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Welcome to the Jungle: The Kidney during Sepsis

What do we know about acute kidney injury (AKI)? Many might intuitively think we know a lot. We do know that sepsis-induced AKI affects up to 60% of patients in the intensive care unit (1, 2), but remarkably, we do not understand its pathophysiology at all, as a study in this issue of the *Journal* by Maiden and colleagues (pp. 692–700) now suggests (3). In fact, the pathophysiology of AKI during sepsis represents one of the big mysteries in critical care medicine.

For as long as we read our textbooks, septic AKI was thought to be a result of systemic hypotension and the resultant decrease in renal blood flow. Acute tubular necrosis was thought to be both consequence and histopathological correlate of insufficient blood flow (4), despite the fact that it has hardly ever been observed in human studies (5). As a consequence, standard therapy favored to increase cardiac output, arterial blood pressure, and renal perfusion. With the beginning of this millennium, however, first reports suggested a paradigm shift by convincingly demonstrating diminished renal function upon increased renal blood flow in animal models of hyperdynamic sepsis (6). These findings opened the gates for speculations and required a novel hypothesis. At that time, a *post mortem* analysis histologically demonstrated dead tubular cells and marked capillary leukocyte infiltration in human samples (7). Because dead tubular cells mostly undergo immunogenic cell death, such as necroptosis or ferroptosis (8), those findings raised the possibility of therapeutically interfering with regulated necrosis, as previously suggested for nonseptic AKI (9). But what drives the disease? Is it the perfusion by itself, the cell death by itself, ill-defined soluble factors, combinations of these, or a new kid on the block?

Maiden and colleagues provide a unique approach to elucidate the pathophysiology of septic AKI (3). Fifteen sheep were

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anesthetized for 48 hours, and mean arterial pressure and central venous pressure were monitored and corrected with parenteral fluids and norepinephrine. The beauty of this study comes from its controls. Renal function was thoroughly controlled, and renal perfusion was continuously measured by an ultrasonic flow probe. Renal oxygen consumption, lactate flux, urine production, and serum creatinine were evaluated regularly: a state-of-the-art setup. In 10 animals, septic shock was induced with intravenous *Escherichia coli* injection, and five sheep served as controls. At baseline and after 24 and 48 hours, kidney biopsies were obtained for quantification of histological changes. For the first time, this experimental setting allowed visualization of the early changes in septic AKI in an appropriate animal model without distortion by preexisting renal pathologies and *post mortem* artifacts. Except that there were (almost) no such changes.

The septic sheep developed oliguria and a marked increase in serum creatinine without any signs of hypoperfusion, hypoxia, renal blood flow, lactate flux, or all other parameters measured, apart from a statistically significant difference in the grade of mesangial expansion in the intervention group. The absence of severe damage is the remarkable finding of this approach. Therefore, despite the small sample size (not so small, in fact, for a study with sheep), the comparably short duration of the model, and lack of examination of the renal medulla, Maiden and colleagues added an important piece of data to our understanding of the pathophysiology of septic AKI (3). However, here is the trouble that raises questions about the experimental setup: necrotic cells in the lumen of the tubules occurred equally in the septic and the control mice. It is certainly challenging to evaluate the relative contribution of necrosis to the pathophysiology in a setting like this, but more important, the unknown "other factors" that cause a decline in renal function without killing cells have probably shown their ugly face here. That is one of the reasons why, as with every good study, it provides more questions than answers. What are these "other factors" that functionally drive septic AKI? Maiden and colleagues' approach might represent a model to screen for them (3). Cells, cytokines, dead cells, damage-associated molecular patterns, the "microenvironment"? Can we ever interfere with this very early phase, and how do we routinely detect it in the intensive care jungle? At least there is something to study here! The piece cannot rule out the cell death hypothesis resulting from the setup, but will the detection of dead cell corpses and urine sediment along with the detection of damage-associated molecular patterns in the urine and plasma really add to the early diagnosis, and if so, what would we do next? Would it make sense to stop the necrosis? Proper clinical studies to investigate this might involve progression trials of AKI. As we learned from Maiden and colleagues, the blood pressure is not the key. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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A Protease Inhibitor Tackles Epithelial Sodium Channels in Cystic Fibrosis

Early-onset and progressive lung disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene continues to determine most of the morbidity and mortality in patients with cystic fibrosis (CF). In the lungs, airway surface dehydration leading to a hyperconcentrated, highly viscous mucus causing impaired mucociliary clearance has been established as a key disease mechanism in CF that turns the airways vulnerable to mucus plugging, chronic polymicrobial infection, and nonresolving neutrophilic inflammation (1). The underlying defects leading to dehydrated airway surfaces are defined by genetically impaired CFTR-dependent anion (Cl^- and bicarbonate) and fluid secretion, combined with increased absorption of Na^+ and fluid that is mediated by the hyperactive amiloride-sensitive epithelial Na^+ channel (ENaC) (1).

