Published in final edited form as:

Crit Care Med. 2016 June ; 44(6): e420–e431. doi:10.1097/CCM.0000000000001615.

Use of biotelemetry to define physiology-based deterioration thresholds in a murine cecal ligation and puncture model of sepsis

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Abstract

Objective—Murine models of critical illness are commonly used to test new therapeutic interventions. However, these interventions are often administered at fixed time intervals after the insult, perhaps ignoring the inherent variability in magnitude and temporality of the host response. We propose to use wireless biotelemetry monitoring to define and validate criteria for acute deterioration and generate a physiology-based murine cecal ligation and puncture (CLP) model that is more similar to the conduct of human trials of sepsis.

Design—Laboratory and animal research

Setting—University basic science laboratory

Subjects—Male C57BL/6 mice

Interventions—Mice underwent CLP, and an HD-X11 wireless telemetry monitor (DSI) was implanted that enabled continuous, real-time measurement of heart rate, core temperature, and mobility. We performed a population-based analysis to determine threshold criteria that met face validity for acute physiologic deterioration. We assessed construct validity by temporally matching mice that met these acute physiologic deterioration thresholds with mice that had not yet met deterioration threshold. We analyzed matched blood samples for blood gas, inflammatory cytokine concentration, Cystatin C, and alanine aminotransferase.

Measurements and Main Results—We observed that a 10% reduction in both heart rate and temperature sustained for ≥ 10 minutes defined acute physiologic deterioration. There was significant variability in the time to reach acute deterioration threshold across mice, ranging from 339 to 529 minutes after CLP. We found adequate construct validity, as mice, which met criteria for acute deterioration had significantly worse shock, systemic inflammation (elevated TNF α ,

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All work was performed at the University of Pittsburgh

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Dr. Angus disclosed that he does not have any potential conflicts of interest.

p=0.003; IL-6, p=0.01; IL-10, p=0.005), and acute kidney injury when compared to mice that had not yet met acute deterioration criteria.

Conclusion—We defined a murine threshold for acute physiologic deterioration after CLP that has adequate face and construct validity. This model may enable a more physiology-based model for evaluation of novel therapeutics in critical illness.

Keywords

sepsis; animal model; telemetry; physiology; wireless technology; mice

Introduction

Sepsis is common, deadly, and now accounts for almost 1 in every 2 to 3 deaths in the hospital (1–4). Numerous trials test novel therapeutics in humans that are derived from animal-based models, but the majority are notable for their failure to translate into improvements for patients (5). Among the many reasons, one could be that contemporary animal models lack relevance to the delivery of care at the bedside of the septic patient.

For example, contemporary murine models administer, and thus test, interventions at *fixed* time intervals after the septic insult. This approach ignores the inherent variability in magnitude and temporality of the host response (6). Particularly since human trials often use physiologic triggers to administer and test new treatments, murine models using fixed treatment schedules may lack clinical relevance (5).

Among the many murine models of sepsis, cecal ligation and puncture (CLP) is validated and used widely (7–10). This model of host defense and barrier breakdown incorporates several components to create a dynamic and complex model of sepsis: tissue trauma from the surgical procedure, tissue necrosis from the cecal ligation and devascularization, and polymicrobial infectious insult from cecal puncture(s) (11). Although relatively simple, CLP has significant variability due to puncture size and quantity, fasting state or diet of the animals, anesthetic differences, extruding different amounts of stool from the cecum after puncture, time of day, and inter-operator variability in incision size and surgical technique (8, 12). Historically this is overcome by increasing sample size. These issues often result in a heterogeneous population at varying stages of the host response to sepsis (13).

An ideal animal model of sepsis would incorporate the physiology of the host response to septic insult. To accomplish this goal, we will use novel implantable biotelemetry technology, an emerging technique in small animal studies of sepsis (14–40). We hypothesize that implantable biotelemetry will aid in the identification of thresholds for acute physiologic deterioration after CLP that have both face and construct validity. Such a model that incorporates real-time assessment of physiology may allow the design of animal trials more relevant to the physiologic inclusion criteria of human clinical trials in sepsis, as treatments could be then administered at the precise point of physiologic deterioration.

Materials and Methods

Ethics Statement

All experiments were performed in accordance with the National Institutes of Health guidelines under protocols approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh (Protocol #13021581).

Mice

Male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) aged 8 to 12 weeks (mass 25–30 g) were utilized for all experiments. Mice were housed in specific pathogen-free rooms under 12 hour light/12 hour dark conditions cycled on at 0700 and off at 1900. Ambient temperature in animal housing areas ranged from 21 to 24°C. Animals were given *ad libitum* access to water and LabDiet Prolab Isopro RMH 3000 diet pellets (LabDiet, St. Louis, MO). Experiments were conducted in the morning to control for circadian variations.

Wireless Telemetry Technology

Mice were monitored using DSI HD-X11 implanted wireless telemetry (Data Sciences International, St. Paul, MN). The HD-X11 is a reusable 2.2 gram, 1.4 cc wireless telemetry device capable of continuous measurement of one biopotential (e.g., electrocardiogram), systemic blood pressure, core body temperature, and animal activity. Monitors were implanted within the peritoneal cavity according to manufacturer instructions. Data collection and analysis was performed using Ponemah version 5.20 (Data Sciences International, St. Paul, MN).

