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## Use of Organ Dysfunction as a Primary Outcome Variable Following Cecal Ligation and Puncture: Recommendations for Future Studies

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## USE OF ORGAN DYSFUNCTION AS A PRIMARY OUTCOME VARIABLE FOLLOWING CECAL LIGATION AND PUNCTURE: RECOMMENDATIONS FOR FUTURE STUDIES

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### Abstract

Outcomes variables for research on sepsis have centered on mortality and changes in the host immune response. However, a recent task force (Sepsis-3) revised the definition of sepsis to “life-threatening organ dysfunction caused by a dysregulated host response to infection.” This new definition suggests that human studies should focus on organ dysfunction. The appropriate criteria for organ dysfunction in either human sepsis or animal models are, however, poorly delineated, limiting the potential for translation. Further, in many systems, the difference between “dysfunction” and “injury” may not be clear. In this review, we identify criteria for organ dysfunction and/or injury in human sepsis and in rodents subjected to cecal ligation and puncture (CLP), the most commonly used animal model of sepsis. We further examine instances where overlap between human sepsis and CLP is sufficient to identify translational endpoints. Additional verification may demonstrate that these endpoints are applicable to other animals and to other sepsis models, for example, pneumonia. We believe that the use of these proposed measures of organ dysfunction will facilitate mechanistic studies on the pathobiology of sepsis and enhance our ability to develop animal model platforms to evaluate therapeutic approaches to human sepsis.

### Keywords

Animal model; cecal ligation and puncture; gold standard; mice; organ dysfunction; organ injury; rats; sepsis; Sepsis-3

## INTRODUCTION

Translational research is dependent on the 2-way conduit connecting bench and bedside. In this paradigm, the characteristic features and common outcomes of a disease, syndrome or disorder are recognized in patients. A model system is constructed, most often in an animal. The model is then used to explore the underlying pathobiology of the disorder, and perhaps to preliminarily assess potential therapeutic approaches. If successful, these interventions can then be tested in patients and their effect on outcomes determined. Clearly, success is critically dependent on the correlation between animal model and human disorder. It is also essential that assessment tools and outcomes measurements in animals have human correlates. And, there are few disorders where this imperative is more important than in sepsis.

The first formal definition of the clinical syndrome we call sepsis was developed by a consensus conference convened in 1991 (1). The participants identified sepsis as “the systemic inflammatory response to infection.” “Systemic inflammation” was clinically identified via the criteria that comprise the “Systemic Inflammatory Response Syndrome”. Ten years later a second consensus conference affirmed the definition and expanded the list of clinical criteria (Sepsis-2) (2). Over a similar period, cecal ligation and puncture (CLP) in rodents (and, on occasion, larger animals) emerged as the most-frequently used animal model of sepsis (3). The approach has been extensively refined over the years (4). The use of CLP and other models has provided a great deal of insight into the pathobiology of sepsis. These models have not, however, resulted in the development of clinically useful interventions. Simply put, despite in excess of 150 randomized clinical trials of therapies for sepsis, many first tested in animal models, no specific, clinically effective intervention has been identified (5). The failure to translate findings from CLP to clinical sepsis has limited confidence in the use of animal models for this deadly and common disorder.

An important potential contributor to our inability to translate therapies developed in animals to human sepsis may lie in the identification of sepsis as “infection + inflammation.” This approach has led to a focus on immune/inflammatory markers, most often cytokines. However, “systemic inflammation” may be present in many conditions, including uncomplicated infection. Compounding this issue is a concern regarding correlations between immune responses in rodents and humans (6). In addition, mortality has been the most commonly used outcome variable in animal studies and, indeed, in most clinical trials. But improved clinical support of critically ill patients has shifted the focus away from simple survival. Indeed, reduced mortality has uncovered a substantial cohort of sepsis survivors who are plagued by persistent abnormalities in organ function, particularly in cognition (7–10). Limited studies have demonstrated similar abnormalities in long-term survivors of CLP (11, 12).

Fortunately, there is a readily available remedy. The 3rd consensus conference on the definition of sepsis (Sepsis-3) redefined sepsis as “life-threatening organ dysfunction caused by a dysregulated host response to infection” (13). This new formulation emphasizes that sepsis is more than an immunological disorder. Although the immune system is indeed a profoundly important element of the “dysregulated host response”, the Sepsis-3 definition

posits that the defining characteristic of sepsis is organ dysfunction rather than systemic inflammation. Thus, it is logical that organ dysfunction (perhaps including dysfunction in the “immune organ”) be used as an endpoint in studies on sepsis in patients and in animal models (14). Indeed, function in some organ systems can be measured in both patients with sepsis and mice subjected to CLP, providing an enhanced ability to make comparisons and seek correlations. In most systems, however, the terms “dysfunction” and “injury” are used interchangeably. Clearly, the two are not equivalent; dysfunction may not reflect demonstrable injury whereas injury does not necessarily lead to dysfunction. Often, however, it is by recognizing that the other can be identified. In general, dysfunction can be identified in vivo whereas injury requires either examination of tissue or the existence of a validated biomarker. Obtaining tissue is often problematic; biopsy may be life-threatening in coagulopathic patients and is technically difficult in small animals, which are often sacrificed to allow tissue sampling. Nonetheless, seeking commonalities between feasible measurements of dysfunction or injury between human sepsis and animal models of the disorder offers a promising avenue from the development of translatable therapies.

Therefore, in this review, we identify measures of organ dysfunction and/or injury in human sepsis and in rodents subjected to CLP, the most commonly used animal model. Where correlations are possible, they are identified. Where limited commonality exists, we emphasize the opportunity to either seek correlations between existing variables or to seek new, more applicable measures.

## APPROACH

We undertook a comprehensive review of the literature using PubMed. We surveyed articles published between Jan 1, 2000 and the present and used the search terms “Sepsis-3, sepsis, organ dysfunction, organ injury, human, mice, rats, cecal ligation and puncture, and murine model.” We sought to identify metrics of dysfunction and/or injury in each of the systems discussed below. Selected articles were reviewed and commonalities and correlations between human and animal data were identified. Sections were generated on the basis of the following template.

- Clinical presentations/quantifiable measures of organ dysfunction/injury in patients with sepsis.
- Quantifiable measures of organ dysfunction/injury following CLP.
- Correlations between measures used in clinical sepsis and those used in CLP.
- Indication of areas where additional investigation might identify currently unrecognized correlations and commonalities.
- Specific limitations and caveats, where applicable.
- Tabulated recommendations for parameters to be assessed in animals subjected to CLP.

## RESULTS

Measures of dysfunction/injury in individual organ systems are detailed below. Table 1 contains a compendium of measures of organ dysfunction in human sepsis and following CLP in rodents.

