Septic Acute Kidney Injury

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Chapter 1: Introduction

Rationale

Acute kidney injury, formerly known as acute kidney failure, is a syndrome characterized by the rapid loss of the kidney´s excretory function. This is normally diagnosed by the accumulation of urea and creatinine, decreased urine output, or both. Acute kidney injury affects approximately 40% of critically ill patients and one third of them die within the first 90 days of admission (1). Despite the importance of this disease, no evidence-based treatment recommendations exists and the pathogenesis is debated. The concept of acute renal failure has undergone a modification the recent years. Traditionally acute renal failure has been related to ischemia and development of acute tubular necrosis. Mounting evidence suggests that acute, mild injury to the kidneys causes alterations to the urine output and blood chemistries. This could potentially have serious clinical consequences. The syndrome of acute kidney injury also includes patients without functional damage, but with impairment relative to physiologic demand. This means that AKI includes both injury and impairment. This is a more holistic approach to the clinical challenge, including patients that could benefit from early intervention. Sepsis is one of the main factors contributing to the development of acute kidney injury. Sepsis and septic shock account for approximately 50% of acute kidney injury cases in the ICU (2). Though, the pathophysiology behind this development is inadequately understood. With the intention to optimize treatment of critically ill patients in the ICU, it is pivotal to understand current research and the foundation of which the current treatment guidelines are based on.

Objectives

The overall objective of this thesis is to give an introduction to the current literature and the ongoing research on the topic of septic acute kidney injury. The aim of study is the current understanding and hypothesis of the pathophysiology of septic AKI. With an overview of existing literature, the objective is two-fold. First, to learn to collect and evaluate research papers with emphasis on literature review methodology. Second, to obtain a thorough understanding of the physiology and pathophysiology of the kidney, sepsis and acute kidney injury. Through an in-depth literature research I will try to present different views and theories behind septic AKI. First, I will review basic important concepts of renal physiology together with definitions of sepsis and acute kidney injury. Second, I will present methods of literature review. Thirdly, I will discuss key ideas on the pathophysiology of septic AKI, and lastly, possible new concepts and areas of future research.

Abstract

Systemic hypotension, renal vasoconstriction and ischemia-reperfusion injury as the mechanism behind septic acute kidney injury has been challenged. Septic acute kidney injury can occur during a hypo- and hyperdynamic circulatory state. It is associated with a high flow, low-pressure renal circulation. New histological findings indicate a lack of acute tubular necrosis and apoptosis during septic AKI, with only a minor influence of the tubular cells. Mounting evidence suggests exposure of the nephron and tubular cells to inflammatory

mediators. This could alter the permeability of the glomerular filtration barrier through glycocalyx shedding, decreased fenestrae density and increased fenestrae diameter. Alterations to the permeability could possibly activate the tubuloglomerular feedback mechanism and result in a decrease in kidney function. Further research should focus on the intergrative glomerular barrier complex, with emphasis on glycocalyx, endothelial surface layer and fenestrae. New technology like PET and nanomechanics could be beneficial in the search for the pathogenesis behind septic AKI.

Chapter 2: The Kidney, Sepsis and Septic Acute Kidney Injury

In this section I will first be giving a brief overview of the main components of the kidneys and there function. The intention is to give pertinent information to the reader unfamiliar with the kidney and its physiology, together with reinforcing old knowledge for the experienced few. The scope of this section is to elaborate on the important components involved in glomerular filtration, indicating a deep dive into molecular structures of the glomerular filtration barrier. To understand the impact of sepsis on the glomerular filtration barrier I will elaborate on definitions and give a short overview of current understanding of the pathophysiology of the disease. This thesis evolved due to the unknown pathophysiology of septic acute kidney injury. A thorough understanding of the current knowledge and evolving theories on the subject is pivotal before introducing new concepts and theories.

Gross Anatomy

The kidneys are paired organs that are situated in the retroperitoneal space on either side of the vertebral column. In an adult human the weight of the kidney is approximately 115g to 170g and is about 11 cm long, 6 cm wide and 3 cm thick (3). The medial side of the kidney is where the renal artery, vein and nerves enters and exits. This concave surface, which is called the hilus, is also where the renal pelvis exits the kidney. If cut in two, the kidney is divided into two distinct regions: the outer darker region is called the cortex and the inner paler region is called the medulla. The cortex and medulla is made up of nephrons, which is the functional unit of the kidney. The cortex and medulla is also composed of blood vessels, lymphatics and nerves. The medulla is composed of renal pyramids and the base of each renal pyramid originates at the corticomedullary border. The apex of the pyramid ends in a papilla. Every papilla lies within a minor calyx and they collect urine from the papilla and drain it into the major calyces. The major calyces stretch into the renal pelvis, which is the upper end part of the ureter. One ureter from each kidney carries urine from the kidney to the urinary bladder.

The Nephron

The basic functional unit of the kidney is the nephron. Each human has approximately 1-1,5 million nephrons (4). The nephron is a hollow tube composed of a single cell layer with a blind end forming a capsule around a knot of blood capillaries, the glomerulus. The other end forms the collecting duct system. Between the glomerulus and the collecting duct the nephron consists of the proximal tubule, loop of Henle and the distal tubule. The glomerulus has the

function of producing an ultrafiltrate of plasma. The proximal and distal tubules and loop of Henle have the function of secretion, metabolization and reabsorption of fluids and substances (3). The collecting duct system has important functions in pH regulations, ion reabsorption and excretion, together with water reabsorption (4).

Renal Vasculature and Nerves

The kidneys are supplied with blood from the renal artery. Approximately 20-25% of the cardiac output flows to the two kidneys (3, 4). The renal artery divides into two or three segmental arteries, which again branch into several interlobular arteries. These will eventually turn into afferent arterioles, which will supply the glomerulus. The glomerulus is the site of filtration of fluid into Bowman`s capsule. The blood exits the glomerulus and drains into the efferent arteriole. The efferent arteriole from nephrons in the outer 2/3 of the cortex will branch into a great peritubular capillary network (4). These capillaries will surround the cortical tubular elements. The inner 1/3 of the nephrons will have an efferent arteriole that will give rise to peritubular capillaries, but also the vasa recta. The vasa recta is a capillary network lying adjacent to the loop of Henle and the collecting tubules. The vasa recta has important functions. Giving oxygen and important nutrients to the nephron segments, delivering substances to the segments for secretion, serving as a pathway for the return of reabsorbed water and solutes to the circulatory system and concentrating and diluting the urine (3). The vessels of the renal venous system runs parallel to the arteries. al axons, which accompany the intrarenal arteries and the afferent and efferent arterioles. They originates in the celiac plexus and the kidney has no parasympathetic innervation (3). The nerves are monoaminergic and noradrenaline and dopamine have been identified (5). In addition, several other neuropeptides have been found together with noradrenaline. Tubules in connection to the juxtaglomerular apparatus and the apparatus itself are densely innervated by terminal axons, more than other sites (5). Little is known about the afferent nerves of the kidney. They are believed to be sparse, but it is still an unresolved issue (5).

Glomerular Filtration

The kidney functions through filtration of plasma over the glomerular filtration barrier, creating ultrafiltrate. The ultrafiltrate consists of salts, organic molecules and a very low concentration of protein. There is also no cellular elements in the filtrate (6). In healthy adults the glomerular filtration rate, GFR, ranges from 90 to 140 mL/min for men and 80 to 125 mL/min. In 24 hours as much as 180 L of plasma is filtered by the glomeruli (6). The estimation of glomerular filtration rate is based on the concept of renal clearance. The clearance of any substance excreted by the kidney is the volume of plasma which is cleared of the substance in unit time (4). The renal clearance is used to assess the renal function in disease. The renal clearance is based on Ficks principle of mass balance relationship.

$$P_x^a \times RPF^a = (P_x^v \times RPF^v) + (U_x \times \dot{V})$$

(6)

The equation is based on substances that are neither metabolized nor synthesized by the kidney. The amount of any substances into the kidney is equal to the amount excreted in the urine plus the amount that leaves the kidney through the renal vein. It is a proportional relationship between the plasma concentration of a substance and the rate of urinary excretion. To calculate the rate of which substance x is removed from the plasma, one has to calculate the clearance. Clearance is given by the formula:

$$C_x = \frac{U_x V}{P_x}$$

(4)

In this formula the Cx is the clearance of substance x. Ux is the urine concentration of x, Px is the plasma concentration of x and V is the urine flow (mL/min). Measuring the renal clearance require a constant and accurate concentration of substance x in the plasma. This means that measuring the renal clearance can only be done under steady states of GFR and renal blood flow. Another challenge is that a steady flow of urine is needed for collection during the clearance period. This is not possible in conditions with oligo- or anuria (4).

