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PART II: MINIMUM QUALITY THRESHOLD IN PRE-CLINICAL SEPSIS STUDIES (MQTiPSS) FOR TYPES OF INFECTIONS AND ORGAN DYSFUNCTION ENDPOINTS.

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Abstract

While the clinical definitions of sepsis and recommended treatments are regularly updated, a systematic review has not been done for pre-clinical models. To address this deficit, a Wiggers-Bernard Conference on pre-clinical sepsis modeling reviewed the 260 most highly cited papers between 2003 and 2012 using sepsis models to create a series of recommendations. This Part II report provides recommendations for the types of infections and documentation of organ injury in pre-clinical sepsis models. Concerning the types of infections, the review showed that the cecal ligation and puncture model was used for 44% of the studies while 40% injected endotoxin. Recommendation #8 (numbered sequentially from part I): endotoxin injection should not be considered as a model of sepsis; live bacteria or fungal strains derived from clinical isolates are more appropriate. Recommendation #9: microorganisms should replicate those typically found in human sepsis. Sepsis-3 states that sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection, but the review of the papers showed limited attempts to

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document organ dysfunction. Recommendation #10: organ dysfunction definitions should be used in pre-clinical models. Recommendation #11: not all activities in an organ\system need to be abnormal to verify organ dysfunction. Recommendation #12: organ dysfunction should be measured in an objective manner using reproducible scoring systems. Recommendation #13: not all experiments must measure all parameters of organ dysfunction, but investigators should attempt to fully capture as much information as possible. These recommendations are proposed as “best practices” for animal models of sepsis.

Keywords

acute lung injury; acute kidney injury; animal models; endotoxin

INTRODUCTION

Preclinical sepsis models vary widely in terms of the pathogens used to induce sepsis, site of infection and how organ injury is quantified. This heterogeneity creates difficulties when comparing results from different studies. A 2017 review highlighted the need for using different pathogens and sources of infection in pre-clinical models of sepsis (1). While this prior paper did not propose a universal, standardized model of infection, it did advocate for creating standards in two specific areas. The first area concerns the types of infections, including the pathogens and the site of infection. The second area involves measurements of organ injury, effectively replicating the new Sepsis-3 definitions for patients (2) in pre-clinical models. A 2018 paper provided the scientific premise for attempting to create standardized sepsis models that recognized that a single model will not be sufficient to recapitulate the heterogeneity of sepsis (3).

To address these topics and other issues, an international Wiggers-Bernard Conference on Sepsis Modeling was organized in May 2017 in Vienna. The goal of that meeting was to identify the limitations of the pre-clinical models and to propose a set of guidelines, defined as the “*Minimum Quality Threshold in Pre-Clinical Sepsis Studies*” (MQTiPSS; the references to the executive summary in Shock will be inserted), to enhance the translational value of the available and future sepsis models. Prior to the conference, participants conducted a review of the literature between 2003 and 2012. We identified the 260 most highly cited scientific articles on sepsis models as basis for the conference discussions by six pre-defined working groups. The time period 2003 to 2012 was selected to allow sufficient time for the papers to be referenced. The conference used the concept that the most highly cited papers should provide the baseline information on the use of animal models, in effect “crowd sourcing” which papers were felt to be the most important. These 260 papers were referenced over 29,000 times in aggregate, demonstrating the power of this approach. The analysis of the pre-clinical sepsis literature revealed many inadequacies in the use of models of infection and organ dysfunction assessment protocols in sepsis research.

Overall, the Wiggers-Bernard initiative created three joint publications (insert the references to papers I and III) that we intend to serve as a MQTiPSS guideline for establishing the basic conditions in modeling of sepsis to improve their translational relevance. The current Part II

paper makes specific recommendations for preclinical models of sepsis within the areas of types of infections and organ injury endpoints. The goal of the conference was to create quality thresholds for future studies so that findings from models are more clinically applicable and the studies themselves may be more comparable between laboratories and species.

METHODS

The Wiggers-Bernard Conferences on Shock, Sepsis and Organ Failure is an opinion-exchange platform for international scientists organized by the Ludwig Boltzmann Institute of Experimental and Clinical Traumatology in the AUVA Research Center (LBI Trauma), Vienna, Austria (http://trauma.lbg.ac.at/en). The conference series was named after two outstanding scientists, one from the "New World" (Dr. Carl Wiggers) and one from the "Old World" (Dr. Claude Bernard) who devoted their careers to critical care medicine and experimental sciences. LBI Trauma is responsible for the topic selection while the Austrian Society of Advancement of Research in Shock and Tissue Engineering provides sponsorship for each Wiggers-Bernard conference.

To address the deficits regarding management guidelines and standardization in the field of pre-clinical sepsis research, in May 2017 LBI Trauma organized the 9th iteration of the Wiggers-Bernard Conferences titled: "*Pre-clinical Modeling in Sepsis: Exchanging Opinions and Forming Recommendations*". The key goal of the conference was to create publishable material that identifies essential elements that should be included in pre-clinical sepsis studies and defined by the MQTiPSS descriptor (4). A total of 31 experts from 12 countries (including five members of the Sepsis-3 definitions task force (2) were invited to participate in the initiative based on their experience in experimental, clinical and translational research.

The initiative consisted of three phases: a) three month preparatory phase where participants performed a systematic review of the 260 top cited publications from 2003–2012 and identified the key modeling topics to be discussed, b) discussions in Vienna (two days), during which the participants drafted a list of guidelines and c) post-conference refinement of the created works.

The preparatory phase review was conducted using ISI Web of Knowledge database (using the query: "*sepsis model*"). The 260 most cited papers (the citation range 50–743, over 29,000 citations in aggregate) featuring total of 374 animal experiments were identified. The time frame was subjectively defined as 10 consecutive years beginning with 2003 as the year of publication of the second iteration of sepsis definitions (2). The results of that survey pertinent to the topics covered in this paper are collated in Tables 1 and 3. Since the first analysis showed that mice were used in 79% of the 2003–12 papers, a secondary smaller search was performed and included all 2013–2017 studies (total of 190; irrespective of the number of citations) with mouse sepsis models only (using PubMed and the query: "*sepsis AND mice*"); to compare to selected endpoints reviewed in the main review that spanned 2003–2012. Overall, the preparatory phase aimed at identification of the most important concepts in animal sepsis modeling to be addressed at the Viennese Wiggers-Bernard

Conference. All participants were allocated into six specific thematic Working Groups (WGs): 1) Study Design, 2) Humane Modeling, 3) Infection Type, 4) Organ Failure/ Dysfunction, 5) Fluid Resuscitation and 6) Antimicrobial Therapy Endpoints. Both analyses were used during the meeting.

At the conference phase, each WG separately drafted a set of guideline points that formed the basis for discussion and refinement in WGs or dismissed from further consideration (day 1). After improvements, the proposed points were voted on by all participants to reach consensus (day 2). Overall, the Wiggers-Bernard Conference participants reached consensus on 29 points; 20 at “recommendation” strength and 9 at “consideration” strength (the WG-3/4 points are listed in Tables 2 and 4). Following the format used by the Sepsis-3 task force (2), at least 2/3 (over 65%) of the votes were required for approval of a proposed point. All consensus points were reached either unanimously or with no more than 2 abstentions per point (i.e. Recommendation 8). The “recommendation” strength indicates virtually unanimous agreement among the 31 participants, regarding both the content as well as the need for rapid implementation. Issues that require additional discussion before final recommendations could be made were classified as considerations.