Cecal Ligation and Puncture

Anesthesia was induced by isoflurane (2–4%) followed by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (6 mg/kg). A 21-gauge, double puncture cecal ligation and puncture (CLP) was performed as previously published, utilizing a cecal ligation of 1 cm as measured from the cecal tip (9, 10). Care was taken to avoid occluding the ileocecal junction. A small amount (1 mm) of cecal contents was extruded using gentle pressure after punctures were created. The cecum was then returned to the abdominal cavity and placed in the upper central abdomen.

At the time of laparotomy, skin flaps were raised lateral to the incision on both sides of the abdomen for placement of biopotential leads. The telemetry device was then implanted in the peritoneal cavity as per manufacturer instructions. Biopotential leads were brought through the rectus bilaterally, and the fascia was closed with interrupted 4-0 Vicryl suture (Ethicon, Somerville, NJ). Biopotential leads were then tunneled subcutaneously to achieve positioning analogous to Lead II in human electrocardiography. The skin was closed with simple interrupted 4-0 PDS sutures (Ethicon). After completion of the procedure, mice were immediately administered warmed 0.9% normal saline (30 cc/kg SQ), as described by Chaudry et al (8).

One primary objective was to develop a testable murine model of sepsis more translatable to the sepsis experienced by a person prior to the presentation for or administration of medical

care. Thus, no antibiotics were administered during the course of the study. Mice were placed in individual cages, which were situated on a heating pad until the mice righted themselves and had regained independent mobility. All mice received buprenorphine analgesia (0.1 mg/kg SQ Q12H) as per our protocol established in conjunction with University of Pittsburgh IACUC guidelines (41–43). This dose was not found to produce any cardiac or activity depression. Data acquisition began immediately after an animal exhibited full emergence from anesthesia (i.e., upright and independent mobility and foraging). During the period of monitoring, heart rate (beats per minute), core temperature (degrees Celsius), and activity data were acquired every minute. Physiologic monitoring was continued for 24 hours after surgery in initial descriptive groups, and up to 7 days in later descriptive cohorts of mice. Sham animals underwent laparotomy and bowel manipulation without CLP, telemetry monitor implantation, and data acquisition for a 24-hour period. At the times indicated, mice were euthanized and blood samples were obtained by cardiac puncture (right ventricle).

Variability of Mice at Fixed Time Intervals after CLP

To investigate the suspected variability in inflammatory response and organ dysfunction between individual mice at fixed time points, two groups of mice (n=6 for each group) underwent CLP and were sacrificed at either 6 or 12 hours after surgery. The time points of 6 and 12 hours after CLP were chosen as representative points where investigators have typically elected to test potential therapies (24, 44, 45). Plasma samples were analyzed for markers of inflammation (TNF α , IL-6, IL-10) and organ dysfunction (Cystatin C).

Definition and Validation of Acute Physiologic Deterioration

We performed a population-based analysis of the descriptive cohort data to identify physiologic (i.e., heart rate, core temperature) thresholds that best classified mice into those that had acutely deteriorated after CLP versus those who had not. Mice were determined to meet a threshold for acute deterioration after experiencing sustained declines in both heart rate and temperature of at least 10% from their peak values. After demonstrating that the newly defined thresholds for deterioration had adequate face validity, we tested construct validity of our 'acute deterioration' definition by sacrificing mice at the acute physiologic deterioration threshold, temporally matched to another CLP mouse that had not reached deterioration threshold. We analyzed matched blood samples for markers of inflammation (TNF α , IL-6, IL-10), organ dysfunction (Cystatin C and ALT concentrations), and shock (mixed venous blood gas analysis). Quantitative bacterial cultures from blood and peritoneal lavage fluid were also compared between CLP mice at the defined deterioration threshold and those that had not experienced physiologic decline.

Bacterial Culture

At the time of sacrifice after CLP, the peritoneal cavity was irrigated with 1 mL of sterile PBS solution, and samples were collected in a sterile fashion. Heparinized whole blood was also obtained by cardiac puncture using sterile technique. Peritoneal lavage and serum samples were serially diluted, plated on 5% sheep blood agar (Teknova, Hollister, CA), and incubated at 37°C overnight. Colony forming units (cfu) were quantified by manual counting.

Inflammatory Cytokine and Organ Dysfunction Analysis

Heparinized blood samples were centrifuged at 2000g for 15 minutes, serum was collected, and samples were stored at -80°C for future analysis. Peritoneal fluid samples remaining after plating bacterial cultures were stored at -80°C for future analysis. Thawed samples then underwent quantitative analysis for markers of inflammation (IL-6, $\text{TNF}\alpha$, IL-10) and renal failure (Cystatin C) using enzyme immunoassay kits (R&D Systems, Minneapolis, MN). Cystatin C has emerged as a more precise marker of glomerular filtration rate and has been validated in murine and human studies (46–48). Liver injury was quantified by measuring alanine aminotransferase (ALT) using a Heska DRI-CHEM 4000 automated dry chemistry analyzer (Heska, Loveland, CO).

Blood Gas Parameters of Shock

Heparinized blood collected at animal sacrifice immediately underwent venous blood gas analysis using an i-STAT portable handheld device (Abbott, Princeton, NJ).