Glomerular filtration rate

The GFR in the kidney is the total sum of filtration from all the nephrons. Therefore the GFR is a measurement of kidney function. A change in GFR indicates a change in kidney function. Today our most frequently used substance for measuring GFR, is Creatinine. Creatinine is a byproduct of skeletal muscle metabolism and is freely filtered across the filtration barrier (6). It is not metabolized, secreted or reabsorbed in the kidney, making it a good substance for evaluation of kidney function. Using the above-mentioned equation for renal clearance, it is possible to calculate the clearance of Creatinine and indirectly estimating glomerular filtration rate.

$$\mathrm{GFR} = \mathrm{K_f}\left[(\mathrm{P_{GC}} - \mathrm{P_{BS}}) - \sigma(\pi_{\mathrm{GC}} - \pi_{\mathrm{BS}})\right]$$

(6)

The forces influencing the filtration of fluids from plasma are the same as in all the capillary beds. The Starling forces with the hydrostatic and oncotic forces regulating the filtration rate (6). The GFR is proportional to the Starling forces that exits across the membrane multiplied with the ultrafiltration coefficient (Kf). Kf is the sum of capillary permeability and the surface area of filtration. The renal blood flow is regulated by adjusting the vascular resistance in response to arterial pressure. The auto regulation of the kidney keeps the blood flow relatively constant between arterial pressures between 90 mmHg and 180 mmHg (6). The auroregulation is controlled by two mechanisms. The tubuloglomerular feedback mechanism is a sodium-chloride dependent mechanism controlled by the macula densa and the juxtaglomerular apparatus. Another mechanism controlled by the arterioles themselves is the myogenic mechanism; the intrinsic property of vascular smooth muscle to contract when stretched. Both mechanisms regulate the tone of the afferent arteriole (6). The macula densa and juxtaglomerular apparatus regulates the afferent arteriole by increasing production of ATP and adenosine stimulating to vasoconstriction. By decreased production of ATP and adenosine together with nitric oxide

production the afferent arteriole will be stimulated to vasodilatation. Other factors that affect GFR and RBF are sympathetic stimulation, angiotensin 2, prostaglandins, nitric oxide, endothelin, bradykinin, natriuretic peptide, glucocorticoids, histamine and dopamine (6).

Glomerulus and Glomerular Filtration Barrier

The glomerulus consists of a network of capillaries arising from an afferent arteriole and the blood is drained by an efferent arteriole (7). A framework of mesangial tissue supports the capillaries. Each capillary loop consists of a basement membrane covered by glomerular endothelium and on the visceral side covered by podocytes. The podocytes possess foot processes that are separated by filtration slits, also called pores (7). These pores have a specialized diaphragm which covers the filtration slit (8). As earlier mentioned the glomerulus has the function of filtrating plasma and creating ultrafiltrate. This process is done by the glomerular filtration barrier, GFB. The barrier consists of podocytes, the basement membrane, glomerular endothelium and the endothelial surface layer (8).

Podocytes

The podocytes which is also called visceral epithelial cells, have large cell bodies and long extending cytoplasmic foot processes which support the glomerular capillary loop from the visceral aspect of Bowman´s space (9). The podocytes function as vascular support cells and providing vascular growth factors necessary for endothelial health and survival (10). The podocyte foot processes are separated by filtration slits, which are cell-to-cell junctions. The filtration slit is covered by a diaphragm made up by different proteins (8). The slits are 25-60 nm wide (8, 11, 12). The slit diaphragm dictates the permselectivity on the basis of molecular size, charge and physical configuration (8, 13). Within the slit diaphragm there are small pores postulated to smaller than albumin, and thus contributing to the permselectivity of the GFB (8, 11, 12). A key feature of the podocyte that differentiates it from other cells in the glomerulus is that it is unable to replicate itself. Thus, if injured the only way to replace function is by hypertrophy of the remaining cells (12, 13). The podocyte is attached to the glomerular basement membrane by intergrins on its basal side (13). On the apical side it is strongly negative charged due to the glycoprotein glycocalyxin(10, 13). The podocyte produce structural components of the glomerular basement membrane. On its basolateral surface it also produces transmembrane proteins involved in communication with the glomerular basement membrane (10). The podocyte is a large and important part of the glomerular filtration barrier, but its direct effect on fluid restriction and protein transport is unclear (8).

Glomerular basement membrane

The basement membrane consists of a fibrous network composed of type 4 collagen, laminin, nidogen/entactin and proteoglycans (8). It is synthesized from both the podocytes and the glomerular endothelium (10, 14). The basement membrane of the glomerular endothelium is much thicker than other vascular beds (10). The collagen 4 network is considered the backbone of the GBM, but the membrane consists of large amount of proteoglycans as well. It is primarily heparan sulfate chains attached to the polysaccharide (8). The role of the GBM in

permselectivity has been under investigation for duration of time. The charge selective component of the membrane has been challenge the recent years, questioning the role of the GBM (7, 15). It is clear that defects to the membrane can cause proteinuria and nephrotic syndrome (14), so it does not rule out the GBM as a contributor to the GFB.

Glomerular endothelial cell

Glomerular endothelial cells are thin, unusually flat cells with a height of 50 to 150 nm (8). The glomerular endothelial cells are different from other epithelial cells in the fact that they are highly fenestrated. Fenestrations are round transcellular holes that goes through the endothelial cell cytoplasm (16). 20-50% of the epithelial surface consists of fenestrae and they are located peripherally in the cell (17). The glomerular fenestrae is approximately 50-100 nm in diameter (9, 10, 18). In the healthy adult the fenestrae do not have diaphragms, but this may be altered in disease and present in the embryonic stage of development (18). The glomerular endothelial fenestrae do not express "plasmalemmal vesicle-associated protein-1", PV-1 which is a component of fenestrae slit diaphragms in other endothelial cells (16). The fenestrations have been thought to not provide much restriction to the permselectivity of proteins. This is due to albumin measuring 3,6 nm, and in regards to size, indicating a free flow of albumin across the GFB. However the endothelial surface layer were explored with transmission electron microscopy by Ryan and Karnovsky (19). Studies indicated that albumin does not cross the GFB and is confined to the glomerular capillaries during normal conditions. It is now believed that the endothelial surface layer that covers the fenestrations contributes to the permselectivity (20-22). The endothelial cells have receptors for vascular endothelial growth factor A (VEGF-A) and angiopoetin that are produced by podocytes (23, 24). These mediators are very relevant for maintenance and development of the glomerular endothelium (25-28). There

Endothelial surface layer

The endothelial surface layer, which is located on the luminal side of the glomerular endothelial cell, consists of two layers; the glycocalyx and the endothelial cell coat. The endothelial surface layer is involved in blood coagulation, modulation of angiogenesis, rheology and capillary barrier function (8). The ESL is also involved in filtration of fluids and the restriction of proteins (29-31). The glycocalyx is composed of membrane-bound proteoglycans and glucosaminoglycans, such as hyalorunan, chondoitin sulfate and heparan sulfate with terminal sialic acids (31, 32). The glycocalyx is a negatively charged barrier and will therefore create a permselectivity based on charge and size, contributing to the glomerular filtration barrier (31, 33, 34). The glycocalyx extends into the lumen and its components are anchored to actin stress fibers in the cytoskeleton and are believed to transmit shear stress, a force created by the blood stream (34). The ESL also covers the endothelial fenestrations with slit diaphragm like "sieve plugs" (35). The thickness of the ESL has been debated, but with intravital microscopy the thickness of the ESL has been calculated to 200-400 nm (8, 29, 36). The cell coat is composed of plasma proteins such as albumin and orosumicoid, as well as proteins produced by the endothelium, proteoglycans, glycoproteins and glucosaminoglycans (37). The ESL is not static,

but rather a dynamic layer, constantly replacing molecules. It is not a significant boundary between locally synthesized and associated elements (38).

Sepsis

Sepsis is an old and unfortunately common disease. 2% of the patients admitted to hospital in the United States have recorded severe sepsis (39). The definition of sepsis has for a long time been debated in the medical community (40). A summary of current definitions and understanding based on the International Sepsis Definitions Conference gives the following explanations: (40, 41).

- Infection: a pathologic process caused by the invasion of normally sterile tissue, fluid, or body cavity by pathogenic or potentially pathogenic microorganisms
- Sepsis: infection, documented or suspected, and some of the signs and symptoms of an inflammatory response.
- Severe sepsis: sepsis complicated by organ dysfunction
- Septic shock: severe sepsis plus acute circulatory failure characterized by persistent arterial hypotension despite ad- equate volume administration, unexplained by causes other than sepsis

The concept Systemic Inflammatory Response Syndrome, SIRS was introduced in 1992 by the ACCP/SCCM Consensus Conference as an acronym for the complex findings that result for a systemic activation of the innate immune response, regardless of the cause (42). The SIRS criteria are:

- >38°C or <36°C
- Heart rate >90/min
- Respiratory rate of >20/min or a PaCO2 of <32 mmHg
- white blood cell count of >12,000 cells µL or <4,000 µL

Severe sepsis is a result of infections acquired in the community and in the hospital. The most common cause is pneumonia, counting for about half of the cases and intra-abdominal infection and urinary tract infection as the second and third most common causes for sepsis (39, 43-45). Staphylococcus aureus and streptococcus pneumoniae are the most common gram-positive isolates, and Escherichia coli, Klebsiella species and Pseudomonas aeruginosa predominate among gram-negative isolates (43, 46). In the Vincent et al study, microbiological identification results were positive in 70% of the infected patients, 62% of the positive isolates were gram-negative organisms, 47% gram-positive and 19% were fungi. In the same study the ICU mortality rate of infected patients was more than twice that of non-infected. It must be added that in only 1/3 of the cases the blood culture is positive (43, 44, 46). The pathophysiology behind sepsis and SIRS is complex and not yet completely understood. Over the years it has become apparent that an infection triggers a host response in which both pro-inflammatory and anti-inflammatory mechanisms work to clear the infection and contribute to tissue recovery. The same mechanisms are involved in organ dysfunction and secondary infections. (39). The specific response to the infection is dependent on the pathogen with its load and virulence, as well as the host with its genetic characteristics and coexisting illnesses (39, 40).