ORIGINAL ARTICLE

Structure and Function of the Kidney in Septic Shock

A Prospective Controlled Experimental Study

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Abstract

Rationale: It is unclear how septic shock causes acute kidney injury (AKI) and whether this is associated with histological change.

Objectives: We aimed to determine the nature and extent of changes in renal structure and function over time in an ovine model of septic shock.

Methods: Fifteen sheep were instrumented with a renal artery flow probe and renal vein cannula. Ten were given intravenous *Escherichia coli* to induce septic shock, and five acted as controls. Animals were mechanically ventilated for 48 hours, while receiving protocol-guided parenteral fluids and a norepinephrine infusion to maintain mean arterial pressure. Renal biopsies were taken every 24 hours or whenever animals were oliguric for 2 hours. A renal pathologist, blinded to tissue source, systematically quantified histological appearance by light and electron microscopy for 31 prespecified structural changes.

Measurements and Main Results: Sheep given *E. coli* developed septic shock, oliguria, increased serum creatinine, and reduced creatinine clearance (AKI), but there were no changes over time in renal blood flow between groups ($P > 0.30$) or over time within groups ($P > 0.50$). Renal oxygen consumption increased only in nonseptic animals ($P = 0.01$), but there was no between-group difference in renal lactate flux ($P > 0.50$). There was little structural disturbance in all biopsies and, although some cellular appearances changed over time, the only difference between septic and nonseptic animals was mesangial expansion on electron microscopy.

Conclusions: In an intensive care–supported model of gram-negative septic shock, early AKI was not associated with changes in renal blood flow, oxygen delivery, or histological appearance. Other mechanisms must contribute to septic AKI.

Keywords: acute kidney injury; histology; pathophysiology; animal models

Acute decreases in renal function define acute kidney injury (AKI). AKI occurs in up to two-thirds of intensive care unit (ICU) patients and is independently associated with health-care use and mortality (1–3). Sepsis is the most common condition associated with AKI, and increasing sepsis severity is associated with more severe AKI (4–6).

Several mechanisms have been proposed to explain septic AKI. Systemic hypotension during septic shock has been thought to decrease renal blood flow and impair renal function (7–10). However, reliable measures of renal blood flow during septic shock are not available in humans (11) and relevant animal studies have contradicted this theory, revealing that blood flow to the

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At a Glance Commentary

Scientific Knowledge on the Subject: It is unclear how sepsis acutely alters renal function. Experimental studies have illustrated that glomerular filtration decreases with sepsis despite preservation of renal blood flow. Acute tubular necrosis and glomerular microthrombi have been proposed to contribute, based on observational *postmortem* studies.

What This Study Adds to the Field: This is the first prospective controlled study to simultaneously assess renal function, histology, and glomerular ultrastructure over time in septic shock. In a clinically relevant animal model, septic shock provoked a marked decrease in glomerular filtration, while renal blood flow was maintained. Systematic histological assessment of each functional component of the renal cortex revealed no cellular changes to account for the marked reduction in glomerular filtration. This study supports the notion that early septic acute kidney injury represents a functional disturbance within the kidney.

kidney is actually preserved or increased (12–15).

Structural changes to the kidney have also been considered to occur, with "acute tubular necrosis" frequently thought responsible for septic AKI (7–10, 16, 17). However, acute tubular necrosis is somewhat ill-defined and is infrequently reported in human and experimental septic AKI studies (18–20). Other processes such as glomerular thrombosis, renal parenchymal inflammation, microvascular congestion, and apoptosis have also been proposed (7–10, 21), but the clinical significance of these is uncertain given that they have been noted only in *post-mortem* specimens. Studies of septic AKI histology are further confounded by selection bias, subjective grading scales, inconsistent definitions of AKI, variable use of parenteral fluids and catecholamines, the impact of the dying process itself, and an inability to determine whether abnormalities were present before the septic insult.

Given the major clinical impact of septic AKI, a better understanding of its pathogenesis is of vital importance. However, renal morphology studies in humans with septic AKI are not possible because of the risk of hemorrhage after biopsy, and functional studies are limited by an inability to reliably measure renal blood flow in the critically ill. Accordingly, using a controlled experimental model that closely simulated human septic AKI and its ICU treatment, we sought to concurrently determine the evolution of renal structural and functional changes. Some of the results of these studies have been previously reported in the form of an abstract (22).

Methods

Study Design

We conducted a prospective, blinded, controlled study of renal histology and function in an ovine model of septic shock. Approval for the study was obtained from institutional animal ethics committees and conducted according to the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (23).

