

PART III: MINIMUM QUALITY THRESHOLD IN PRE-CLINICAL SEPSIS STUDIES (MQTiPSS) FOR FLUID RESUSCITATION AND ANTIMICROBIAL THERAPY ENDPOINTS.

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ABSTRACT

As outlined in the “International Guidelines for Management of Sepsis and Septic Shock: 2016”, initial fluid resuscitation and administration of antibiotics are key steps in the early management of sepsis and septic shock. However, such clear guidelines do not exist for pre-clinical sepsis models. To address these shortcomings, the Wiggers-Bernard conference on pre-clinical sepsis models was held in Vienna in May, 2017. The participants reviewed 260 of the most highly cited papers between 2003 and 2012 that used sepsis models. The review demonstrated that over 70% of experiments either did not use or failed to report resuscitation and/or antibiotic treatment. This information served as the basis to create a series of recommendations and considerations for pre-clinical sepsis models; this Part III report details the recommendations for fluid resuscitation and antibiotic treatment that should be addressed in sepsis models. Similar to human sepsis, fluid resuscitation is recommended in the experimental setting unless part of the study. Iso-osmolar crystalloid solutions are preferred. The administration route and its timing should be adjusted to the specific requirements of the model with preference given to dynamic rather than static hemodynamic monitoring. Pre-defined endpoints for fluid resuscitation and avoidance of fluid overload should be considered. Pre-clinical sepsis studies display serious inconsistencies in the use of antimicrobial protocols. To remedy this, antimicrobials are recommended for preclinical studies, with choice and dose adjusted to the specific sepsis model and pathogen(s). Ideally, the administration of antimicrobials should closely mimic clinical practice, taking into account the drug’s pharmacokinetic profile, alterations in absorption, distribution and clearance, and host factors such as age, weight, and co-morbidities. These recommendations and considerations are proposed as “best practices” for animal models of sepsis that should be implemented.

Keywords: sepsis; septic shock; sepsis model; Minimum Quality Threshold in Pre-Clinical Sepsis Studies (MQTiPSS); fluid resuscitation; antimicrobial therapy

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INTRODUCTION

“*Panta rhei*” (everything flows) - according to Heraklit (500 BC); even the ancient Greeks were aware of the importance of this aphorism. Although this was used in relation to the continuous fluctuation of nature, it also reflects the ongoing changes in humans and the necessity to maintain flow by fluid homeostasis. Sepsis is currently defined as a life-threatening organ dysfunction caused by dysregulated host response to infection (1;2). Consequently, eradication of the infection-causing microorganisms by source control and antibiotic treatment is mandatory. Septic patients can be rapidly identified as being more likely to have a poor outcome by the new bedside score termed quickSOFA (qSOFA) (2). Septic shock, as the most severe subset of septic patients, reveals circulatory and cellular/metabolic dysfunction associated with a higher risk of mortality (1). Septic shock patients can be clinically identified by vasopressor requirement to maintain a mean arterial pressure (MAP) of 65 mmHg (or greater) and a serum lactate level greater than 2 mmol/L (18 mg/dL) in the absence of hypovolemia (1). As outlined in the “International Guidelines for Management of Sepsis and Septic Shock: 2016”, initial fluid resuscitation and administration of antibiotics are the crucial steps in the early management of sepsis and septic shock in humans. To maintain an adequate circulation, resuscitation should begin promptly, using at least 30 ml/kg of crystalloid fluid *i.v.* within the first 3 hours, with frequent reassessment of hemodynamic status (3). The latest guidelines also recommend initiation of empiric *i.v.* antimicrobial therapy within one hour of sepsis/septic shock diagnosis (3). Notwithstanding the lack of confirmation of benefit from fixed resuscitation protocols such as early goal-directed therapy (EGDT) (ProCESS 2014; ARISE 2014; ProMISe 2015) (4-6), the use of appropriate antibiotic treatment and fluid resuscitation is beyond dispute. Nevertheless,

the potential risks of resuscitation-induced fluid overload (7;8) and increasing antimicrobial resistance should not be ignored.

To address the above topics, an international Wiggers-Bernard Conference on Sepsis Modeling was organized in May 2017 in Vienna. The goals of the meeting were to identify the limitations of pre-clinical models and to propose a set of guidelines, defined as the “*Minimum Quality Threshold in Pre-Clinical Sepsis Studies*” (MQTiPSS; reference to Executive Summary to be co-published in Shock), to enhance the translational value of current and future sepsis models. Prior to the conference, participants conducted a literature review of the 260 most highly cited scientific articles on sepsis models published between 2003 and 2012 as the basis for conference discussions within six pre-defined working groups. This scrutiny revealed many inadequacies in the use of fluid resuscitation and antimicrobial protocols. For example, over 70% of experiments either did not use or failed to report fluid resuscitation and/or antibiotic treatment (Tables 1 and 2). Such a discrepancy between experimental and clinical management significantly limits the scientific impact and translational relevance (9;10). This is amplified further by the lack of comparability among the available sepsis animal models due to the heterogeneity of their study design and management protocols (11).

