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Different Preclinical Animal Models for Sepsis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Sepsis is a significant cause of illness and death, primarily due to the multiple organ dysfunction it can induce. Despite promising results in preclinical studies, most clinical trials testing new treatment strategies for sepsis have failed to show effectiveness. One possible reason for this discrepancy is the misinterpretation of preclinical data, particularly when using animal models that do not adequately mimic human sepsis. This review discusses the potential and limitations of various animal models used in sepsis research, aiming to determine the extent to which these findings are applicable to human sepsis. These animal models encompass different methods such as intravascular infusion of endotoxin or live bacteria, bacterial peritonitis, cecal ligation and perforation, soft tissue infection, pneumonia, and meningitis models. Animal models are crucial in developing new sepsis therapies because they provide essential information about pharmacokinetics, toxicity, and the mechanisms of drug action that cannot be obtained through

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other means. They offer insights into drugs and initial treatments interact with the body, assess the safety and efficacy of drugs, and investigate the underlying mechanisms of sepsis. However, it is important to acknowledge the limitations of animal models. Animals used in research may not fully replicate the complexity and heterogeneity of human sepsis, leading to differences in treatment response. Ethical considerations also restrict certain invasive procedures in human subjects that can be performed in animal models. These factors contribute to challenges in translating preclinical findings into successful clinical trials.

Keywords: Sepsis; cecal ligation and puncture model; lipopolysaccharide; colon ascendens stent peritonitis.

1. INTRODUCTION

Sepsis is a syndrome that can be fatal and has significant clinical symptoms such as pyrexia, hypotension, hyper-lactacidemia, coagulopathy, severe inflammation, and multiple organ dysfunction. The host's reaction to infection leads to the illness known as sepsis. Septic shock is sepsis connected to systemic arterial hypotension, while severe sepsis is sepsis that indicates acute multiple organ failure [1]. The WHO predicts that six million individuals died and over thirty million people get complications from septicemia annually; nevertheless, these numbers may be understated due to low disease monitoring in nations with low and middle incomes [2,3]. The past eighty years have witnessed the establishment of an experimental sepsis model to better comprehend the causes and mechanisms of actual sepsis. In rich countries, there are frequently fresh outbreaks of sepsis. For example, complications from septicemia contribute to thirty percent of every fatality in healthcare facilities in the USA [4], and in hospitals, infections are a significant manifestation of sepsis due to the emergence of antimicrobial drug resistance among the primary pathogens involved [5,6]. Sepsis usually comes on by infections caused by strains of *Escherichia coli, Staphylococcus aureus, K. pneumonia, A. baumannii, P. aeruginosa, and streptococci spp.,* yet it can also be caused by normal microbes when barrier integrity of tissues suffers [7-9]. Individuals older than 65, babies, immunocompromised individuals, and individuals with ongoing medical conditions (Malignancies, kidney problems, pulmonary conditions, etc.) have the greatest likelihood of getting sepsis [10]. To prevent the faulty functioning of host tissues like the pulmonary system, liver function. or the kidneys, it is necessary that pro-inflammatory and opposing anti-inflammatory processes are in homeostasis. To develop customized plans for therapy, it is also essential to categorize individuals. The first infection cannot be halted for some immune-compromised individuals, which leads to septic shock [11]. The infections can get to the host via different internal sites as the outcome of a broken barrier defense. The respiratory system and intestines are two of the access points that frequently arise. Sepsis can occur when pathogens penetrate the abdominal cavity if the intestines leak because of illness, trauma, or surgery, resulting in peritoneal inflammation. Therefore, through the past thirty years, multiple animal models which resemble the development of this kind of disease have been set up. Examples include "cecal ligation and puncture" (CLP) and "colon ascendens stent peritonitis" (CASP) [12]. Experimental animal models of sepsis have sparked a lot of discussions lately, specifically in context of the way they connect with human disease and are employed to create imaginative biological remedies [13]. While some argue that there are biological similarities, others emphasize the importance of carefully selecting appropriate models. It is not appropriate to assume that a single genetically homogeneous breed of rodents can fully represent the complex characteristics of sepsis in humans [14]. Approaches that cause all experimental animals to succumb swiftly and evenly are likely only comparable to just a few percent of sepsis patients, wherein mortality becomes more prevalent and more inclined to occur days instead of hours after the onset of the infection [15,16]. In addition, people with infections lack the privilege of obtaining healthcare prior to acquiring a life-threatening infection [17]. Regardless of the ease of testing, the accessibility of transgenic creatures, and the comparatively inexpensive price, mouse strains of sepsis are frequently used amongst the animal models of the condition. There are frequent changes, issues, and restrictions of the sepsis model so we offer an overview of the mouse models of sepsis that are currently available. Considering the variety and complexity of the disease, the notion that there is an ideal mouse model of sepsis is unrealistic; nevertheless,