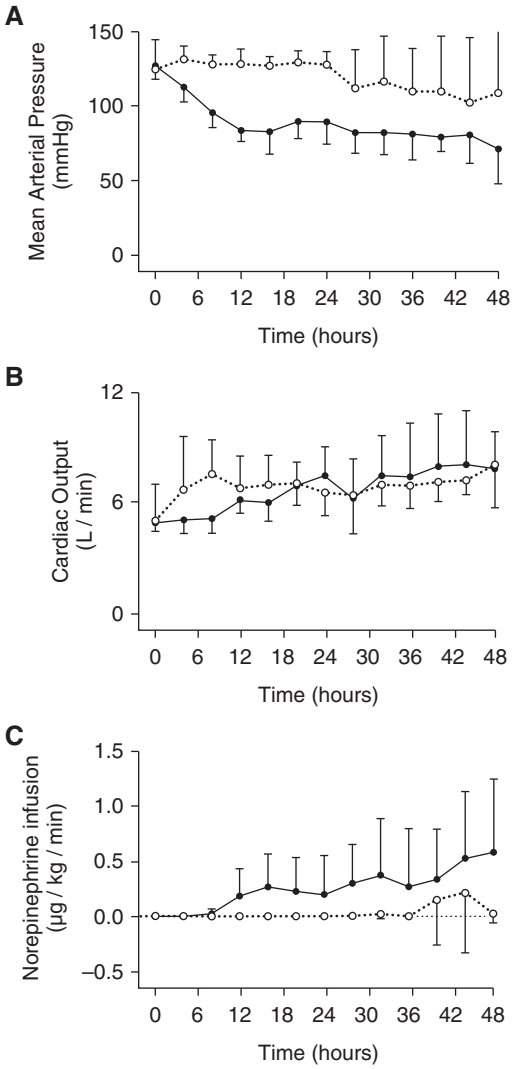

Figure 1. Hemodynamic variables in nonseptic (*open circles*, n = 5) and septic (*solid circles*, n = 10) sheep managed with protocol-guided intensive care support for 48 hours. One animal died in the nonseptic group (45 h) and five died in the septic group (18, 18, 24, 29, 33 h). Results are presented as means ± 95% confidence intervals. (*A*) Mean arterial pressure decreased significantly over time ($P < 0.01$) and was lower in the septic group of sheep ($P < 0.01$). (*B*) Cardiac output did not differ over time ($P > 0.5$) or between groups ($P > 0.3$). (*C*) Group-by-time interaction was significant for norepinephrine dose ($P = 0.02$).

Ovine Model

Description of the ovine model of septic shock is included in the online supplement. In brief, 15 mature female sheep (65–70 kg) were studied individually and anesthetized for insertion of carotid and pulmonary artery catheters, tracheostomy, and a urinary catheter. An incision was made in the left flank with dissection to the renal hilum and an ultrasonic flow probe placed around the left renal artery. The wound was closed with removable clips allowing subsequent access to the kidney for sequential renal biopsies under direct vision. Using radiological guidance, a cannula was placed via the jugular vein into the left renal vein.

After instrumentation, animals received protocol-guided sedation, ventilation, parenteral fluids, and norepinephrine infusion titrated to maintain normal mean arterial pressure of sheep (75 mm Hg) (24) (*see* Table E1 in the online supplement). Ten animals were rendered septic with intravenous *Escherichia coli* (10^8 organisms/kg) infused over 1 hour, while five sheep acted as nonseptic controls. Animal euthanasia occurred after 48 hours.

Assessment of Renal Function

Renal blood flow was measured continuously and recorded at 10-minute intervals. Urine production was measured hourly and pooled into 12-hour samples. Serum and urine biochemistry (sodium, creatinine, urea) were analyzed at baseline and repeated every 12 hours. Creatinine clearance is equivalent to the glomerular filtration rate in sheep (25), and fractional excretion of sodium was used as a measure of tubule function (26). Renal oxygen consumption and lactate flux were determined every 4 hours by measuring levels in arterial and renal venous blood multiplied by mean renal blood flow over the corresponding time period.

Renal Biopsies

A 16-gauge biopsy needle was used for sampling renal tissue at baseline, 24 hours, and 48 hours; or earlier if urine output was less than 0.2 ml/kg for two consecutive hours. Each biopsy comprised two samples of tissue, collected remote from previous biopsy sites and placed immediately into specimen containers of glutaraldehyde and formaldehyde.

Tissue Preparation

Renal tissue for light microscopic assessment was embedded in paraffin, sliced at 2 μm, and stained with hematoxylin and eosin. For ultrastructure assessment, 100-nm epoxy resin sections were stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (*see* METHODS in the online supplement).

Assessment of Renal Tissue

Functional components of the kidney (i.e., glomerulus, tubule, interstitium, nonglomerular blood vessels) and 31 predetermined histological changes (Table E2) were systematically assessed on every renal biopsy. A clinical pathologist expert in renal disease, unaware of timing and sepsis status of each sample, scored each histological change on a visual analog scale (range: 0 cm, absent; 10 cm, severe) for every biopsy in one sitting.

