physiology is undertaken. Thus, to limit the evaluation of kidney function to short-term changes in GFR could obscure truly beneficial effects. More intricate ways to describe and define the damage occurring to the kidneys during severe infections, may be needed.

Sammanfattning på svenska

En snabb försämring av njurfunktionen, akut njursvikt, är en vanligt förekommande komplikation vid livshotande sjukdom hos vuxna som behandlas på intensivvårdsavdelning. Nästan hälften av alla intensivvårdade drabbas och livshotande infektioner med effekter på hela kroppen, sepsis, är den vanligaste orsaken till detta. Akut njursvikt diagnosticeras med hjälp av kriterier där njursviktens svårighetsgrad klassificeras i stadierna 1, 2 och 3. Förändringar i antingen en markör i blod, kreatinin, som avspeglar njurens reningsförmåga(filtration), eller en nedsatt urinproduktion över tid utgör de 2 olika sätten att diagnosticera akut njursvikt. Ökad svårighetsgrad av njursvikten ökar risken för död under vårdtiden och dödligheten vid intensivvårds tillstånd som komplicerats av den högsta graden av njursvikt, grad 3, är i vissa undersökningar närmare 40%. Akut njursvikt är ofta självläkande för de patienter som överlever sin akuta sjukdom men trots det medför den akuta njursvikten en ökad risk att över tid drabbas av kronisk njursvikt samt även en ökad risk att dö av andra sjukdomar under den närmaste tiden efter. Behandlingsmöjligheterna som står till buds är främst understödjande, behandling av den underliggande sjukdomen och möjlighet till akut dialysbehandling för att undvika grava rubbningar i kroppens vätske- och saltbalans samt delvis avlägsna slaggprodukter som bildas i kroppen.

Sjukdomsprocesserna som leder till njursvikt vid svåra infektioner är komplexa, där en del utgörs av inflammatoriska processer med sitt ursprung i immunförsvarets reaktion på infektionen. Detta medför flertalet rubbningar i njurens cellers normala funktioner samt rubbningar i njurens cirkulation. Rubbningar i blodcirkulationen uppstår också i de minsta blodkärlen, kapillärerna, bl.a. i proximala tubuli som är lokaliserad i njurens bark som normalt återtar mycket av de salter och vatten som filtreras ut och står för mycket av njurens normala syrgaskrävande arbete. Njuren får normalt sett en förhållandevis stor mängd blod till sig för att möjliggöra en effektiv filtration av blodet. Trots det finns det delar av njuren, märgen, som får en förhållandevis liten del av detta och är känslig för nedsatt cirkulation till njuren och riskerar att drabbas av syrebrist.

Syftet med detta doktorandprojekt var att studera sjukdomsmekanismer relaterade till cirkulation och syresättning i njuren vid akut njursvikt i samband

med livshotande infektioner, och har inkluderat experiment på försöksdjur samt patientnära studier.

I delarbete I har en försöksdjursmodell med får använts. Fåren har varit sövda och respiratorvårdade på ett sätt som liknar modern intensivvård och randomiserats till att antingen få sepsis med hjälp av en infusion levande bakterier eller att vara kontroller. Mätningar av njurblodflöde har gjorts med hjälp av en flödesmätare och syresättning i njuren har mäts med hjälp av en kateter i njurens ven. Cellprover för att undersöka funktionen i njurbarkens mitokondrier, som är den del av cellen som använder syrgas för tillgängliggöra energi till resten av cellen, har tagits i början och slutet av försöket. Resultaten visar att njurfunktionen försämras påtagligt i försöksdjuren som får sepsis. Mitokondriernas funktion påverkas av detta på så sätt att mitokondriernas reglering av energiproduktionen rubbas och att ett viktigt proteinkomplex, komplex I, fungerar sämre när hög energiproduktion stimuleras. Njurarnas syrgashantering rubbas på så sätt att konsumtionen av syrgas fortsatt är hög, trots att återtagandet av salt minskar påtagligt. Detta kan dock inte förklaras med en sämre effektivitet i mitokondrierna eller att mitokondrierna försätts i friläge där syrgas används utan att skapa energi tillgänglig för cellerna.

I delarbete II har akut njursvikt vid den nya infektionssjukdomen COVID-19 beskrivits och karaktäriserats. I en prospektiv kohortstudie på ett och samma sjukhus har 57 intensivvårdade patienter inkluderats i studien efter samtycke. De båda kriterierna för akut njursvikt har beskrivits i relation till tid och från 52 av studiedeltagarna har markörer för skador i tubuliceller mätts i ett urinprov taget i början av intensivvårdstillfället. Resultaten visar att 89% av studiedeltagarna hade akut njursvikt någon gång under de två första veckorna efter ankomst till intensivvården och att nedsatt urinproduktion var vanligt och uppstod tidigt under intensivvårdstillfället. De fanns också skillnader i vilka kriterier som blev uppfyllda. En fjärdedel av patienterna hade enbart nedsatt urinproduktion utan allvarlig påverkan på filtrationen. Markörerna i urinen var ofta förhöjda dock med endast mindre skillnader mellan patienter indelade efter framtida svårighetsgrad av njursvikten.

I delarbete III har 19 patienter vårdade på intensivvårdsavdelning till följd av COVID-19 efter samtycke undersökts med magnetkamera avseende njurarnas blodflöde, syresättning och vatteninnehåll. En grupp patienter med akut njursvikt (10st) enligt filtrationskriteriet har jämförts med en grupp patienter utan akut njursvikt (9st). Undersökningen visar att patienter med akut njursvikt vid undersökningstillfället oftare hade lägre blodflöde till njurarna, både i barken och märgen jämfört med de utan njursvikt. Dock kunde inte några skillnader avseende syresättning eller vatteninnehåll påvisas.

I delarbete IV undersöks huruvida en ökning av cirkulerande blodvolym med hjälp av infusion av vätska intravenöst kan påverka blodflödet till njurarna hos 17st av studiedeltagarna i delarbete III. Ringer Acetat i mängden 7,5ml/kg kroppsvikt infunderades och mätningen med magnetkamera upprepades. Resultaten visar blodflödet inte ökade i någon undersökt aspekt, var sig till patienter med eller utan njursvikt, trots att ökningen av cirkulerande blodvolym resulterade i högre blodtryck. Patienter utan njursvikt hade dessutom en lägre perfusion i njurens märg efter infusionen.

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