based on the study's significance, there are actually plenty of excellent modeling alternatives available. This review provides an overview of the numerous experimental models of sepsis including an emphasis on their benefits and drawbacks. We are also going to focus on approaches that might assist the application of findings from animal experiments to sepsis in patients.

2. DIFFERENT MODELS OF SEPSIS

Experimental sepsis can often be caused by employing any of the three types of models: injection of a toxic agent (lipopolysaccharide known as LPS, CpG, zymosan, or another PRR ligand), the injection of live pathogens (bacteria or intestine material; induction of pneumonia, etc.), and deterioration of barrier integrity of tissues (intestinal perforation, wound sepsis models, etc.) [18]. The first group is a surgical method, and the next two groups are primarily minimally invasive or nonsurgical models. The different types of sepsis models are discussed in Table 1 along with their mechanism [19].

This Table 1 provides an overview of different types of animal models commonly used in sepsis research. The models are categorized based on the basic mechanism of induction, type of model (surgical or nonsurgical/chemical), and specific types of models within each category

2.1 Sepsis Induction by Impairing the Integrity of Barrier Tissue

These are the surgical methods in which the gut's architecture becomes compromised in such clinically applicable current models, mimicking polymicrobial conditions, and enabling microbiome constituents to penetrate the cavity of the peritoneum [20].

2.1.1 Cecal ligation and puncture (CLP)

Cecal ligation and puncture (CLP) mimic the medical scenario of an abdominal infection and polymicrobial peritonitis in appendicitis accompanying tissue damage as the infection foci have developed and microbes progressively penetrate the cavity in the abdomen [21]. In addition, the model exhibits various characteristics of sepsis, including the activation of both pro-inflammatory and anti-inflammatory immune responses, early hyperdynamic and late hypodynamic stages, multiple organ damage, hypothermia, metabolic changes, and a consistent pattern of inflammatory mediator response [22,23]. Unlike other methods that involve the administration of toxins or live pathogens, the cecal ligation and puncture (CLP) technique used in this simulation does not require such interventions. It allows for the preservation of the intestinal microbiota diversity by avoiding sample preparation. The progression of sepsis can be controlled by adjusting parameters such as the length of the ligated cecal region, the size of the needle used (18-25G), the number of punctures (to some extent), and by implementing infusion therapy, administering antibiotics, or simulating an appendectomy through the removal of the necrotic cecal region during a subsequent surgical procedure [24,25]. Mechanism involved in precipitation of sepsis by use of CLP model is demonstrated in Fig. 1 in which the puncturing and ligation of the cecum causes the leakage of various forms of bacteria inside the systemic circulation that releases endotoxin and thus the activation of TLR rdeceptor takes place and thus the phosphorylation of NFκB receptor takes place. This leads to the gene transcription of pro-inflammatory cytokines and the elevation of cytokines such as TNF-α, IL-1β, and IL-6 inside the body takes place. The variability in outcomes observed in different experimental settings can be attributed to several factors, including but not limited to: the extent of cecal ligation, the number and size of punctures made, the amount of stool released during cecostomy, the choice of anesthetic agent, the use of antibiotics (if any) and the specific drug regimen, the timing and volume of fluid resuscitation following the cecal ligation and puncture (CLP) procedure as shown in Fig. 1, the surgical expertise of individual operators in terms of operation duration and tissue trauma, and the dietary status of the mice (e.g., fasting or fed, type of feed provided) [26]. While these factors are relevant in most models, the severity and duration of organ injury, particularly acute kidney injury (AKI), may vary between young and aged mice [27-29]. The incidence of AKI tends to increase with time after CLP, particularly as the mice approach death. Furthermore, the aging process itself can lead to alterations in many biological processes, which may affect clinically significant outcomes [30].