Statistical Analysis

Continuous data over time were analyzed by general estimating equations with robust

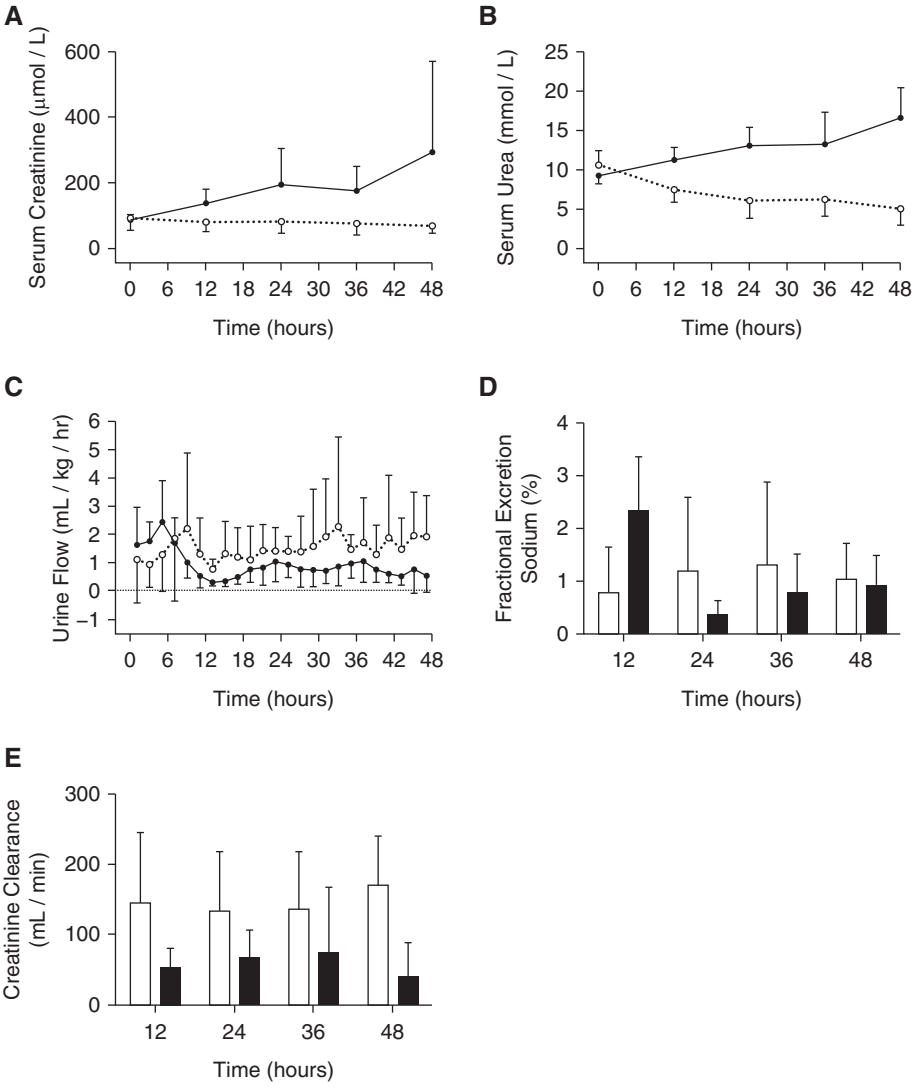

Figure 2. Measures of renal function in nonseptic (*open circles* and *bars*) and septic sheep (*solid circles* and *bars*). Animal deaths occurred as described in Figure 1. Results are presented as means ± 95% confidence intervals. There was a significant group-by-time interaction for (*A*) serum creatinine ($P = 0.01$), (*B*) serum urea ($P < 0.01$), (*C*) urine flow ($P < 0.01$), and (*D*) fractional excretion of sodium ($P = 0.03$). (*E*) Creatinine clearance was lower in septic sheep ($P < 0.01$) and did not change over time ($P > 0.50$).

standard errors. Histology scores were log-transformed and analyzed for a group-by-time interaction, group effect, and time effect with random-effects linear mixed models (27). Analyses were undertaken with Stata/MP (version 14.0; StataCorp, College Station, TX). Statistical significance was taken as $P < 0.05$. Data are described as means (±SD in tables, ±95% confidence intervals on graphs).

Results

Group Characteristics

The septic group of sheep received *E. coli* at 0.9 (±0.2) × 10^8 organisms/kg and subsequently developed arterial hypotension requiring increasing amounts of norepinephrine to maintain target mean arterial pressure (Figure 1). Septic animals developed coagulopathy, elevated serum lactate, and a decrease in systemic vascular resistance (Table E3 and Figure E1). Cardiac output did not significantly change over time and was not different between nonseptic and septic sheep (Figure 1). There was no difference between groups in the amount of parenteral fluids administered (ml/kg/h; nonseptic, 3.5 ± 0.8 vs. septic, 4.0 ± 0.7; $P = 0.21$) or in central venous pressure (Figure E1).

