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Sepsis-3 on the block: what does it mean for pre-clinical sepsis modeling?

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Sepsis-3 Guidelines in a Nutshell

In February 2016, new definitions for sepsis and septic shock were published, the first update in more than 20 years (1) resulting from a rigorous evaluation of the literature. The validity of the criteria for the new definitions were tested against clinical databases to ensure that a patient fulfilling the criteria had an increased risk of death. The task force also recommended a lay definition of sepsis to better educate the public: “Sepsis is a life-threatening condition that arises when the body’s response to infection injures its own tissues.” This concise definition will assist basic scientists and clinicians to explain their work to the public, friends and families.

The clinical definition of sepsis was also succinct: “life-threatening organ dysfunction caused by a dysregulated host response to infection.” Severe sepsis was no longer considered to be a discrete entity as it was deemed ill defined. Organ dysfunction is defined as an increase in >2 points in the Sequential (Sepsis) Organ Failure Assessment (SOFA) score. An important aspect of the new definitions is a clear SOFA scoring system that allows rapid, unbiased determination that the patient’s disease has progressed so that they are at increased risk of death. The SOFA score is based on assessing the patient’s respiratory, coagulation, hepatic, cardiovascular, central nervous system (CNS) and renal systems. The score uses objective laboratory measurements except for the cardiovascular system where blood pressure and pharmacological support are the determinants and the CNS that uses the subjective (but reproducible) Glasgow coma score. Patients meeting the new criteria for sepsis have a 10% mortality. One significant challenge was the current lack of a diagnostic test for sepsis.

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Sepsis-3 Guidelines and Pre-clinical Modeling of Sepsis

While Sepsis-3 guidelines have fueled controversy in the high-profile clinical sepsis environment (see commentaries under original article), our attention turns to a somewhat overlooked aspect: Should Sepsis-3 drive meaningful change in pre-clinical investigations? Based on the translational premise alone, we view its influence as inevitable since animal models must be tailored to patients, not *vice versa*. The nature of in vivo experimentation gives us some enviable advantages over clinical science, i.e., it is known that the animals are septic and timeline/origin of infection are defined. Thus, the conundrum of the correct sepsis definitions frustrating our clinical colleagues does not cause us concern.

Yet, sepsis-3 brings a considerable shift regarding the experimental focus: we move away from inflammatory states (think SIRS/CARS) to organ failure (single, multiple) as the decisive factor. This shift, appropriate or not, exposes shortcomings of current animal sepsis modeling. We believe that the following issues are the most critical: 1) lack of an established scoring system for sepsis severity in general and/or for organ dysfunction/failure in particular (resembling clinical SOFA and other scores), 2) high variability of organ dysfunction phenotypes (among animal species/strains), 3) difficulty in reproducing/maintaining severe, ICU-grade of organ dysfunction for prolonged periods (due to resistance of animals to injury/infection and technical difficulties), 4) type I errors resulting from over-interpretation of organ-related readouts, 5) a frequently misplaced comparator approach focusing on the healthy vs. septic comparison (rather than surviving septic vs. lethal septic).

Starting simply: MODS-like score

The lack of a widely accepted in vivo organ injury scoring system resembling the ones used in clinics represents a very serious deficiency. Its implementation would enable better normalization and comparability of the wide array of animal MODS studies across laboratories. This need has been marginalized; only a handful of attempts to devise such a score(s) have recently emerged, primarily to eliminate death as an unethical endpoint. Huet and colleagues were first to propose “the mouse clinical assessment score for sepsis” (M-CASS), based on eight selected physiological/behavioral points and verified in *Klebsiella* pneumosepsis (2). Notably, M-CASS correlated well with the magnitude of organ damage/dysfunction (by AST, ALT, urea and creatinine) and inflammation. Soon after, two similar murine scores were tested in fecal-induced peritonitis (3) and cecal ligation and puncture (CLP) (4) models demonstrating a high predictive capacity for outcome. In the latter, biotelemetry-based measurements (i.e. heart rate, body temperature, mobility) approximate qSOFA criteria (blood pressure, respiratory rate, mentation) opening a possibility for such a pre-clinical score to serve an analogous role as clinical SOFAs.

Better standardization using score(s) based on organ dysfunction would also help categorize existing models based on severity and phenotype. Some species are preferable (due to clinical resemblance) to study certain types of system/organ deregulation (e.g. cardiovascular dysfunction in pigs with septic shock). Other models have been criticized given their high resistance to infection and technical limitations (e.g. mice). In rodents, monitoring of MODS/MOF is difficult but not impossible. Multiple repeated organ-related

readouts can be obtained non-invasively by echocardiography (5,6), telemetry (4), or novel micro-CT/magnetic resonance techniques (7). Repeated low-volume sampling of blood (5,8) and urine (9) is also possible.