Acute Kidney Injury

Acute kidney injury (AKI) is a syndrome characterized by rapid loss of urine output and accumulation of urea and Creatinine (azotemia) (47). A consensus definition was made by the Acute Dialysis Quality Initiative through the RIFLE criteria (risk, injury, failure, loss, end stage) (48). From the RIFLE criteria the KDIGO criteria has emerged, being the updated international guidelines on acute kidney injury (49).

AKI is defined as any of the following:

- Increase in SCr by >0.3 mg/dl (>26.5mikromol/l) within 48 hours; or
- Increase in SCr to >1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; o
- Urine volume <0.5ml/kg/h for 6 hours.

AKI affects approximately 40% of critically ill patients and one third of them die within the first 90 days of admission (1). Septic shock induced AKI accounts for approximately 50% of all the patients with AKI (2). AKI is also triggered by major surgery (especially open heart surgery) and acute decompensated heart failure (47). Particular risk factors for the critical ill patient in development of AKI are older age, diabetes, higher baseline Creatinine, heart failure, sepsis/SIRS, use of nephrotoxic drugs, higher severity of disease scores, use of vasopressors/inotropes, high risk surgery, emergency surgery, and possibly hypertension (50). The pathophysiology of septic AKI is inadequately understood. The common pathophysiological theory on the subject involves systemic hypotension, renal vasoconstriction and ischemia reperfusion injury (47, 51). The last years these theories have been challenged (52-54). New and recently proposed theories embrace dominant efferent arteriole dilatation and subsequent decrease in glomerular filtration pressure (intra-glomerular hypotension), intra-renal hemodynamic alterations (periglomerular shunting) and excessive inflammatory activation (55, 56).

Chapter 3: Methods and process

The methodology and structure of this thesis is inspired by the Preferred Reporting Items for Systematic Reviews and Meta-analysis: The PRISMA Statement (57). The statement has been guidance, not a fixed protocol. Due to the unknown pathophysiology of septic acute kidney injury and the controversies surrounding current knowledge and evolving theories, following a strict protocol in regards to systematic reviews has not been the scope of this thesis. My intention is to elaborate on evolving theories and discuss new key ideas on the subject, forcing through a more pragmatic approach. From the PICOS approach (57), the aim of study was identified together with population, interventions, comparator/comparison, outcomes and study design.

PICOS approach

1. Population/Problem

- Key words: Human, Animal, Rodent, Primate
- Explanation: Experimental studies on septic AKI have been performed on different animal models. Sparse human clinical data exists on the subject, but there have been done several systematic reviews, multicenter trials

etc. Since experimental animal models have such a significant value in the investigation of the pathophysiology of septic AKI, it is not beneficial to limit search and literature to one particular population.

2. Intervention/Exposure
   - Key words: Sepsis, septicemia, bacteremia,
   - Explanation: AKI has multiple etiologies, but sepsis is the scope of study.
3. Comparator/Comparison
   - Explanation: Nothing to compare.
4. Outcome
   - Key words: Azotemia and reduced urine output. Histopathology, glomerular filtration pressure, glomerular filtration barrier, glomerular endothelial surface layer, glomerular endothelial cell, intra-glomerular pressure, tubular injury, acute tubular necrosis.
   - Explanation: Effect of sepsis on kidney function and development of septic AKI. Alterations to microcirculation, GFR and in particular the glomerular filtration barrier.
5. Study design
   - Key words: experimental study, multicenter trial, randomized control trial, systematic reviews, observational study, and metaanalysis.
   - Explanation: Every relevant study has been included, irrespective of study design.

Protocol and registration

Method of analysis and inclusion criteria where specified in advance, but a thorough review protocol did not exist. The intention was to evaluate studies concurrently with the data collection and adapt and review studies published by recognized researchers and medical journals.

Eligibility criteria

Primary search strategies focused on literature from the last 10 years, but when necessary, papers older then 2005 were included. Only literature in English was analyzed and no study designs were excluded. As mentioned briefly in the PICOS approach, due to the nature of septic AKI and current research, it was not beneficial to limit search and literature to one particular population. No restriction was made in regards of participants included. Abstracts from conferences were not included in this thesis. This was done due to inadequate study information. It was several abstracts that were relevant for the thesis, but excluded due to no publication.

Information sources

Studies were identified by searching electronic databases and scanning reference lists of articles. The following databases were searched:

- PubMed
- Embase
- Bibsys, University of Tromsøs database.

Last search: 01.05.15.

Search and study selection

The search was performed together with specialist librarian Elin Strand at Nordlandsykehuset. We searched all databases with sepsis, endotoxemia, glomerular filtration barrier, glomerular endothelium and other relevant search terms. The studies selected were based on title and abstract review. This was done independently by the author alone. Selection of articles only done by the author is a source of selection bias and ideally the study selection should have been performed independently by two or more reviewers to enhance objectivity and reduce mistakes.

Risk of Bias

It´s desirable to comment on the risk of bias in this thesis. As a single author and reviewer, it is room for error. Selection bias, selective reporting within studies and inadequate knowledge of study design and methodology are the most pertinent ones. Further, the great share of single experimental studies that has been evaluated in this thesis, are potentially a great source of bias.

The Process

As part of the mandatory requirements of the master thesis booklet, I will comment on the working process. The working process can be divided into three main parts. First, theory and hypothesis discussion. Through conversations with my supervisor, Professor Ytrebø, the current theories and hypothesis on acute kidney injury was discussed, and I was enlightened on the theoretical and clinical aspects and challenges of acute kidney injury and sepsis. This began back in 2013, and over several cups of coffee the foundation for this thesis was made. In the beginning the goal was to come up with new hypothesis on the pathogenesis of AKI and sepsis, but through careful considerations, a more holistic approach was taken. The intention was to gain a deeper understanding of the medical conundrum itself, as well as to learn more about the method of literature review and data collection. Second, literature search. Together with specialist librarian Elin Strand we conducted a pragmatic search in relevant databases on different occasions. She also conducted several searches during this stage. Third, data extraction and review. Through careful evaluation of papers and data, current theories have been systemized and evaluated. Giving a deeper understanding of sepsis and acute kidney injury.

Chapter 4: Current Theories and Understanding

The common pathophysiological theory on septic acute kidney injury involves systemic hypotension, renal vasoconstriction and ischemia reperfusion injury (47, 51). The last years these theories have been challenged (52-54). In this section I will elaborate on current theories and discuss their strengths and weaknesses. I will discuss hemodynamic alterations, histopathological alterations, immune system influence, organ cross talk and glomerular filtration barrier.

Hyperperfusion in Sepsis – it also includes the Kidneys

The classical clinical and biochemical signs of septic AKI are the reduction in blood pressure, subsequent decrease in urine output together with an increased

level of Creatinine and urea. This had led to the dogma that sepsis leads to a systemic vasodilatation with subsequent fall in blood pressure. The fall in systemic blood pressure and alterations in kidney hemodynamics induces AKI and the final outcome would be acute tubular necrosis (ATN). In this section I will elaborate on recent findings that has challenged this dogma and given new insight into understanding the kidney during septic AKI.