In the nonseptic group, one animal died at 45 hours, associated with significant renal hemorrhage. In the septic group, five animals died before 48 hours (18, 18, 24, 29, and 33 h after *E. coli* infusion) (Figure E2).

Renal Function

Septic sheep developed AKI with a fourfold increase in serum creatinine and urea concentrations, and a significant decrease in creatinine clearance and urine output (Figure 2). The fraction of filtered sodium excreted in urine had a variable pattern within the first 24 hours of sepsis, but thereafter was similar to that in nonseptic sheep (Figure 2).

Renal blood flow did not change over time and did not differ between septic and nonseptic animals (ml/min; nonseptic, 417 ± 36 vs. septic, 387 ± 49; $P = 0.39$). Renal oxygen consumption increased in nonseptic animals over time (ml/min; nonseptic, 7.0 ± 2.3 vs. septic, 5.6 ± 1.4; $P = 0.01$) (Figure 3), while lactate flux across the kidney did not change over time or differ between groups (Figure 3).

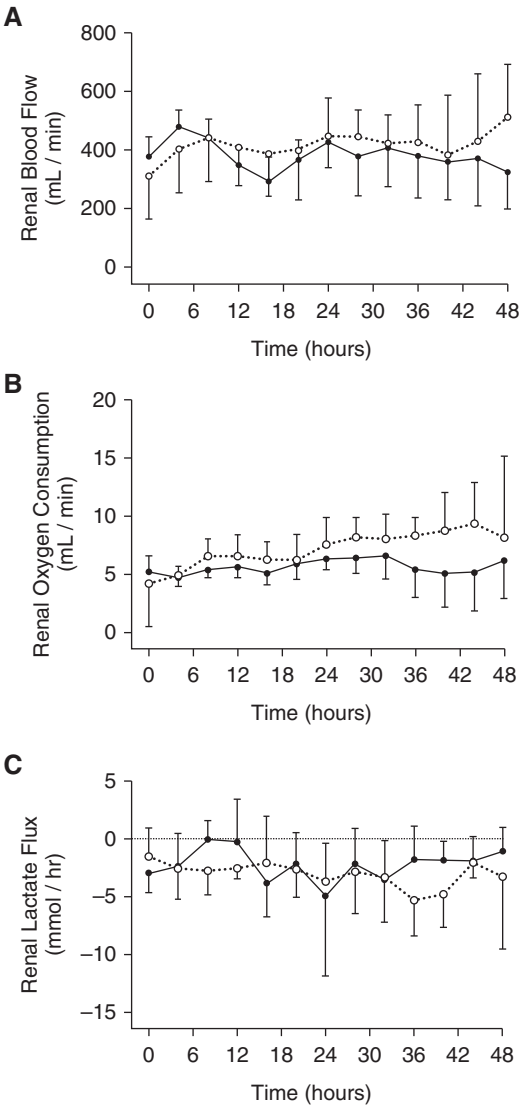

Figure 3. Renal metabolic indices in nonseptic (*open circles*) and septic sheep (*solid circles*). Animal deaths occurred as described in Figure 1. Results are presented as means ± 95% confidence intervals. (*A*) Renal blood flow did not differ between groups ($P > 0.30$) or over time ($P > 0.50$). (*B*) Renal oxygen consumption increased in nonseptic animals over time ($P = 0.01$). (*C*) Lactate flux did not differ over time ($P > 0.50$) or between groups ($P > 0.50$). A negative lactate flux represents renal lactate consumption.

Kidney Histopathology

Timing of the renal biopsies is outlined in Table 1. Overall, there was little histological disturbance in the renal biopsies (Figure 4). None of the histological parameters showed a significant group-by-time interaction, indicating that the two groups did not differ over the three time points. Histology scores for luminal necrotic cells and podocyte foot-process effacement increased over time ($P < 0.01$ for both) but were no different between groups ($P > 0.5$ for both). Mesangial expansion (electron microscopy) scored higher in the septic group ($P = 0.01$) but did not change over time ($P > 0.50$). Of note, there were no microthrombi observed and little evidence of inflammation, tubular cell necrosis, or vascular hemorrhage. A panel of light and electron histomicrographs representing the range of cellular changes observed is available in the online supplement (Figures E3 and E4).