### CARDIAC DYSFUNCTION FOLLOWING CECAL LIGATION AND PUNCTURE

#### Clinical presentation/quantifiable measures of organ dysfunction/injury in patients with sepsis

The defining characteristics of cardiovascular dysfunction in human sepsis are vasodilatation and myocardial depression. This latter can be definitively identified using echocardiography. Specific indices include decreased shortening in both long and transverse axes and in contraction velocity (15, 16). The net functional result may be a depressed ejection fraction (EF) and circulatory inadequacy (16) but both are influenced by fluid resuscitation. Incomplete resuscitation is associated with modest increases in heart rate (HR), decreases in systolic (SVP) or mean arterial blood pressure (MAP) and central venous pressure (CVP), elevations in pulmonary artery pressure (PAP) (17). HR changes may result in an increase in cardiac output (CO) despite a low-to-normal stroke volume (SV) (16). With resuscitation, SVP and MAP decrease, CVP and PAP become quite elevated and calculated vascular resistance reflects a profound loss of systemic tone. SV and CO may increase substantially (18). The increase in SV usually reflects dramatic left ventricular (LV) dilation and an increase in LV end-diastolic volume (LVEDV) that may compensate for the reduced EF (19). Some correction of blood pressure may occur in the terminal phase of sepsis, but patients become bradycardic and LV dysfunction may progress to outright failure (20). In recovery, EF, HR, MAP, CVP, PAP, and SV normalize and resuscitation fluid and interstitial edema are eliminated. Some myocardial depression may persist and, in extreme cases, never fully resolve (16). Importantly, all cited parameters can be serially measured with echocardiography.

#### Measurable abnormalities following cecal ligation and puncture in rodents

Although myocardial depression remains the hallmark of cardiac dysfunction in both CLP and human sepsis, the presentations differ. Specifically, rodents develop bradycardia following CLP, returning to normal only in animals that survive and recover. MAP and CVP in mice reflect resuscitation, whereas PAP is elevated. Use of speckle-tracking echocardiography can identify early systolic and diastolic dysfunction analogous to changes in shortening along both axes and reduced contraction velocity (21) (Capone C, Fernandes T, Abraham M, Taylor M, Deutschman C, unpublished observations).

#### Correlations between changes induced by clinical sepsis and cecal ligation and puncture

As stated, contractility in both humans and rodents is easily identified using echocardiography. The effects of human sepsis and murine CLP on SV, EF, and CO may differ but echocardiographic parameters of systolic dysfunction—longitudinal and circumferential shortening/strain, conduction velocity—appear to correlate (22, 23). Importantly, measurements in mice are altered by the need for anesthesia, also a myocardial

depressant. These parameters can be quantified from echocardiography using readily available software, eliminating variability among echocardiographers.

### Recommendations

- Cardiac dysfunction following CLP should be quantified using echocardiography with particular emphasis on parameters of systolic dysfunction, especially strain and fractional shortening.
- Changes in SV and CO may or may not correlate with findings in human sepsis but should be followed.

### LIMITATIONS AND CAVEATS

Echocardiographic measurements may sometimes be subjective and dependent on technical experience of the clinical examiner.

### LUNG DYSFUNCTION FOLLOWING CECAL LIGATION AND PUNCTURE

#### Clinical presentations/quantifiable measures of organ dysfunction/injury in patients with sepsis

The Acute Respiratory Distress Syndrome (ARDS) is a common and often deadly form of pulmonary dysfunction in human sepsis and other inflammatory disorders. ARDS is complex and involves dysregulation and injury in many different cells, pathways, and processes (24). Dysfunction is primarily reflected in abnormal gas exchange and, following the initiation of mechanical ventilation, changes in lung volumes and compliance. Hypoxemia and abnormalities in ventilation/perfusion matching are defining characteristics. Indeed, severity is often quantified on the basis of the magnitude of hypoxemia as reflected in the  $\text{PaO}_2/\text{FiO}_2$  ratio (Table 2) (25). As fatigue develops, initial hypocarbia that results from tachypnea quickly gives way to elevations in  $\text{PaCO}_2$ . Pulmonary artery pressure rises, often to extremes. Increases in extravascular lung water (edema), both interstitial and alveolar, result from enhanced pulmonary vascular permeability, with loss of aerated lung tissue (25). These latter changes are reflected in decreases in lung compliance and loss of functional reserve capacity (FRC). Injury is reflected in broncho-alveolar lavage (BAL) fluid that contains inflammatory cells and cytokines, shed type I pulmonary epithelial cells, other evidence of cellular debris and high levels of protein. In addition to the primary disorder, management may also contribute to the pathobiology of ARDS. Positive pressure mechanical ventilation alone causes lung injury (ventilator-induced lung injury [VILI]) (26), whereas fluid restriction may be problematic because it effects the need to optimize perfusion in other organs and tissues. Thus, it is often impossible to determine what abnormalities represent sepsis-induced lung injury, what role is played by fluid administration, and what changes reflect VILI (27).

#### Measurable abnormalities following cecal ligation and puncture

CLP-induced changes on the lungs of mice and rats mimic, to some degree, the changes seen in clinical ARDS (28, 29). Animals develop hypoxemia, initially with hypocarbia but eventually with a respiratory acidosis (30–33). Broncho-alveolar lavage reveals an intense

inflammatory infiltrate, primarily of neutrophils, elevated levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, and an increase in detached pneumocytes and other cellular debris. Most other abnormalities are identified histologically or using homogenates of lung tissue. Initial changes include interstitial edema, a neutrophilic infiltrate, and shed epithelial cells in the alveolar space (34). Later abnormalities include airway edema, alveoli filled with cellular debris, white blood cells and proteinaceous fluid, overgrowth of type II pneumocytes in areas where type I cell loss has denuded the basal lamina, and eventual patchy consolidation. Fluid, sodium and protein leak into the airways (33). Studies on type I cells and gap junctions reveal impaired transport of salt and water out of airways (28). Increased lung water may be quantified using wet/dry weights (35). Ultimately there is progression to intra-alveolar inflammation or hyaline membrane deposition, important developments in the pathogenesis of human ARDS (33).

### **Correlations between changes induced by clinical sepsis and cecal ligation and puncture**

Although a number of ARDS-associated abnormalities can be determined in both septic patients and mice subjected to CLP, most are nonspecific. In part, this limitation reflects the fact that ARDS is induced by disorders other than sepsis. Blood gas changes will be present in virtually any lung disorder. BAL findings of protein, neutrophil accumulation, cytokines, and detached pneumocytes are near-universal findings in ARDS but are by no means pathognomonic. More characteristic abnormalities require either airway access (ie, endotracheal intubation) which is not the norm in rodents and other small animals, or histologic analysis, which is possible in euthanized animals but problematic in patients with ARDS. The use of some tests (eg, MRI scanning, flux of salt, and water into the alveolar space) that can be measured in both patients and rodents is limited by expense and the need for specialized equipment (36–38).