In the post-conference phase, the work was primarily focused on the finalization of the MQTiPSS recommendations and considerations. This task was accomplished by teleconferences and electronic-based discussion among WGs using a modified Delphi method. Finally, a writing committee (formed at the conference) together with all participants developed an Executive Summary for MQTiPSS (insert the reference for the executive summary) and three full length publications (Insert the references for papers I and III). Each (of the three) publication focuses on two related working groups; the current Part II paper provides detailed discussion on the guideline points for Types of Infections and Organ Injury/Dysfunction Endpoints.

CHAPTER 1: TYPES OF INFECTIONS

Sepsis 3.0 defines human sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection (2). Septic shock is defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone (2). Thus, the presence of an infection constitutes one of the defining elements for the development and progression of sepsis. Infection in sepsis is usually bacterial, most often located in the lungs, peritoneum or urogenital system (5). When using animal models that replicate human sepsis, therefore, infectious challenges using bacteria are most appropriate and preferred over bacterial components such as molecules from the cell wall. The conference carefully reviewed the most highly cited papers using pre-clinical models of sepsis to ascertain the types of infection(s) that were used (Table 1). This analysis showed that most studies used the cecal ligation and puncture (CLP) peritonitis model (44%), followed by the LPS model (40%), and single infection models such as pneumonia (16%). Our subsequent smaller review of 190 murine sepsis studies in the years 2013–2017 showed that CLP was employed in 64% of the studies while LPS was the second most common model used in 23% (both as single and 2-hit combination). The popularity of the CLP model was obvious in this review, but the

extensive use of LPS (as a sepsis model) should be discouraged. Moreover, the relatively few studies using organ-specific sepsis models (lung, urogenital system, blood stream) resulted in a specific recommendation by the working group. The relatively simple model of septic peritonitis induced by CLP (6, 7) was viewed favorably, especially when performed in combination with resuscitation, antibiotics and infectious burden control. The model begins with gut flora, which may or may not be in a dysbiotic condition (8). This model replicates some but not all features of human sepsis (e.g. no acute lung injury (9)).

Specific infection models may be more informative, because they allow pathogen dosing, selection of the route of infection and strain of bacteria. Because not all animal species respond with the same intensity to given bacterial strains, some bacterial species and strains are only applicable in certain mammalian model systems (10).

The choice of the bacterial strain requires careful consideration. Although the bacterial isolates from septic patients differ between neonatal, pediatric and adult sepsis patients, a number of the same species are frequently found in clinical isolates. These include the Gram- negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacteroides fragilis* and the Gram-positive bacteria *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Using these species as infectious agents in sepsis animal models would be a logical choice. One should realize that many strains of a certain species are available to the researcher, however, in comparison to clinical isolates, these strains may differ greatly in their virulence (11), their capacity to form biofilms, and their antibiotic resistance profile (12, 13). Thus, working with bacterial isolates from sepsis patients, provided that these are well characterized and pure populations, may be a choice to consider. The route of infection is important and will depend on the specific interest of the study, but should also follow simple concepts, e.g. infecting an animal intratracheally with a bacterial species exclusively found in the lungs of human with pneumonia.

Specific recommendations for Types of Infections

The conference discussed several specific recommendations for preclinical models of sepsis. The following recommendations and considerations from the Types of Infections working group are numbered consecutively from the preceding companion paper Part I and start with recommendation 8.

Recommendation 8: We recommend that challenge with LPS is not an appropriate model for replicating human sepsis.

Lipopolysaccharide (LPS, endotoxin) is one of the most highly-studied molecules in modern medical history (13). LPS, derived from the cell wall of Gram negative bacteria, features many bacterial species-dependent variations. The toxin is inexpensive and can be reliably administered even by a junior researcher. The response to LPS in several mammalian species is robust, rapid and precisely characterized both in terms of pathophysiological reactions (tachycardia, fever, circulatory failure after high doses) as well as biochemical alterations (leukocytosis, cytokine release). Additionally, the LPS-induced response is highly reproducible, opening a defined and stable window of opportunity in which interventions can be tested (14). However, sepsis is a highly lethal clinical entity with a complex

pathophysiology that evolves over days rather than hours. Multiple consensus conferences were convened to develop a reasonably functioning diagnostic ‘umbrella’ definition for human sepsis. Of note, the report from the 1992 conference (15) acknowledged that there are multiple causative infectious agents for sepsis including bacteria but also parasites, fungi or viruses, exposing the misleading assumption of LPS as the central trigger in the onset of the disease.

The widespread use of LPS can be attributed, among other things, to the fact that its administration mimics some of the acute clinical features of the sepsis syndrome, including a systemic inflammatory response syndrome (e.g. fever, leukocytosis, cytokine release). Our evaluation of the most cited recent literature on animal sepsis models showed that LPS was used in one-third of the reviewed studies (Table 1). The current body of evidence, however, clearly demonstrates that administration of LPS should not be accepted as a relevant model of sepsis given the many differences between the sepsis phenotype and that induced by LPS. For example, the robust but transient character of the inflammatory response after LPS strongly differs from milder and protracted release of cytokines in the CLP model (16). Studies demonstrated that anti-TNF treatment was beneficial in an LPS but not a CLP mouse model (17). Furthermore, comparison of TNFα release profiles in human volunteers injected with a low LPS dose (18, 19) and severely septic patients (20, 21) shows that peak TNFα concentration in the latter group never exceeded the one in the former. An emergence of a new patient phenotype of “persistent critical illness” (PCI) (22, 23), further negates the translational usefulness of (acute) LPS-based protocols. At best, researchers may claim that some characteristics of the early septic reaction can be replicated by a challenge with LPS.

However, there continue to be studies using LPS as a model of sepsis. A researcher with limited time, money and perhaps understanding of clinical sepsis may be tempted to use LPS-based models, arguing that at least some patients with a Gram-negative infection causing sepsis have been shown to have an elevated level of LPS in the blood (24), and that such an LPS-exposure is an important part of the illness. Species differences in sensitivity to LPS exist (25), with humans being very sensitive. A way forward is to continue accepting data from research on LPS as relevant for the study of the first 24 hours of the septic response. However funding agencies and the readership should always be reminded of the unavoidable limitations in extrapolating any conclusions drawn in a study on LPS to clinical sepsis.

Recommendation 9: We recommend that microorganisms used in animal models preferentially replicate those commonly found in human sepsis.

Coze and Feltz (26) demonstrated for the first time in 1866 that the bacteria contained in the blood of a sepsis patient could infect and kill rabbits, providing a clear example of a clinical isolate used for a pre-clinical study. Currently, many investigators use laboratory bacterial strains rather than clinical isolates. Studies with these laboratory strains do not account for virulence genes (11) and the growing antibiotic resistance (27) that greatly impact sepsis severity and outcome (13, 28). Laboratory strains subcultured long-term (e.g. *E. coli* K12 and *P. aeruginosa* PAO1) may lose important pathophysiological characteristics and will fail to reflect “real world” pathogenesis (29). Bacterial genomes evolve during serial *in vitro*

passages as a result of specific growth conditions (29) and may actually become avirulent. Laboratory strains gradually lose their ability to form a biofilm reducing their pathogenic capability and antimicrobial resistance (30). Furthermore, virulent isolates and common laboratory strains can display major metabolomic differences (31), possess different capacity to bind host proteins, produce exotoxins and virulence factors. For example, laboratory Gram-negative bacteria may modify their endotoxin to produce reduced acylation associated with a poorly activating capacity compared to the LPS prepared from clinical isolates (32). Of note, conducting planktonic versus biofilm cultures of bacteria may also result in endotoxin modifications (33). Another important criterion that cannot be ignored is the viral reactivation observed in sepsis patients (34) since several synergies between asymptomatic viral infection and microbial products have been reported (35, 36).