Discussion

We performed an experimental study to assess the changes in renal function and

Table 1. Timing of Renal Biopsies Taken from Septic and Nonseptic Sheep

	Biopsy 1		Biopsy 2		Biopsy 3	
	Nonseptic	Septic	Nonseptic	Septic	Nonseptic	Septic
Time of biopsy after *Escherichia coli* administration	0 h	0 h	24 h	21 ± 4 h	48 h	42 ± 10 h
Number of animals biopsied*	n = 5	n = 10	n = 5	n = 9	n = 4	n = 7
Urine output preceding each biopsy, ml/kg/h			1.5 ± 0.8	0.5 ± 0.4†	1.8 ± 1.1	0.3 ± 0.3†

*One renal biopsy in the nonseptic group at each time point had an insufficient number of glomeruli to assess.
†$P < 0.05$ for difference between nonseptic and septic groups.

histology over the first 48 hours of septic shock in an animal model that closely mimicked many of the features of a septic human in ICU. Septic shock induced a marked reduction in glomerular filtration despite preservation of renal blood flow, and no changes in oxygen consumption or lactate flux. Importantly, despite the marked loss of glomerular filtration, the histological appearance of the kidneys was largely the same as in controls. The lack of histopathological change is particularly remarkable given the severity of disease, dramatic increase in serum creatinine, need for high doses of norepinephrine, and lethality of the model.

Preservation of relatively normal histology despite marked changes in renal function may appear surprising. However, from as early as 1957, small observational studies noted relatively little structural change in the kidneys from septic patients who died with severe renal dysfunction (28–30). Consistent with our controlled study, these case reports all remarked on the apparent discrepancy between profound functional impairment and little structural change.

More recent studies have observed histopathological changes in *post-mortem* renal tissue from septic patients with AKI, when compared with samples from nonseptic patients with normal renal function. Lerolle and colleagues noted acute tubular lesions, intense leukocytic infiltrate, tubular apoptosis, and occasionally glomerular microthrombi in 19 *post-mortem* renal biopsies (21). Takasu and colleagues found features of tubule injury in 39 septic AKI samples, but this occurred in only a small proportion of tubules (31). Interpreting the significance of abnormalities reported in these studies is confounded by the effect of premortal hemodynamic injury, age differences, preexisting renal disease, the potential influence of supportive therapies, and most of all, by selection bias (only the sickest people who had been in ICU for a long time and died despite treatment were chosen for *post-mortem* assessment). Indeed, Takasu and colleagues noted that their septic patient group had more features typical of chronic renal disease including glomerulosclerosis, interstitial fibrosis, and arteriolar hyalinosis. Furthermore, any histological changes observed in these *post-mortem* studies do not necessarily represent the pathological mechanisms that initiate or perpetuate septic AKI.

AKI histology studies to date have largely focused on renal tubular changes. Given that AKI is typically defined by a reduction in glomerular filtration, assessing glomerular histology is particularly salient. Our study is unique, having systematically assessed and reported glomerular histology and ultrastructure in septic AKI. With light microscopy, glomerular appearance was indistinguishable between septic and nonseptic sheep. At an ultrastructural level, podocyte foot processes effaced over time, but this was not exacerbated by sepsis. Mesangial expansion was greater in the septic group, but the clinical significance of this is uncertain as it did not change over time and was not accompanied by similar changes at light microscopy. However, it is possible that the mesangium may contribute to the pathogenesis of septic AKI by changing intraglomerular vessel tone and blood flow. Mesangial cells provide structural support for the glomerular capillaries, are involved in regulating glomerular flow and ultrafiltration surface, and contain receptors to a wide array of inflammatory mediators and vasoactive agents (32). Mesangial expansion is typically seen in diabetic nephropathy and certain glomerulonephritides but its contribution to septic AKI is currently unknown.

In contrast to the early and marked reduction of glomerular filtration, tubular function was largely preserved. Although there was a higher fraction of filtered sodium excreted after sepsis induction, this had resolved by 24 hours, and was consistent with the unchanged histological appearance of the cortical tubules. Transient early natriuresis and diuresis have been noted in other models of sepsis with subsequent preservation of sodium reabsorption (14, 15, 33–35).

A noteworthy aspect of this study was that renal blood flow and metabolic function were monitored concurrently with structural assessment. In this model, there was no change in renal blood flow or lactate flux and in septic animals, renal oxygen consumption did not increase. These findings are consistent with other experimental acute septic AKI studies that have also reported unchanged renal oxygen consumption (20, 36, 37), normal lactate flux (33, 36), and preserved renal ATP levels (38). Given these findings and the lack of sepsis-induced histological changes, it appears that in early septic AKI, the kidneys are not grossly ischemic from a biochemical or structural perspective.

More recently, a prespecified analysis of the Protocolized Care for Early Septic Shock (ProCESS) trial reports that the incidence, duration, severity, and recovery from AKI was not altered by the more frequent application of hemodynamic monitoring, parenteral fluids, catecholamines, and blood transfusion (39). This is consistent with the premise that gross hemodynamic changes alone do not contribute to the AKI of sepsis.