Statistical Analysis

We analyzed continuous data using non-parametric rank-sum and categorical data using Chi-squared analysis. A p-value < 0.05 was considered significant.

Results

Baseline Physiology Parameters and Biomarker Analysis

We observed an early bradycardia in the initial postoperative and post-implantation phase (Figure 1A), with gradual recovery over the first two to three hours (median time 131 minutes, range 56 to 301 minutes) to a normal range: 500 to 700 bpm (49). We observed a corresponding mild hypothermia after surgery (Figure 1B), with core temperature recovering to normal values, typically after recovery of bradycardia. Early hypothermia after CLP due to ketamine/xylazine anesthesia has been previously described in the literature (29). We show baseline values for biomarkers of inflammation and organ dysfunction in C57BL/6 male mice not undergoing CLP, both with and without telemetry monitor implantation, in Table 1.

Variability of the Murine Response to Sepsis

Our clinical correlate was the early (initial 24 hours) period of sepsis that a person may experience prior to receiving medical care. Thus, we initially studied and characterized the murine physiology during the first 24 hours after CLP. We observed considerable heterogeneity in the physiologic and thermoregulatory responses to sepsis among mice subjected to CLP (Figures 2A and 2B). Five of 16 mice (31.3%) died in the initial 24-hour period. Five mice (31.3%) exhibited minimal physiologic disturbance after CLP, with heart rate and core temperature values remaining near normal. The remaining six (37.5%) mice exhibited intermediate (moderate to severe) levels of physiologic derangement. At the traditional 6 and 12 hour time points of evaluation after CLP, we observed a wide range of physiologic responses with some mice exhibiting little physiologic alterations and others being nearly moribund (Figures 2A and 2B).

Reports in the literature have described biological differences between mice that die “early” and those dying “later” after CLP (50). Thus, we further characterized the physiology of mice that survived the initial 24 hours of CLP sepsis. We observed considerable heterogeneity in each mouse’s physiologic trajectory (Figures 3A and 3B). All mice ultimately reached their threshold physiologic deterioration threshold, and 83% died during the 7 day period of observation (Figure 4). However, although mice experienced physiologic decline at widely varying times, the sharp decline in core temperature and bradycardia was a conserved event preceding death.

To better and more objectively characterize the heterogeneity in the biologic response to sepsis, we sampled mice at two time points commonly used for testing interventions in murine CLP models: 6 and 12 hours. As shown in Figure 5, we found widely varying inflammatory responses of mice at both 6 and 12 hours after CLP. For example, at the 6-hour time point after CLP, IL-6 ranged from 15063 to 49220 pg/mL (Figure 5B). In addition a wide range of magnitudes of AKI was also observed, as Cystatin C concentrations 6 hours after CLP ranged from 390 to 879 ng/mL (Figure 5D).

Defining Thresholds for Acute Deterioration

Noting the temporal heterogeneity in each animal’s physiologic response to CLP, we explored whether the biotelemetry parameters could be modeled to identify a similar state in the host response to sepsis for each animal, independent of time after CLP. We investigated longitudinal physiology curves justified to either the time of death or the time of last observation (1440 minutes post-CLP). We noted that for those mice dying prior to 1440 minutes, the heart rate and temperature trajectory were nearly superimposable (Figures 6A and 6B). However, individual mice reached a point of acute physiologic deterioration at varying times after CLP, ranging from 356 to 1344 minutes. Scanning the population of temperature and heart rate trajectories, we defined thresholds for acute deterioration that had adequate face validity -- a 10% drop in heart rate from the observed maximum combined with a fall in core temperature (10% of the difference between maximum temperature and 25°C) that were sustained for at least 10 minutes.

As aforementioned, several animal studies highlight that the biology of late deaths is different than that of deaths occurring early (< 24 hours) after CLP (50). They further note the resultant limitations imposed by these biological differences on the generalized use of biomarker prognostication (51). By contrast, we observed in the cohort of mice monitored for 7 days that the heart rate and temperature trajectories, when justified to death, were similar to those of animals dying early (Figures 6C and 6D). Thus, our ‘acute deterioration threshold’ definition appeared similarly applicable to and valid for deaths occurring at later time points.

Construct Validity

We sacrificed mice in matched pairs consisting of one mouse that had met criteria for acute physiologic deterioration and another control CLP mouse that had not met criteria (n=12 pairs). The average time for mice to meet criteria for acute deterioration after surgery was

433 minutes (range 340 to 532 minutes), which was no different than mean time to sacrifice for matched non-threshold CLP mice (426 minutes, range=347 to 502 minutes, $p=NS$).

We determined the construct validity of our threshold definitions as representing a different biological trajectory and measured inflammatory cytokines, blood gas parameters of shock, and markers of kidney and liver dysfunction. Mice that met criteria for acute physiologic deterioration had significantly greater systemic concentrations of TNF α , IL-6, and IL-10 when compared to mice that had not yet met criteria for deterioration after CLP (Table 2 and Figure 7). For example, a mouse at threshold, on average, had an IL-6 concentration of 26,148 pg/mL compared to a matched non-threshold CLP mouse with a mean IL-6 concentration of 10424 pg/mL (Figure 7B). Similarly, mice that reached the threshold deterioration criteria exhibited significantly worse shock than mice not meeting criteria, as evidenced by worse acidemia and elevated base deficit (Table 2). Finally, mice that had met threshold for acute deterioration exhibited higher concentrations of Cystatin C by comparison to mice that had not: 600 ng/mL vs. 480 ng/mL, $p=0.09$ (Table 2 and Figure 7D). There was no significant difference in alanine aminotransferase (ALT) as a marker of liver injury (Table 2).