Table 1. Three types of Sepsis models and their basic mechanism

S. No.	Basic Mechanism of Model	Method	Type of Model
1.	Sepsis Induction by compromising the integrity of barrier tissues	Surgical	**a)** Cecal ligation and puncture (CLP)
			b) Colon ascendens stent peritonitis (CASP)
			c) Cecal ligation and incision (CLI)
2.	Administration of Live pathogens for sepsis induction	Mostly by nonsurgical	**a)** Bacteria introduced in the body
			b) Intraperitoneal administration of cecal slurry or fecal solution
			c) Intraperitoneal implantation of a fibrin clot infected with bacteria
3.	Sepsis induction by the administration of toxic agents.	Nonsurgical/Chemical	**a)** Systemic LPS administration
			b) Lipopolysaccharide (LPS) and D-Galactosamine (D-GalN) induced

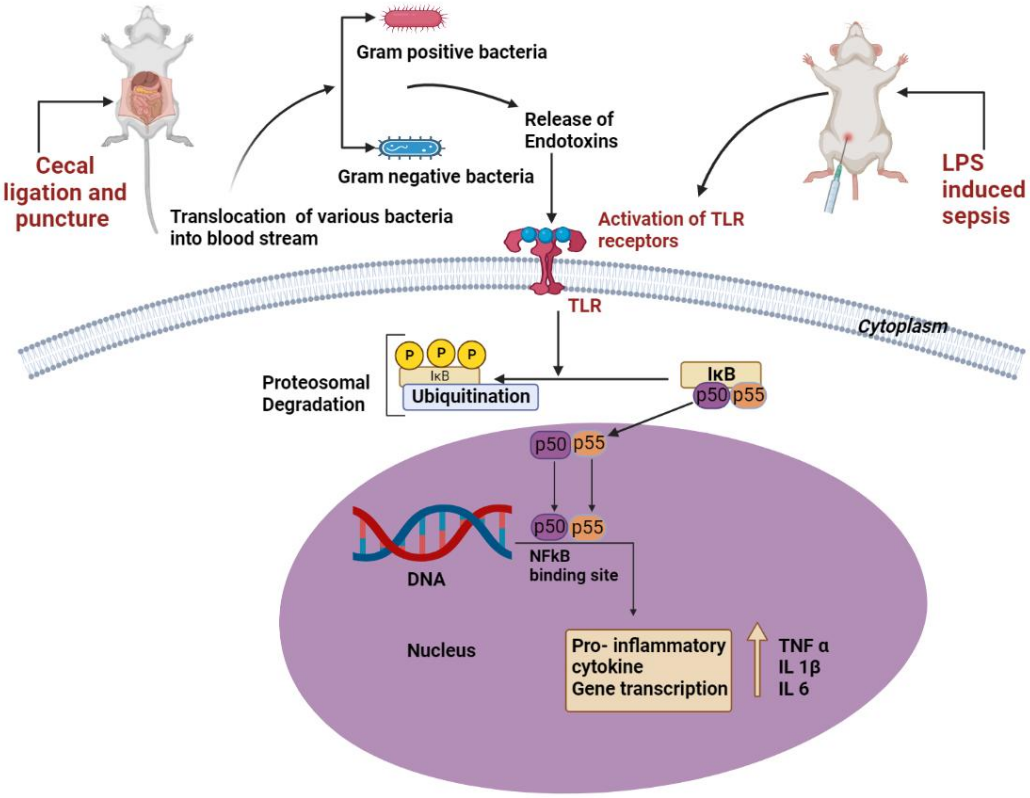

Fig. 1. Overview of Toll-like receptor signalling pathways in LPS and CLP-induced model of sepsis