Identifying the molecular processes leading to cell death is an area of burgeoning interest in many diseases including acute kidney injury (40, 41). Cells can regulate their own death and involve a combination

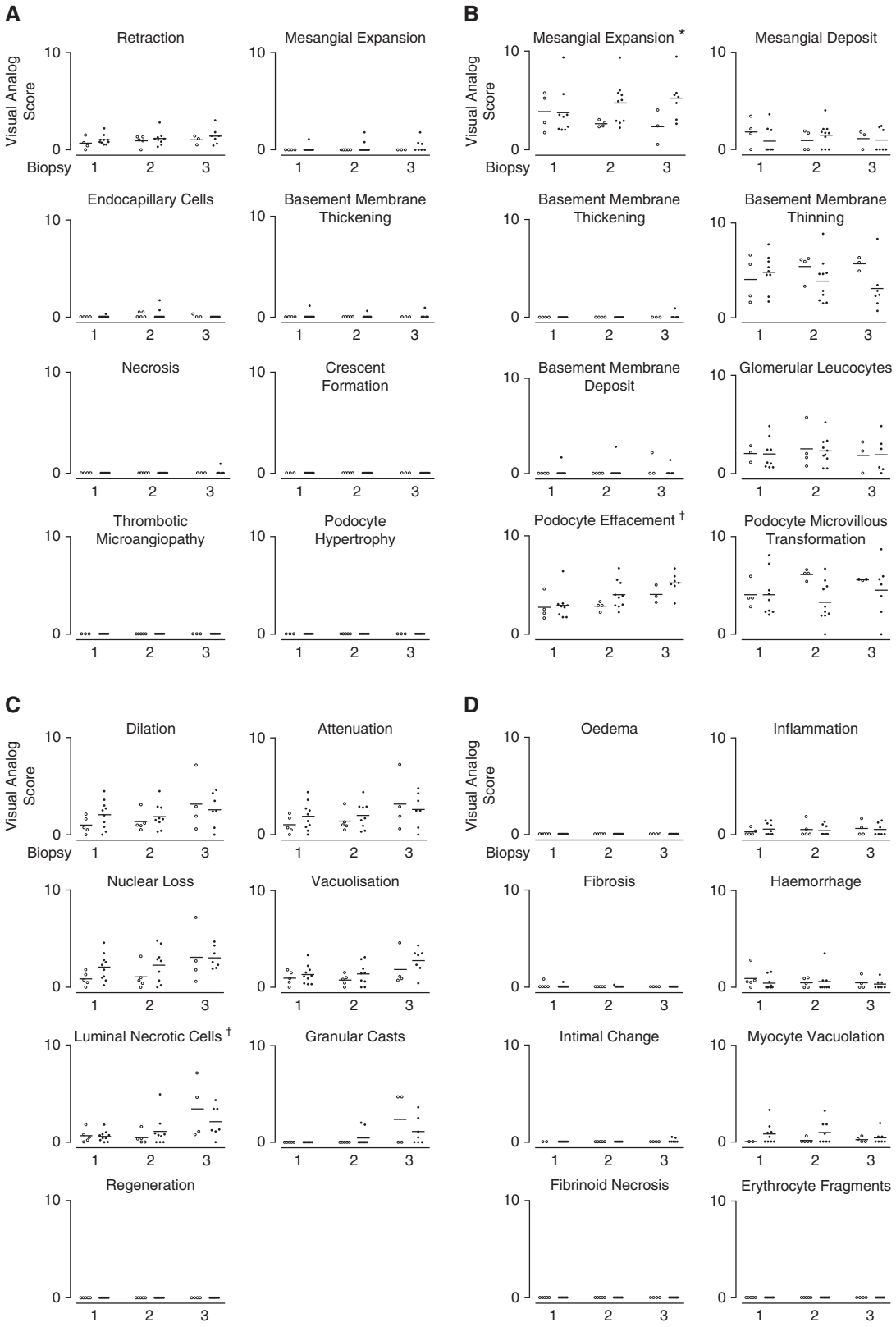

Figure 4. Histology scores of renal biopsies taken from nonseptic sheep (*open circles*) and septic sheep (*solid circles*), represented as a scatter plot with a *horizontal line* representing the mean. Extent of histology abnormality was scored on a visual analog scale from 0 (absent) to 10 (severe). (*A*) Assessment of glomerulus by light microscopy. There was no difference in histology scores over time or between groups. (*B*) Assessment of glomerulus by electron

of processes including apoptosis, necroptosis, altered mitochondrial permeability, parthanatos, and ferroptosis. Each of these processes involves a unique series of molecular changes and target molecules such as phosphorylated mixed-lineage kinase domain–like (MLKL), receptor-interacting protein kinase 3 (RIPK3), cleaved caspases, poly(ADP-ribose) polymerase-1 (PARP-1), glutathione peroxidase 4, and cyclophilin D. Identifying these cell death markers was not possible on ovine tissue with the immunohistochemical techniques currently available. Although histological features of cell death were infrequent in this study, and no different between the septic and nonseptic groups, further research is required to develop experimental techniques that will allow us to understand how cells die in septic AKI.