Bacterial Clearance

After determining that mice meeting our criteria for acute deterioration showed higher levels of inflammation, shock, and renal dysfunction, we sought to elucidate potential mechanisms for these observed differences. We initially focused on differences in bacterial clearance. As shown in Figure 8, mice that met the threshold for deterioration had higher bacterial growth in both the peritoneal cavity, as well as, in the blood than mice that had not met deterioration threshold.

Discussion

Murine models have long been key to advances in understanding the host-pathogen interaction during sepsis. However, multiple recent studies of novel therapeutics have failed to translate evidence from animal models into meaningful changes in patient outcomes. One potential limitation is that contemporary murine models test interventions at fixed time points, ignoring the inherent temporality of the host response to severe infection. What's more, most human trials use physiology-based enrollment criteria and triggers for intervention. We address this important issue by developing a physiology- and biology-oriented murine model of sepsis that identifies the point of acute physiologic deterioration after CLP using wireless biotelemetry. This model more precisely identifies a similar point in the spectrum of host response to sepsis for each animal, independent of lapsed time after the septic insult. In doing so, our model yields a more homogeneous cohort of testable mice at a similar physiologic state and accurately excludes mice that do not meet physiologic inclusion criteria; these are fundamental characteristics of the conduct of human sepsis trials.

For more than a decade, there have been calls to develop more clinically relevant animal models in sepsis (52). Many modifiable features of animal models introduce bias: choice of model and technique (e.g., CLP vs. LPS injection, CLP puncture size and quantity), choice of anesthetic and analgesics, varying fluid resuscitation practices, routine use of antibiotics,

and administration of treatments at fixed time intervals. Thus, in the context of time after CLP, there exists considerable heterogeneity in both the magnitude and timing of the murine response to CLP sepsis. Indeed, we observed both mice exhibiting a profound host response to CLP resulting in death within the first 24 hours, as well as, those with only a minor disturbance that approximated normal physiology. These data highlight the inherent limitations imposed when testing treatments at fixed time intervals: animals are being treated at very different points in their biological response.

To address these limitations, a considerable amount of investigative work has been conducted using biomarker measurements, such as cytokines, to prognostically stratify severity of illness (51, 53, 54). In one notable study, IL-6 measured six hours post-CLP and dichotomized at 2000 pg/mL, effectively classified animals as non-survivors vs. survivors, which exhibited mortality rates of approximately 90% vs. 30% (53). The group of predictive cytokines was expanded to also include TNF- α , KC, MIP-2, IL-1 receptor antagonist, TNF soluble receptor I, and TNF soluble receptor II (51). The investigators further demonstrated that stratification of mice into survivors and non-survivors based on cytokine measurements allowed them to select a cohort more appropriate for testing of experimental therapies (54). However, in this study, a considerable proportion (approximately 30%) of mice categorized as 'survivors' based upon low IL-6 concentration still progressed to death (53). Perhaps this misclassification is one limitation of a single biochemical assessment performed at an early time point after the insult.

By contrast, biotelemetry enables the continuous monitoring of each animal, and thus accounts for inter-animal variability. It has been used in multiple studies to describe physiologic changes in response to candidate sepsis therapies; however, this differs from utilizing the technology to define physiologic inclusion criteria to test a therapeutic, as performed here (14, 19, 21, 22, 24, 26, 29, 33–35, 38, 39). In our model, coupling of biotelemetry with a CLP model helped quantify physiologic differences in the murine response to sepsis that were indicative of significant biological differences, including inflammation and degree of shock and organ dysfunction. We were able to define a physiologic threshold for 'acute deterioration' that identified a similar physiologic point of decompensation for each animal, independent of time after insult. While meeting these criteria did not guarantee that the mouse will have a fatal outcome in the first 24 hours after CLP, it effectively excluded the possibility that the mouse would be a mild or non-responder to the CLP insult. Indeed, nearly all animals that reached our deterioration threshold died, and all that did not survived. These data highlight the accuracy of our technique in predicting the binary outcome of death. Thus, these data facilitate the standardization of experiments and enable the investigator to deliver treatments at specific biologic points along the host-pathogen sepsis response curve. Rather than waiting on biochemical assays, which may take several hours to process before mice can be stratified, we gain the ability to stratify in real-time. Furthermore, our model reduces the population variability, producing a model that may be more 'sensitive' to identifying therapeutic differences in tested treatment regimens. We propose that our model enables prognostication, but does so dynamically and specifically to each animal's unique temporal response.

Several publications have highlighted that mice dying later exhibit variable responses in terms of inflammatory cytokine production compared to mice dying earlier (50, 51, 53). They further note the resultant limitations imposed by these biological differences on the generalized use of biomarker prognostication (51). Yet our acute deterioration threshold criteria were equally valid in predicting late death. These data underscore a notable advantage of our real-time model: a high capacity for prognosticating decompensation and death independent of time after the initial insult. By contrast to a single or even repeated blood sampling that is predicated upon choosing *a priori* a specific time(s), our model is continuously ‘sampling’ each animal, and thus is more dependent upon each host’s unique response to sepsis. In this vein, we believe that many of the animals miscategorized as ‘survivor’ using the cytokine/biomarker threshold, but which ultimately died, would be accurately identified as ‘non-survivor’ using biotelemetry methodology.

