Reviewer's report

Title: Injurious mechanical ventilation causes kidney apoptosis and dysfunction during sepsis but not after intra-tracheal acid instillation: an experimental study.

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Reviewer: Ciro Esposito

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In the present manuscript Kuiper et al. expand their previous findings on the different effects of low tidal (LT) volume vs high tidal (HT) volume mechanical ventilation (MV) evaluating the kidney damage in different models of acute lung injury (direct by acid instillation vs indirect by cecal puncture induced sepsis). They interestingly found that MV was associated with kidney apoptosis evaluated by TUNEL technique in sepsis but not in the model of local lung injury, furthermore they observed a positive correlation between PAI-1 serum levels and the degree of kidney apoptosis. The paper is well written and the findings are promising, however they are very limited to be suitable for publication.

Major concerns:
- The present study did not show any significant morphological or functional kidney alteration, neither differences between the models (acid instillation vs sepsis) described or MV technique. In fact kidney function evaluation by creatinine clearance is not reliable in the studied animal models because creatinine is actively secreted by kidney in rats (Pharm Res. 1991 Oct;8(10):1318-22.). Although the gold standard to evaluate GFR in rats is inulin clearance, blood urea nitrogen (BUN) could give more precise information on kidney function. Proteinuria/aluminuria ( Crit Care. 2011;15(6):R277) may add relevant data to the manuscript. Considering the absence of histological damage, evaluation of early urinary markers of kidney injury, as KIM-1/NGAL, should be considered.

- In their previous paper the authors showed that PAI-1 increased systemically after a pro-inflammatory insult (acid instillation or cecal puncture). Of note circulating PAI-1 increased in parallel to kidney apoptosis in the septic model. PAI-1 is known to exert direct and indirect antiapoptotic effect (Thromb Haemost. 2008 Dec;100(6):1037-40), and the production of PAI-1 by tubular cells is increased in hypoxic condition (Clin Exp Nephrol. 2011 Feb;15(1):34-40). It is likely that PAI-1, rather than being the cause of the increased kidney apoptosis, could be produced directly by kidney tubular cell in response to the ischemic damage caused by dysregulation of kidney vasoactive mechanism induced by sepsis and worsened theoretically by mechanical ventilation.

- The explanations the Authors give to justify the changes observed in the kidneys and renal function of the HVt sepsis group are not supported by the results of this study and are the same suggested almost ten years ago by other
Investigators, thus this paper does not add anything to the current knowledge;

- Moreover, to support the notion of regional alterations in renal hemodynamics despite constant systemic MAP, the Authors could have measured NO in blood and urines of the animals to detect changes in the vasoconstrictor-vasodilator balance; in addition, or alternatively, they could have stained kidney sections by immunohistochemistry for NOS or other molecules involved in the regulation of vascular tone;

- It seems that apart from HVt sepsis group that showed an increased tubular epithelial cell apoptosis the other experimental groups did not suffer from kidney injury; nevertheless, serum creatinine, although universally acknowledged, is a widely used but late marker of kidney damage and the observation period of the animals in this study is quite short, thus other, more precocious, markers of kidney function (such as serum cystatin C) could help detecting the earliest changes in renal function during ALI.

Minor issues:

I would suggest the Authors to include control animal data in the graphs of figure 2;

It would be useful to show micrographs of H&E staining of the renal tissue
The Authors should include in the manuscript a picture of the experimental design to facilitate the readers to have an idea of the experimental plan at a glance;

The Authors should include in the manuscript a table with the data (even if not statistically significant) that in the actual version of the paper are only listed in the Results section.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I have no competing interests in relation to the paper I have just reviewed