### **Recommendations**

- The criteria that comprise the Berlin criteria for ARDS—respiratory distress, radiographic abnormalities, increased lung water not because of cardiac failure or fluid overload, and hypoxemia as reflected in the PaO<sub>2</sub>/FiO<sub>2</sub> ratio—can be measured in both clinical sepsis and in rodents subjected to CLP. Practical considerations, however, suggest that arterial blood gases (including PaO<sub>2</sub>, PaCO<sub>2</sub>, and pH) be obtained in animals subjected to CLP.
- BAL should be performed, despite the fact that airway access is required. Fluid should be examined for protein, neutrophils, and detached epithelial cells.
- The lung wet:dry ratio should be determined to provide an index of extravascular lung water content. Similar measurements can be made in patients, although special techniques are required.
- Histologic changes, easily assessed in animals but not often examined in patients, should be assessed, as the classic histologic characteristics of human ARDS have long been established (39). Tissue should be examined for evidence of cellularity, fibrosis, edema, hyaline membrane formation, and apoptosis/necrosis.



- Histologic findings in rodents can to some extent be compared with findings on patient MRI scans. Similarly, MRI scanning of the lung can be performed on rodents. Thus, lung imaging might become useful in the future (38, 40).

## LIMITATIONS AND CAVEAT

All studies on mice are limited by size. In particular, the small blood volume of mice makes serial blood gas determinations untenable. Similarly, measuring lung compliance or performing BAL may not be viable or repeatable in such small animals. The use of rats may overcome some of these limitations. Histologic studies must be made postmortem following CLP and are not truly feasible in patients. However, it may become possible to correlate the results with imaging abnormalities, providing a modality that might 1 day be practical in both patients and rodents.

## LIVER DYSFUNCTION FOLLOWING CECAL LIGATION AND PUNCTURE

### Clinical presentations/quantifiable measures of organ dysfunction/injury in patients with sepsis

The clinical presentation of sepsis-induced liver dysfunction reflects underlying hepatocellular dysfunction. Under normal conditions, activity in these cells involves (but is not limited to) synthesis of biomolecules that serve as enzymes in pathways that modulate the substrates used to support organism-wide metabolism, production of proteins involved in transport and coagulation, detoxification of endogenous and exogenous “poisons”, and 4) maintenance of acid-base balance (41). Many of these processes are directly altered by sepsis-induced changes in other systems. For example, hepatic glucose production is dependent on precursors provided by skeletal muscle or adipose breakdown, or by metabolism of lactate through the Cori cycle. In addition, hepatocyte activity is hormone-dependent and is thus altered by the endocrinopathy of sepsis. As a result, hepatic gluconeogenesis is elevated relative to basal, unstimulated conditions and is driven in part by high levels of precursor delivery. However, glucose production is far less than appropriate for the amount of available substrate and the hormonal milieu that characterize sepsis. Similarly, serum levels of the transport proteins (prealbumin [transthyretin], transferrin, and albumin) and of coagulation factors are inappropriately low, due in part to decreased synthesis. The bio-transformation and conjugation of toxins and drugs for excretion into bile or urine is impaired. As a result of the impaired detoxification of bilirubin, normal and abnormal bile acids may be detected in the blood, whereas impaired conjugation and excretion of bilirubin lead to jaundice. Finally, stressed hepatocytes become “leaky” and elevated circulating levels of transaminases are very common (41). However, the changes are nonspecific and may not even reflect cell injury.

Unfortunately, most of these measures are not characteristic of sepsis but rather represent the hepatic response to any of a number of abnormalities. Further, the liver has tremendous reserve capacity. Indeed, clotting indices may remain normal with coagulation factor levels as low as 15% of normal.

### Measurable abnormalities following cecal ligation and puncture

Investigations using mice subjected to CLP exhibit the same alterations in fuel metabolism, reductions in hepatic protein synthesis, impaired detoxification and decreased membrane integrity observed in septic humans. In addition, studies using CLP and other models of sepsis have provided important information about the pathobiology of sepsis-induced liver dysfunction. Unfortunately, all measurements may reflect not just intrinsic hepatic dysfunction but also the influence of substrate availability/utilization and endocrine effects.

Abnormalities in substrate utilization/synthesis take 2 basic forms; decreased expression of the genes encoding key proteins, especially gluconeogenic, ketogenic and ureagenic enzymes (42–46); and attenuated responses to hormones (42–45, 47). Decreases in transport proteins similarly reflect impaired expression/synthesis but also result from extrahepatic consumption and leak into the interstitium (48). Decreased levels of coagulation factors may well reflect reprioritization of protein synthesis as well as increased utilization. Finally, altered detoxification of endogenous and exogenous toxins and drugs is reflected in hyperbilirubinemia (49) and may result from changes in the microsomal (P450) system (50) and in transport both into and out of hepatocytes (51).

Hepatic function is an important determinant of lactate recycling, a process that is impaired following CLP (47). However, lactate levels also reflect tissue perfusion, increased glycolysis (“aerobic glycolysis”) (52) that may result from mitochondrial dysfunction (53) and/or from infused catecholamines (54). Thus, abnormal lactate levels, and even clearance, are poor indicators of hepatic function. Similarly, increases in serum transaminase levels occur in cases of “hepatocyte stress” that are not necessarily dysfunctional.

### Correlations between changes induced by clinical sepsis and cecal ligation and puncture

Most of the clinical indices used to identify liver dysfunction are either nonspecific (transaminases) or are late biomarkers (bilirubin). Changes in serum levels of transport proteins, coagulation factors or endogenous toxins such as bilirubin, as noted above, are altered by nonhepatic factors. Thus, correlations between these findings in patients with sepsis and mice subjected to CLP may exist but more likely than not are not specific for hepatic dysfunction. Recent studies, however, suggest that the appearance of normal or abnormal bile acids in the serum is a more sensitive marker of hepatic dysfunction (41). In addition, clearance studies on drugs (eg, lidocaine) or toxins may, in the long run, prove to be most useful. Importantly, the liver has extensive reserve capacity that may limit the value of biomarkers of any sort in the diagnosis of sepsis-induced liver dysfunction. Ultimately, biopsies that can be processed for examination of gene expression may be required.

### Recommendations

- Although the limitations of most currently used indices of hepatic dysfunction have been discussed, measurement of transaminases and bilirubin, reflecting cellular integrity, are nearly universally obtained in clinical sepsis and therefore should be performed following CLP.

- Despite only rarely being examined in humans, analysis of hepatic histology and gene expression should be determined. Future development of imaging modalities in patients may provide an avenue for comparison.
- Hepatic protein synthesis should be investigated. It can be approximated by measuring serum levels of albumin, transferrin, and transthyretin.
- Tests with potential value requiring validation that should be considered. These include lactate clearance, serum bile acid levels, measurement of drug clearance and impaired gene expression.