Other groups have developed and validated scoring systems for use in murine models of sepsis, usually based off a combination of behavioral and physical observations (23, 55, 56). While these scoring systems are able to separate mice that are significantly ill from sepsis from those that have a lesser sick response, they lack sensitivity in determining the exact point of physiologic deterioration. The resolution of these scoring systems is dependent on the frequency of mouse analysis and scoring, which requires handling or manipulation of the mouse and introduces bias from an animal’s fear or escape responses (40). Furthermore, scoring systems require a significant amount of experience with the grading scale to maintain consistency in scoring between individual laboratory personnel and minimize subjectivity. Additionally, activity and behavior of mice in pain from surgery are similar to that of mice that are becoming septic, making judgment of mouse behavior in the postoperative period of a surgical model of sepsis difficult. Thus, we perceive our model to be more objective and less biased in quantifying and qualifying an individual mouse’s response to sepsis.

There are several limitations to our study. Our threshold definitions were initially chosen using face validity and further tested for construct validity. Though we observed significant separation at the threshold for acute deterioration, alternative thresholds are possible – particularly those that identify earlier time points in the host response. However, having characterized the physiology of the murine response, one can now model a variety of thresholds representing various points along the host septic response. We propose that the defined deterioration thresholds herein serve as an experimental analogue to human patients calling for emergency assistance: the murine “9-1-1.” It is at this moment a subject may also be screened for trial inclusion. As a primary objective was to develop a testable murine model better resembling human clinical trial design, we specifically chose to not include routine antibiotic administration. It was perceived that the absence of antibiotics more closely parallels the natural course of human sepsis, which initially proceeds without antibiotic treatment until sufficient physiologic derangement has occurred to prompt the patient to seek medical care. We studied mice aged 8 to 12 weeks, a relatively young cohort, and we acknowledge the host response to CLP is different in older mice (9, 57). The use of a single strain and gender of mice represents an additional limitation, though plans are being developed to expand this analysis to female mice and to add additional commonly-used

strains. Finally, we cannot rule out an inflammatory reaction to the biotelemetry device. However, in our control CLP mice there was only a modest increase in baseline values.

Conclusions

In summary, a CLP murine model of sepsis coupled with implantable biotelemetry can identify the variability in the host response to sepsis and time of acute physiologic deterioration with adequate construct validity. This new approach avoids administering treatments to mice which do not become ill, or suffer only mild physiologic changes at fixed time points used in prior models. It is anticipated that this model can serve as a platform for investigation of novel therapeutics in sepsis administered in a fashion more analogous to the conduct of human trials.

Acknowledgements

The authors wish to thank the Surgical Infection Society and the Surgical Infection Society Foundation for fellowship award funding to support this work.

Financial support was provided by a Basic/Translational Research Training Fellowship Grant awarded by the Surgical Infection Society (MRR) and R01 GM082852 (MRR) from the National Institutes of Health.

Dr. Rosengart received support for article research from the National Institutes of Health (NIH) and the Surgical Infection Society Foundation Resident Research Fellowship. Dr. Lewis received support for article research from the NIH and the Surgical Infection Society Foundation Resident Research Fellowship. Dr. Yuan disclosed receiving support from the Xiangya Third Hospital (Living expenses and tuition when Dr. Yuan does research in United States). Dr. Zhang received support for article research from the NIH. Dr. Seymour received support for article research from the NIH and received funding from Beckman Coulter consultancy. His institution received funding from the NIH/NIGMS.

References

1. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med*. 2001; 29:1303–1310. [PubMed: 11445675]
2. Martin GS, Mannino DM, Eaton S, et al. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med*. 2003; 348:1546–1554. [PubMed: 12700374]
3. Dombrovskiy VY, Martin AA, Sunderram J, et al. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. *Crit Care Med*. 2007; 35:1244–1250. [PubMed: 17414736]
4. Kumar G, Kumar N, Taneja A, et al. Nationwide trends of severe sepsis in the 21st century (2000–2007). *Chest*. 2011; 140:1223–1231. [PubMed: 21852297]
5. Fink MP. Animal models of sepsis. *Virulence*. 2014; 5:143–153. [PubMed: 24022070]
6. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. *Physiol Rev*. 2013; 93:1247–1288. [PubMed: 23899564]
7. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock--a review of laboratory models and a proposal. *J Surg Res*. 1980; 29:189–201. [PubMed: 6997619]
8. Hubbard WJ, Choudhry M, Schwacha MG, et al. Cecal ligation and puncture. *Shock*. 2005; 24:52–57. [PubMed: 16374373]
9. Howell GM, Gomez H, Collage RD, et al. Augmenting Autophagy to Treat Acute Kidney Injury during Endotoxemia in Mice. *PLoS One*. 2013; 8:1–7.
10. Zhang X, Guo L, Collage RD, et al. Calcium/calmodulin-dependent protein kinase (CaMK) I α mediates the macrophage inflammatory response to sepsis. *J Leukoc Biol*. 2011; 90:249–261. [PubMed: 21372190]