(The external administration of lipopolysaccharides and the cecal ligation and puncture procedure both activate TLR, leading to the phosphorylation of IkB. Phosphorylated IkB then facilitates the translocation of P50 and p55 into the nucleus, where they bind to the NFκB binding site, triggering gene transcription and an increase in pro-inflammatory cytokines, ultimately contributing to the development of sepsis)

2.1.2 Colon ascendens stent peritonitis (CASP)

Contrary to CLP, the CASP model of peritoneal, polymicrobial sepsis is rarely employed. The "gold benchmark model" CLP was only lately displaced or rather complemented by this version. This shows unequivocally the continued reliance of the research community on CLP as a peritoneal sepsis model [31]. The model was just

recently entirely detailed, including a captivating and informative video [32]. A fixed stent (catheter) is inserted into the colon ascending by laparotomy during the procedure to induce CASP, to put it briefly. Faecal material is being milked into the stent out of the cecum and can eventually flow into the peritoneal space, inducing polymicrobial peritonitis or even sepsis [33]. The size of the stent, which varies from Fourteen gauge causes complete mortality to twenty gauge leads to partial lethality and reflects the degree of severity of the disease. As already mentioned, all the rodents who had a fourteen Gauge stent succumbed to the infection and died within inside first forty-eight hours after the procedure [34]. CASP does not seem to rely solely on TNF-α release as it is in the case of CLP [35]. TLRs, particularly the TLR2 receptor and TLR4, play a crucial part in the development of the natural immune system in the CASP model. In this experimental model, a rodent is first administered general anaesthesia, after which a midline laparotomy is performed. Subsequently, a plastic stent made from an IV catheter is inserted into the ascending colon of the rodent, approximately 1 cm away from the ileocecal border as shown in Fig. 2.

By connecting the plastic stent to the ascending colon using two separate sutures, a continuous passage of stools from the colon to the intestines is established. This setup leads to a persistent fecal outflow from the colon, resulting in bacteremia and the dissemination of microorganisms to distant organs, ultimately leading to extensive peritonitis [36,37]. This discovery may be beneficial for those with septicemia since severe hemorrhaging problems are common complications of some recombinant therapy. Together, these data points indicate the crucial role of CASP plays in mimicking real sepsis. The academic community, nevertheless, remains not convinced by its ongoing utilization [38]. Table 2 shows the characteristics of Cecal Ligation and Puncture (CLP) and Colon Ascendens Stent Peritonitis (CASP) Models in Sepsis Research [39-41].