Increased and decreased Flow

Back in 2005,Langenberg et al. did a literature review on renal blood flow in sepsis (58). The following year a controlled experimental study on septic sheep and blood flow (58, 59). The systematic review by Langenberg et al. on septic AKI showed that approximately 30% of animal studies reported an unchanged or even an increase in renal blood flow. In this review it was emphasized that previous studies had been heterogeneous in design and had many confounders. In the experimental study the following year flow probes were planted around the pulmonary and left renal artery and monitored the blood flow during development of septic AKI. Key finding in this study was increased total renal blood flow and renal vasodilatation during septic AKI. Also, despite well-maintained renal perfusion, the glomerular filtration rate decreased. This was contrary to common belief that septic AKI developed due to inadequate perfusion. Also fractional sodium and uric acid excretion were reduced indicating a normal kidney function. This was the first continuous assessment of renal blood flow during septic AKI, and questioned the medical dogma. The follow up study by Langenberg et al. in 2007 gave an even deeper understanding of the hemodynamic alterations in septic AKI (52). Nine sheep were induced with E.coli and monitored with flow probes and intra-arterially and intravenously catheters. Again they observed a hyperdynamic circulatory state with increased cardiac output and decreased blood pressure together with septic AKI. An interesting finding was that in the recovery phase of septic AKI, the renal blood flow decreased during functional improvement despite increasing MAP. This indicated that the vascular bed could be involved in the both the loss and recovery of GFR in septic AKI (60). Now, new preclinical evidence questioned the long believed theory that fall in blood pressure directly led to decreased urine output. But, preclinical evidence is one thing, human experiment is another. It has been questioned the applicability of preclinical studies in animals to humans in sepsis (61). In 2012 Prowle et al. investigated the possibility of measuring RBF during phase-contrast MRI in patients admitted to the ICU with septic AKI (62). With a noninvasive technique they found a reduced total renal blood flow as fraction of cardiac output in 10 patients with AKI, compared with 11 normal volunteers. This was within 1-7 days after the diagnosis of AKI was made. This is an interesting finding since it shows the opposite of what is noted in previous mentioned clinical studies.

Angiotensin II and Adenosine Triphosphate

For a duration of time it has been hypothesized that during septic AKI glomerular filtration pressure decreases due to afferent but also efferent vasodilatation (63). To get closer to answering this question Wan et al. (64) induced hyperdynamic sepsis in sheep by the intravenous administration of E. Coli. Thereafter randomly infused the vasopressor Angiotensin II with the intention of increasing the intra-glomerular filtration pressure and increase

urine output. This had never been done before due to concerns about the intense effect on renal blood flow and kidney function (64). The result of the study was that with a dose of AT II titrated to restored levels of MAP, it caused a systemic vasoconstriction, but with a limited effect on mesenteric, coronary and iliac vessels. The RBF and renal conductance decreased to normal levels. Urine output increased 7-fold and the Creatinine clearance increased by 70% as well as the fractional natriuresis. Yet again Bellomo and his research group implicated that the vascular bed and intra-glomerular pressure was involved in the pathogenesis of septic AKI. But not all questions were answered. When infusing AT2 to a MAP level similar to baseline, the urine output and factional natriuresis were much greater. This indicating that the increase in urine output was not simply secondary to an increase in glomerular filtration pressure. Something else was also going on. The authors implicated that use of AT II could have a role in treatment of septic AKI. But with a new treatment came a new concern. Increasing the urine output during AKI may not be beneficial, and in worst case harmful, depleting the kidney of ATP during sodium reabsorption. Redfors et al. (65) did a prospective, two-group comparative study looking into renal oxygenation and sodium reabsorption during AKI. In sodium reabsorption during AKI the oxygen consumption increases and this may be harmful if it leads to bioenergetic failure. Trying to assess the safety and benefit of AT II as vasoconstrictor therapy during AKI, May et al. (66) measured the ATP levels using magnetic resonance spectroscopy. During early hypotensive gram-negative sepsis they found no evidence of ATP depletion despite reduced RBF. The animals were in the scanner for 6 hours in total. They were monitored for 2 hours with infusion of E.coli and thereafter 2 hours during infusion of AT II. Because of the immobilization and positioning of the animals within the scanner they had no possibility to measure urine output, Creatinine clearance or other biomarkers. These data only indicates that during early hypotensive gram negative sepsis there is a sufficient ATP level despite reduced RBF and use of a powerful vasoconstrictor. How this correlates with the clinical picture with reduced urine output and Creatinine clearance is unknown. It is still possible that later in the disease development of sepsis, ATP depletion may develop.

Nitric Oxide Synthase

It has been extensively reported that hypotension during sepsis is caused by a systemic vasodilatation caused by an up regulation of nitric oxide synthase and release of nitric oxide. This is also the case within the kidney (67, 68). Nitric oxide synthase comes in different isoforms; endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). iNOS is thought to have a key role in host defense and inflammation (69). One theory that has evolved over time is that an increased expression of nitric oxide leads to vasodilatation of the afferent and the efferent arteriole. This vasodilatation decreases the glomerular filtration pressure and causes a fall in glomerular filtration. This has been studied by Ishikawa et al (70, 71). They induced hyperdynamic sepsis in sheep and manipulated the nitric oxide synthase expression by a nonspecific nitric oxide inhibitor. With an intra-renal nonspecific nitric oxide inhibitor (N-nitro-L-arginine methyl ester) infusion, the renal blood flow decreased, but did not increase glomerular filtration. With a follow up study Ishikawa et al. induced a highly selective inducible nitric oxide synthase inhibitor (iNOS) with minimal

effect on RBF and glomerular filtration. These findings indicate that an increased release of NO during septic AKI may not be the cause of decreased renal function, as well as it is no clearly correlation between RBF and renal function. On the other hand, one cannot exclude the possibility that inhibition of NOS can cause constriction of both afferent and efferent arteriole, thus not increasing the glomerular filtration pressure. With selective iNOS inhibition it is also possible that eNOS and nNOS produce sufficient amount of NO, that inhibition of iNOS has no effect in regards of increased vasoconstriction.

In summary

Measuring the macrocirculation of the kidney does not permit an adequate understanding of the hemodynamic alterations in septic AKI. Nevertheless, current research indicates that septic AKI may develop during a hyperdynamic state and increase in renal blood flow, as well as with reduced renal blood flow. The autoregulation of renal blood flow has been shown to be impaired in critical illness (72) and AKI (62), so the renal blood flow can vary with cardiac output. This may also differ between preclinical and clinical studies and may also differ in time during the disease process. What is also interesting is that during decreased urine output the fractional sodium and uric acid excretion decreases, indicating that the kidney function is intact during early stages of the disease. If septic AKI can develop during reduced and increased total renal blood flow, one can question ischemia and bioenergetics/ATP depletion as a primary cause of septic AKI. To support this, experimental data done by May et al. showed that cortical and medullary blood flow, renal vascular conductance, and adenosine triphosphate levels remained unchanged in hyperdynamic septic sheep (66). In another study by Porta et al. they looked at mitochondrial respiration during endotoxemia. The found that during 24 hours RBF, renal mitochondrial respiration, nor renal lactate/pyruvate ratio did not change in experimental endotoxemia (73). An interesting question not answered in these studies is whether the changes in renal blood flow are linked to the changes in glomerular filtration. It does also not answer the question regarding intra-renal blood flow distribution during septic AKI. It is still not clear if this is a macro- or microcirculatory challenge. So, if ischemia or bioenergetics is not the primary cause of septic AKI, what is? Is there an alteration to the kidney and do it at a later stage develop acute tubular necrosis as hypothesized?

Histopathology – alterations to the kidney during AKI

For a long duration of time the general key idea behind prerenal acute kidney injury has been acute tubular necrosis (ATN). The ATN has been described as an intrinsic acute kidney injury due to severe and persistent hypoperfusion that leads to decrease in oxygen delivery and development of cell necrosis. The complex clinical syndrome of septic acute kidney injury and the ethical aspects of the procedure make it difficult to verify the histopathological condition by biopsy. In this section I will elaborate on current knowledge on the histopathology in septic AKI.

A systematic review was done by Langenberg et al. in 2008 looking into current knowledge on histopathology in septic AKI (74). Of a total of 184 patients, only 26 (22%) had features suggestive of acute tubular necrosis. In four primate

studies and 13 rodent studies only 37% and 23% respectively had ATN. The majority of the studies reported normal histology and mild to non-specific changes, ATN was relatively uncommon. To draw a conclusion from these numbers is hasty, but it questions the medical dogma that septic AKI is caused by ATN. The number of patients in these studies, limited data regarding hemodynamic status and the duration of the experiments are limitations to these studies. That makes one question their liability. On the other hand there is a significantly limited human data existing on the histopathological changes associated with septic AKI. In 2014 Langenberg et al. investigated the histology, immunohistochemistry, markers of apoptosis and expression of nitric oxide synthase isoforms and hypoxia-inducible factor-1 alfa (53) in septic AKI. Investigations were performed by inducing septic AKI by continuous infusion with E.coli and analysis was done on kidneys from normal sheep, sheep with septic AKI and sheep after recovery from septic AKI (infusion with gentamycin). As previous studies they found a lack of ATN or increased apoptosis within the animals with septic AKI. They also did not find evidence of macrophage or myofibroblast infiltration, as well as caspase-3 cleavage to suggest activation of apoptotic pathways. Nor did they find any increase in neutrophil gelatinase-associated lipocalin that could suggest tubular injury. This study focused on the first 48 hours of the condition and it is still unclear if these findings are relevant to a more sustained septic state as often seen in humans. Though this is the first time there has been done a comprehensive investigation into the histopathology of septic AKI. Indicating again that hypoperfusion does not play a significant role in the development of the condition, together with minimal histological changes in the first 48 hours. This indicating a functional, not a structural change responsible for alterations in renal functions.