11. DeJager L, Pinheiro I, Dejonckheere E, et al. Cecal ligation and puncture: The gold standard model for polymicrobial sepsis? *Trends Microbiol.* 2011; 19:198–208. [PubMed: 21296575]
12. Rittirsch D, Hoesel LM, Ward Pa. The disconnect between animal models of sepsis and human sepsis. *J Leukoc Biol.* 2007; 81:137–143. [PubMed: 17020929]
13. Poli-de-Figueiredo LF, Garrido AG, Nakagawa N, et al. Experimental models of sepsis and their clinical relevance. *Shock.* 2008; 30(Suppl 1):53–59. [PubMed: 18704008]
14. Doi K, Hu X, Yuen PST, et al. AP214, an analogue of alpha-melanocyte-stimulating hormone, ameliorates sepsis-induced acute kidney injury and mortality. *Kidney Int.* 2008; 73:1266–1274. [PubMed: 18354376]
15. Fairchild KD, Saucerman JJ, Raynor LL, et al. Endotoxin depresses heart rate variability in mice: cytokine and steroid effects. *Am J Physiol Regul Integr Comp Physiol.* 2009; 297:R1019–R1027. [PubMed: 19657103]
16. Garver J, Bermeo-Blanco Oa, Gibson N, et al. Implantation and Monitoring of a Novel Telemetry Unit in the Syrian Golden Hamster Model. *J Investig Surg.* 2012; 25:186–196. [PubMed: 22583016]
17. Granger JI, Ratti PL, Datta SC, et al. Sepsis-induced morbidity in mice: Effects on body temperature, body weight, cage activity, social behavior and cytokines in brain. *Psychoneuroendocrinology.* 2013; 38:1047–1057. [PubMed: 23146654]
18. Lustig MK, Bac VH, Pavlovic D, et al. Colon ascendens stent peritonitis--a model of sepsis adopted to the rat: physiological, microcirculatory and laboratory changes. *Shock.* 2007; 28:59–64. [PubMed: 17483746]
19. Schuerholz T, Doemming S, Hornef M, et al. The anti-inflammatory effect of the synthetic antimicrobial peptide 19–2.5 in a murine sepsis model: a prospective randomized study. *Crit Care.* 2013; 17:R3. [PubMed: 23302299]
20. Seely, Ka; Holthoff, JH.; Burns, ST., et al. Hemodynamic changes in the kidney in a pediatric rat model of sepsis-induced acute kidney injury. *Am J Physiol Renal Physiol.* 2011; 301:F209–F217. [PubMed: 21511700]
21. Zanotti-Cavazzoni SL, Guglielmi M, Parrillo JE, et al. Fluid resuscitation influences cardiovascular performance and mortality in a murine model of sepsis. *Intensive Care Med.* 2009; 35:748–754. [PubMed: 19066851]
22. Nandi M, Kelly P, Torondel B, et al. Genetic and pharmacological inhibition of dimethylarginine dimethylaminohydrolase 1 is protective in endotoxic shock. *Arterioscler Thromb Vasc Biol.* 2012; 32:2589–2597. [PubMed: 22995517]
23. Nemzek, Ja; Xiao, H-Y.; Minard, AE., et al. Humane endpoints in shock research. *Shock.* 2004; 21:17–25. [PubMed: 14676679]
24. Remick DG, Call DR, Ebong SJ, et al. Combination immunotherapy with soluble tumor necrosis factor receptors plus interleukin 1 receptor antagonist decreases sepsis mortality. *Crit Care Med.* 2001; 29:473–481. [PubMed: 11373406]
25. Saia RS, Anselmo-Franci Ja, Carnio EC. Hypothermia during endotoxemic shock in female mice lacking inducible nitric oxide synthase. *Shock.* 2008; 29:119–126. [PubMed: 17621253]
26. Sand, Ca; Starr, A.; Nandi, M., et al. Blockade or deletion of transient receptor potential vanilloid 4 (TRPV4) is not protective in a murine model of sepsis [Internet]. *F1000Research.* 2015; 4 Available from: <http://f1000research.com/articles/4-93/v1>.
27. Starr A, Sand Ca, Heikal L, et al. Over-expression of GTP cyclohydrolase 1 feedback regulatory protein is protective in a murine model of septic shock. *Shock.* 2014; 42:432–439. [PubMed: 25046538]
28. Wang Y, Wang DH. TRPV1 ablation aggravates inflammatory responses and organ damage during endotoxic shock. *Clin Vaccine Immunol.* 2013; 20:1008–1015. [PubMed: 23637043]
29. Xiao H, Remick DG. Correction of perioperative hypothermia decreases experimental sepsis mortality by modulating the inflammatory response. *Crit Care Med.* 2005; 33:161–167. [PubMed: 15644664]
30. Xu H, Wang Y, Garver H, et al. Vascular BK Channel Deficiency Exacerbates Organ Damage and Mortality in Endotoxemic Mice. *J Cardiovasc Pharmacol.* 2012; 59:207–214. [PubMed: 21997262]