Consequently, the Wiggers-Bernard initiative has led to the creation of three joint publications (references to Part I and II papers to be simultaneously published in Shock) to serve as a MQTiPSS guideline for establishing the basic conditions for modeling of sepsis to improve their translational relevance. This current Part III paper makes specific recommendations for preclinical models of sepsis concerning fluid resuscitation and antimicrobial treatment. 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 are better comparable across laboratories and/or 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 science. 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 preclinical sepsis research, in May 2017 LBI Trauma organized the 9th iteration of the Wiggers-Bernard Conferences addressing “*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 preclinical sepsis studies as defined by the MQTiPSS descriptor (12). 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, translational and clinical research.

The initiative consisted of three phases: a) a three month preparatory phase where participants performed a systematic review of the 260 top cited publications from 2003-2102 and identified the key modeling topics to be discussed, b) development of guideline points and subsequent discussion in Vienna over 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 the ISI Web of Knowledge database (using the query: “*sepsis model*”). The 260 most cited papers (the citation range 50-743; referenced over 29,000 times in aggregate), featuring a total of 374 animal experiments, were identified. The time frame was subjectively defined as 10 consecutive years beginning from 2003 as the year of publication of the second iteration of the International Sepsis Definitions (13;14). The results of that survey pertinent to the topics covered in this paper are collated in Tables 1 and 2. Since the first analysis showed that mice were used in 79% of the 2003-12 papers, a secondary smaller search was performed using PubMed and this included all 2013-2017 studies (total of 190; irrespective of the number of citations) focusing on murine sepsis models only (using the query: “*sepsis AND mice*”). This was used to compare selected endpoints within the 2003-2012 period. Both analyses were used during the meeting. Overall, the preparatory phase aimed at identification of the most important concepts in animal sepsis modeling to be addressed at the Vienna Wiggers-Bernard Conference. 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.

At the conference phase, each WG separately drafted a set of guideline points that was subsequently subjected to general discussion and streamlined either for further refinement in WGs or dismissal (day 1). After improvements, the proposed points were voted upon by all

participants to see if a clear consensus could be gained (day 2). Overall, the Wiggers-Bernard Conference participants reached consensus on 29 points; 20 at “recommendation” strength and 9 at “consideration” strength (the WG-5/6 points are listed in Tables 3 and 4). Following the format used by the Sepsis-3 task force (14), 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 (in the opinion of the participants) 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 arguments/narrative to be included in the final MQTiPSS publications. 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 (reference to Executive Summary to be co-published in Shock) and three full-size publications (references to Part I and II papers to be simultaneously published in Shock). Each (of the three) publication focuses upon the deliberations of two related WGs; this current Part III paper provides detailed discussion on recommendations made for Fluid Resuscitation and Antimicrobial Therapy Endpoints.

CHAPTER 1: FLUID RESUSCITATION

Sepsis is associated with disturbances in fluid homeostasis. In general, treatment guidelines recommend correction of hemodynamic abnormalities by administration of fluids as standard

care (16-18). There is increasing evidence that successful fluid therapy needs to be tailored to individual patients and/or their defined subgroups. Sepsis models closely mimicking the clinical setting need to implement fluid resuscitation to avoid cardiovascular deterioration to septic shock. Experimental studies provide evidence for the therapeutic efficacy of fluid resuscitation in improving a number of physiological variables and the septic shock state (19-21). However, the majority of findings have been obtained in models featuring a hypodynamic circulation. This makes direct data extrapolation to humans (and *vice versa*) difficult, given that septic patients typically develop hyperdynamic shock conditions (22). In animals, the definition of clinically relevant resuscitation volumes that mimic hyperdynamic sepsis is difficult, although such hyperdynamic models have been reported (23;24). This, and the compartmentalization and complexity of the pathophysiological responses seen during sepsis, call for clinically relevant animal models.

Although fluid administration protocols for sepsis are clinically well-established, our literature survey revealed that this therapeutic principle frequently lacks reverse translation to animal models. Indeed, 70% of the scrutinized experiments (years 2003-2012) lacked any fluid resuscitation regimen (Table 1). In those that reported fluid use in sepsis models, the majority relied upon a single dose of fluids to address the expected fluid imbalance (Table 1). Of note, in only 3% of the experiments a circulatory support such as catecholamines or specific organ support was used and adjusted to fluid resuscitation endpoints (Table 1). Our subsequent smaller review of 190 murine sepsis studies in the years 2013-2017 showed that fluid resuscitation was missing in 73% of the experiments. Of note, the lack of fluid administration can be justified in certain types of sepsis studies but their investigative premise should be always clearly delineated.

These survey data clearly underline the need to define various minimum quality recommendations (displayed in Table 2 and addressed in detail below). Summarizing, adequate fluid administration is crucial during sepsis. Due to the loss of endothelial integrity within the capillary bed, the restoration of intravascular volume and, in consequence, the enhancement of tissue oxygenation represents the major aim of fluid therapy.