To elaborate on the existing human data, Lerolle et al performed postmortem biopsies in 2010 on 19 patients who died of septic shock (75). Significant findings in this study were that all patients had acute tubular lesions, intense leukocyte infiltration and tubular cell apoptosis. This study is interesting in regards of end stage kidney damage, and indicates that in humans that dies from septic shock may have fibrin disposition in the glomeruli, ATN and leukocyte infiltration. The discrepancy from previous studies comes most likely from the fact that these patients were old, severely hemodynamic unstable, used vasopressors and had prolonged illness. They were anuric for two days and had systolic blood pressure of 60 mmHg for the last 4-6 hours. Another study by Takasu et al. came out in 2013 (76). They looked into the effect of sepsis on myocyte and tubular cells by rapid post mortem biopsies. They found that injury to the tubular cells were quite common, but presented focally. Tubular injury occurred in 30 of 39 (77%) septic patients, affecting 10.3±9.5% corticomedullary junction tubules and 5.9±9.8% cortical tubules. They defined tubular injury as tubular dilatation, epithelial flattening, cell sloughing or coagulative necrosis. Furthermore, they did find focal coagulative-type tubular necrosis in 17 of 39 patients (44%), involving less than 5% of tubular cells. Also interestingly medullary tubular epithelial cell sloughing occurred in 35 of 36 (97%) patients, affecting 18.7±11.7% of tubules. Apoptosis was seen in only 0,3% of the cells investigated.

Takasu et al. findings differ from Lerolle et al in regards to the effect of sepsis on tubular cells. Takasu et al. did not report on leukocyte infiltration and fibrin deposition as a result of septic AKI. Furthermore, the most important difference is the postulation by Lerolle et al. that apoptosis is a major mechanism of sepsis-induced AKI. Takasu et al question this.

These studies differ in many ways from previous studies, mainly because they are post mortem human study and not an experimental animal study. Further, this is the end stage of septic AKI and describes the histopathology post mortem, giving relevant information about the end result of the disease. Previous studies like Langenberg et al. have focused on the early phase of the condition and therefore the results cannot be compared. These studies are completely different in design and must be interpreted thereafter. These data indicates a lack of ATN and leukocyte infiltration in the early phases of the condition, but may develop during septic shock and within death.

The current research is not conclusive when it comes to the effect of septic AKI on tubular cells. Though, what is interesting is that the majority of the evidence points in the direction of minor influence of the tubular cells, which can not explain the dramatic fall in urine output and Creatinine clearance. Further, the key idea of acute tubular necrosis being the reason for loss of renal function must be rejected. The kidney does not show signs of major alterations, but what about the immune system and the massive activation of inflammatory mediators, could it have any effect on the kidney?

The immune system and septic AKI

Sepsis as a disease activates the immune system and challenges organs and tissues not just through reduced perfusion, but also with toxins, cytokines and inflammatory mediators that are produced by the host and the bacteria. In this section I will elaborate on theories involving the immune system and its effect on the kidney. Also I will introduce the concept of DAMPs and PAMPs. Gomez et al. presented a possible unifying theory in 2014, which combined hemodynamic alteration and the effect of inflammation (54). Through a literature review they proposed a theory that would explain the alterations to kidney function during sepsis. The theory is presented here since it embraces many of the theories involved in inflammation and septic AKI

The classic histological picture of septic AKI is patchy, heterogenous tubular cell injury with apical vacuolization, with absence of acute tubular necrosis or extensive apoptosis (77). With an absence of acute tubular necrosis, Gomez et al. emphasize the presence of three main alteration that are pivotal in the development in septic AKI; inflammation (78, 79), diffuse microcirculatory flow abnormalities (80) and cell bioenergetic adaptive response to injury (81). Gomez et al. speculates that septic AKI is a clinically and biochemically manifestation of an adaptive response of the tubular cells to an inflammatory danger signal. Further, they hypothesize an interplay between inflammation and microvascular dysfunction with a response of the mitochondria within the tubular cells to down regulate and reprioritize cell processes which will favor cell survival. This process, which involves maintenance of membrane potential and cell cycle

arrest, will be at the expense of kidney function i.e. tubular absorption and secretion of solutes.

Inflammation

During sepsis inflammatory mediators (cytokines, LPS etc.) derived from pathogens and host cells are distributed into the circulatory system. They are also known as damage- or pathogens associated molecular patterns, DAMPs or PAMPs. These mediators guide the immune system in its fight against infection. Allegedly these mediators can exert their function on tubular cells through the peritubular circulation or through filtration in the glomerulus, affecting tubular cells in the lumen. Through toll like receptors (TLR-4 and TLR-2) on tubular cells DAMPS and PAMPS can be recognized (82), though only patches of cells seems to display signs of distress (83). Gomez et al. speculate that the patchy areas affected by DAMPS/PAMPS are in connection with areas with heterogeneous blood flow caused by microcirculatory dysfunction and that these to events are closely connected.

Microcirculatory flow

The authors try to explain the pathogenesis behind the heterogeneous histopathological changes seen during septic AKI. They argument that sepsis cause microcirculatory dysfunction and subsequent sluggish peritubular flow.
Anatomically there is allegedly a reduction in proportion of capillaries with normal function. A reduced number of capillaries with normal flow, an increase in vessels with intermittent or no blood flow (78, 84, 85). Further, the inflammation causes areas of sluggish blood flow. Holthoff et al. (85) investigated the velocity of erythrocyte during CLP. They found a marked reduced velocity after 6 hours with intravital microscopy. Also Goddard et al. (86) experienced reduced leukocyte velocity during endotoxemia in cardiac muscle cells. Through decreased capillary velocity Gomez et al. arguments that tubular cells have a prolonged exposure to cytokines, DAMPS/PAMPS and leukocytes. This in turn will up regulate inflammatory pathways and induce oxidative stress and vacuolization. Oxidative stress is believed to be a hallmark of sepsis-induced tubular injury and is especially widespread among cells that are not in direct connection with peritubular capillaries. Supporting this is increased RNS and NOS activity 4 h after CLP in cells that are not in direct contact with capillaries (78, 79). Furthermore, Gomez et al. emphasize the fact that the only uniformly identified feature of septic AKI is oxidative stress in apical vacuoles (83). Another aspect that allegedly affects the microvasculature is the heterogeneous expression of iNOS during septic AKI. This will again result in heterogeneous regional concentrations of NO and contribute to a local depravation of NO, which could affect vascular beds and lead to shunting and hypoxia (87).

Microcirculatory dysfunction in sepsis has in several articles been characterized by heterogeneous abnormalities in RBF in which some capillaries are under-perfused, while others have normal or abnormally high blood flow (88-90). As previous elaborated, Langenberg et al. (59) is of the understanding that there could also be a hyperdynamic state during sepsis. In regards to microcirculatory alterations, there is not yet possible to draw any conclusion. Tubular injury during sepsis could be affected not only by DAMPS and PAMPS, but also by

hypoxia. So the tubular injury seen during septic AKI could be an adaptive response to reduced oxygen supply (83, 88, 91).

Tubular metabolic down regulation

Another interesting mechanism presented by Gomez et al. is the allegedly response of tubular cells to danger signals. A metabolic down regulation and reprioritization of cellular functions occur as a response to DAMPS/PAMPS. This is done by the mitochondria to ensure energy balance and prevent DNA damage.
The oxidative stress and inflammation allegedly affects tubular function as well as histology. This is through reduced endocytic capacity from the tubular lumen (92) and reduced bicarbonate reabsorption in the medullary thick ascending limb of the loop of Henle (93). Further, the early adaptive response in the tubular cells is done by mitophagy, the removal of and digestion of dysfunctional organelles from the cytoplasm (94). The intention is to protect the cell by removal of dysfunctional mitochondria, which will lead to a decrement of O2 consumption and conservation of energy. Also, the authors speculate that mitochondria is involved in cell cycle arrest (95) which will affect kidney function.

Tubular injury and GFR

The elaboration on the effect of sepsis on tubular influence and injury does not explain the massive decline in urine output and Creatinine clearance. A theory that has been proposed is that the relationship between tubular injury and the decreased GFR is due to an up regulation of the tubuloglomerular feedback mechanism (TGF). The TGF works within each nephron through the juxtaglomerular apparatus. It acts to stabilize the nephron function and distal delivery of solutes (96). With increased GFR or reduced reabsorption, the NaCl concentration increases in the tubules. A Na/K/2Cl transporter in the tubules will increase the reabsorption of sodium and trigger the glomerular feedback mechanism (96). This again will cause macula densa to express signaling substances that will constrict afferent arteriole and decrease the intraglomerular pressure and decrease GFR.