## LIMITATIONS AND CAVEAT

The liver's immense reserve capacity may limit the ability to truly identify dysfunction or, absent tissue sampling, injury. Conversely, abnormalities in some frequently—used measures (eg, serum transaminase levels) may be too sensitive to truly function as biomarkers. Substrate levels and enzyme expression/activity are subject to changes in systems other than the liver (eg, skeletal muscle, endocrine). Correlations between changes in gene expression, determinations of protein abundance and histologic changes in patients with sepsis and rodents subjected to CLP require validation. Levels of bile acid in serum have not been examined in rodents (who have different bile acids than humans), whereas validation in patients is lacking and no correlations between patients and rodents have been identified.

## KIDNEY DYSFUNCTION FOLLOWING CECAL LIGATION AND PUNCTURE

### Clinical presentations/quantifiable measures of organ dysfunction/injury in patients with sepsis

Sepsis is often accompanied by an abrupt, poorly understood decrease in renal function that is termed sepsis-induced acute kidney injury (SIKI) (55). Identification of SIKI is often problematic. At one time, it was believed that SIKI resulted from an ischemic injury secondary to reduced renal perfusion. However, the demonstration of SIKI in normotensive patients, often in the presence of increased renal blood flow (RBF) coupled with infrequent demonstration of post mortem ischemic injury in patients with SIKI cast doubt on this hypothesis (56, 57). SIKI is characterized by a decrease in the glomerular filtration rate (GFR) and/or urine output but these measures incompletely reflect renal function. The use of serum creatinine levels to quantify changes in GFR is compromised by decreased creatinine production in skeletal muscle and high-volume intravenous fluid administration (58). Thus, seemingly small increases in creatinine may not reflect a precipitous loss of renal function (58). In addition, SIKI epitomizes the difficulties in distinguishing between functional decline and actual injury (59, 60). This discrepancy makes it difficult to separate maladaptive renal dysfunction from adaptive reductions in performance. Indeed, it has been suggested that recovery of function, rather than the severity of decline, maybe the most important prognostic factor (61). To address these challenges, experts in SIKI have examined biomarkers for their ability to distinguish renal injury from renal functional decline (often called renal “stress”) (55). The currently accepted paradigm postulates that SIKI originates with a DAMP-induced injury in the early proximal tubule.

The effects of injury are amplified by local inflammation, microvascular abnormalities, metabolic downregulation and mitochondrial dysfunction (60). Ultimately, these abnormalities lead to cell-cycle arrest at the G1-S checkpoint that reduces GFR. At what point injury becomes functionally important, that is, at what point “dysfunction” develops, is unclear. Importantly, not all renal dysfunction in clinical sepsis is the result of SIAKI. Indeed, septic patients are at high risk for renal ischemia, microangiopathy, and/or iatrogenic nephrotoxicity.

There is no gold-standard for diagnosing SIAKI. The current Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend identifying acute kidney injury (AKI) based on changes in serum creatinine, urine output, and the need for renal replacement therapy (62), grading the disorder into 3 stages that have short and long-term prognostic validity. To account for premorbid status, only changes in creatinine and urine output from baseline, and not absolute levels, are considered (62). As a surrogate for the early proximal tubular injury that, per current theory, initiates SIAKI, KDIGO criteria include biomarkers such as serum levels of Kidney Injury Molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) (63, 64). Renal stress is quantified using serum levels of insulin-like growth factor-binding protein-7 (IGFBP7) and tissue-inhibitor of metalloproteinases-2 (TIMP2) (65). The fact that these markers often rise before changes in creatinine and urine output become clinically evident underscores the inability to identify the point where “injury” and “stress” give way to “dysfunction.” The Food And Drug Administration recently approved use of the TIMP2/IGFBP7 ratio as an index of AKI risk; a ratio <0.3 indicates very low risk whereas a value >2.0 suggests a high probability of developing AKI (66, 67).

### **Measurable abnormalities following cecal ligation and puncture**

Histopathologic abnormalities in CLP-induced AKI include an absence of acute tubular necrosis, patchy foci of heterogeneous tubular injury, indicated by apical vacuolization and minimal apoptosis (60). The presence of ischemic injury suggests inadequate resuscitation (68–70). The stress indicators TIMP2 and IGFBP7 can be measured in urine or using immunohistochemistry (71). The injury markers KIM-1 and NGAL can be measured in urine, serum, and/or immunohistochemically (70). KIM-1 is likely a better marker in CLP because it is specific to the proximal tubule, is expressed only during injury, and persists until the cellular injury resolves (72). GFR following CLP has been determined using clinically relevant but somewhat unreliable surrogates such as creatinine, cystatin C or plasma inulin clearance (73). Other quantifiable abnormalities include assessments of RBF (74, 75). Although many measures have been used following CLP (76), heterogeneous microvascular dysfunction and preserved overall RBF are characteristic of SIAKI.

### **Correlations between changes induced by clinical sepsis and cecal ligation and puncture**

Clinically accepted measures of SIAKI correlate variably with findings following CLP. The most commonly measured clinical indices that readily translate to CLP include serum creatinine and BUN concentrations. Urine output can also be compared. In contrast, histopathology is often analyzed following CLP but is rarely available in clinical settings. Biomarkers such as KIM-1, NGAL, IGFBP7, and TIMP2 can be measured in both patients

and rodents; small urine volumes in rodents favor the use of serum levels. Detection of biomarkers using immunohistochemistry on fixed tissue sections is feasible following CLP but not in patients. Renal hemodynamics in clinical sepsis and following CLP are similar but their measurement may be limited by technical concerns. For example, interpretation of renal ultrasonography (US) in clinical sepsis may be problematic but findings are readily translatable. That said, no currently available evidence support the clinical utility of renal US in SIAKI.

### Recommendations

- Urine output should be quantified daily.
- CLP studies should include at least 1 clinically relevant measure from 3 domains. These are:
  - A GFR surrogate such as creatinine or cystatin C that can also be measured in patients. The validity of these proxies can be augmented using other functional measures, such as BUN and urine output, that are also routinely clinically determined, and with more direct measures of GFR. These last, however, are rarely measured in patients.
  - At least 1 injury biomarker—KIM-1 (our preference) or NGAL—measured in urine, serum, or using histopathology. These markers can also be determined in patients, and it is to be hoped that their clinical use will increase in the near future.
  - Model verification—some evidence that the injury is not secondary to ischemia. This can be accomplished via demonstration of histopathologic findings classical for SIAKI or by showing that RBF is preserved. Although demonstrating absence of ischemic injury is currently problematic in clinical practice, it is essential in model systems.

### LIMITATIONS AND CAVEAT

Small volumes in rodents limit the measurement of urine output or determination of biomarkers in urine. In addition, urine collection requires the use of metabolic cages. As mentioned, the relationship between actual dysfunction and “stress” markers (TIMP2, IGFBP7) is somewhat controversial and has not been validated in rodents. Finally, it is currently difficult to rule out the presence of ischemic injury in patients, where tissue is not available.