Specific recommendations for Fluid Resuscitation

The conference discussed several specific recommendations for preclinical models of sepsis to advance the use of these models. The following recommendations and considerations from the Fluid Resuscitation Endpoints working group are numbered consecutively from the preceding companion papers and start with recommendation 14.

Recommendation 14: Fluid resuscitation is essential unless part of the study.

Since the importance of fluid administration has been clearly demonstrated in the clinical setting, and volume resuscitation is a central part of established current human therapies (3), the incorporation of fluid resuscitation into animal sepsis studies should approximate that given to human care, thereby increasing the clinical relevance of such models. A large body of evidence exists in support of this apparently obvious concept. Fluid administration should thus be an essential part of the experimental design in order to separate sepsis-related events from pathological events resulting solely from a circulatory decline due to protracted hypovolemia (25-27). Furthermore, cardiovascular parameters are important determinants of (micro)perfusion and organ function, and thus strongly influence the applicability of the model. Fluid administration remedies (at least partly) hypovolemia and anesthesia-induced perfusion pressure alterations. Application of more complex and invasive monitoring techniques enhances

comparability to the human setting (28-31). The hemodynamic profile of human sepsis is characterized by an initial hyperdynamic phase followed by a hypodynamic period. Thus, fluid resuscitation is needed in sepsis models to replicate the hyperdynamic cardiovascular state seen in the early, resuscitated phase of human sepsis (23;32;33). In addition, fluid resuscitation ensures a more standardized experimental environment, enables reproducible and comparable measurements, and is essential to ensure scientific quality (27;34). Concerning the 3Rs principles, fluid administration is an effective means of reducing animal suffering and unnecessary mortality (35;36).

Recommendation 15: Administer fluid resuscitation based on the specific requirements of the model.

In septic patients, the optimal rate and volume of fluid resuscitation still remain uncertain (37-39). In most animal studies fluid bolus treatment has generally improved sepsis survival (23;40). Experimental animals can receive variable amounts of fluids through a variety of administration routes including intravenous, subcutaneous and intraperitoneal. The different kinetics in the systemic volume load by these routes need appropriate consideration in animal sepsis experiments.

In small animal models with limited intravenous access both peritoneal and subcutaneous routes are frequently used for fluid resuscitation. Subcutaneous fluid bolus treatment, typically used in small animal models, simulates an intravenous continuous infusion rather than a bolus dose. For example, in geriatric patients subcutaneous rehydration has proved as effective as intravenous therapy (41). However, the subcutaneous route, despite its ease, has limitations, for example, the risk of variable absorption rates dependent on microperfusion disturbances

occurring during sepsis (42). In order to avoid electrolytes moving from the intravascular space to the extravascular (subcutaneous) space, isotonic solutions are recommended for subcutaneous application. Considering irritation at the application locus, an unbuffered 0.9% saline with a pH of 5.0 is irritating and painful when administered subcutaneously. Buffered systems such as lactated Ringer's solution (pH: 6.5) or Plasmalyte (pH: 6.5-8.0) are less irritating and thus recommended for subcutaneous administration. However, to closely simulate the clinical setting, fluid resuscitation should ideally be given via the intravenous route. The miniaturization of experimental equipment enables a feasible use of *i.v.* catheters even in small rodents. If an *i.v.* line is absent, fluid is recommended to be administered in intermittent repetitive doses, preferentially via an intraperitoneal route, to correct or prevent hypovolemia (35).

Recommendation 16: Consider the specific sepsis model for the timing of the start and continuation of fluid resuscitation.

In order to avoid both fluid deficit and fluid overload, appropriate monitoring of therapeutic interventions is needed.

Post- versus Pre-/Co-/administration. The timing of fluid resuscitation should consider the type of model and the objectives of the study. The 2016 SSC Guidelines recommended that treatment and initial fluid resuscitation should begin immediately on diagnosis (3). However, this occurs after presentation of a sick patient with sepsis and organ dysfunction, and not from the time of onset of the infection. Models of sepsis exhibit considerable time variability in the development of (patho)physiological responses, which may negatively affect the value of the obtained results, especially when the interventions are performed using fixed treatment regimens. Some

experimental interventions may be executed too early (e.g. before sepsis has developed), which likely results in some protective effects. In many models, the onset of fluid support is synchronized with administration of the infectious insult, which is justifiable only if the focus of study is on the transition state from pre-sepsis development to the early stages of sepsis and shock (43). In the clinical setting, patients rarely present *de novo* during the very early stages of sepsis, and the impact of fluid resuscitation in relation to timing requires further research. In this context, clinical studies do not illustrate the same uniformly positive response to fluid resuscitation. Post-treatment fluid resuscitation can be initiated at a later time point, e.g., after infection becomes clinically evident, thus more closely mimicking the downstream pathological characteristics of severe clinical sepsis (44). The use of implantable biotelemetry technology may help to identify in real-time the thresholds for acute physiologic deterioration (e.g., after CLP in rodents), enabling initiation of treatment at the precise point of physiologic deterioration (45). The impact of high-, intermediate-, and low-volume resuscitation regimens on cardiovascular performance, the development of edema, capillary leakage, organ damage, and overall outcome at various time points during sepsis all need investigation to fill the existing knowledge gap (25). Fluid therapy has a dose-effect dependency; side effects should be treated in the same way as other medications, with adjustments in timing, type and dosing of fluid (46). Overall, if the study design allows, fluid administration and/or vasopressor/inotropic support should be administered in a post-treatment (rather than a pre- or *co*-treatment) fashion, using predefined target endpoints, to avoid both fluid overload and ongoing hypovolemia.