However, pre-clinical sepsis modeling with non-identical pathogens should not be completely dismissed. In some cases, non-identical pathogens can produce similar disease phenotypes in animals when compared to patients. For example, a renal disease caused by a *Citrobacter* infection in animals is identical to the one caused by *E. coli*-produced Shiga toxin in humans. Similarly, *P. berghei* ANKA in C57BL/6 mice (partly) recapitulates cerebral malaria caused by *P. falciparum* in humans.

Consideration e) Consider modeling sepsis syndromes that are initiated at sites other than the peritoneal cavity (e.g. lung, urinary tract, brain).—Sepsis in patients may originate from infections in different body compartments. Over the past 20 years the sites of infection in humans have remained essentially unchanged, with lungs being the most frequent site followed by the abdominal cavity, urinary tract, and soft tissue (37, 38). In sharp contrast to these clinical data, nearly 60% of preclinical sepsis models rely on infections originating from the abdominal cavity (Table 1). As such, the poly-microbial models of CLP and colon ascendens stent peritonitis (CASP) or mono microbial settings including the direct intraperitoneal or intravenous injections of bacteria are frequently employed (38, 39). While these models provide important information, peritonitis models do not replicate the most frequent site of infection in patients (the lung), a problem has been addressed in prior publications (6, 40).

Considering these epidemiological clinical data, we propose that multiple preclinical models would be appropriate for the study of sepsis. The specific model should attempt to closely mirror clinical reality in order to identify relevant pathways and pathophysiological changes at specific body sites. A better understanding of the dysregulated pathways will aid in the design of more appropriate therapeutic interventions (1). Sepsis models where the infection occurs in the lung have the greatest clinical relevance and such infections should be initiated with causative organs such as *Streptococcus pneumoniae, Klebsiella pneumoniae*, or *Pseudomonas aeruginosa* (40)to potentially identify distinct features. In a similar fashion, models of urinary tract infection (41) or soft tissue infections, e.g. /Group A Streptococcus (42) will provide valuable information. Using different models with different sites of infection will allow the study of the natural course of infection(s) that may culminate in sepsis.

CHAPTER 2: ORGAN INJURY AND DYSFUNCTION

The 2016 Sepsis-3 clinical definitions succinctly states that sepsis is " … *life-threatening organ dysfunction caused by a dysregulated host response to infection*." (2). This definition provides an imperative for pre-clinical studies to include an evaluation of organ dysfunction. Measuring organ dysfunction in sepsis studies has the potential to provide insights into the pathogenesis. The relevance of a particular pathway dysregulated in sepsis will be more important if there is an established cause and effect relationship between that pathway and organ dysfunction. The conference participants did a careful analysis of whether the most highly cited papers documented organ dysfunction, and whether an organ injury scoring system was used. Unfortunately, many of the highly cited papers on sepsis did not measure organ dysfunction. As shown in table 3, out of the 260 most highly cited papers, only 204 (i.e. less than 60%) measured some aspect of organ dysfunction. Of these 204 papers, only 10 used a scoring system to quantify the injury, such as the quick Sequential Organ Injury Score (43). Looking at the table more closely, 77 papers using sepsis models examined the lungs but less than half (23 out of 77) included some measure of lung function, for example, contents of the bronchoalveolar lavage fluid. The American Thoracic Society has published guidelines on measurements of acute lung injury in animals (44), so criteria and methods are readily available. The renal system was studied in 48 papers in table 3, but none measured creatinine, an important element in the Risk Injury, Loss of kidney function and End stage kidney disease scoring system (45). Using clinical scoring systems similar to those used in patients would be a logical place to start, but there will need to be modifications so that the measurements accurately reflect organ dysfunction in experimental animals. Failing to measure organ dysfunction represents a lost opportunity to understand if the organs are injured in sepsis models. Additionally, measuring organ dysfunction will add to the scientific rigor of the experiments, an important scientific initiative (46).

Specific recommendations for Organ Injury and Dysfunction

The following recommendations from the Organ Injury and Dysfunction Endpoints working group are numbered consecutively from the preceding chapter and start with recommendation 10.

Recommendation 10: Organ/system dysfunction is defined as life threatening deviation from normal for that organ/system based on objective evidence.

Organ dysfunction may be defined as a maladaptive response to a potentially harmful danger signal or pathogen. Importantly, organ dysfunction caused by sepsis fundamentally differs from adaptive responses that attempt to minimize harm. It is essential to develop objective, readily measured criteria for dysfunction in individual organ systems and utilize these criteria as outcome variables.

Both clinical and experimental investigations in sepsis have focused on two outcome criteria; survival and/or biomarkers (47–50). In experimental animals, biomarkers are often the measure of immune function, such as cytokine concentrations (51), e.g., IL-6 (52, 53) or a white blood cell (WBC) count. Human biomarkers usually consist of composites of commonly measured variables such as vital signs or standard laboratory tests such as WBC

counts. For example, the systemic inflammatory response syndrome (SIRS) criteria measure body temperature, heart rate, respiratory rate, and the WBC count (15, 54, 55). The Sepsis – 3 clinical criteria re-directed the focus towards organ dysfunction by making it one of the two essential characteristics that define sepsis (2). Unfortunately, measures used to estimate the presence of organ dysfunction are poor, failing to provide specificity. For example the Sepsis - 3 task force used the Sequential Organ Failure Assessment (SOFA) score (56), a composite of clinical measures that has prognostic value (57), as a proxy for global “organ dysfunction”. However, the task force noted that points used by SOFA only linked to a given organ system and did not specifically identify dysfunction in that organ. Consequently, the task force also recommended derivation of an updated tool (2). Human studies are further hampered by the lack of “gold standard” criteria that unequivocally identify sepsis; instead surrogates that have either outcome (predicting mortality) or construct (identifying patients that develop characteristics that “look like” sepsis, e.g., refractory lung injury) validity are used to identify septic patients post-hoc (58, 59). Animal studies eliminate the need to use an outcome to identify sepsis because both infection and sepsis are known to be present. Therefore, preclinical models may be used to identify potentially translatable criteria for organ dysfunction. In addition to assessing the effects of interventions on survival and the development of the long-term abnormalities that plague sepsis survivors, animal studies provide a unique opportunity to evaluate organ dysfunction, which is now required for the clinical diagnosis of sepsis (2).

Recommendation 11: Not all activities in an individual organ/system need to be abnormal for organ dysfunction to be present.

While the progression of acute illness to Multiple Organ Dysfunction Syndrome (MODS) and the pattern of organ dysfunction has been addressed in clinical studies since the first description of the syndrome by Tilney et al. in 1973 (59), many animal studies only measure survival. However, quantifying conventional markers (platelets, creatinine, bilirubin etc.) of organ dysfunction similar to the SOFA score is feasible (60–63), even in rodents. If conventional biochemistry markers are used to describe organ dysfunction, they are typically only used as a single endpoint assessment, unlike the clinical SOFA score measured sequentially (the S in SOFA). Animal studies lack a validated and standardized panel of markers similar to SOFA.

