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LETTER

Preclinical Model in Sepsis: Should We Abandon the CLP? [Letter]

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Dear editor

We read with interest the study by Garcia et al, "Common Variables That Influence Sepsis Mortality in Mice".[1] Based on their own experience, authors identified some factors specific to sex and season that significantly influence sepsis mortality in the CLP model, therefore questioning the status of this model as the gold standard in preclinical research on sepsis.

In support of this report, we performed a systematic review of 497 studies using CLP (Medline-indexed studies mentioning CLP for the years 1999, 2004, 2009, 2014, and 2019 arbitrarily) in mice aged 6 to 16 weeks old, to analyzed the surgical technique, the puncture needle size, drugs used in the protocol (class of anesthetic, analgesics, fluids, and antibiotics), and their effect on survival at 48 hours. We observed tremendous variability in procedures and outcomes: Needle size ranged from 10 to 30 gauge (21.04 ±2.8), and 15 different anesthetic regimens (single or multiple agents) were recorded. Analgesics were used in only 19.3% of studies, and their use increased in recent years (Figure 1A and 1). Antibiotics and fluid resuscitation were reported in 15.5%and 62% of the studies respectively (Figure 1C). In multivariate analysis, the puncture needle size was the only factor significantly associated with mortality at 48h (Figure 1D and 1).

In addition to these potentially standardizable parameters, we advocate that bacterial load burden, intensity and timing of caecum necrosis, occult bleeding, and inter-operator variability are non-standardizable contributors to variability in survival rate (Table 1). Indeed, CLP causes three insults to the host: surgical trauma, tissue ischemia and necrosis from the ligated cecum, and polymicrobial sepsis from fecal spillage after needle puncture(s). Subsequently, in the few studies measuring the CFU /mL in blood or peritoneal lavage using the complete spectrum of host enteric bacteria, significant differences are reported (respectively from 10^1 to 2.5*10^8 CFU/mL in the blood and from 10^2 to 10^8 CFU/mL in the peritoneal lavage at 16–24h).[2] Furthermore, in the CLP model, bacteria responsible for the infection are derived from the host enteric microbiome. This ensemble includes more than 400 different strains of bacteria, viruses, parasites, and fungi whose abundance, variability, and relative distribution are eminently variable. Moreover, heterogeneous peritoneal stool spillage can lead to localized or diffuse infection. Also, the germs in blood culture are extremely different from a study to another.[3] Overall, each step of the model is hardly standardizable and induces a risk of inter- and intra-experiment variability, which ultimately explains the literature's lack of homogeneity and external validity.

Finally, although the CLP model is undoubtedly valuable for studying sepsis pathophysiology, we believe it cannot meet the standard of "*a rigorous, controlled, and standardized preclinical animal model*" for preclinical studies