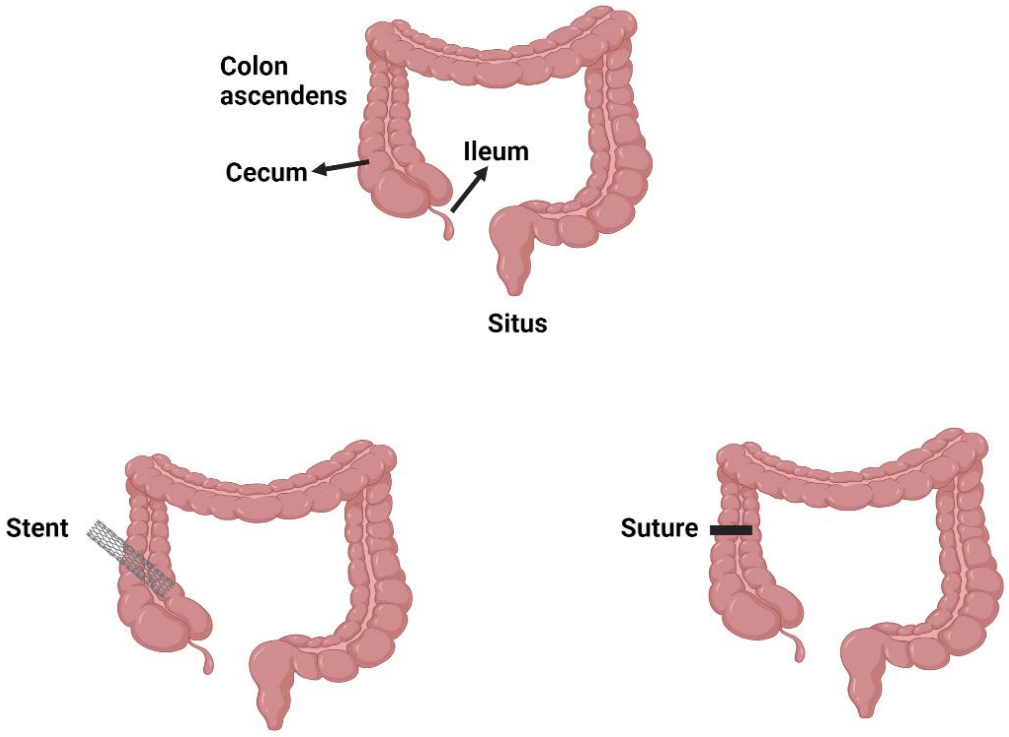

Fig. 2. Schematic representation of the CASP model

(A stent is intently installed into the ascending colon directly adjacent to the cecum to cause CASP. A catheter is attached to the healthy wall of the intestine throughout a sham procedure. After CASP induction (which takes less than 9 hrs), CASPI is determined by the surgical elimination of the stent)

Table 2. Characteristics of CLP versus CASP models of sepsis

Characteristics	Cecal ligation and puncture (CLP)	Colon ascendens stent peritonitis (CASP)
Advantages	Time consumption is less	SIRS can be predicted
	Resemble the human condition	Comparable stages of the disease to human
	Inflammatory cell analysis is feasible	Resemble the human condition
	Easy Procedure	Bacteremia and sepsis development can be assessed.
Disadvantages	Cecum undergoes necrosis	In respect to experimental skills, this approach is more challenging
	Limitation to the peritoneum and persistent bacterial spread	A high number of subjects are involved
	Invariability is highly observed	Time consumption is higher
	A high number of subjects are involved	Invariability is there but less compared to CLP
Best use of model	Investigation of the development of peritoneal abscesses alongside peritoneal penetration, inflammatory processes, and local intraperitoneal infection	Assess the course of sepsis, SIRS, and enterobacterial peritoneal infection

This Table 2 compares the characteristics of two surgical models commonly used in sepsis research: Cecal Ligation and Puncture (CLP) and Colon Ascendens Stent Peritonitis (CASP). The advantages and disadvantages of each model, as well as their best use, are outlined.

2.1.3 Cecal ligation and incision (CLI)

Recent research has shown that CLI resembles more of the acute phase of sepsis compared to the CLP model. CLI has not been frequently used, however, and its metabolic processes, hemodynamic, and immunological manifestations are still not sufficiently elucidated [42–44].

2.2 Administration of Live Pathogens to Induce Sepsis

Inducing sepsis through the administration of either Gram-positive (*S. aureus, S. pneumoniae*) or Gram-negative (*E. coli, K. pneumoniae, A. baumannii, and P. aeruginosa*) microorganisms can be done without conducting surgery and is a reproducible and low-invasive approach [45,46]. Mainly LPS induced approach is used for the induction of sepsis. Different bacteria frequently stimulate PRRs, because they fluctuate in LPS physiological activity, regardless of whether clinically isolates or laboratory variants are being utilized [47-49].