31. Vlach KD, Boles JW, Stiles BG. Telemetric evaluation of body temperature and physical activity as predictors of mortality in a murine model of staphylococcal enterotoxin shock. *Comp Med.* 2000; 50:160–166. [PubMed: 10857007]
32. Ray N, Kuwahara M, Takada Y, et al. c-Fos suppresses systemic inflammatory response to endotoxin. *Int Immunol.* 2006; 18:671–677. [PubMed: 16569682]
33. Ganopoulos JG, Castellino FJ. A protein C deficiency exacerbates inflammatory and hypotensive responses in mice during polymicrobial sepsis in a cecal ligation and puncture model. *Am J Pathol.* 2004; 165:1433–1446. [PubMed: 15466407]
34. Souza LL, Duchene J, Todiras M, et al. Receptor MAS protects mice against hypothermia and mortality induced by endotoxemia. *Shock.* 2014; 41:331–336. [PubMed: 24430551]
35. Remick DG, Garg SJ, Newcomb DE, et al. Exogenous interleukin-10 fails to decrease the mortality or morbidity of sepsis. *Crit Care Med.* 1998; 26:895–904. [PubMed: 9590320]
36. Leon LR, White aa, Kluger MJ. Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice. *Am J Physiol.* 1998; 275:R269–R277. [PubMed: 9688988]
37. Ebong SJ, Call DR, Bolgos G, et al. Immunopathologic responses to non-lethal sepsis. *Shock.* 1999; 12:118–126. [PubMed: 10446892]
38. Remick D, Manohar P, Bolgos G, et al. Blockade of tumor necrosis factor reduces lipopolysaccharide lethality, but not the lethality of cecal ligation and puncture. *Shock.* 1995; 4:89–95. [PubMed: 7496903]
39. Newcomb D, Bolgos G, Green L, et al. Antibiotic treatment influences outcome in murine sepsis: mediators of increased morbidity. *Shock.* 1998; 10:110–117. [PubMed: 9721977]
40. Kort WJ, Hekking-Weijma JM, TenKate MT, et al. A microchip implant system as a method to determine body temperature of terminally ill rats and mice. *Lab Anim.* 1998; 32:260–269. [PubMed: 9718473]
41. Guarnieri M, Brayton C, DeTolla L, et al. Safety and efficacy of buprenorphine for analgesia in laboratory mice and rats. *Lab Anim (NY).* 2012; 41:337–343. [PubMed: 23079917]
42. Tubbs JT, Kissling GE, Travlos GS, et al. Effects of buprenorphine, meloxicam, and flunixin meglumine as postoperative analgesia in mice. *J Am Assoc Lab Anim Sci.* 2011; 50:185–191. [PubMed: 21439211]
43. Hugunin KMS, Fry C, Shuster K, et al. Effects of tramadol and buprenorphine on select immunologic factors in a cecal ligation and puncture model. *Shock.* 2010; 34:250–260. [PubMed: 20803785]
44. Guan S, Guo C, Zingarelli B, et al. Combined treatment with a CXCL12 analogue and antibiotics improves survival and neutrophil recruitment and function in murine sepsis. [Internet]. *Immunology.* 2014 Available from: <http://doi.wiley.com/10.1111/imm.12382>.
45. Vianna RCS, Gomes RN, Bozza FA, et al. Antibiotic Treatment in a Murine Model of Sepsis: Impact on Cytokines and Endotoxin Release. *Shock.* 2004; 21:115–120. [PubMed: 14752283]
46. Song S, Meyer M, Türk TR, et al. Serum cystatin C in mouse models: A reliable and precise marker for renal function and superior to serum creatinine. *Nephrol Dial Transplant.* 2009; 24:1157–1161. [PubMed: 19004848]
47. Herget-Rosenthal S, Feldkamp T, Volbracht L, et al. Measurement of urinary cystatin C by particle-enhanced nephelometric immunoassay: precision, interferences, stability and reference range. *Ann Clin Biochem.* 2004; 41:111–118. [PubMed: 15025800]
48. Herget-Rosenthal S, Marggraf G, Hüsing J, et al. Early detection of acute renal failure by serum cystatin C. *Kidney Int.* 2004; 66:1115–1122. [PubMed: 15327406]
49. Ho D, Zhao X, Gao S, et al. Heart Rate and Electrocardiography Monitoring in Mice. *Curr Protoc Mouse Biol.* 2011; 1:123–139. [PubMed: 21743842]
50. Xiao H, Siddiqui J, Remick DG. Mechanisms of mortality in early and late sepsis. *Infect Immun.* 2006; 74:5227–5235. [PubMed: 16926416]
51. Osuchowski MF, Welch K, Siddiqui J, et al. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. *J Immunol.* 2006; 177:1967–1974. [PubMed: 16849510]
52. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A.* 2013; 110:3507–3512. [PubMed: 23401516]

53. Remick DG, Bolgos GR, Siddiqui J, et al. Six at six: interleukin-6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. *Shock*. 2002; 17:463–467. [PubMed: 12069181]
54. Osuchowski MF, Connett J, Welch K, et al. Stratification is the key: inflammatory biomarkers accurately direct immunomodulatory therapy in experimental sepsis. *Crit Care Med*. 2009; 37:1567–1573. [PubMed: 19325479]
55. Shrum B, Anantha RV, Xu SX, et al. A robust scoring system to evaluate sepsis severity in an animal model. *BMC Res Notes*. 2014; 7:233. [PubMed: 24725742]
56. Huet O, Ramsey D, Miljavec S, et al. Ensuring animal welfare while meeting scientific aims using a murine pneumonia model of septic shock. *Shock*. 2013; 39:488–494. [PubMed: 23603767]
57. Saito H, Sherwood ER, Varma TK, et al. Effects of aging on mortality, hypothermia, and cytokine induction in mice with endotoxemia or sepsis. *Mech Ageing Dev*. 2003; 124:1047–1058. [PubMed: 14659593]

Figure 1.