Fluid Dosing according to hemodynamic targets. The goal of fluid resuscitation is to combat hypovolemia and restore perfusion (47). The 2016 SSC Guidelines recommend restoring euvoolemia with *i.v.* fluids, more urgently initially, and then more cautiously as the patient

stabilizes. Fluid challenge is advised as long as hemodynamic factors continue to improve (3). Unlike previous iterations, the new guidelines do not provide specific initial hemodynamic target values, with the exception of mean arterial pressure. A few studies have addressed the impact of resuscitation volume on sepsis outcomes, but have often focused on the impact of aggressive or high-volume fluid resuscitation (27).

Sepsis is characterized by vasoplegia with loss of arterial tone, vasodilation with sequestration of blood in the unstressed blood compartment, and changes in ventricular function with reduced compliance and reduced preload responsiveness. Data suggest that a physiologic, hemodynamically-guided restricted approach to fluid therapy would be prudent and could improve sepsis outcomes (44). Future research should focus on the design of hyperdynamic animal sepsis models (better recapitulating human sepsis), and on studies evaluating minimal fluid strategies in the resuscitation phase.

Hemodynamic monitoring. Hemodynamic monitoring guides not only therapeutic interventions, but also the diagnosis of shock, assessment of volume status, fluid responsiveness, and the need for vasopressor and/or inotropic support. However, only few experimental settings offer broad hemodynamic monitoring. For example, echocardiography in a murine sepsis model demonstrated that a hyperdynamic state could be achieved with adequate fluid resuscitation (23). In a fluid resuscitated long-term (3-day) rodent model of sepsis, outcome could be determined from early hemodynamic readouts. Significant differences in stroke volume and heart rate measured 6h post-insult predicted 3-day mortality with high accuracy (48). Other monitoring methods include: a) pressure-volume catheter measurements for comprehensive cardiac hemodynamics, b) transit-time volume flow measurement in blood vessels, c) ultrasound-dilution for cardiac function and blood volume measurements, d) non-invasive Doppler flow velocity

measurements to assess cardiac output, filling and ejection velocities and e) ultrasonic pulse wave velocity which can be determined in large and small animals. As a standard for freely moving rodents, radiotelemetry or mobile tethered systems can be used for blood pressure, oxygenation, temperature and other physiological parameters. Microcirculatory monitoring can be performed by sidestream dark field imaging, laser Doppler, or laser speckle contrast-based techniques, and is typically performed under anesthesia in a variety of vascular beds (49).

Dynamic versus static monitoring. SSC guidelines suggest that, when available, dynamic variables should be used over static variables to predict fluid responsiveness (3). Elevation of central venous pressure correlates with an exponential rise of pressures in the right atrium and thus CVP-driven protocols are at risk of causing cardiac failure (47). In a meta-analysis Marik et al. could not show that a static CVP value predicts fluid responsiveness (50). In addition, elevated CVP correlated to acute renal failure in sepsis (49). MAP-driven strategies also failed to show advantages over perfusion-driven protocols in reducing morbidity and mortality (51-54).

The decision whether to administer fluid or not can be best guided by using dynamic variables such as pulse pressure variation, stroke volume variation or the passive leg raising test (19-21;25;26), all of which can be realistically applied at present to anesthetized large animals only. Besides hemodynamic parameters, lactate clearance can be considered for the guidance of fluid therapy (55) as it correlates with the success of fluid therapy. Functional hemodynamics have been described to a very limited extent in animal models of sepsis; validation of this approach has been mainly reported in large animal models assessing the response to a fluid challenge. In both normo- and hypovolemic conditions of LPS-induced rat pneumonia, peripherally derived pulse pressure variation (PPV) was not reflected by centrally measured stroke volume variation (SVV) in the setting of increased total arterial compliance (56). In conclusion, it appears

reasonable to transfer the recent clinical findings in the field of intravenous volume therapy to animal models, with preference given to dynamic rather than static hemodynamic monitoring (57).

Recommendation 17: Resuscitation is recommended by the application of iso-osmolar crystalloid solutions.