The validity of conventional markers of organ impairment (platelets, creatinine, bilirubin etc.) to quantify the injury to cells and tissues in this context is augmented by the ability to study organ-specific dysfunction. This measurement of injury may be done using histology, immunohistochemistry and other modalities, allowing in depth analysis of organ integrity since tissue samples would be available. These techniques allow assessment of the severity of dysfunction, to gather insights into the mode of injury, such as necrosis as opposed to various forms of programmed cell death (64–66). In addition these techniques provide information regarding the spatial distribution of injury. The option of “reverse translation”, i.e. comparing patterns and severity of tissue damage in animal models, allows conventional clinical markers to be more useful when interpreting the degree of protection provided by a therapeutic intervention (61, 62, 65). Interestingly, in murine CLP it was shown that while parameters indicative of organ dysfunction were greater in dying compared to surviving

mice, they never exceeded the changes in surviving CLP mice in which cisplatin or carbon tetrachloride were used to induce non-lethal hepatotoxicity and nephrotoxicity (67). In other words, standard organ injury models which are non-lethal induce substantially more organ injury than that observed in mice dying from CLP-induced sepsis. Given the central role of the cardiovascular system in failing organs, a longitudinal assessment of clinical and hemodynamic parameters using wireless biotelemetry monitoring is particularly promising (68, 69). In the murine CLP model, telemetry has been used to define and validate criteria for acute deterioration to mimic human sepsis studies (69). In addition, in small animal models techniques applying *in vivo* microscopy allow assessment of microcirculatory disturbances beyond macrohemodynamic assessment even in internal organs such as liver and kidney (63). Extrapolating from patients, reduced organ function in individual organs would meet the definition of an inappropriate host response, albeit experimental studies suggest that several tissues and organs are only mildly affected. ICU-specific interventions, including mechanical and cardiovascular support, might be required to allow the development of severe MODS (70).

This specific recommendation states that not all functions of an organ need to be disrupted or abnormal in order to state that organ dysfunction is present. This recommendation is specifically included because pre-clinical models offer opportunities to study multiple parameters and provide tissue for in depth studies. The standard for organ dysfunction would be too high if it was required that all functions were abnormal.

Recommendation 12: To define objective evidence of the severity of organ and system dysfunction, a scoring system should be developed, validated and used, or use an existing scoring system.

In disease and pathological conditions in patients, where individual or multiple organs are involved e.g. sepsis, acute lung injury, or severe traumatic injury, diagnostic scoring systems have been developed and validated which describe the organ(s) morbid status. Scoring systems such as SOFA, qSOFA, SAPS, APACHE 2, (1, 2, 5) have been developed as an index of the organ(s) function. Such scoring systems largely serve to provide a uniform reference for the individual clinician's assessment of organ function. With this common basis, decisions can be made about the patient's status which drive clinical decisions. Databases of organ dysfunction have been created to allow study of the disease. In a similar manner, scoring systems could be developed for experimental models of sepsis to allow sharing of results. These scoring systems, similar to those used for patients, would consist of biochemical indices, physiological indices, blood chemistry, coagulation system status, discrete pathological changes (both gross and microscopic), and behavioral alterations. Comorbid conditions, such as age, gender, nutritional status, etc. could also be included (2, 56, 71–76). Such scoring systems have been especially useful for stratifying the severely injured (74, 75). Scoring systems for preclinical studies have not been applied in a consistent manner. Consequently, they provide limited data for detailing the clinical condition of sepsis they were attempting to model (4, 77). At the 9th Wiggers-Bernard- Conference on "Pre-clinical Modeling in Sepsis" we reviewed 260 manuscripts looking at experimental sepsis which included a total of 374 experimental studies that assessed the organ(s) for dysfunction. There were 116 published studies that examined only a single organ injury/

dysfunction endpoint, while 88 measured multiple organ dysfunction endpoints and 170 simply did not report any endpoints. Further, of those 374 experimental studies only 10 used some form of clinical-like scoring system, while 364 did not. Finally, of those 374 experimental studies 118 used histological assessment of organ damage and 256 did not. Although most of the recent studies did not use an organ injury score, guidelines have already been published on which parameters should be measured, such as the American Thoracic Society's guidelines on experimental acute lung injury (44). These guidelines were an attempt to encourage investigators to adopt standard approaches to measure dysfunction in experimental models of lung injury. Measuring a common set of organ injury parameters will allow better comparison between the animal models. If such guidelines exist or can be established through scientific consensus for not only an organ (72, 76), but at a system level, such as a rodent sepsis severity score (78), investigators attempting to document their animal model of sepsis should incorporate such scoring systems.

Recommendation 13: Not all experiments must measure all parameters of organ dysfunction but animal models should be fully exploited.

Conducting research and developing therapies and treatments for sepsis relies heavily on *in vivo* studies. Models of sepsis, however, can cause significant discomfort, pain, and distress to the animals. Therefore, investigators should attempt to collect as much information as their budgets allow and thus make the best possible use of the animals, while attempting to reduce the numbers of animals needed.

A number of simple behavioral and physiologic measurements can be obtained non-invasively and without the need for sophisticated equipment. Cage activity, body weight, and rectal temperature, for example, can be easily obtained and prior studies show that these correlate with hypothalamic levels of proinflammatory cytokines (79). Vital signs such as arterial blood pressure, heart rate, respiratory rate, and blood oxygen saturation can also be easily measured non-invasively (80). These vital signs can be used to indicate septic shock, increased sympathetic tone, metabolic acidosis, and pulmonary shunt. Furthermore, investigators can use a sepsis severity score for later correlation with other variables (78).

Maximizing sample collection is also recommended. For example, if an animal is sacrificed, plasma or serum should be collected, frozen, and stored for subsequent studies. Animals euthanized due to humane endpoints or at the end of the study should be subjected to necropsy, including external examination, exsanguination, exploratory dissection, gross anatomy, weighing and preservation of key organs, including the heart, lungs, spleen, gut, liver, and kidneys in fixative solution for later studies. Necropsy studies should be planned before each study, as some organs require unique collection and fixation techniques (81, 82). Ideally, the use of the animal data and samples can also be improved by collaboration among investigators in various fields. An investigator studying sepsis-associated kidney injury, for example, could share sepsis severity scores, blood oxygen saturation, and lung tissues with investigators interested in lung injury, as well as body weight, serum, and gut tissue with investigators interested in sepsis-associated gut dysfunction.

It is recognized that not all investigators will have the time, equipment or expertise to fully define dysfunction in each organ. For example, it is possible to measure pulse oximetry and

respiratory rate non-invasively in a mouse, although these measurements do not provide the same level of detail as arterial blood gases, whole body plethysmography and histologic examination of the lungs. However, if an investigator's studies focus on the kidney, it would be unreasonable to expect a detailed pulmonary study. It may be reasonable for the investigator to non-invasively measure blood oxygenation and respiratory rate.

Consideration f) Avoid hypoglycemia—Metabolic disorders occurring in sepsis often lead to hyperglycemia, which is frequently associated with worsened outcome in septic patients (83) and experimental models of sepsis (84). The definition of acute hyperglycemia varies by study ranging from 8 to 15 mmol/l (144 to 270 mg/dl) (85, 86). It has been shown that the severity of sepsis is impacted by the occurrence of hyperglycemia, which appears as a risk marker of morbidity and mortality (87). The latter is the reason for insulin therapy in septic patients. However, intensive insulin therapy has repeatedly been associated with increased incidence of hypoglycemia, defined as a blood glucose < 70 mg/dl (88). Two studies in large observational cohorts have identified sepsis as an important risk factor for the development of severe hypoglycemia predominantly due to insulin therapy (89, 90). Even a single episode of severe hypoglycemia can substantially influence the severity of sepsis (91) and significantly increase risk of mortality. Apart from hyperglycemia and hypoglycemia, increased glycemic variability has been associated with substantially increased mortality rate in septic patients (91–93). The effect of increased glycemic variability was unexpectedly high; it was associated with nearly 10 fold increased mortality in critical care patients (91).

The available data suggest that the levels of blood glucose critically influence the sepsis outcome and should be considered in preclinical models with particular attention to the glucose variability. If the effects of hyperglycemia and hypoglycemia are not in the scope of a study, then it can be recommended keeping glucose levels in a normal range (79.2 to 110 mg/dL), and the daily variations should neither drop below 70 mg/dL nor exceed 140 mg/dL.