The observation of rapid AKI development, lack of histologic injury, and preserved global renal blood flow in septic animals implies a redistribution of blood flow within the kidney during sepsis. This interpretation is supported by other studies that have reported higher oxygen content in the renal vein than the efferent arteriole (42), preservation of renal blood flow in the septic cortex but a decrease in the medulla (35), and by the lack of change in renal oxygen and lactate consumption. Previous scanning electron microscopy studies have revealed glomerular bypass vessels, most prevalent in the juxtamedullary region of the kidney, which may provide an anatomical basis for intrarenal shunt (43–45). Such shunting and diminished glomerular filtration may be protective. It may help preserve circulating volume, limit exposure to filtered injurious mediators, limit tubular solute load, spare renal metabolic function, and avoid exposure to reactive oxygen species (46, 47).

This study addressed many limitations of earlier reports of renal structure and function in sepsis, being conducted in a controlled setting, with a relevant model of septic AKI (48). The model was typical of the clinical scenario, given that two-thirds of ICU patients with altered renal function develop these changes within 48 hours of admission (17) and that 80% with severe AKI receive mechanical ventilation and catecholamines (4, 5). The study controlled for variables that may alter renal function such as parenteral fluids, use of norepinephrine, timing of sepsis, and animal age. Renal morphology, hemodynamics, and function were assessed simultaneously over multiple time points and compared with control animals subject to the same protocol. Histological assessment was systematic, quantified the magnitude of prespecified cellular changes on a continuous scale, and was performed blindly by a single expert renal pathologist, avoiding bias and interobserver variation.

Despite the above-described strengths, several complex limitations remained and must be considered. This was a nonhuman study and involved only one model of sepsis. Inducing sepsis with *E. coli* bacteremia allowed timing of the sepsis challenge to be standardized, but may not represent changes that could occur with other infectious sources or organisms. Other factors that alter renal structure or function such as nephrotoxins and comorbidities were not incorporated in this controlled study. Nevertheless, many pathophysiological features of the ovine model, including previously published data on systemic markers of inflammation (49), appear consistent with human sepsis. This model replicated only the first 48 hours of sepsis. Persistent renal dysfunction may involve other pathological processes that were not identified in this short-term model. Biopsies were limited to cortical tissue to limit the risk of renal hemorrhage from deeper biopsy; hence medullary tissue was not examined. Medullary perfusion and tissue oxygenation decrease during experimental sepsis (35), supporting the possibility that tubular injury may have been more noticeable in this region of the kidney. Cortical tubule ultrastructure was not assessed as cellular changes are patchy and foreshortened views make finding a representative cell prone to sampling error. Despite these limitations, changes isolated to medullary tubules or cortical tubule ultrastructure would be unlikely to account for the early and marked impairment of glomerular filtration of septic AKI. Although we describe the development of hemoconcentration with sepsis, we are unable to comment in detail on its effects on microvascular viscosity and how this may have contributed to the reduction of glomerular filtration. Biomarkers of renal tubule injury such as neutrophil gelatinase-associated lipocalin (NGAL) or kidney injury molecule 1 (Kim-1) were not available for assay in sheep. We detected some early changes in tubular function related to sodium excretion, but because of the pooling of urine over 12 hours, were unable to provide a more detailed understanding of its time course.

Incorporating a positive histological control into the study may have enhanced the validity of the study. However, it is uncertain what should represent a positive control and its potential to bias histological assessment. We considered that the use of a positive histological control was not warranted and negated by (*1*) the pathologist's years of experience in analyzing renal tissue, (*2*) blinding to the sample origin and physiological parameters, (*3*) sensitivity of electron microscopy to detect cellular change, and (*4*) use of objective histological assessment. Finally, with a relatively small number of animals studied, the lack of appreciable histological difference between groups may represent a type II error. Although the histological appearance of individual elements did not significantly change with sepsis, it is possible that AKI may result from a combination of multiple small changes. Nevertheless, the aggregate of our findings, in concert with earlier observational studies, supports the notion that there is little structural injury in early septic AKI.

In conclusion, a large animal experimental model of septic shock that closely simulated the human condition allowed a controlled study of renal structure and function in early septic AKI. We found

Figure 4. (Continued). microscopy. Scores for mesangial expansion were higher in the septic group ($P < 0.01$) but did not significantly change over time. Podocyte effacement scores increased over time ($P < 0.01$) but did not differ between groups. (*C*) Light microscopy assessment of cortical tubules. The only significant change was an increase in scores for luminal necrotic cells over time ($P < 0.01$). (*D*) Light microscopy assessment of interstitium and arterial profiles. There was no difference in histology scores over time or between groups. $^{*}P < 0.01$ difference between groups; $^{\dagger}P < 0.01$ difference over time.

marked reduction of glomerular filtration despite preservation of renal blood flow and lack of histological changes in the kidney. Our findings support the view that early manifestations of AKI during septic shock are functional rather than structural in nature. ■

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