## GASTROINTESTINAL TRACT DYSFUNCTION FOLLOWING CECAL LIGATION AND PUNCTURE

### Clinical presentations/quantifiable measures of organ dysfunction/injury in patients with sepsis

Under nonpathologic conditions, the GI tract serves several essential functions. Involuntary sequential contractions—peristalsis—propel ingested material from the stomach to the rectum. The bowel wall is a nearly impenetrable barrier that separates the host from luminal contents. This barrier allows the host to selectively absorb nutrients via enzymes located in the brush border of the villi that characterize intestinal microstructure. The impediment also separates the host from the microbiome, a large repository of bacteria. Finally, organized lymphoid centers (Peyer’s patches) provide immune surveillance of intestinal content.

Sepsis disrupts all of these functions. Peristaltic contractions may be “mistimed,” failing to move ingested substrate forward, or may disappear altogether (“ileus”) (77). Tight junctions are disrupted by mechanical forces and reduced expression of their constituent proteins. Similar changes reduced absorption via brush border enzymes and induce villus atrophy. In addition, epithelial cells are lost to necrosis/apoptosis and regeneration is limited. Overall barrier function is reduced, allowing some intestinal debris and bacterial products access into the lymphatics and the blood stream (77). Immune activity in Peyer’s patches mirrors systemic effects; cytokine production is enhanced but engulfment and breakdown damaged cells, proteins, or microorganisms are impaired (78). Sepsis itself, or the antibiotics used to treat it, induce massive changes in the microbiome that Alverdy et al (80) have described as a “pathobiome” (79). The impact of these changes (“dysbiosis”) on organ function and survival are incompletely understood, highlighting an essential gap in our understanding of sepsis (81, 82).

Intestinal motility, which is disrupted in sepsis, can be measured clinically via several methods. The lactulose breath hydrogen test indirectly measures motility via quantification of exhaled hydrogen, the product of lactulose metabolism by colonic bacteria. Manometry may be used to measure pressure alterations at various points along the GI tract. Innovative methods include wireless capsule technology, where ingestible capsules perform endoscopic and other assessments of GI functionality (83).

GI absorptive capacity can be measured using a differential sugar absorption test, most often using sucrose and xylose. Because D-xylose does not require enzymatic cleavage before transport across the mammalian epithelium, the ratio of D-xylose to other sugars in blood provides an index of absorption. Clinical studies in septic patients subjected to the xylose absorption test suggest decreased absorption (84).

Epithelial integrity and barrier function of the GI are markedly decreased in sepsis. However, separating dysfunction and injury is problematic because it is unclear if the intestinal barrier can become leaky in the absence of actual damage. Barrier dysfunction can be identified by the presence of bacterial products such as endotoxin or plasma D-lactate, a fermentation product of intestinal bacteria, in the circulation (85–87). Injury to cells may be reflected in high plasma and/or urine levels of epithelial cell proteins such as intestinal fatty-

acid binding protein, whereas damage to tight junctions will elevate serum levels of claudin-3 (85, 88). Serum zonulin, a regulator of tight junctions, has been measured in patients with sepsis (89). A single study of 9 patients identified increased colorectal permeability in patients using rectal administration of <sup>51</sup>Cr-labeled ethylene diamine tetraacetic acid (EDTA) (90). This study has not been verified, and administration of other markers used following CLP (see below) has not been reported in patients with sepsis.

Dysbiosis in patients with sepsis is characterized by a decrease in commensal microorganisms with a concomitant rise in pathogenic strains. The extent of these microbiomic alterations can be measured via fecal sampling and bacterial genomic sequencing (91).

### **Measurable abnormalities following cecal ligation and puncture**

CLP significantly alters GI function in a manner that appears to resemble the changes seen in clinical sepsis (92–94). Motility can be examined using intracolonic balloon-tipped catheters or tracking of ingested solid beads (95, 96). Tests of absorption described in human sepsis can be used following CLP. Multiple studies have demonstrated an increase in flux across intestinal tight junctions following CLP. Orally administered fluorescein isothiocyanate-conjugated-dextran (FD-4), or, less frequently, <sup>51</sup>Cr-labeled EDTA or ovalbumin have been detected in the blood following CLP (93, 97, 98). CLP also enhances direct injury. For example, CLP decreased the abundance of the tight junction proteins claudin-2, claudin-5, occludin, and JAM-A in intestinal wall samples (93, 99). Post-CLP atrophy and shortening of villi may reflect decreased crypt proliferation and increased epithelial cell apoptosis (100).

Few studies have evaluated changes in the microbiome post-CLP. Chen et al noted that CLP in wildtype mice caused a 25.87% increase in Bacteroidetes abundance and a 36.68% decrease in Firmicutes abundance, along with significant alterations in other phyla (101). The use of antibiotics that altered the microbiome led to a significant increase in CLP-induced mortality. It should be noted that microbiome profiles vary greatly on the basis of the vendor providing the animals and may also be altered by diet (102).

### **Correlations between changes induced by clinical sepsis and cecal ligation and puncture**

Although GI dysfunction can be assessed in clinical sepsis and following CLP, only a limited number have been used in both. Motility measurements in each condition are quite different and what is useful in 1 may not be appropriate for the other (84, 95, 96). Similarly, although clinical tests measuring absorption of sugars or food coloring can be used following CLP, more precise measurements can be made via administration of fluorescent or radiolabeled molecules. Clinical administration of radiolabeled substances such as <sup>57</sup>Cr EDTA is problematic in the US, whereas FD-4 has not been evaluated in patients. Although biomarkers indicative of tight junction or cellular injury can be identified in the blood of patients, CLP affords the opportunity to determine their abundance directly in intestinal cell homogenates or fixed tissue samples. This approach is clinically viable only in biopsy or postmortem analyses.



Microbiomic alterations can be determined in septic patients and post-CLP using 16S rRNA sequencing, shotgun metagenomics, or other techniques. Thus, a more direct comparison of microbiome homeostasis between CLP and clinical sepsis is possible. However, the endogenous flora of the 2 species may be markedly different, requiring comparison based on similarities (eg, gram-negative bacteria, anaerobes).

## Recommendations

Despite current limitations on the clinical use of methodology that has been immensely valuable in animals, taking full advantage of the opportunities to examine intestinal dysfunction/injury available following CLP appears to be advisable. Should novel clinical methods be developed, the availability of robust data on intestinal dysfunction/injury following CLP will enable comparisons. Complete GI evaluation following CLP might include the following

- Intestinal integrity using xylose absorption.
- Barrier function should be examined via measurement of
  - Serum/urine levels of bacterial products (eg, endotoxin), claudin-3, intestinal fatty binding protein, or D-lactate
  - Administered D-xylose or Blue Dye #1
- Motility can be assessed using solid beads
- Measures of barrier function that cannot be performed in patients, at least at this time, should be considered. These might include:
  - tight junction proteins (claudins, occludin, JAM-A) abundance in tissue homogenate or sections
  - serum levels of orally administered FD-4 or <sup>57</sup>Cr EDTA
- Performance of bacterial genomic sequencing to identify changes in the microbiome in colonic samples

## LIMITATIONS AND CAVEAT

Concerns regarding the current lack of tests that can be used in both patients with sepsis and following CLP have been discussed. The normal microbiota in humans and rodents are different and either may be affected by factors other than sepsis. Further, differences in diet, endogenous flora, and other conditions across studies might have bearing on the results.