As elaborated earlier in previous mentioned studies, septic AKI can occur in both hypo- and hyper perfused kidneys, therefore questioning TGF activation as a primary cause of decrease urine output. Also recent human and clinical experimental data shows that there is a possible vasodilation, not a vasoconstriction leading to a decrease in glomerular pressure. With a TGF activation the afferent arteriole should constrict and decrease the glomerular filtration pressure, but experiments shows the opposite. Also, one could question if the TGF mechanism is powerful enough to maintain anuria. TGF reduces GFR on average by approximately 45% (97). Using AT2 or norepinephrine in sufficiently high enough doses would produce total vasoconstriction (97). On the other hand sepsis could potentially damage or alter the proximal tubular cells in regards of reabsorption. There is an increase in urine output in the early phases of septic AKI (52). With increased GFR and subsequently increased amount of NaCl in the distal tubule, the TGF would be activated leading to a fall in GFR. Further, it has been speculated that this effect could be pathological, but also physiological (98). Due to the excessive volume loss caused by for example increased permeability or reduced reabsorption, the kidney tries to retain

volume through the activation of the TGF. With increased filtered sodium the oxygen consumption increases, damaging the kidney even further in a time of stress. Activating the TGF, constricting the afferent arteriole with the effect of decreased GFR will hinder just that.

Organ cross talk

An interesting theory that has not been investigated properly in terms of septic AKI is the organ cross talk theory. Sepsis arises from infections often in the respiratory- and urine tract. It is not unlikely that organs and in particularly the kidney can be affected by damage to other organs and will adjust itself or be influenced by the bodies response.

A typically known example of a possible organ cross talk is the hepato-renal syndrome involved in liver failure (99). Back in 2003 Imai et al (100) looked into injurious mechanical ventilation during ARDS and its effect on end-organ epithelial cell apoptosis and organ dysfunction. They proposed that injurious mechanical ventilation strategies would lead to end-organ cell apoptosis and that circulating factors in the plasma may be involved in this process. Therefore, 24 rabbits with acid-aspiration were mechanically ventilated with injurious- and non-injurious strategy. Injurious strategies are in this case an increase in tidal volume and a low peak end expiratory pressure. After 8 hours the animals were killed and samples from lung, liver, kidney and intestines were collected. They further assessed the effect of plasma from ventilated animals on development of apoptosis on rabbit renal tubular cells. They found an increase in renal tubular cells apoptosis in animals with injurious ventilation strategies, 10,9%. Furthermore, rabbit renal tubular cells that were exposed to plasma from animals that were ventilated with injurious strategies did also show increase induction of apoptosis at 4 and 8 hours. Imai et al. questioned the role of Fas ligand, a potent pro-apoptotic molecule in development of end-organ apoptosis in the kidney. Therefore they measured the molecule in rabbits during the experiment. Also they measured Creatinine and Fas ligand in plasma from patients included in a randomized clinical trial looking into the effect on inflammatory mediators by mechanical ventilation (101). They found a significant correlation between Creatinine rise and Fas ligand in patients with ARDS.
The theory behind organ cross talk is intriguing in regards to septic AKI due to, as of now, a lack of knowledge in regards to etiology of this devastating disease. ARDS is of course not sepsis and in many ways these two diseases cannot be compared. Though, the subtle changes in other organs during ARDS is interesting, in the fact that mediators in the blood stream from a disease process in one place of the body can affect another organ not in close proximity of the disease. As of now, one can not rule out the possible effect of inflammatory mediators from disease processes elsewhere in the body being the primary cause of changes to renal function during septic AKI. Another aspect of this study is that the degree of apoptosis seen in rabbit kidney during ARDS does not explain the changes in kidney function. Further, the rise in Creatinine and Fas ligand after 24-30 hours and 36-40 hours does not support Fas ligand as the primary cause of the Creatinine rise. Also, Creatinine is known to be a late and inaccurate predictor of kidney function. Septic AKI is known to have subtle

histopathological changes, though not a great deal of apoptosis. In ARDS there is allegedly subtle changes to the renal tubular cells and Imai et al. proposes an effect on the kidneys by inflammatory mediators excreted by the lungs during ARDS and mechanical ventilation. This indicates an effect on the kidneys by inflammatory markers, but do not explain the changes in kidney function seen for example during septic AKI.

Glomerular filtration barrier and GFR

Sepsis is an endothelial alternating disease in the whole body. Why should it not be the same in the kidney? Key concepts necessary to understand the glomerular filtration barrier were mentioned previously in this thesis, though a short reinforcement is necessary. Vascular permeability in renal glomeruli is determined by the glomerular filtration barrier (GFB) which are made up by glomerular capillary endothelium, podoctyes and the basement membrane (102). Another important structure not always emphasized is the glycocalyx, which is covered by a thicker cell coat composed of plasma proteins such as albumin and orosomucoid, as well as proteins and hyaluronan produced by the endothelium (8, 37). Glomerular endothelial cells contain glomerular fenestrae, which are circular transcellular pores, 50-100 nm in diameter (9, 10, 16). One of the main functions of the GFB is to prevent leakage of lager molecules like albumin and other plasma proteins into the urine (8). Alteration to the GFB has been suggested in some studies to lead to albuminuria, which is the main characteristic of GFB damage (103-105). During a LPS model of sepsis several cytokines are released into the blood stream, affecting endothelial cells. Tumor necrosis factor alfa (TNFalfa) has been shown to be a key mediator in LPS induced AKI through effects on tumor necrosis factor receptor-1 (TNFR-1) (106, 107). Another molecule, which also exerts its effect on the endothelial cell, is the vascular endothelial growth factor (VEGF). VEGF is known as an important inducer of fenestrae and is exert its effect on vascular endothelial growth factor receptor 2 (VEGFR2). In the kidney VEGF is produced by podocytes (108). Plasma levels of VEGF has been directly associated with changes in glomerular fenestrations where increased levels induces fenestrations (109). VEGF has also been found to markedly increase endothelial permeability (110).

In 2011 Adembri et al. came out with a paper that focused on another aspect of septic AKI that had not been extensively investigated before. The effect of sepsis on the glomerular filtration barrier (104). They looked into the early stages of septic AKI and tried to determine whether albuminuria developed in the early stages of the disease and if it was associated with alterations to the GFB. With cecal ligation and puncture (CLP) they induced sepsis in rats and measured TNF-alfa, growth of microorganisms in the peritoneal fluid and took kidney specimens for assessment, at 0, 3 and 7 hour. They also measured serum Creatinine and Creatinine clearance for kidney function and albuminuria. They found that urinary albumin significantly increased after 7 hours. Further, they observed increased levels of TNFalfa. Also they saw diffuse alterations to the glycocalyx of the GFB, together with reduced syndecan-1 expression and decrease in hyaluronan and sialic acid contents. This was without an increase in serum Creatinine or Creatinine clearance. Another interesting observation was in the alteration of the sialic acid content. They observed a higher degree of acetylation

(a reaction that introduces a acetyl functional group into the compound) to different forms of the sialic acid. Specifically sialic acid with acetyl in C9 position is known to have a specific role in defense against neuraminidase (111), which cleaves the glycosidic linkage of neuraminic acid. Sialic acid is a derivative of neuraminic acid. Therefore, the authors speculate that the acetylation is a defense against bacterial degradation, with a masking of recognition sites on the sialic acids (112). The endothelium and podocytes could therefore be involved in the defense against circulating pro-inflammatory mediators. The glycocalyx is rich in anions and sialic acid is an important component. With an alteration to the sialic acid content, the charge barrier is disrupted and could therefore contribute to the increased loss of albumin (8, 113)

Another interesting study that needs further elaboration is the study by Xu et al. in 2014 (26). They investigated the effect of sepsis and LPS on the glomerular endothelium and endothelial surface layer. TNFR1 knockout mice and normal mice were infused with E.coli to induce sepsis. This to evaluate if the effect of LPS on glomerular epithelium was mediated by TNFalfa activation of TNFR1. They measured urea levels as indicator of GFR and urine Albumin/Creatinine ratio to assess injury to GFB. Through immunofluoresence they could detect VEGFR2 and heparanase-1 levels indicative of endothelial surface layer alterations, and with TEM analyses the GFB were assessed. Xu et al. observed that LPS induces AKI and an increase in urine concentration of albumin. Furthermore, they observed that mice deficient in TNFR1 are resistant to LPS-induced AKI and albuminuria. In normal mice LPS induced AKI led to a detachment of glomerular endothelial cells from the basement membranes. Also, they observed a 5-fold decrease in fenestrae density, whereas the average fenestrae diameter was 3-fold higher in mice treated with LPS compared with controls. Intravenous TNF injection caused AKI and similar changes to the glomerular endothelial cell fenestra as with LPS. Further, kidney VEGF level was decreased during LPS-induced AKI, but VEGFR2 levels were not changed. Also, LPS- and TNF-induced AKI did lead to degradation of the endothelial surface layer, ESL. This was further investigated through glomerular heparanase expression, which is known to degrade the ESL. This is done through removal of heparan sulfate for heparan sulfate glycoproteins, which contribute to the glomerular filtration barrier (114).