Administration of 0.9% (“physiological”) saline may result in metabolic acidosis as a result of chloride overload (58;59). The mechanism of this so-called “hyperchloremic acidosis”, which occurs despite the alkalizing effect of hemodilution-induced hypoalbuminemia (58), is the result of the interplay between an extracellular strong ion difference and the concentration of non-volatile weak acids (58;60). Fluid resuscitation with saline aggravated organ injury and increased mortality in rodent models of hemodilution (61) and sepsis (59). This suggests that iso-osmolar balanced crystalloid solutions rather than saline should be used for resuscitation. Albeit its role in human sepsis/septic shock is not yet definitely settled (62), albumin should be the only colloid resuscitation fluid used, accompanied by adequate monitoring of proteinemia and/or albuminemia. Finally, given the fundamentally different metabolic response to stress in rodents (63-65), attention should be paid to avoid hypoglycemia. Depending on the underlying hypothesis to be investigated, vasopressor and/or inotropic support should be used to allow for “...*experiments with more advanced supportive care...*” which “...*would allow for better mimicry of ...multi-organ failure...*”(66).

Consideration g) Consider using pre-defined endpoints for fluid resuscitation.

The 2016 Surviving Sepsis Campaign recommends clinical examination, hemodynamic assessment, and the use of dynamic variables in estimating fluid responsiveness during the initial

fluid resuscitation of septic patients (3). In the 2008 Surviving Sepsis Campaign guidelines different endpoints for the initial resuscitation were mandated (67), such as a central venous pressure (CVP) of 8-12 mmHg, MAP >65 mmHg, and central venous or mixed venous oxygen saturation above 70% or 65%, respectively. Rivers et al. recommended the above static variables for the monitoring of fluid administration during sepsis (68), however their findings were not subsequently confirmed by several multicenter clinical trials (4-6). Of note, in the current SSC guidelines (3), individualized endpoints for fluid resuscitation are suggested in accordance with underlying comorbidities (e.g. such as arterial hypertension) in order to improve immediate outcomes. Additionally, in clinical practice guidelines for resuscitation from traumatic shock (69), the proposed resuscitation endpoints are categorized into global and regional. Adaptation of such complex reverse translation approaches to small laboratory animal models of sepsis is technically challenging; their daily use implementation does not appear realistic in the near future. Conversely, in larger animals (e.g. pig, sheep), dynamic responses rather than static monitoring (such as pulse pressure variation) may help to implement fluid resuscitation endpoints. In septic patients, for example, cardiac output monitoring, pulse pressure and stroke volume variation, and IVC diameter and stroke volume assessment by echocardiography have been suggested as dynamic predictors of responsiveness to fluids and to guide fluid administration (70). These and other measurement tools should be verified and utilized in large animal models of sepsis. This would not only allow testing new approaches for clinical translation but also ensure a parallel development of targeted fluid resuscitation strategy between septic patients and animals. The three recent EGDT randomized trials have not confirmed benefits of targeting specific physiologic parameters in a general population. Thus, the key clinical problem for fluid resuscitation remains in identifying proper endpoints that suit an

individual patient, and defining optimal thresholds for them. In this context, large animal sepsis models appear to constitute an ideal testing platform for determination of valid and reliable pre-defined endpoints and development of micro-technical tools for their monitoring. Properly designed animal models of septic shock testing various sets of pre-defined endpoints could meaningfully advance progress in this particular field.

Consideration h) Avoid fluid overload.

While the deleterious effects of fluid overload *per se* are well-established (8;47), there are no readouts available to define possible threshold values for fluid overload, in particular under conditions of sepsis-induced barrier dysfunction and increased vascular permeability. Clearly, fluid resuscitation may achieve a hyperdynamic hemodynamic state (23;27;32;33;71;72) and, thereby, more closely mimics the clinical scenario of resuscitated hyperdynamic sepsis or septic shock. Nevertheless, aggressive fluid resuscitation, despite a more stable, hyperdynamic circulation, aggravated organ dysfunction and ultimately increased mortality (71). This was most likely due to interstitial edema resulting from barrier disruption (73). Hence, investigators should pre-define the maximum amount of fluids to be administered, and, if hemodynamic target values are to be achieved, to incorporate vasopressor/inotropic treatment into the experimental design. Furthermore, the animal should be closely observed for the development of edema.

CHAPTER 2: ANTIMICROBIAL THERAPY

Antimicrobial therapy plays a central role in the clinical management of sepsis (3). The Surviving Sepsis Campaign (SSC) guidelines recommend that *i.v.* antimicrobials be administered as soon as possible, ideally within 1 hour of diagnosis (3). They further recommend that empiric broad-spectrum antimicrobials be administered to cover likely pathogens, and subsequently be

narrowed to cover identified pathogens based on their antimicrobial sensitivities (3). However, antimicrobials are not consistently used in animal sepsis studies. Indeed, our systematic review of the 260 most cited papers using sepsis models published between 2003-2012 suggest that antibiotics are underutilized. The majority of studies using infection models (74%) either did not use antimicrobials or did not describe their use. Carbapenems were used most frequently (50%), followed by other β -lactams (22%). Other agents included metronidazole (6%), aminoglycosides (5%), polymyxin (5%), fluoroquinolones (3%), vancomycin (2%), clindamycin (2%), clarithromycin (2%), and trimethoprim (2%). Our review of 190 murine sepsis studies in the subsequent 5 years (2013-2017) showed that antimicrobials were still only used in a minority of infection models (14%), with β -lactams being used most frequently (90%).