2.2.1 Bacteria introduced in the body

The model, in general, does a poor job of portraying the clinical picture of sepsis as there is no focal point of local infection where bacteria disseminate continually in a particular manner instead bacterial infection is achieved by a single huge injection. Additionally, particularly in cases of early sepsis, the cytokine profile has a quicker kinetics. Sepsis is frequently polymicrobial, but only one bacterial strain is frequently utilized [50]. In order to avoid endotoxemia, lesser amounts of bacteria should be administered; this can be accomplished by utilizing strains of bacteria that are especially harmful or by adding additional additives (such as sterilized feces). Depending on the strain of the bacterial infection, different pathways may contribute to the development of sepsis; for example, IFN enhances survival in infections caused by P. aeruginosa and S. pneumoniae despite decreasing mortality in infections caused by S. aureus and E. coli [51,52]. The pathogen's means of administration may also have an impact on the pathogenesis mechanism; for example, IL-10 has an inhibitory effect when microorganisms are administered intraperitoneally but increases disease progression when bacterial pneumonia is induced. Different organisms can be differentially affected by bacteria; for instance, mice are more easily infected by Salmonella typhimurium than humans [53].

2.2.2 Implantation of a bacteria-laden fibrin clot

Unlike the systemic administration of bacteria, the method of placing a fibro plug filled with bacteria in the peritoneal space involves a surgical procedure performed under general anesthesia. This approach leads to a continuous release of bacteria over time [54]. The inflammatory response kinetics, hemodynamic, and metabolic alterations replicate the medical scenario for microbial peritonitis [55]. The approach is appropriate for studying early antibiotic use throughout pathological progression. Only when the laparotomy and preparation of the bacteria-laden clot are standardized then the results of the model become reproducible [56]. By modifying the fibrin clot thickness and choosing the right quantity of microbes, one can regulate the pace at which sepsis occurs [57]. In addition, through the elimination of the clot with a second procedure, peritonitis advancement may be blocked [58].

2.2.3 Intraperitoneal injection of a fecal solution or cecal slurry (CS)

As specimens must be initially examined and their bacterial makeup should be established, the models are easier to collect and provide a low-invasive way to trigger polymicrobial sepsis [59-61]. Massive intestinal contents injection, in contrast to conventional sepsis, may cause a potent immune response that results in either early mortality or a complete recovery [62]. The models have variable expression patterns of genes and cytokine production profiles, as well as do a poor job of mimicking the hemodynamic and metabolic changes during infection [63]. Although it partially fixes the matter of sample consistency in some assessments, freezing the product about to be delivered kills some sensitive cultures [64]. When mice become resistant to their own microbiota, an extra adjuvant, such as barium sulfate, is needed [65].

2.3 Sepsis induction by Administering Toxins

The induction of low-invasive sepsis with toxic substances, the administration of pathogen-associated molecular patterns (PAMPs) such as zymosan, LPS, peptidoglycan, lipoteichoic acids, etc., is often necessary [66]. The two most used methods are direct LPS toxicity, which involves systemic administration of LPS, and acute liver toxicity, which involves systemic infusion of LPS combined with D-GalN [67].

2.3.1 Administration of LPS

These regulated accurate models imitate certain features of endotoxemia or the initial stages of Gram-negative sepsis, such as not having an infection determination, expansion of a hypo-dynamic phase with no earlier hyper-dynamic phase, acidosis caused by lactic acid, the immediate and plentiful release of cytokines that are proinflammatory, higher levels of DAMPs, and strong reactivation of innate immune reactions. In addition, meningococcemia, bacterial infection, and antimicrobial treatment have been linked with elevated blood LPS levels, rendering the simulations clinically relevant [68]. The major stimulants of sepsis in the animal models are cytokines that are anti-inflammatory and distributed upon activating the TLR4 signaling system, and their generation corresponds with the extent of sepsis in the models and patients with the condition [69-71]. In modifying the LPS dosage or employing LPS formulations with various biological activities, the immune response can be regulated [72,73]. It ought to be highlighted that different species significantly vary in their LPS susceptibility. As an example, in comparison to mice, humans have multiple times magnitudes and more prone to LPS [74].