However, the glycemic response in septic mice is different from that in patients. CLP mice typically develop a prolonged and profound hypoglycemia, whereas the hyperglycemia often reported in patients does not occur in rodents (94). Hypoglycemia in rodents was associated with increased sensitivity to insulin resulting in hypoglycemia at normal insulin levels (95). However, even in rodents that are hypoglycemic insulin reduces inflammation and multiple organ dysfunction (96). For these reasons investigators should consider preventing marked changes in blood glucose levels in pre-clinical models of sepsis. We recognize that other parameters are typically monitored in septic patients, e.g. blood pressure, urine output, and these issues should be addressed in future discussions.

SUMMARY

This Part II manuscript details the recommendations and considerations of the two working groups from the Wiggers-Bernard conference on pre-clinical models of sepsis. The intent was to determine the current state of pre-clinical models by reviewing the most highly cited articles on pre-clinical models. Analysis of the data showed great heterogeneity with regards

to types of models and how the animals were monitored to evaluate organ injury. The participants were aware of the 2016 Sepsis - 3 definition stating that sepsis was organ dysfunction due to an infection (2). The working group made specific recommendations about the types of infections as well as monitoring organ dysfunction. We hope that these recommendations and considerations will serve to bring a level of standardization to pre-clinical models of sepsis and ultimately improve the translation of pre-clinical findings. We acknowledge that new challenges based on new information from the clinical and bench studies will continue to arise. A close collaborative work between basic scientists and clinicians is critical for a thoughtful (re)interpretation of any existing and newly posited principles.

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References

1. van der Poll T, van de Veerdonk FL, Scicluna BP and Netea MG: The immunopathology of sepsis and potential therapeutic targets. Nat Rev Immunol 17(7):407–420, 2017. [PubMed: 28436424]
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Angus DC: The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 315(8): 801–10, 2016. [PubMed: 26903338]
3. Remick DG, Ayala A, Chaudry IH, Coopersmith CM, Deutschman CS, Hellman J, Osuchowski MF: Premise for Standardized Sepsis Models Shock In press(in press):in press, 2018.
4. Osuchowski MF, Thiemermann C and Remick DG: Sepsis-3 on the Block: What Does It Mean for Preclinical Sepsis Modeling? Shock 47(5):658–660, 2017. [PubMed: 28410332]
5. Angus DC and van der Poll T: Severe sepsis and septic shock. N Engl J Med 369(21):2063, 2013.
6. Dejager L, Pinheiro I, Dejonckheere E and Libert C: Cecal ligation and puncture: the gold standard model for polymicrobial sepsis? Trends Microbiol 19(4):198–208, 2011. [PubMed: 21296575]
7. Hubbard WJ, Choudhry M, Schwacha MG, Kerby JD, Rue LW, 3rd, Bland KI and Chaudry IH: Cecal ligation and puncture. Shock 24 Suppl 1:52–7, 2005. [PubMed: 16374373]
8. Lankelma JM, van Vught LA, Belzer C, Schultz MJ, van der Poll T, de Vos WM and Wiersinga WJ: Critically ill patients demonstrate large interpersonal variation in intestinal microbiota dysregulation: a pilot study. Intensive Care Med 43(1):59–68, 2017. [PubMed: 27837233]
9. Iskander KN, Craciun FL, Stepien DM, Duffy ER, Kim J, Moitra R, Remick DG: Cecal ligation and puncture-induced murine sepsis does not cause lung injury. Crit Care Med 41(1):159–70, 2013. [PubMed: 23222255]
10. Warren HS, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L, Tesini B, Cavaillon JM: Resilience to bacterial infection: difference between species could be due to proteins in serum. J Infect Dis 201(2):223–32, 2010. [PubMed: 20001600]
11. Mora-Rillo M, Fernandez-Romero N, Francisco CN, Diez-Sebastian J, Romero-Gomez MP, Fernandez FA, Mingorance J: Impact of virulence genes on sepsis severity and survival in Escherichia coli bacteremia. Virulence 6(1):93–100, 2015. [PubMed: 25654604]
12. Fux CA, Costerton JW, Stewart PS and Stoodley P: Survival strategies of infectious biofilms. Trends Microbiol 13(1):34–40, 2005. [PubMed: 15639630]

13. Seboxa T, Amogne W, Abebe W, Tsegaye T, Azazh A, Hailu W, Henriksen TH: High Mortality from Blood Stream Infection in Addis Ababa, Ethiopia, Is Due to Antimicrobial Resistance. PLoS One 10(12):e0144944, 2015. [PubMed: 26670718]

14. Chen TY, Warren HS, Greene E, Black KM, Frostell CG, Robinson DR and Zapol WM: Protective effects of anti-O polysaccharide and anti-lipid A monoclonal antibodies on pulmonary hemodynamics. J Appl Physiol (1985) 74(1):423–7, 1993. [PubMed: 8444723]

15. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Sibbald WJ: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine [see comments]. Chest 101:1644–1655, 1992. [PubMed: 1303622]

16. Remick DG, Newcomb DE, Bolgos GL and Call DR: Comparison of the mortality and inflammatory response of two models of sepsis: lipopolysaccharide vs. cecal ligation and puncture. Shock 13(2):110–6, 2000. [PubMed: 10670840]

17. Remick D, Manohar P, Bolgos G, Rodriguez J, Moldawer L and Wollenberg G: Blockade of tumor necrosis factor reduces lipopolysaccharide lethality, but not the lethality of cecal ligation and puncture. Shock 4(2):89–95, 1995. [PubMed: 7496903]

18. van der Poll T, Coyle SM, Kumar A, Barbosa K, Agosti JM and Lowry SF: Down-regulation of surface receptors for TNF and IL-1 on circulating monocytes and granulocytes during human endotoxemia: effect of neutralization of endotoxin-induced TNF activity by infusion of a recombinant dimeric TNF receptor. J Immunol 158(3):1490–7, 1997. [PubMed: 9013996]

19. van der Poll T, Coyle SM, Levi M, Jansen PM, Dentener M, Barbosa K, Lowry SF: Effect of a recombinant dimeric tumor necrosis factor receptor on inflammatory responses to intravenous endotoxin in normal humans. Blood 89(10):3727–34, 1997. [PubMed: 9160678]

20. Mera S, Tatulescu D, Cismaru C, Bondor C, Slavcovici A, Zanc V, Oltean M: Multiplex cytokine profiling in patients with sepsis. APMIS 119(2):155–63, 2011. [PubMed: 21208283]

21. Oberholzer A, Oberholzer C and Moldawer LL: Cytokine signaling--regulation of the immune response in normal and critically ill states. Crit Care Med 28(4 Suppl):N3–12, 2000. [PubMed: 10807312]

22. Gentile LF, Cuenca AG, Efron PA, Ang D, Bihorac A, McKinley BA, Moore FA: Persistent inflammation and immunosuppression: a common syndrome and new horizon for surgical intensive care. J Trauma Acute Care Surg 72(6):1491–501, 2012. [PubMed: 22695412]

23. Sauaia A, Moore EE, Johnson JL, Chin TL, Banerjee A, Sperry JL, Burlew CC: Temporal trends of postinjury multiple-organ failure: still resource intensive, morbid, and lethal. J Trauma Acute Care Surg 76(3):582–92, discussion 592–3, 2014. [PubMed: 24553523]

24. Levin J, Poore TE, Zauber NP and Oser RS: Detection of endotoxin in the blood of patients with sepsis due to gram-negative bacteria. N Engl J Med 283(24):1313–6, 1970. [PubMed: 5478453]

25. McCuskey RS, McCuskey PA, Urbaschek R and Urbaschek B: Species differences in Kupffer cells and endotoxin sensitivity. Infect Immun 45(1):278–80, 1984. [PubMed: 6376358]

26. Coze L and Feltz Vt: Recherches expérimentales sur la présence des infusoires et l'état du sang dans les maladies infectieuses. Gazette Medicale de Strasbourg 6:115–125, 1866.