Consistent with clinical practice, we recommend that antimicrobials be considered for pre-clinical studies assessing potential human therapeutics (Table 4). The inclusion of appropriate antimicrobials should allow for assessment of such therapies under clinically relevant conditions. However, as discussed below, it is important to recognize that some antimicrobials can impact significantly on the host which should be taken into account when designing a given study. Finally, there may be experimental situations that make it unnecessary or even inappropriate to use antimicrobials, or that preclude use of a specific agent. Examples include studies testing the antimicrobial properties of an experimental agent, or mechanistic studies designed to understand a pathway or the role of a specific mediator.

Specific recommendations for Antimicrobial Therapy

The following recommendations from the Antimicrobial Therapy Endpoints working group are numbered consecutively from the preceding chapter and start with recommendation 18.

Recommendation 18: Antimicrobials are recommended for pre-clinical studies assessing a potential human therapeutic.

Adequate early source control and early administration of appropriate antimicrobials are considered central to the management of human sepsis (3). Source control is, however, rarely undertaken in preclinical sepsis studies, the majority of which involve peritoneal contamination with bacteria and abscess formation (e.g. CLP model). While the benefit of early, appropriate antimicrobials may not be so great as generally supposed (74), it is nevertheless a standard of care in clinical practice that antimicrobials should be administered promptly (3). Administration of antimicrobials is therefore recommended when studying putative therapeutics, as they are routinely administered in humans with sepsis (3). The routine administration of antimicrobials in sepsis models may alter the efficacy of the therapeutic agent being evaluated, perhaps offering synergism (75-80). Thus, the absence of an antibiotic treatment arm may potentially skew the final conclusions. Studies in animal models do show improved survival with antibiotic treatment (23;81-83). However, the impact is minimal or absent in aged animals (81), which are more reflective of human septic populations that are heavily skewed towards the elderly. It is also important to define an optimal dosing regimen to provide adequate but not excessive dosing of antimicrobials over the duration of the experiment, a topic that is also pertinent to human ICU patients (83;84). Shorter duration therapy has been shown to be effective in the CLP model (83). Antimicrobial dosing will likely be both species- and insult severity-dependent. This should ideally take into account the altered pharmacokinetics that occur during sepsis, for example related to altered metabolism and excretion, volumes of distribution and protein binding and, potentially, augmented renal clearance (85). Antibiotic pharmacodynamics are generally poorly understood in sepsis (85;86) and are not well characterized in animal models.

Antimicrobial toxicity is increasingly recognized, even in healthy subjects. Antimicrobials affect the microbiome, modulate inflammatory pathways and immune function, bind and neutralize bacterial toxins such as LPS, affect cellular metabolism and mitochondrial function, and can affect the CNS (82;87-93). These effects may be potentially amplified during sepsis. Antimicrobials have also been postulated to augment sepsis pathophysiology by generating Jarisch-Herxheimer reactions and cytokine release that are well-described with first dose administration, particularly of cidal antibiotics that destroy the bacterial cell wall (94). The large-scale, rapid release of cell constituents such as endotoxin and DNA can significantly enhance the host PAMP (pathogen-associated microbial pattern) inflammatory response. However, the functional relevance of antibiotic-induced endotoxin release in animal models is unclear (95). Although improving survival, cidal antibiotics temporarily increased inflammation and worsened acute kidney injury in an experimental sepsis model (82). Further study is needed in these areas to better understand the benefits and risks of antimicrobial therapy, and establishment of correct dosing regimens.

Recommendation 19: Antimicrobials should be chosen based on the model and likely/known pathogen.

In humans, the failure to provide appropriate antimicrobial therapies expeditiously has been associated with increased morbidity and mortality (96-98). Antimicrobials for animal studies should be chosen with careful attention to the particular model being utilized for a given study and the causative pathogen(s). The timing of the first dose of antimicrobials should also be chosen carefully (see also R-18), taking into account that the interval between the exposure (to pathogen) to the development of clinical infection varies between infections (e.g.: pneumonia, peritonitis, primary bacteremia, fungal infection). Thus, in some situations it may be appropriate

to provide antimicrobials early (e.g. a *Neisseria*-induced meningococcal model), whereas delayed administration may be appropriate for other models (e.g.: polymicrobial CLP peritonitis).

While it may not be feasible to fully recreate the antimicrobial choices given to humans, whenever possible it is recommended that the same or equivalent agents be used. The SCC guidelines recommend that antimicrobials be tailored to the pathogen(s), which vary widely between patients (3). A similar approach should be considered in animal sepsis models, with individualization of the antimicrobial regimen based on the likely specific pathogen(s). Several basic concepts of antimicrobial treatment follow.