Apart from the incorrigible species/strains variability, the hesitation of relying on organ dysfunction/failure may be due to the uncertainty whether MODS endpoints in animal models adequately reflect the true clinical MODS phenotype(s). This hesitation is additionally aggravated by excessive MODS-related conclusions featured in many pre-clinical publications. In animal sepsis studies, deterioration of organ function defined by incomplete, snap-shot readouts may be over-interpreted regarding its true impact upon the end sepsis phenotype and outcome (10). These concerns can be partially alleviated by a) performing confirmatory functional organ tests coupled with b) a robust study design, e.g., surviving vs. dying comparison. Several rodent studies incorporating the above elements demonstrated that reproducible sepsis models will produce MODS-related findings. For example, the dynamics of functional liver impairment were similar in patients and septic rats (5), while in two independent mouse studies, CLP led to a similar deterioration of renal function in mice predicted-to-die (10,11). These (and other) relevant sepsis modeling elements appear as a good starting point to create a model for MODS standardization, and could be used in the future to study the impact of pre-existing co-morbid conditions.

Getting organized: Minimum Quality Threshold in Pre-clinical Sepsis Studies (MQTiPSS)

Sepsis-3 should also serve as an incentive for an overdue pre-clinical advancement: developing a standardized approach in sepsis modeling to maximize its translational potential. Using Sepsis-3 as an example, creation of a working group (WG) focused on the pre-clinical guidelines appears rational. Its chief goal: creating recommendations for 1) the best modeling choices for particular sepsis syndromes (along with “must-do” study design elements) and 2) specific types of organ dysfunction (e.g. heart, kidney, liver) that satisfactorily resemble clinical scenarios. Such an undertaking would likely help to “clean up” the sepsis modeling act. Similar guidelines are effectively utilized in various disciplines. The Functional Genomics Data Society established clear-cut specifications for microarray (MIAME), qPCR (MIQE) and *in-situ* hybridization (MISFISHIE) experiments (http://fged.org/projects/) while the MouseAGE consortium (COST Action) recently published modeling recommendations for age-related diseases including sepsis (12). Formation of the proposed WG could be propelled by the European COST Actions (http://www.cost.eu/COST_Actions) and/or North American R13/U13 NIH programs (http://grants.nih.gov/grants/funding/r13/index.htm). The WG effort would primarily require a systematic scrutiny of already existing model data rather than launching new complex animal studies.

Hypothetical *MQTiPSS* are not meant to mimic the report-oriented ARRIVE guidelines. By integrating the existing knowledge on specific complexities in sepsis modeling, (updatable) *MQTiPSS* would recommend a suitable model-design quality a given animal sepsis experiment should implement to be deemed clinically relevant. Additionally, *MQTiPSS* guidelines could identify/encourage a package of optional experimental design “upgrades”

(e.g. outcome stratification (13), low-volume repeated sampling (8) that further enhance the translational value of a given sepsis study. In the MODS context, *MQTiPSS* would recommend reliable protocols for induction of dysfunction/failure of a given organ (either as single or multiple targets).

The ethical challenge

The focus on MODS-related endpoints triggered by the Sepsis-3 guidelines puts animal investigators on a collision course with the ever stricter regulations of use of animals in medical research (*vide* the EU directive 2010/63). There has been growing recognition of the importance of maximally translateable modeling designs and acknowledgement of organ dysfunction as a more useful endpoint compared to all-cause mortality. Both push pre-clinical sepsis research towards more complex and burdensome combinations of 2-hit, co-morbidity and chronic exposure protocols. Such protocols are in stark conflict with the policies promoted by the ethics of 3R professed by many countries (14). As the scientific community, we need to wisely select, justify and clearly communicate why such complex modeling choices are necessary and constitute a viable support of clinical research. Recommendations from a recognized working group emphasizing the importance of survival and multiple-hit models would likely make justification of such procedures to respective national institutional animal care and use committees more understandable and effective. Finally, development of systematic guidelines would effectively help reduce useless experimentation and deflect public criticism.

Conclusions

Animal sepsis experiments should approach the design and reporting quality demands of clinical studies. We strongly believe we must not only achieve those demands but also develop a systematic testing framework for sepsis modeling. Such a framework should rely on a high-quality approach in which a given therapeutic intervention is tested in a series of optimal (e.g. *MQTiPSS* pre-defined) sepsis models and across different species, possibly in an incremental order (e.g. mouse-rat-rabbit-pig). Pre-clinical findings subjected to such a systematic scrutiny would more reliably support decision-making for any subsequent animal-to-human transition. Failure to adopt robust modeling protocols will result in a continuous increase of “noise” (and confusion) in the scientific literature and further deviation of animal experiments away from the clinical reality and potential therapeutic relevance. An internationally-coordinated standardization effort in sepsis modeling would constitute a pro-active riposte to those mounting vulnerabilities.

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