Baseline physiology: heart rate and core temperature. C57BL/6 mice underwent laparotomy and implantation of a DSI HD-X11 telemetry device and were then monitored for 24 hours.

A, Heart rate. B, Core temperature. Each line represents the measurements of a single mouse (n=8).

Figure 2.

Physiology early after CLP: heart rate and core temperature. C57BL/6 mice underwent laparotomy, CLP, and implantation of a DSI HD-X11 telemetry device and were then monitored for 24 hours or until death. A, Heart rate. B, Core temperature. Each line represents a single mouse (n=15). Vertical red lines represent time points 6 and 12 hours after CLP.

Figure 3.

Physiology late after CLP: heart rate and core temperature. C57BL/6 mice underwent laparotomy, CLP, and implantation of a DSI HD-X11 telemetry device. Twelve mice surviving past the initial 24 hours after CLP were monitored for up to 7 days or until death. A, Heart rate. B, Core temperature. Each line represents a single mouse.

Figure 4.

Survival of mice after CLP. C57BL/6 mice underwent laparotomy, CLP, and implantation of a DSI HD-X11 telemetry device. Twelve mice were monitored for up to 7 days or until death.

Figure 5.

Biologic response to CLP. C57BL/6 mice underwent laparotomy, CLP and then were sacrificed at 6 (n=6) or 12 (n=6) hours after CLP. Serum was isolated and assayed for cytokine concentrations and Cystatin C. A, TNF α . B, IL-6. C, IL-10. D, Cystatin C. Boxplots represent 25–75% interquartile range (IQR). Whiskers extend to 1.5 IQR. Horizontal bar = median. Numerical values = mean.

Figure 6.

Population-based analyses of heart rate and core temperature. A, Heart rate (24 hours). B, Core temperature (24 hours). C, Heart rate (7 days). D, Core temperature (7 days).

Longitudinal measurements of heart rate and core temperature for each mouse were zeroed at either time point of death or time point of last observation. Population-based analyses using fractional polynomial methodology was performed to generate means and 95% confidence intervals of longitudinal panel data for the three cohorts: Sham control (Blue), CLP-Dead (Red), and CLP-Alive (Orange). Single data points are represented by individual

dots; average slope for each group is plotted with a bold line. Groups separated by color as indicated.

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Figure 7.

Construct validity of deterioration thresholds. C57BL/6 mice underwent laparotomy, CLP, monitor implantation and then were sacrificed in pairs: 1 mouse meeting deterioration threshold paired temporally with 1 mouse not meeting threshold but at identical time post-CLP (n=12 pairs). Serum was isolated and assayed for cytokine concentrations and Cystatin C. A, TNF α . B, IL-6. C, IL-10. D, Cystatin C. Boxplots represent 25–75% interquartile range (IQR). Whiskers extend to 1.5 IQR. Horizontal bar = median. Numerical values = mean.

Figure 8.

Animals meeting deterioration threshold have greater bacterial load. C57BL/6 mice underwent laparotomy, CLP, monitor implantation and then were sacrificed in pairs: 1 mouse meeting deterioration threshold paired temporally with 1 mouse not meeting threshold but at identical time post-CLP (n=6 pairs). Peritoneal washings and blood were obtained, serially diluted, and plated on 5% sheep blood agar. A, Peritoneal Fluid. B, Blood. Boxplots represent 25–75% interquartile range (IQR). Whiskers extend to 1.5 IQR. Horizontal bar = median. Numerical values = mean.

Table 1

Baseline murine biomarkers

	No Implanted Biotelemetry Device (n=6)	Implanted Biotelemetry Device (n=6)
IL-6 (pg/mL)	26.88 (0.89)	635.91 (212.01)
TNF- α (pg/mL)	< 1.88	18.34 (6.88)
IL-10 (pg/mL)	6.82 (0.66)	69.40 (17.8)
Cystatin C (ng/mL)	395 (7.31)	543 (26.09)

Values expressed as mean (s.d.)

Table 2

Comparison of parameters among matched CLP mice that did and did not meet acute physiologic deterioration threshold

	Met Threshold (n=12)	Did not meet threshold (n=12)	p value
<i>Inflammation</i>			
IL-6 (pg/mL)	26148 (15789)	10424 (6654)	0.01
TNF-α (pg/mL)	49.2 (52.3)	17.3 (10.5)	0.003
IL-10 (pg/mL)	1405 (1813)	407 (121)	0.005
<i>Organ Dysfunction</i>			
Cystatin C (ng/mL)	600 (163)	480 (154)	0.09
ALT (IU/L)	191 (29.8)	147 (18.7)	0.13
<i>Parameters of Shock</i>			
pH	6.95 (0.01)	7.02 (0.02)	0.047
HCO₃⁻ (mmol/L)	17.7 (0.65)	20.0 (0.72)	0.047
Base Excess (mmol/L)	-14.4 (0.75)	-11 (0.95)	0.03

Values expressed as mean (s.d.)