With other words, LPS reduces the endothelial surface layer, resulting in mild albuminuria, reduced glomerular filtration rate and fewer endothelial fenestrae. Furthermore, TNFalfa seems significant with its effect on TNFR1 in this process. This indicates that the ESL has a greater role in the filtration barrier than first anticipated, especially in regards to proteins, but also the GFR. The authors speculate that the fall in GFR and hence urine output is due to a reduced hydraulic conductance. This could be through a reduction of the density of fenestrae, but also through alterations to the composition of the ESL, alternating the hydraulic conductance. The mild and transient proteinuria observed during induction of LPS has been attributed to the effect of LPS on podocytes (115), but with Xu et al. new data, the alterations to the ESL must be taken into account. This indicating that there are several components in the GFB responsible and essential to the permselectivity during LPS and TNFalfa induction. Similar renal

pathology has been observed in patients with pre-eclampsia (116) and in patients with Diabetes type 2 (117).

Glycocalyx

The endothelial glycocalyx is a complex macromolecular network which has many functions (118) and cover every blood vessel in the body. Sepsis leads to degradation of the glycocalyx, alterations to the endothelial permeability with the result of hypovolemia, hypoalbuminemia and edema (103, 119). During sepsis the glycocalyx gets exposed to different pro-inflammatory mediators such as interleukin 1 (IL-1), IL-2, IL-6, TNFalfa and other mediators such as Bradykinin, VEGF, Histamine and Thrombin. These mediators activate the glomerular endothelial cell and increases glycocalyx expression of endothelial leukocyte adhesion molecule 1, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1). These proteins promote leukocyte rolling, adherence, and migration, which initiate the inflammatory damage to endothelium (120). Inflammation injury during sepsis is linked to increased paracellular permeability and outflow of albumin and fluids in the interstitial space (121). It has been hypothesized that the increased permeability is due to loss of anionic charges, changes in geometry of the clefts, and direct endothelial injury (120). The glycocalyx can be affected by other mediators as well as interleukins and TNFalfa. During sepsis there is produced a large amount of reactive oxygen species, ROS and cytokines. ROS and cytokines are known to depolymerize glycosaminoglycan, GAGs, chains of proteoglycans, which are crucial for the barrier function of glomerular endothelial cell, GEnC (122, 123). ROS disrupts the GEnC`s glycocalyx through a direct mechanism of action (124), and therefore could be relevant during septic AKI.

Chapter 5: Discussion

As of our discussion so far, it is questionable how big of an impact bioneregetic failure or ischemia have on the kidney during septic AKI. Acute tubular necrosis and apoptosis is almost not present and does not explain the alterations to the kidney in regards of renal blood flow and reduced renal function. Also, there is no conclusive information regarding the role of microvascular changes and glomerular filtration pressure. The immune system is activated and the tubular cells show signs of oxidative stress, but one cannot conclude if, or in what degree, the alterations to tubular cells influence kidney function. Thus, current theories do not fully explain the alterations to kidney function and give answer to the primary cause of septic AKI.

Septic AKI is most likely a result of different factors that influences the kidney. Though, some factors contribute more to the disease process than others. During my study of current research on the topic of septic AKI, some ideas and theories has emerged. I will in this section present my key ideas on the mechanism behind the development of septic AKI. The theory behind the pathophysiology of septic AKI can be summarized like this: Induction of sepsis through bacteria with a systemic effect, causes an alteration to the macro- and microcirculation in the body. Renal blood flow is affected and shifts between a hyper dynamic and hypo dynamic state. Sepsis with its inflammatory mediators affects the endothelium

and especially the glomerular filtration barrier. Through alterations to the GFB the permselectivity is affected, triggering an abnormal response by the glomerulus itself and the glomerular endothelium. The glomerulus and its hemodynamics are affected, causing a decline in GFR. The endothelium modifies its filtration component; resulting in a marked fall in urine output, increase in serum Creatinine and urea, and the situation progresses to what we physicians see as septic AKI.

An interesting phenomenon was reported in Langenberg et al. (52) study. They reported that the urinary output increased briefly after induction of sepsis before steadily decreasing. This increase happened at about 4 hours after induction of sepsis. Gupta et al. (92) reported that after 3 hours an apparent leakage of low molecular weight dextran, LMWD, and high molecular weight dextran, HMWD were present and indicated an increase in endothelial permeability. Adembri et al. (104) observed an increase in albumin/urinary Creatinine ratio by 152% and 288% at 3 and 7 hours respectively. These data implies that there is an alteration to the GFB and its permselectivity already after 3 and 7 hours post induction of sepsis. Langenberg reported that 8-10 hours after induction of sepsis the urinary output was at it´s lowest. The same was reported in their previous study the year before (59). So, the permselectivity increases, before drastically decreasing in literally just a few hours. This occurs despite an increased renal blood flow and satisfactory MAP. What causes this drastic decline in urinary output?

Before elaborating on the cause of decreased GFR during septic AKI, it is interesting to discuss the overall view on the pathology behind septic AKI. One could speculate on the response of the kidney to sepsis. Is this a physiological response or is it a pathological response to injury? I think this is interesting in that sense that we can look at the response from two different angles. First, the physiological response: As mentioned earlier in this thesis there are theories that state that the tubular cells and glomerulus is under an attack during sepsis. This is from circulating inflammatory mediators such as DAMPS and PAMPS. The glycocalyx is also believed to be under influence of shear stress, and shedding of the glycocalyx is reported (125, 126). This attack on the GFB could increase the permeability through alternating the glycocalyx and the GFB composition. One could imagine that the nephron itself is under attack and response to injury and uncontrolled loss of fluids and proteins would be to promptly decrease the GFR. This could be done rapidly by decreasing the intra-glomerular pressure by a vasodilatation of the afferent and efferent arteriole. On a long-term basis through alternating the composition of the GFB and its permselectivity with a decline in fenestrae density. A cellular alteration in a matter of just a few hours is difficult to imagine, but with a decrease in intra-glomerular pressure, regulating the afferent and efferent vasoconstriction, the nephron has the ability to affect the degree of glomerular filtration rate in a matter of minutes. In a prolonged disease process it is interesting to speculate on the kidneys ability to adjust and adapt, alternating the filtration barrier with one key effect: restoring blood volume and plasma proteins in response to a generalized sepsis induced systemic vasodilatation.

This theory is intriguing, though it is not applicable to the clinical setting of septic AKI. If this were a physiological response then we would expect septic AKI to develop in every patient with the condition. Interestingly, this is not the fact. So, if we look at the same alterations from a pathological view, it gets even more interesting. Yet again the nephron and the renal glomerular endothelial cells are under attack from inflammatory mediators such as DAMPS and PAMPS. The attack and shedding of the glycocalyx and the GFB increases the permeability and alters its composition. This is manifested by increased albumin excretion (104) and increased urine output (52), a symbol of a damaged barrier. The increased albumin excretion is reported to occur just few hours after the induction of sepsis as already mentioned. Now, simultaneously the nitric oxide expression is increased (67) causing a vasodilatation of the afferent and efferent arteriole, decreasing the intra-glomerular filtration pressure and subsequently the urine output. As a response to the attack on the glycocalyx and the GFB the glomerulus tries to repair itself. Now, this is where it all gets interesting; could this process be altered? Could the decreased fenestrae density and increased diameter observed by Xu et al. (26), be a consequence of maladaptive response to injury? Is it possible to think that the endothelial cell alters its composition due to an extrinsic stimulus, which in turn will cause a fall in conductivity? Causing an accumulation of Creatinine and urea, a fall in urine output and the condition we call septic AKI?

Now, let´s elaborate on one of the main components involved in the disease process: the glycocalyx. The glycocalyx and its alterations during septic AKI have not been studied as well as endothelial cells and glycocalyx in other parts of the body during sepsis. Though, I think the theories regarding alterations to the endothelial cell during sepsis can be adapted to the glomerulus during AKI. It is known that the enzymatic selective thinning of the endothelial glycocalyx will promote hyper permeability (37, 127) and expose endothelial adhesion molecules like intracellular adhesion molecule 1, ICAM-1 and vascular cell adhesion molecule 1, VCAM-1. This will allow neutrophils to adhere to the endothelial surface (128, 129) and commence their inflammatory response and damage to the endothelium (130). Subsequent damage will increase permeability (121). This may also be true for sepsis (131). In 2013 Wiesinger et al. looked into the Nano mechanics of endothelial glycocalyx during sepsis. They investigated the inflammation-induced damage to the glycocalyx during sepsis with atomic-force microscopy (131). Endothelial cells from a rodent aorta were harvested 18 hours after administration of E.coli. They found that during endotoxemia the glycocalyx was reduced in regards of thickness and stiffness. This is in concordance with previous studies (132, 133). This was investigated ex vivo and in vitro, using human pulmonary microvascular endothelial cells. An interesting finding was that when Wieninger et al. used TNFalfa and thrombin, the glycocalyx was reduced with 48% and 55% respectively. This indicating that different septic mediators can alter the glycocalyx rapidly in an in vitro model. Also, it may not be confined to sepsis, but also to other critical illness where there have been reported increased amount of glycocalyx constituents; Acute lung injury (134), major trauma (135) and major vascular surgery (126).