27. Alicino C, Giacobbe DR, Orsi A, Tassinari F, Trucchi C, Sarteschi G, Icardi G: Trends in the annual incidence of carbapenem-resistant Klebsiella pneumoniae bloodstream infections: a 8-year retrospective study in a large teaching hospital in northern Italy. BMC Infect Dis 15:415, 2015. [PubMed: 26464061]

28. Wang JT, Hsu LY, Lauderdale TL, Fan WC and Wang FD: Comparison of Outcomes among Adult Patients with Nosocomial Bacteremia Caused by Methicillin-Susceptible and Methicillin-Resistant Staphylococcus aureus: A Retrospective Cohort Study. PLoS One 10(12):e0144710, 2015. [PubMed: 26690351]

29. Fux CA, Shirtliff M, Stoodley P and Costerton JW: Can laboratory reference strains mirror "real-world" pathogenesis? Trends Microbiol 13(2):58–63, 2005. [PubMed: 15680764]

30. Cassat J, Dunman PM, Murphy E, Projan SJ, Beenken KE, Palm KJ, Smeltzer MS: Transcriptional profiling of a Staphylococcus aureus clinical isolate and its isogenic agr and sarA mutants reveals global differences in comparison to the laboratory strain RN6390. Microbiology 152(Pt 10):3075–90, 2006. [PubMed: 17005987]

31. Bundy JG, Willey TL, Castell RS, Ellar DJ and Brindle KM: Discrimination of pathogenic clinical isolates and laboratory strains of Bacillus cereus by NMR-based metabolomic profiling. FEMS Microbiol Lett 242(1):127–36, 2005. [PubMed: 15621429]
32. Hajjar AM, Ernst RK, Tsai JH, Wilson CB and Miller SI: Human Toll-like receptor 4 recognizes host-specific LPS modifications. Nat Immunol 3(4):354–9, 2002. [PubMed: 11912497]
33. Ciornei CD, Novikov A, Beloin C, Fitting C, Caroff M, Ghigo JM, Adib-Conquy M: Biofilm-forming Pseudomonas aeruginosa bacteria undergo lipopolysaccharide structural modifications and induce enhanced inflammatory cytokine response in human monocytes. Innate Immun 16(5): 288–301, 2010. [PubMed: 19710099]
34. Mansfield S, Griessl M, Gutknecht M and Cook CH: Sepsis and cytomegalovirus: foes or conspirators? Med Microbiol Immunol 204(3):431–7, 2015. [PubMed: 25788396]
35. Fejer G, Szalay K, Gyory I, Fejes M, Kusz E, Nedieanu S, Duda E: Adenovirus infection dramatically augments lipopolysaccharide-induced TNF production and sensitizes to lethal shock. J Immunol 175(3):1498–506, 2005. [PubMed: 16034087]
36. Nansen A, Christensen JP, Marker O and Thomsen AR: Sensitization to lipopolysaccharide in mice with asymptomatic viral infection: role of T cell-dependent production of interferon-gamma. J Infect Dis 176(1):151–7, 1997. [PubMed: 9207361]
37. Opal SM, Laterre PF, Francois B, LaRosa SP, Angus DC, Mira JP, Group AS: Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. JAMA 309(11):1154–62, 2013. [PubMed: 23512062]
38. Wheeler AP and Bernard GR: Treating patients with severe sepsis. N Engl J Med 340(3):207–14, 1999. [PubMed: 9895401]
39. Lewis AJ, Seymour CW and Rosengart MR: Current Murine Models of Sepsis. Surg Infect (Larchmt) 17(4):385–93, 2016. [PubMed: 27305321]
40. Rittirsch D, Hoesel LM and Ward PA: The disconnect between animal models of sepsis and human sepsis. J Leukoc Biol 81(1):137–43, 2007. [PubMed: 17020929]
41. Olszyna DP, Florquin S, Sewnath M, Branger J, Speelman P, van Deventer SJ, van der Poll T: CXC chemokine receptor 2 contributes to host defense in murine urinary tract infection. J Infect Dis 184(3):301–7, 2001. [PubMed: 11443555]
42. Castiglia V, Piersigilli A, Ebner F, Janos M, Goldmann O, Dambock U, Kovarik P: Type I Interferon Signaling Prevents IL-1beta-Driven Lethal Systemic Hyperinflammation during Invasive Bacterial Infection of Soft Tissue. Cell Host Microbe 19(3):375–87, 2016. [PubMed: 26962946]
43. Wang JY, Chen YX, Guo SB, Mei X and Yang P: Predictive performance of quick Sepsis-related Organ Failure Assessment for mortality and ICU admission in patients with infection at the ED. Am J Emerg Med 34(9):1788–93, 2016. [PubMed: 27321936]
44. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Acute Lung Injury in Animals Study G: An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol 44(5): 725–38, 2011. [PubMed: 21531958]
45. Lopes JA and Jorge S: The RIFLE and AKIN classifications for acute kidney injury: a critical and comprehensive review. Clin Kidney J 6(1):8–14, 2013. [PubMed: 27818745]
46. Hsieh T, Vaickus MH and Remick DG: Enhancing Scientific Foundations to Ensure Reproducibility: A New Paradigm. Am J Pathol 188(1):6–10, 2018. [PubMed: 28958817]
47. Chavan SS, Huerta PT, Robbiati S, Valdes-Ferrer SI, Ochani M, Dancho M, Diamond B: HMGB1 mediates cognitive impairment in sepsis survivors. Mol Med 18:930–7, 2012. [PubMed: 22634723]
48. McCullough PA, Shaw AD, Haase M, Bouchard J, Waikar SS, Siew ED, Ronco C: Diagnosis of acute kidney injury using functional and injury biomarkers: workgroup statements from the tenth Acute Dialysis Quality Initiative Consensus Conference. Contrib Nephrol 182:13–29, 2013. [PubMed: 23689653]
49. Singer M: Biomarkers in sepsis. Curr Opin Pulm Med 19(3):305–9, 2013. [PubMed: 23411577]

50. Walley KR, Lukacs NW, Standiford TJ, Strieter RM and Kunkel SL: Balance of inflammatory cytokines related to severity and mortality of murine sepsis. Infect Immun 64(11):4733–8, 1996. [PubMed: 8890233]

51. Osuchowski MF, Welch K, Siddiqui J and Remick DG: Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. J Immunol 177(3):1967–74, 2006. [PubMed: 16849510]

52. Remick DG, Bolgos GR, Siddiqui J, Shin J and Nemzek JA: Six at six: interleukin-6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. Shock 17(6):463–7, 2002. [PubMed: 12069181]

53. Turnbull IR, Javadi P, Buchman TG, Hotchkiss RS, Karl IE and Coopersmith CM: Antibiotics improve survival in sepsis independent of injury severity but do not change mortality in mice with markedly elevated interleukin 6 levels. Shock 21(2):121–5., 2004. [PubMed: 14752284]

54. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Sccm/Esicm/Accp/Ats/Sis: 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Critical Care Medicine. 31(4):1250–6, 2003. [PubMed: 12682500]

55. Shapiro N, Howell MD, Bates DW, Angus DC, Ngo L and Talmor D: The association of sepsis syndrome and organ dysfunction with mortality in emergency department patients with suspected infection. Ann Emerg Med 48(5):583–90, 590 e1, 2006. [PubMed: 17052559]

56. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Thijs LG: The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 22(7):707–10, 1996. [PubMed: 8844239]

57. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, Blecher S: Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on “sepsis-related problems” of the European Society of Intensive Care Medicine.[see comment]. Critical Care Medicine. 26(11):1793–800, 1998. [PubMed: 9824069]

58. Seymour CW, Coopersmith CM, Deutschman CS, Gesten F, Klompas M, Levy M, Angus DC: Application of a Framework to Assess the Usefulness of Alternative Sepsis Criteria. Crit Care Med 44(3):e122–30, 2016. [PubMed: 26901560]

59. Tilney NL, Bailey GL and Morgan AP: Sequential system failure after rupture of abdominal aortic aneurysms: an unsolved problem in postoperative care. Ann Surg 178(2):117–22, 1973. [PubMed: 4723419]

60. Arulkumaran N, Sixma ML, Jentho E, Ceravola E, Bass PS, Kellum JA, Singer M: Sequential Analysis of a Panel of Biomarkers and Pathologic Findings in a Resuscitated Rat Model of Sepsis and Recovery. Crit Care Med 45(8):e821–e830, 2017. [PubMed: 28430696]

61. Coldewey SM, Rogazzo M, Collino M, Patel NS and Thiemermann C: Inhibition of IkappaB kinase reduces the multiple organ dysfunction caused by sepsis in the mouse. Dis Model Mech 6(4):1031–42, 2013. [PubMed: 23649820]

62. Ozer EK, Goktas MT, Kilinc I, Bariskaner H, Ugurluoglu C and Iskit AB: Celecoxib administration reduced mortality, mesenteric hypoperfusion, aortic dysfunction and multiple organ injury in septic rats. Biomed Pharmacother 86:583–589, 2017. [PubMed: 28024294]

63. Recknagel P, Gonnert FA, Westermann M, Lambeck S, Lupp A, Rudiger A, Bauer M: Liver dysfunction and phosphatidylinositol-3-kinase signalling in early sepsis: experimental studies in rodent models of peritonitis. PLoS Med 9(11):e1001338, 2012. [PubMed: 23152722]

64. Jia P, Wu X, Dai Y, Teng J, Fang Y, Hu J, Ding X: MicroRNA-21 Is Required for Local and Remote Ischemic Preconditioning in Multiple Organ Protection Against Sepsis. Crit Care Med 45(7):e703–e710, 2017. [PubMed: 28437377]

65. Shindo Y, Unsinger J, Burnham CA, Green JM and Hotchkiss RS: Interleukin-7 and anti-programmed cell death 1 antibody have differing effects to reverse sepsis-induced immunosuppression. Shock 43(4):334–43, 2015. [PubMed: 25565644]

66. Unsinger J, Kazama H, McDonough JS, Griffith TS, Hotchkiss RS and Ferguson TA: Sepsis-induced apoptosis leads to active suppression of delayed-type hypersensitivity by CD8+ regulatory

T cells through a TRAIL-dependent mechanism. J Immunol 184(12):6766–72, 2010. [PubMed: 20483771]

67. Drechsler S, Weixelbaumer KM, Weidinger A, Raeven P, Khadem A, Redl H, Osuchowski MF: Why do they die? Comparison of selected aspects of organ injury and dysfunction in mice surviving and dying in acute abdominal sepsis. Intensive Care Med Exp 3(1):48, 2015. [PubMed: 26215812]
68. Lambden S, Kelly P, Ahmetaj-Shala B, Wang Z, Lee B, Nandi M, Leiper J: Dimethylarginine dimethylaminohydrolase 2 regulates nitric oxide synthesis and hemodynamics and determines outcome in polymicrobial sepsis. Arterioscler Thromb Vasc Biol 35(6):1382–92, 2015. [PubMed: 25857313]
69. Lewis AJ, Yuan D, Zhang X, Angus DC, Rosengart MR and Seymour CW: Use of Biotelemetry to Define Physiology-Based Deterioration Thresholds in a Murine Cecal Ligation and Puncture Model of Sepsis. Crit Care Med 44(6):e420–31, 2016. [PubMed: 26862708]
70. Albuszies G, Vogt J, Wachter U, Thiemermann C, Leverve XM, Weber S, Barth E: The effect of iNOS deletion on hepatic gluconeogenesis in hyperdynamic murine septic shock. Intensive Care Med 33(6):1094–101, 2007. [PubMed: 17458540]
71. Biron BM, Ayala A and Lomas-Neira JL: Biomarkers for Sepsis: What Is and What Might Be? Biomark Insights 10(Suppl 4):7–17, 2015. [PubMed: 26417200]
72. Edmark C, McPhail MJ, Bell M, Whitehouse T, Wendon J and Christopher KB: LiFe: a liver injury score to predict outcome in critically ill patients. Intensive Care Med 42(3):361–9, 2016. [PubMed: 26820880]
73. Fan SL, Miller NS, Lee J and Remick DG: Diagnosing sepsis - The role of laboratory medicine. Clin Chim Acta 460:203–10, 2016. [PubMed: 27387712]
74. Knaus WA, Wagner DP, Draper EA, Zimmerman JE, Bergner M, Bastos PG, et al.: The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. Chest 100(6):1619–36, 1991. [PubMed: 1959406]
75. Le Gall JR, Lemeshow S and Saulnier F: A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 270(24):2957–63, 1993. [PubMed: 8254858]
76. Valette X and du Cheyron D: A critical appraisal of the accuracy of the RIFLE and AKIN classifications in defining “acute kidney insufficiency” in critically ill patients. J Crit Care 28(2): 116–25, 2013. [PubMed: 22981530]
77. Stortz JA, Raymond SL, Mira JC, Moldawer LL, Mohr AM and Efron PA: Murine Models of Sepsis and Trauma: Can We Bridge the Gap? ILAR J 58(1):90–105, 2017. [PubMed: 28444204]
78. Shrum B, Anantha RV, Xu SX, Donnelly M, Haeryfar SM, McCormick JK and Mele T: A robust scoring system to evaluate sepsis severity in an animal model. BMC Res Notes 7:233, 2014. [PubMed: 24725742]
79. Granger JI, Ratti PL, Datta SC, Raymond RM and Opp MR: Sepsis-induced morbidity in mice: effects on body temperature, body weight, cage activity, social behavior and cytokines in brain. Psychoneuroendocrinology 38(7):1047–57, 2013. [PubMed: 23146654]
80. Mella JR, Chiswick E, Stepien D, Moitra R, Duffy ER, Stucchi A and Remick D: Antagonism of the Neurokinin-1 Receptor Improves Survival in a Mouse Model of Sepsis by Decreasing Inflammation and Increasing Early Cardiovascular Function. Crit Care Med 45(2):e213–e221, 2017. [PubMed: 27632670]
81. Braber S, Verheijden KA, Henricks PA, Kraneveld AD and Folkerts G: A comparison of fixation methods on lung morphology in a murine model of emphysema. Am J Physiol Lung Cell Mol Physiol 299(6):L843–51, 2010. [PubMed: 20935232]
82. Williams JM, Duckworth CA, Vowell K, Burkitt MD and Pritchard DM: Intestinal Preparation Techniques for Histological Analysis in the Mouse. Curr Protoc Mouse Biol 6(2):148–68, 2016. [PubMed: 27248432]
83. Schuetz P, Kennedy M, Lucas JM, Howell MD, Aird WC, Yealy DM and Shapiro NI: Initial management of septic patients with hyperglycemia in the noncritical care inpatient setting. Am J Med 125(7):670–8, 2012. [PubMed: 22608986]