Models involving mono-microbial bacterial infection should be treated with a single antibiotic that likely covers the pathogen. For instance, methicillin-sensitive *S. aureus* can be treated with an appropriate β -lactam, but methicillin-resistant *S. aureus* should be treated with vancomycin or similar. *E. coli* could be treated with a second or third generation cephalosporin or an aminoglycoside. Polymicrobial infections, such as would be expected to arise from bowel perforation (e.g.: CLP and CASP models) or fecal slurry injection, can be treated with either a broad-spectrum single agent such as a carbapenem, or a combination of agents that cover Gram-positive and Gram-negative aerobic and anaerobic bacteria. Fungal infections should be treated with an appropriate anti-fungal agent. Antimicrobial resistance for a given pathogen should be factored into decisions about antimicrobials.

The site of infection may also influence the choice of antimicrobial agent. Some antimicrobials are not effective for certain infections despite *in vitro* pathogen sensitivity. For instance, aminoglycosides are inactivated by low pH, and thus may not be effective for treating

abscesses (99). Similarly, antimicrobials that do not effectively cross the blood brain barrier may be inappropriate for CNS infections. Finally, whether an antibiotic is cidal *versus* static may also be an important factor; cidal antibiotics are often chosen for life-threatening human infections, and thus may be appropriate for animal models.

Finally, minimum inhibitory concentrations (MICs) are used by diagnostic laboratories to assess the resistance of microbes to antimicrobial agents (susceptible, intermediate-susceptible and resistant) (100). As compared with non-ICU settings, infections in ICU patients are often caused by pathogens with higher MICs. Often the MIC of a specific strain of bacteria is known. However, if the pathogen's MIC is not known, consideration should be given to defining the MIC prior to initiating a study. This certainly cannot be demanded within current standard experimental settings but could be considered when specific microbial research aims are tested.

Recommendation 20: Administration of antimicrobials should mimic clinical practice.

Whenever antimicrobials are included in a study, we recommend that their administration mimics clinical practice as closely as possible. The following factors should be considered when deciding on how to administer antimicrobials for a given study.

Route of administration: In the majority of small rodent sepsis studies antimicrobials are administered subcutaneously (typically with fluid resuscitation) and, in seldom cases, intraperitoneally (e.g. in peritonitis). Indeed, in our survey of animal sepsis studies published between 2003-2012, intravenous antimicrobials were only utilized in five (large animal) experiments. This differs from the standard of care for humans with sepsis, which is to deliver antimicrobials intravenously (3). For practical reasons, subcutaneous delivery of antimicrobials is often necessary for small rodent models given that an indwelling venous catheter may be

impractical, while repeated intravenous injection increases the burden on animals. However, it is recommended that antimicrobials be administered intravenously in studies using larger animals (e.g.: rabbits, pigs, dogs and non-human primates), in which more complex instrumentation is common.

Pharmacokinetic profile. Antimicrobial pharmacokinetics differ between species. For example, the elimination half-life of cephalosporins was shorter in mice and rats *versus* rabbits, dogs, and monkeys (101). Clearance of garenoxacin differed in rats, dogs, and monkeys (102), while absorption of moxifloxacin was more rapid in rats, dogs and humans, than in monkeys and minipigs (103).

Alterations in absorption, distribution and clearance of drugs. Numerous factors contribute to the altered pharmacology of antimicrobial agents in septic critically ill patients (104-108). These include an increased volume of distribution, altered protein binding, fluctuations in plasma clearance, the presence of edema which can limit the absorption of drugs, and drug-drug interactions (109-111). These alterations can lead to lesser or higher levels of drug exposure (108;112). Optimal antimicrobial dosing regimens for human sepsis have still not been established. For instance, although broad-spectrum β -lactam antibiotics are considered appropriate for the treatment of ICU-acquired pneumonia (113) optimal administration (e.g.: intermittent dosing versus continuous infusion) remains uncertain (114;115).

Host factors. Advanced age, sex, and co-morbidities are among the most important contributors to mortality in both septic patients and animal models (81;116-120). These factors impact upon pharmacokinetics and pharmacodynamics of antimicrobials, but this is poorly characterized in animal models. Preclinical sepsis studies employing two-hit models and/or various comorbidities

potentially constitute an attractive, clinically translatable testing platform for establishing the influence of such factors upon the efficacy of antimicrobial treatment regimens.

Consideration i) Antimicrobials should be initiated after sepsis is established.

For pre-clinical studies antimicrobial therapy should be initiated once sepsis is established, as is the case in humans, and should take into account evolving clinical practices. Factors to consider when deciding upon the timing of the first dose of antimicrobials include the severity and type of preclinical sepsis model (e.g.: pneumonia, peritonitis, bacterial, fungal, etc), and the animal species.