## BRAIN DYSFUNCTION FOLLOWING CECAL LIGATION AND PUNCTURE

### Clinical presentations/quantifiable measures of organ dysfunction/injury in patients with sepsis

Brain dysfunction in sepsis takes the form of either acute septic encephalopathy (ASE), a catchall that encompasses a diverse array of behavioral and cognitive abnormalities (103), or more chronic and lingering abnormalities. Alterations in consciousness may range from



confusion and disorientation to delirium to deep coma. Seizures and focal neurological signs have also been reported (103). The most commonly invoked state is described as “hypomanic delirium”. Diagnosis is complicated by the frequent use of neuropharmacologic agents. Sepsis survivors report that ASE is associated with vivid nightmares, hallucinations, and paranoid delusions, whereas chronic issues include lingering delirium, PTSD, and significant declines in memory and attention (7). Sepsis survivors are at increased risk to develop dementia (104). Both ASE and dysfunction in long-term sepsis survivors are associated with increased mortality (105).

Acute alterations in consciousness in the ICU have been evaluated with neuropsychological testing, in particular, the Confusion Assessment Method for the ICU (106) and the Intensive Care Delirium Screening Checklist (107), 2 tools that were specifically developed to evaluate the level of consciousness in critically ill patients. Sepsis-induced EEG abnormalities include  $\theta$  waves,  $\delta$  waves, triphasic waves, periodic epileptiform discharges, electrographic seizures, and generalized or burst suppression (103). Abnormalities in MRI scans have also been reported (108).

### **Measurable abnormalities following cecal ligation and puncture**

The ability to collect pathological specimen and perform more involved functional tests has produced an understanding of CLP-induced abnormalities specific to the brain that exceed what has been identified in clinical sepsis. In the first week post-CLP, activation of the orexinergic and basal forebrain cholinergic systems was reduced (109, 110). There is evidence of neuroinflammation (increased cytokine abundance, neutrophil infiltration, inflammasome activation) and oxidative damage (increased nitrite/nitrate ratio, lipid peroxidation, carbonyl protein formation, decreased activity of antioxidants) and reduced expression of brain-derived neurotrophic factor (110–113). The blood–brain barrier is disrupted (112). Structural abnormalities include shrunken cell bodies, nuclear pyknosis and cellular degeneration of hippocampal pyramidal neurons (111, 113), reduction in length and density of dendritic spines in the CA1 and CA2 regions of the hippocampus and in the amygdala have been noted in long-term CLP survivors (12, 114). Limited EEG data suggest reduced EEG delta power density during nonrapid eye movement sleep (115). MRI imaging post-CLP has revealed findings consistent with cytotoxic and vasogenic edema and neuronal damage (116, 117).

Both short and long-term behavioral changes are of particular importance when examining correlations between human sepsis. Abnormalities following CLP include evidence of anxiety and depression-like symptoms (11, 118), impairments in locomotion, spatial and contextual-fear memory, fear conditioning and aversive learning (11, 12, 111, 113, 114, 118).

### **Correlations between changes induced by clinical sepsis and cecal ligation and puncture**

Identifying correlations sepsis-induced defects in brain function and changes following CLP is problematic. MRI studies in humans are too limited for comparison and changes in EEG  $\delta$ -waves are nonspecific. Some structural lesions observed following CLP may reflect ischemia/hypovolemia only. Although histologic changes and evidence of

neuroinflammation have been consistently noted following CLP, similar findings are unlikely to be identified in humans. Short-term behavioral changes have been noted in both septic patients and in rodents during the first-week post-CLP. Limited mobility and lack of response to environmental stimuli following CLP may well represent the murine equivalent of the delirium, agitation and terrifyingly vivid hallucinations and delusions that characterize human hypomanic depression but differences between species make comparisons difficult. The use of drugs that affect the brain in general and that specifically alter behavior in both human sepsis and post-CLP mice further complicates matters. Better correlations may be present when comparing long-term behavioral changes because post-CLP memory impairment is likely the equivalent of cognitive impairment in sepsis survivors, changes in fear conditioning may be analogous to PTSD and depression can be quantitatively assessed in both patients and mice.

### Recommendations

As in previous sections, recommendations reflect the ability to currently correlate sepsis and CLP-induced changes and the possibility that advances in the future will enable correlations. We therefore suggest the following studies be considered in rodents subjected to CLP

- Histologic and immunohistochemical analysis of fixed brain tissue.
- Measures of neuroinflammation, including cytokine abundance and evidence of oxidative stress in different regions of the brain
- Assessment of changes in movement and responses to external stimuli, which may be analogous to human hypomanic depression.
- Changes in MRI scans.
- Measurement of quantifiable indices of memory loss and mood in long-term survivors of CLP.

### LIMITATIONS AND CAVEATS

Some rodent measurements are limited to assessing movement and memory recall of movements. The caveat here is not to parallel that degree of mobility lost or lack of recall with clinical manifestations of memory, mood, anxiety, and depression disorders.

### SKELETAL MUSCLE DYSFUNCTION FOLLOWING CECAL LIGATION AND PUNCTURE

#### Clinical presentations/quantifiable measures of organ dysfunction/injury in patients with sepsis

Skeletal muscle accounts for more than 50% of body cell mass and thus represents the largest organ affected by sepsis. All forms of “stress”/inflammation deplete skeletal muscle, which serves as a source of amino acids for gluconeogenesis and tissue repair (119). More importantly, critical illness in general and sepsis in particular are associated with intensive care unit acquired weakness (ICUAW), a catch-all term that encompasses 2 entities, critical illness myopathy and critical illness polyneuropathy (CIP), that are difficult to separate

(120). The reported incidence of ICUAW varies widely on the basis of the underlying condition and the duration of ICU stay (121, 122). Risk factors include mechanical ventilation, immobilization, sepsis, multiple organ dysfunction, and the use of myotoxic/neurotoxic medications (eg, neuromuscular blocking agents and corticosteroids) (122, 123, 125). ICUAW is characterized by flaccid, symmetrical weakness that is more pronounced in proximal muscle groups. Deep tendon reflexes may be normal or reduced. When CIP is a component of ICUAW, reduced sensation to pain and temperature may be present. ICUAW may interfere with weaning from the ventilator (124) and may persist following recovery from sepsis, leaving survivors with persistent weakness (120, 125).