Xu et al. (26) as mentioned earlier in this thesis, investigated the effect of sepsis and LPS on the glomerular endothelium and endothelial surface layer. The essential finding of their study was that there is a reduction in fenestrae density and an increase in fenestrae diameter during septic AKI. Furthermore, they found that LPS reduced two major components of the glomerular ESL, sialic acid and heparan sulfate glycosaminoglycans. These changes were associated with loss of GFB permselectivity by documentation of albuminuria. These findings indicate that the glycocalyx in the glomerulus is influenced in the same matter as glycocalyx elsewhere in the body. Though, what we must not forget is that the endothelial cell in the glomerulus is different from other endothelium. One of the major components distinguishing glomerular endothelium from other endothelium is the presence of fenestrae. Normally the fluid and solvent shift takes place in a paracellular fashion, but in the glomerulus it is mainly through the fenestrae. In generalized sepsis one of the main challenges is uncontrolled fluid shift, with increased fluid in the interstitial space together with efflux of albumin from the vascular bed. Sepsis leads to degradation of the glycocalyx, alterations to the endothelial permeability with the result of hypovolemia, hypoalbuminemia and edema (103, 119). This phenomenon has not been reported in histological studies of kidney during sepsis. Yet again it is tempting to question if there is an alteration to the conductivity of the GFB. Alterations to the main fluid shift component may be a significant contributor to the development of septic AKI.

One could speculate on the effect of angiotensin 2 during septic AKI and the alteration to the GFB. Wan et al. (64) reported a marked increase in urine output during septic AKI and infusion of AT2. What is interesting with the study by Wan et al. is that this study does only investigate the first 8 hours after induction of sepsis. They report a marked increase in urine output by AT2 during the time period with significantly increased endothelial permeability. These data do not answer the effect of AT2 during a later phase of septic AKI. If there is pronounced alterations to the GFB with a decreased fenestrae density and decreased permeability, one could expect a decreased effect of AT2 on GFR. Further, AT2 is known as a powerful vasoconstrictor, which could significantly increase the glomerular filtration pressure. The normal mean pressure gradient driving ultrafiltrate is only 10mmHg (98). During AT2 infusion one could only speculate on the amount of filtration pressure caused by efferent vasoconstriction. With a powerful vasoconstrictor as AT2, one could easily expect a markedly increase in urine output. Though, with an increase in urine output by a seven fold and an increase in Creatinine clearance to pre-sepsis level(64), it is hard to argue that there may be a decrease in conductivity. Nevertheless, one should not forget the development of the disease. If the GFR declines in the early phases of the disease due to vasodilatation of afferent and efferent arteriole, the effect of AT2 is as expected. If the alternation to the GFB occurs at a later stage then AT2 may not have an effect.

Now, if the ultrafiltrate has reduced filtration capability, alterations to the tubular cells begin. As elaborated earlier in this thesis, the tubular cells gain their oxygen and glucose from peritubular capillaries. If there is a hyperdynamic state in the microcirculation during sepsis, one can speculate that the tubular cells will

not be suffering from hypoxia or the lack of nutrition. This could explain the lack of generalized acute tubular necrosis, apoptosis or any infiltration of macrophages. Though, it does not fully explain the focally tubular damage observed. One can imagine that with a reduction of filtration of fluid across the filtration membrane, this change would alter the conditions of the tubular cells. This brings us to the concept of sodium-retention. The tubular cells are dependent on the sodium gradient which is crucial for the tubular cells as a driving force for reabsorption or secretion of solutes (136). Urine sodium concentration (UNa) and fractional sodium excretion (FeNa) has been used to classify and distinguish prerenal AKI and established AKI. UNa values less than 20 mmol/L has been suggestive of a prerenal AKI and a sodium avid state. UNa values greater than 40 mmol/L have been considered consistent with established AKI (137, 138). This theory has been questioned and the sodium concentration is highly variable and is effected by many co-founders (139, 140). It was further questioned the ability of UNa to distinguish normal kidney function, pre-renal azotemia and ATN (141). Traditionally FeNa has been used to distinguish between prerenal and established AKI, FeNa <1% and FeNa >1% respectively (138, 142). FeNa and its clinical utility has also been questioned (141, 143) with no clear correlation between UNa/FeNa and the severity of AKI. In 2006 Langenberg et al. did a prospective observational animal study on urine biochemistry during septic acute renal failure (144). They observed that the UNa decreased early and remained persistently reduced during the observational period. The authors hypothesized that the sodium retentive state was due to a decrease in intra-glomerular pressure and therefore a loss of glomerular filtration pressure. Interestingly they also observed progressive decreases in UNa were accompanied by decreases in Creatinine clearance. So, with progression of septic AKI there is a loss of clearance and a sodium retentive state. This is an interesting phenomenon if seen in the light of altered glomerular permeability. If sepsis leads to a decrease in fenestrae density and a reduction of permeability, this could be observed as decreased UNa, as well as altered FeNa. The sodium retention could also happen due to an up regulation of the TGF. Early phases of the disease with increased permeability could activate the TGF and cause sodium retention and increased reabsorption.
New research has questioned the applicability of UNa and FeNa in septic AKI. Especially in terms of predicting and diagnosing AKI (145). So, using urine biochemistry in diagnosing and treatment of septic AKI is challenging. The diverse findings in regards of urine biochemistry during septic AKI could be due to a reduction of ultrafiltrate in the tubular lumen or a TGF mechanism activation, which in turn could affect the nephrons ability of reabsorption and excretion.

The clinical practice guideline for acute kidney injury, KDIGO, does not recommend the use of diuretics. Not to prevent AKI, nor as a treatment option. The only situation where there is recommended use of diuretics is during a situation of volume overload (49). Nonetheless, diuretics are still in use during AKI in intensive care units due to the effect in urine output. Diuretics are not associated with improved mortality or rate of independence from RRT, but there is an increase in urine output (146, 147). This effect is difficult to explain put in a

context with decreased intra-glomerular pressure, reduced glomerular permeability and an activated TGF mechanism.

Further recommendations

The pathophysiology behind septic AKI is still not fully understood. For the last decade, AKI and especially septic AKI have gotten renewed attention. Researchers like Bellomo, Prowle, Langenberg and Kellum to name a few, have put in a tremendous amount of work with the intention of unraveling the mystery of septic AKI. Still, there is no consensus and evidence-based treatment of this condition

Hemodynamic management of patients with or at risk of, septic AKI has been a central part of the overall treatment of this patient group. Researchers have focused particularly on understanding the relationship between systemic hemodynamics, renal blood flow and glomerular filtration rate. The interaction between the macrocirculation and the microcirculation has been of great interest. In particular the starling forces, and the kidneys ability to affect these forces have been an area of study. For almost a decade experimental studies have been focusing on the filtration pressure, the intra-glomerular pressure and the interaction between the afferent and efferent arteriole in the nephron. With no clear answers. In recent years, more attention has been given to the glomerular filtration barrier, which is an interesting new aspect. With new research, it is evident that we need to embrace a more holistic approach. Is it time for a new era where we focus on hydraulic conductivity? Haraldsson (148) presents the term "intergrative glomerular barrier complex" which embraces a more all-encompassing concept. Not looking for the single most important component, but a view of the glomerular barrier as a contributing part of the filtration process. Further, is it time to question the ultrafiltration coefficient? Is it possible that the ultrafiltrate coefficient is not constant, and that the GFB is influenced by pathological processes and may be in a shifting state? With Nano mechanics and new technology we have now the possibility of investigating the glomerular filtration barrier in a totally new manner. With insight into the factors affecting hydraulic conductivity, one could possibly unravel the mystery behind septic AKI.

Another concept that could be interesting is the division between early and late phase of septic AKI. The idea that the kidney regulates and adapts to attack and injury in different manners at different times seems rational. The prompt fall i GFR over just few hours with alterations to barrier function at a later time indicate a possible adaptation to injury. Dividing the pathogenesis into early a late stage could be beneficial in the overall understanding of the disease.

Due to the complexity of the pathogenesis of septic AKI and the difficulty of investigating its origin, the research has mainly been confined to experimental animal studies and to patient groups in the ICU. The literature is scarce on patients with sepsis outside the ICU. A large multicenter observational study observed septic AKI in 631 patients of the 1836 included patients with pneumonia (149). 16-25% of the patients with non-severe pneumonia developed septic AKI. This indicates that septic AKI may be more frequent than

expected and many patients develop AKI without getting admitted to the ICU. These data suggests that further research on this topic is needed.

Through careful studying of numerous papers on the topic of septic AKI, it is easy to get persuaded that there are different factors contributing to the development of septic AKI. Our shortcoming in the evaluation of the microcirculation in the kidney is an obstacle that one needs to overcome. The concept of filtration pressure is significant in the search for the pathogenesis of septic AKI, and is currently a prioritized area of research. The role of the endothelium in the glomerular filtration barrier, and especially glycocalyx, ESL and fenestrae development are interesting components for further research. The effect of inflammation on the glomerular endothelium and it´s response to stimulus is intriguing. With new technology like Nano mechanics and PET, it could be possible to visualize components of the GFB in vitro and ex vitro. With a better understanding of the GFB one can investigate the importance of conductivity and possibly micro-damage to the GFB.

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