2.3.2 Systemic administration of LPS in combination with D-GalN

In contrast to the direct LPS toxicity model, D-GalN exposure enables numerous orders of magnitude reductions in the amount of LPS required to trigger sepsis in mice without resulting in fatalities [75]. The LPS/D-GalN acute liver damage model offers the advantages of being affordable, simple to acquire, highly reliable, and simple to calibrate. The activation of TLR4 in hepatic resident macrophages in Kupffer cells, LPS/D-GalN administration causes inflammation and cirrhosis of the liver by inducing the production of cytokines that promote inflammation, particularly TNF-alpha. The inhibition of NF-kappa B in Kupffer cells causes the depletion of liver damage [76,77–79]. Since D-GalN can only be metabolized in hepatic cells, its administration raises the liver's sensitivity to TNF, and stimulation of the TNF-Receptor signaling cascade causes hepatocyte death [80-82]. Despite the way by which hepatocyte apoptotic cell death affects the production of inflammatory mediators is still not fully understood, it has been discovered that inhibiting their apoptosis restricts neutrophils

from migrating into the liver and getting activated [83].

3. MODELS OF SEPSIS: LIMITATIONS AND DEVELOPMENT STRATEGIES

In multiple research studies conducted in the 1980s, a variety of substances (such as steroids, cytokine inhibitors, etc.) were explored as treatments for sepsis; however, only a small number of these substances improved patient survival, while most of them proved useless or even made the condition worse [84]. It is challenging to do active treatment for maintenance (artificial breathing, infusion therapy, renal replacement therapy, parenteral feeding, etc.) and to evaluate hemodynamics using invasive techniques in preclinical investigations of sepsis that use tiny animals. Although it is generally given to real patients, concurrent antimicrobial and vasopressor therapy is frequently disregarded in sepsis experimental models [85-87]. While using bigger animals to represent sepsis would significantly raise study expenditures, it will also result in more effective invasive surveillance and ongoing treatment [88]. It is not probable to refrain from employing rodents in research due to the species' deep study, the identical gene expression patterns that have been observed between mice and humans all through inflammation, the shared similarities in the psychological, hereditary, and biochemical characteristics of rodents and humans, and the fact utilizing rodents in research is inexpensive, simple for carrying out, and boosts few ethical concerns [89-91]. Establishing a standard for the sepsis research models now in use is crucial in establishing novel approaches [92]. Standardization is a prerequisite for a multiparametric procedure intended for assessing the degree of severity of septic ailments in models of animals [93]. Integrating animal models using various sepsis induction strategies is crucial in preclinical research since information gathered from a single model can lead to an erroneous assessment of the roles of factors linked to the pathology of sepsis or the success rate of therapies. For example, investigations have shown that the TLR4-mediated signaling pathway is vital for the onset of polymicrobial sepsis and LPS- or LPS/D-GalN-induced toxic consequences [94–96], but additional investigations have shown that TLR4 has little to no function in the etiology of sepsis in the CLP and CASP models [97–99]. A further instance is the cytokine IL-12, which in the CASP model [100] but not the CLP model causes the development of sepsis [101].

4. CONCLUSION

A significant number of animal ethics and welfare committees allow the utilization of substitute indicators of death instead of using mortality as an endpoint. These investigations, typically lacking the use of modifications in mortality as an indicator of therapy a successful outcome, have been criticized by researchers and funding organizations. For the purpose of considering treatment, many sepsis animal models are taken into account. Improved relevance, translation, and application of findings and conclusions from animal research to what is seen clinically should be the main objectives for the implementation of experimental sepsis models in the future. This necessitates considering the drawbacks of the currently available sepsis models, which include considering factors like age, sex, genetic history, dietary habits, and the environment of the housing facility. It also calls for incorporating concurrent conditions along with supportive treatment options that may be clinically significant, as well as reducing the possibility of bias in sepsis preclinical research using randomization and blinding techniques. In conclusion, despite their limitations, animal models remain essential in sepsis research. They provide crucial information about pharmacokinetics, toxicity, and mechanisms of action for potential therapies. Combining animal studies with other research methods and accounting for the unique characteristics of human sepsis are vital steps towards improving the translation of preclinical findings into successful clinical interventions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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