Guidelines for studying ICUAW in septic patients suggest the use of several functional modalities. Bedside assessment of muscle strength may be quantified using the Medical Research Council (MRC) sum score (120, 126), which evaluates strength in 6 muscle groups. An MRC score of less than 48 is consistent with a diagnosis of ICUAW (127). MRC score may be complemented by handgrip strength (123). The final recommendation is determination of maximal inspiratory pressure, which has been associated with the duration of weaning from the ventilator (123,128). Muscle excitability during needle electromyography (EMG) is characteristically reduced (129, 130). Elevations of serum creatine phosphokinase (CPK) levels, which have been inconsistently documented, provide evidence of muscle injury as do the results of muscle biopsy, which, when performed, characteristically demonstrates a loss of myosin filaments, axonal degeneration and tissue necrosis (131). Finally, mitochondrial number may be reduced, whereas decreased ATP (Adenosine Triphosphate) production likely reflects decreased activity of electron transport chain (ETC) complexes I and IV (132). An increase in the number of damaged mitochondria suggests both direct injury and dysfunction in the processes responsible for mitochondrial removal (mitophagy) and regeneration (biogenesis) (132). It has been suggested that both dysfunction and injury result from inflammation because tissue levels of IL-1, IL-6, IL-10, and TNF- $\alpha$  are elevated (133–135).

### **Measurable abnormalities following cecal ligation and puncture**

Locomotor activity in rodents subjected to CLP is reduced and the animals assume an abnormal posture (136). As in human sepsis, CPK levels are inconsistently elevated, suggesting variable degrees of actual muscle injury. Isolation and direct stimulation of individual motor units in rats post-CLP showed a prolonged muscle contraction time and a reduced maximal muscle force (136, 137). Both primary data and extrapolation from studies on diaphragmatic or cardiac muscle have demonstrated profound reductions in mitochondrial content, whereas function as reflected in a reduction in mitochondrial membrane potential and dysfunction in all 4 ETC complexes, is also decreased (138, 139). As in human sepsis, CLP is characterized by mitochondrial injury, reflected in an increased number of damaged mitochondria and impaired autophagy and biogenesis (140). In addition, cytokine elevations similar to those observed in septic humans have been identified (133–135).

## Correlations between changes induced by clinical sepsis and cecal ligation and puncture

As detailed above, functional changes characteristic of ICUAW in both patients with sepsis and in rodents following CLP include prolonged contraction time and force, abnormalities on EMG, and changes in the number and activity of mitochondria, including reductions in the activity of electron transport complexes I and IV (141, 142). Injury is reflected in muscle proteolysis and, inconsistently, CPK elevations (119, 143). However, although ICUAW preferentially affects myosin filaments in human sepsis (131), myosin, and actin are equally affected in rodents subjected to CLP (144).

## Recommendations

In contrast to a number of other organ systems, dysfunction in patients with sepsis and rodents subjected to CLP are similar, enabling direct comparisons. Therefore, we suggest that the following measurements be considered following CLP

- Assessment of motor activity including postural instability
- CPK levels
- Cytokine abundance in muscle homogenate
- Histologic analysis and quantification of filament loss, mitochondrial number, and biogenesis/mitophagy
- Muscle levels of high-energy phosphate intermediates, mitochondrial respiration, mitochondrial DNA (biogenesis), and ETC complex activity
- Myography (despite potential difficulty in performing the test)

## LIMITATIONS AND CAVEATS

An important caveat in all proposed measures is that myopathy has only been studied in the acute phase post-CLP, whereas ICUAW is most often examined as a chronic condition in patients weeks or even months after recovery from critical illness (123, 143).

## GLOBAL LIMITATIONS, CAVEATS, AND CONCLUSION

In this document, we have outlined indices of organ dysfunction that we believe should be used as outcome measures in assessing rodents subjected to CLP. This approach is consistent with the recent revised definition of sepsis (Sepsis-3), which stresses the importance of organ dysfunction as the key characteristic of sepsis (13). We believe that the use of these proposed measures will facilitate mechanistic studies into the pathobiology of sepsis and enhance our ability to use CLP as a platform to evaluate therapeutic approaches to the human disorder.

There are several key limitations to recommendations made in this article. Perhaps the most important lies in this review's focus on CLP in rodents. It is clear that reliance on CLP is problematic, despite the fact that recent studies indicate that it is the most frequently used animal model of sepsis (145). CLP differs from human sepsis in a substantial number of ways that may well limit its utility as a platform for pre-clinical testing of potential therapies

(6). Although the translational failure of therapeutic approaches developed using CLP is 1 justification for recommending that indices of organ dysfunction be used as endpoints, it may not be the most important. Indeed, it has been argued that the primary value of CLP is “reductionist”, lying in its use to answer specific questions. We suggest that such a specific question might be “What mechanisms underlie sepsis-induced dysfunction in (fill in the organ your organ of choice)?”

We posit that the use of mortality as an endpoint is problematic for 2 reasons; recent work suggests that it may not be possible to completely eliminate mortality from human sepsis (146), and rodents are far more tolerant than humans of insults that lead to sepsis (147). The other endpoint commonly used in studies using CLP involves changes in immune function. This approach is also problematic: clearly, immune dysfunction is not the only form of organ impairment in sepsis; whereas changes in immune function may lead to organism-wide abnormalities, the same can be said of alterations in the endocrine and neuronal systems, which are also profoundly altered early in the course of sepsis (116, 148); and interventions designed to correct sepsis-induced immune dysfunction have failed to improve outcomes (149).

We acknowledge that CLP in rodents is just 1 of many animal models of sepsis. However, an exhaustive recent review of the literature documents that, in spite of its many limitations, it is by far the most commonly used. Indeed, pneumonia is now the most common source of human sepsis (150), accounting for the increasing use of models of lung infection in model systems (93). CLP has many drawbacks, some of which we have identified when reviewing parameters in individual systems. We recognize that what constitutes “organ dysfunction” in mice and rats subjected to CLP may differ substantially from what characterizes “organ dysfunction” in human sepsis. However, although the actual manifestations of organ dysfunction differ between mice and men, organ dysfunction is, in general, present in both. Mice subjected to CLP develop hypothermia, bradycardia, and a reduced respiratory rate, whereas septic humans, at least initially, become febrile, tachycardic, and increase their minute ventilation. But both develop impaired thermoregulation, myocardial contractility, and hypoxemia. Differences in the response of the murine immune system following CLP to those observed in human sepsis have been a key point raised by those who do not believe that mice can be used to model the clinical disorder (6). However, that the immune system becomes dysregulated in both is undisputed. What is key is that there is dysfunction, not the specific manner in which dysfunction manifests itself. We would also point out that not all patients with sepsis exhibit every possible form of organ dysfunction.