Time course of infection/sepsis in animals versus humans. Currently, many animal studies provide antimicrobials immediately or within a few hours following the infectious insult – the period in which clinical symptoms of sepsis are either absent or mild. However, patients are seldom treated in this early window given that antimicrobial treatment is typically triggered at the emergence of clear clinical symptoms. This makes the early administration of antimicrobials less replicative of the human condition (3). Furthermore, late provision of antibiotics starting 12h after severe infection has been reported to allow animals to develop organ dysfunction (44). This suggests that delayed dosing may reasonably replicate the human condition as well as modulating the severity of the sepsis model itself. As discussed in the Part I companion paper (chapter 1; reference to Part I paper to be simultaneously published in Shock), there is uncertainty about the time course of sepsis in animal models relative to humans. For instance, interspecies differences in the interval between exposure to a pathogen and the development of clinical infection are poorly understood. Factors that differ between species, such as metabolic rates (accelerated in healthy rodents compared to bigger species) and differences in leukocyte

distributions could profoundly affect responses to a bacterial insult. Additionally, quorum sensing bacteria may behave differently between species. Thus, it is conceivable that bacteria differentially express virulence genes and/or have different proliferation rates in different species. Finally, many animal studies use highly lethal models (e.g.: high doses of pathogen, 2-hit models) which leads to an earlier onset and more rapid progression of sepsis than seen in patients. These issues make it difficult to recommend definitive time-points for initiating antibiotics. Treatment should however be initiated soon after the animal manifests clinical signs of sepsis (e.g.: lethargy, decreased locomotion, changes in body temperature).

Evolving clinical practices. The evidence base underlying benefit from early antimicrobial administration has been criticized (74). A systematic review and meta-analysis showed no significant mortality benefit from administering antibiotics within 3 hours of emergency department triage or within 1 hour of shock recognition in severe sepsis and septic shock (121). Despite differences in conclusions of various studies, the current standard of care in patients is to provide the first dose of antimicrobials as early as possible after diagnosing sepsis (i.e.: organ dysfunction). It is thus reasonable to use a similar strategy for animal studies (122), particularly if the goal is to mimic current clinical practice. In future sepsis modeling scenarios, the administration of antimicrobials could be matched to the emergence of specific symptoms (that typically prompt evaluation/diagnosis in patients) rather than by the defined number of hours after an insult. There may be other experimental goals that factor into decisions regarding the timing of antimicrobials.

SUMMARY

This Part III manuscript details the recommendations and considerations of two of the six working groups from the 2017 Wiggers-Bernard conference on pre-clinical models of sepsis. Analysis of the top-cited pre-clinical sepsis papers showed substantial heterogeneity with regard to the use of fluid resuscitation and antimicrobial treatment. A number of factors come into play when deciding on antimicrobial and fluid administration in animal sepsis studies. These include the goals of the experiment, the specifics of the model (microorganism, site of infection, comorbidities such as renal or liver dysfunction, age), and the animal species being utilized. Whenever antimicrobial agents or fluids are administered in a preclinical study, we recommend their administration mimics clinical practice as closely as possible. It is hoped that the proposed set of recommendations and considerations will serve to bring a level of standardization to preclinical models of sepsis and, ultimately, improve translatability of preclinical 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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ACCEPTED

Table 1: Fluid Resuscitation Use in Sepsis Models (2003-2012*)

Fluid resuscitation: general	If fluid resuscitation used: frequency of application	Specific circulatory and/or organ support therapy^{&}
used: 111 not used/not stated: 263	1x: 58 2-6x: 14 7-9x: 4 >9x: 11 continuous i.v.: 8 as needed: 3 not stated: 14	used: 12 not used/not stated: 362

*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*”). [&]not fluid resuscitation.

Table 2: Fluid Resuscitation Endpoints Working Group (WG): Recommendations (R) and Considerations (C).

<p>Fluid Resuscitation (WG-5)</p>	<p>14. Fluid resuscitation is essential unless part of the study</p> <p>15. Administer fluid resuscitation based on the specific requirements of the model</p> <p>16. Consider the specific sepsis model for the timing of the start and continuation for fluid resuscitation</p> <p>17. Resuscitation is recommended by the application of iso-osmolar crystalloid solutions</p>	<p>R</p>
	<p><i>g. Consider using pre-defined endpoints for fluid resuscitation as deemed necessary</i></p> <p><i>h. Avoid fluid overload</i></p>	<p>C</p>

Table 3: Antimicrobial Use in Sepsis Models (2003-2012*)

Antimicrobials: general information	Antimicrobials: specific types and number of times used	Antimicrobials: frequency of application
used in infection models (IM): 51 not used/not stated in IM: 198 not applicable (LPS and non-live bacteria models): 125	carbapenem: 33 cephalosporin: 7 penicillin family: 7 metronidazole: 4 polymyxin: 3 aminoglycoside: 2 fluoroquinolone: 2 vancomycin: 1 clindamycin: 1 clarithromycin: 1 trimethoprim: 1 streptomycin: 1	1x: 19 2-6x: 9 7-9x: 7 >9x: 1 pre-operative: 4

*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.

Table 4: Antimicrobial Treatment Endpoints Working Group (WG): Recommendations (R) and Considerations (C).

Antimicrobial Therapy (WG-6)	18. Antimicrobials recommended for pre-clinical studies assessing potential human therapeutic 19. Antimicrobials should be chosen based on the model and likely/known pathogen 20. Administration of antimicrobials should mimic clinical practice	R
	<i>i. Antimicrobials should be initiated after sepsis is established.</i>	C

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