84. Singamsetty S, Shah FA, Guo L, Watanabe Y, McDonald S, Sharma R, McVerry BJ: Early initiation of low-level parenteral dextrose induces an accelerated diabetic phenotype in septic C57BL/6J mice. Appl Physiol Nutr Metab 41(1):12–9, 2016. [PubMed: 26624964]

85. Giugliano D, Marfella R, Coppola L, Verrazzo G, Acampora R, Giunta R, D'Onofrio F: Vascular effects of acute hyperglycemia in humans are reversed by L-arginine. Evidence for reduced availability of nitric oxide during hyperglycemia. Circulation 95(7):1783–90, 1997. [PubMed: 9107164]

86. Schierenbeck F, Wallin M, Franco-Cereceda A and Liska J: Evaluation of intravascular microdialysis for continuous blood glucose monitoring in hypoglycemia: an animal model. J Diabetes Sci Technol 8(4):839–44, 2014. [PubMed: 24876424]

87. Andersen SK, Gjedsted J, Christiansen C and Tonnesen E: The roles of insulin and hyperglycemia in sepsis pathogenesis. J Leukoc Biol 75(3):413–21, 2004. [PubMed: 14657207]

88. American Diabetes A: Standards of medical care in diabetes−−2010. Diabetes Care 33 Suppl 1:S11–61, 2010. [PubMed: 20042772]

89. Krinsley JS and Grover A: Severe hypoglycemia in critically ill patients: risk factors and outcomes. Crit Care Med 35(10):2262–7, 2007. [PubMed: 17717490]

90. Vriesendorp TM, DeVries JH, van Santen S, Moeniralam HS, de Jonge E, Roos YB, Hoekstra JB: Evaluation of short-term consequences of hypoglycemia in an intensive care unit. Crit Care Med 34(11):2714–8, 2006. [PubMed: 16943734]

91. Waeschle RM, Moerer O, Hilgers R, Herrmann P, Neumann P and Quintel M: The impact of the severity of sepsis on the risk of hypoglycaemia and glycaemic variability. Crit Care 12(5):R129, 2008. [PubMed: 18939991]

92. Ali NA, O'Brien JM, Jr., Dungan K, Phillips G, Marsh CB, Lemeshow S, Preiser JC: Glucose variability and mortality in patients with sepsis. Crit Care Med 36(8):2316–21, 2008. [PubMed: 18596625]

93. Preechasuk L, Suwansaksri N, Ipichart N, Vannasaeng S, Permpikul C and Sriwijitkamol A: Hyperglycemia and glycemic variability are associated with the severity of sepsis in nondiabetic subjects. J Crit Care 38:319–323, 2017. [PubMed: 28107738]

94. Hache G, Osuchowski M and Thiemermann C: Does Insulin Protect the Brain in Mice and Man with Sepsis? Shock 44(3):287, 2015. [PubMed: 26274365]

95. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Bouillon R: Intensive insulin therapy in the medical ICU. N Engl J Med 354(5):449–61, 2006. [PubMed: 16452557]

96. Dugo L, Collin M, Allen DA, Patel NS, Bauer I, Mervaala EM, Thiemermann C: GSK-3beta inhibitors attenuate the organ injury/dysfunction caused by endotoxemia in the rat. Crit Care Med 33(9):1903–12, 2005. [PubMed: 16148458]

Table 1.

Infection Type Endpoints in Sepsis Models (2003–2012*)

Types of sepsis-initiating infection	Specific pathogens used
CLP: 134	*Escherichia coli:* 33
LPS/E i.v.: 125	*Pseudomonas aeruginosa:* 19
LPS/Bacteria i.p.: 49	*Streptococcus pneumoniae:* 13
2-hit: 22	*Staphylococcus aureus:* 11
pneumonia: 20	*Streptococcus agalactiae:* 6
P/FP: 6	*Bacillus anthracis:* 5
CASP: 5	*Klebsiella pneumoniae:* 4
bacteria s.c.: 2	*Listeria monocytogenes:* 3
urosepsis: 1	*Salmonella typhimurium:* 3
bacteria p.o.: 1	*Streptococcus GBS:* 3
bacteria mucosa: 1	*Group A Streptococcus:* 2
bacteria urethra: 1	*Salmonella enteritidis:* 2
bacteria bile duct: 1	*Acinetobacter baumannii:* 2
bacteria intranasal: 1	*Candida albicans:* 2
malaria i.p.: 1	*Neisseria lactamica:* 2
fungemia via catheter: 1	*Neisseria meningitidis:* 2
bacteria (undefined) inj.: 3	*Plasmodium chabaudi: 2*
	Proteus mirabilis: 1
	Salmonella suis: 1
	Burkholderia: 1
	Salmonella enterica: 1
	Aspergillus fumigatus: 1
	Bacillus subtilis: 1
	*Citrobacter rodentium:*1
	polymicrobial (undefined): 2

*Collated data is obtained from review of the 260 most-cited papers (featuring total of 374 animal experiments) identified with ISI Web of Knowledge database (using the query:"*sepsis model*"). LPS: lipopolysaccharide; CLP: cecal ligation and puncture; LPS/E: intravenous (i.v.) endotoxemia via lipopolysaccharide or inactivated/live bacterial administration (any live strain); CASP: colon ascendens stent peritonitis; P/FP: pallet and/or fecal/fibrin peritonitis; 2-hit: two different challenges combined with at least one sepsis model included in the list. p.o.: *per os*; i.p. intraperitoneal; s.c.: subcutaneous; inj: injection

Table 2.

Infection Type Endpoints Working Group (WG): Recommendations (R) and Considerations (C)

Infection Types (WG-3)	**8.** We recommend that challenge with LPS is not an appropriate model for replicating human sepsis **9.** We recommend that microorganisms used in animal models preferentially replicate those commonly found in human sepsis	**R**
	i. Consider modeling sepsis syndromes that are initiated at sites other than the peritoneal cavity (e.g. lung, urinary tract, brain)	**C**

Table 3.

Organ Failure/Dysfunction Endpoints in Sepsis Models (2003–2012*)

Organ Injury/Dysfunction studied	Specific organs/systems studied	Any defined clinical-like scoring system used	Histology studied
yes: 204	lungs: 77	used: 10	yes: 118
a) single organ: 116	liver: 62	not used: 364	no: 256
b) multiple organs: 88	leukocytes: 7		
no: 170	kidney: 48		
	circulation: 36		
	spleen: 29		
	gut: 23		
	heart: 22		
	coagulation: 20		
	CNS/brain: 12		
	muscle: 6		
	mitochondria: 5		
	vasculature/endothelium: 4		
	pancreas: 3		
	metabolism: 3		
	skin: 3		
	adrenals: 1		
	thymus: 1		
	thyroid: 1		
	diaphragm: 1		

*Collated data is obtained from review of the 260 most-cited papers (featuring total of 374 animal experiments) identified with ISI Web of Knowledge database (using the query:"*sepsis model*").

Table 4.

Organ Failure/Dysfunction Endpoints Working Group (WG): Recommendations (R) and Considerations (C).

Organ Failure/Dysfunction (WG-4)	**10.** Organ/system dysfunction is defined as life threatening deviation from normal for that organ/system based on objective evidence	**R**
	11. Not all activities in an individual organ/system need to be abnormal for organ dysfunction to be present	
	12. To define objective evidence of the severity of organ and system dysfunction, a scoring system should be developed, validated and used, or use an existing scoring system	
	13. Not all experiments must measure all parameters of organ dysfunction but animal models should be fully exploited	
	f. Avoid hypoglycemia	**C**