We recognize that organ dysfunction in sepsis and following CLP is affected by many additional factors. Manifestations of organ dysfunction may be altered by systemic influences, particularly those resulting from variable degrees of resuscitation or from the influences of both the endocrine and immune systems. Indeed, we have not included either of those systems because both have been reviewed many times (148, 151) and organ dysfunction in either is best recognized on the basis of their interactions with other systems. Perhaps more importantly, conditions that are frequently identified as “comorbidities” have dramatic effects on both clinical sepsis and sepsis-like approaches such as CLP. That age is a determinant of both responses and outcome in both sepsis and following CLP is

unquestioned (152–154). The effects of disorders such as diabetes are more problematic; studies on diabetes have yielded conflicting results such that the impact of the disorder on sepsis outcomes requires further investigation (155, 156). An additional confounder that is implicit but not discussed in detail is the effects of sedations in patients or the need for anesthetics in animals. In addition, we have not included a discussion of vascular dysfunction because it is not readily measured in patients.

There are 2 additional issues regarding the use of CLP that require comment. First, an advantage of this model is that it makes the use of isolated perfused organ preparations possible. Indeed, it was use of this approach that first identified the CLP-induced relative resistance of cells to endocrine stimulation (43, 47). In addition, although there are clear differences between rats and mice, the response to CLP in each is similar. There are 2 important differences that may dictate choice of 1 over the other. The development of transgenic animals is most advanced in mice, whereas the larger size of rats, and in particular, their larger blood volume may enable multiple blood sampling in a single animal, making serial measurements possible.

We have also attempted to distinguish between “dysfunction” and “injury.” The distinction is important; structural damage may have no impact on function, and functional abnormalities may not be reflected in demonstrable damage. Difficulty in separating these 2 entities is often problematic. In some instances, dysfunction independent of injury is easily identified (eg, using ECHO to identify reduced myocardial contractility). Most often, however, some surrogate or proxy for abnormal function is used (eg, oxygen content in the blood, inadequate bilirubin detoxification/elimination by the liver, creatinine clearance by kidneys). At times, these proxies reflect actual injury and not dysfunction (eg, hypoxemia because of lung injury, biomarkers like KIM-1 used to identify AKI). In some cases, they may be too sensitive to actually use (eg, hepatic transaminases, cardiac troponin). Changes in proxies may also be affected by some of the systemic abnormalities previously mentioned. Ultimately, dysfunction must reflect injury on some level (eg, tubular injury in AKI, which likely underlies dysfunction), even if that injury is readily reversible.

Finally, we reiterate that murine CLP is at best a proxy for human sepsis, and not a true model of the disorder. Our attempt to identify measures of organ dysfunction or injury in CLP, and to correlate those measures with similar abnormalities in human sepsis, reflects a desire to encourage the use of organ dysfunction as the most appropriate outcome variable. We hope that this change, which is consistent with the current definition of sepsis, will improve our ability to translate findings from animal models to human sepsis.

Table 1 contains a referenced summary of all suggestions.

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Organ system specific abnormalities in human sepsis and following cecal ligation and puncture

Table 1.

Organ System	Measurements/Tests	Has been determined in clinical sepsis	Has been determined in mice/rats following cecal ligation and puncture
Heart	Echocardiographic abnormalities (21)		
	HR	Y	Y
	SV	Y	Y
	EF	Y	Y
	LVEDV	Y	Y
	LVEDP	Y	Y
	LVESV	Y	Y
	LVESP	Y	Y
	PAP	Y	Y
	Strain	Y	Y
Lung	Fractional shortening	Y	Y
	PaO <sub>2</sub> ; PaO <sub>2</sub> /FiO <sub>2</sub> ratio	Y	Y
	Lung water/wet/dry ratio (35)	Y	Y
	Cell counts/protein (BAL)	Y	Y
	Fixed tissue analyses (33)	Y	Y
	Edema		
	Neutrophils		
	proteinaceous exudate		
	hyaline membrane		
	Tissue homogenate		
Liver	MPO (33)		Y
	Cytokine levels		Y
	Decreased hepatic protein synthesis		Y
	Albumin		Y
	Transferrin		Y
	Transthyretin	Y	Y
	Impaired gene expression (42–45, 47)	Y	Y



Measurements/Tests	Has been determined in clinical sepsis	Has been determined in mice/rats following cecal ligation and puncture
Impaired detoxification (49, 50, 51)		Y
Increased bilirubin		Y
Decreased lidocaine clearance	Y	
Decreased cellular integrity	Y	Y
AST, ALT	Y	Y
Abnormal bile acids (41)		Y
Urine output	Y	Y
RBF (74, 75, 76)	Y	Y
GFR (73)	Y	Y
Inulin		
Creatinine	Y	Y
Cystatin-C	Y	Y
Transcutaneous measurements	Y	Y
Tubular stress markers (71)		
IGFBP7	Y	Y
TIMP2	Y	Y
Tubular injury markers (70, 76)		
KIM-1	Y	Y
NGAL	Y	Y
Motility (83, 95, 96)		
Intracolonic balloon-tipped catheters		
Ingestion/tracking of solid beads	Y	Y
Mucosal barrier integrity	Y	Y
FD-4, EDTA, Ovalbumin (93, 97, 98)		
Microbiome composition (101)	Y	Y
Histology		
Crypt atrophy, villus shortening/atrophy, intestinal apoptosis (100)		
Tight junction proteins (93, 99)		
Claudin-2		Y
Claudin-5		
Occludin		

	Measurements/Tests	Has been determined in clinical sepsis	Has been determined in mice/rats following cecal ligation and puncture
Brain	JAM-A		
	Lethargy	Y	Y
	Anorexia	Y	Y
	Memory, learning, behavior (11, 12)	Y	Y
	Fixed tissue analyses:		Y
	Lateral hypothalamic staining Orexinergic activity (109)		
	Basal forebrain staining Cholinergic activity (110)		
	Hippocampal staining Dendritic spines (12)		
	EEG (115)		
	MRI (116, 117)		
Skeletal muscle	Postural instability (136)	Y	Y
	CPK elevation	Y	Y
	Total mitochondrial content (138)		Y
	Impaired mitochondrial membrane potential (138, 139)	Y	Y
	Myography (129, 130)		
	Tissue cytokine levels (133–135)		Y
	IL-1		Y
	IL-6		
	IL-10		
	TNF- $\alpha$		

CPK, Creatine phosphokinase; EDTA, ethylene diamine tetra-acetic acid; EF, ejection fraction; FD-4, fluorescein isothiocyanate-conjugated-dextran; GFR, glomerular filtration rate; HR, heart rate; IGFBP7, insulin-like growth factor-binding protein-7; KIM-1, Kidney Injury Molecule-1; NgAL, neutrophil gelatinase-associated lipocalin; NGAL, neutrophil gelatinase-associated lipocalin; PAP, pulmonary artery pressure; RBF, renal blood flow; TIMP2, tissue-inhibitor of metalloproteinases-2.

Y denotes YES

**Table 2.**

## Berlin Criteria for ARDS (25)

ARDS Severity	$R = P_{AO_2}/FiO_2$	PEEP
Mild	$300 > R > 200$	5
Moderate	$200 > R > 100$	5
Severe	$R < 100$	5

ARDS, Acute respiratory distress syndrome.

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