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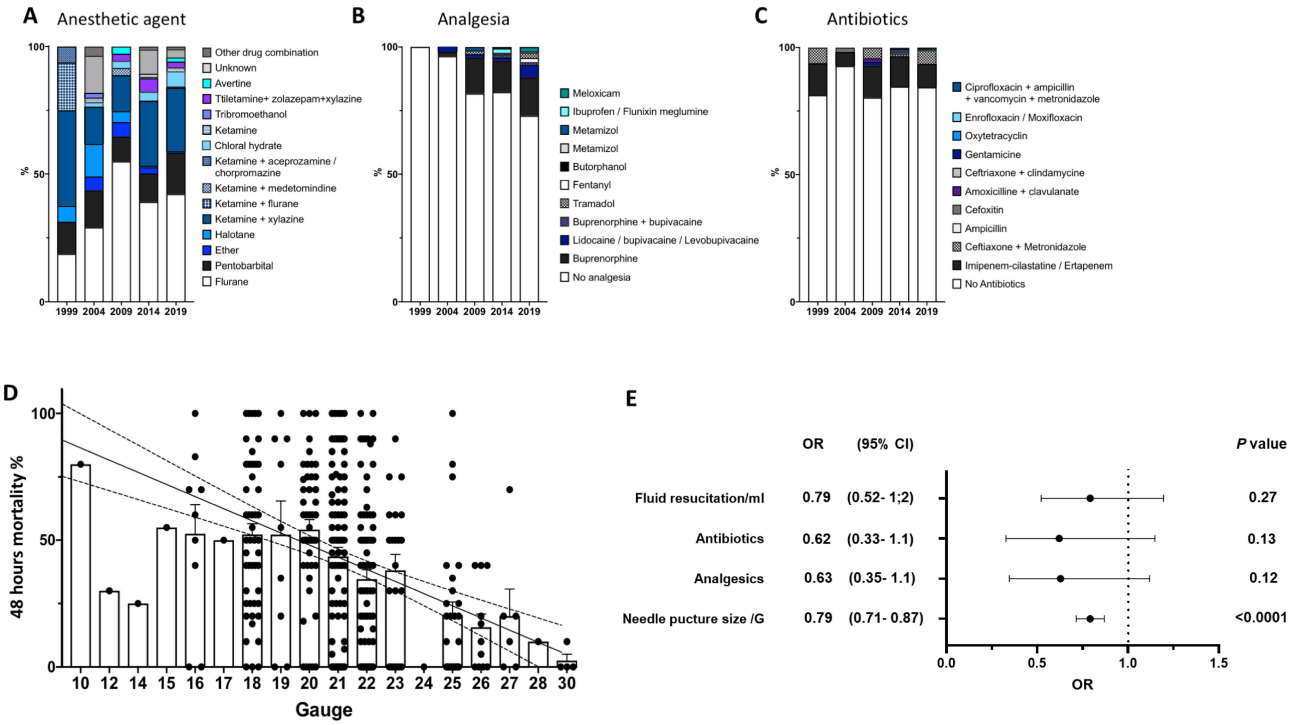

Figure 1 Distribution of anesthetic agents (**A**), analgesic (**B**), and antibiotics (**C**) used in CLP studies in mice according to the year of publication. Respectively 16, 65, 71, 169, and 185 were analyzed for the years 1999, 2014, 2009, 2014, and 2019. (**D**) Puncture needle size negatively correlates with the 48h mortality, (357 studies providing survival data at 48h were analyzed; *P*<0.001, Slope −3.99). (**E**) Multivariate regression analysis of factors associated with 48h mortality (logistic regression). The dots represent the odds ratio; the line through each dot represents the 95% confidence interval. Calibration (AUC-ROC): 0.68 (0.63–0.74); P<0.0001. Goodness of fit (Hosmer-Lemeshow statistic): P value = 0.13.
Abbreviations: OR, odd ratio; G, gauge.

investigating adjuvant therapy for early sepsis.[4] Therefore, one could argue that its "gold standard status"[5] must be challenged and finding an appropriate and reliable mouse model of sepsis and septic shock, which will lead to more successful research, must be a priority of the research agenda in the field.

Table 1 CLP Procedure's Inherent Variability Factors

	Factors accessible to standardization	**Factors that cannot be standardized**
Anesthesia	-Choice of drug -Volume of dilution (weight adjusted) -Injection route	-Duration of anesthesia -Side effects (inflammation, behavior)
Abdominal wall surgery	-Surgical approach -Incision length	Occult bleeding
Cecal ligature	-Length of the caecum	-Volume of the caecum -Caecum necrosis onset and intensity
Puncture and bacterial hit	-Needle gauge size -Number of puncture(s)	-Abondance of stools extruded by cecal pressure -Stool consistency -Stool spillage in the peritoneum (localized/diffuse) -Microbiote species (bacteriobiote, fungus, parasite) -Microbiote variabilty and distribution

(*Continued*)

Table 1 (Continued).

	Factors accessible to standardization	Factors that cannot be standardized
Analgesia	-Local and/or systemic analgesic -Choice of drug -Volume of dilution (weight adjusted) -Injection route -Intervals if multiple dose during follow-up	-Side-effects (Inflammation, behavior, temperature) -Bias if analgesia "on demand"
Antibiotherapy	-Choice of drug -Delay of injection -Volume of dilution (weight adjusted) -Injection route	-Bacterial resistance profile of commensal flora -Side-effects (kidney/liver injury, inflammation)
Fluids	-Choice of solute -Volume of dilution (weight adjusted) -Injection route -Interval if multiple dose during follow-up	
Severity assessment	-Clinical score -Humane criteria requiring euthanasia	-Heterogeneity in humane criteria requiring euthanasia -Subjectivity in scoring

Disclosure

Authors have no conflict of interest to disclose.

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