Author’s response to reviews

Title: Effects of Low Versus Standard Pressure Pneumoperitoneum on Renal Syndecan-1 Shedding and VEGF Receptor-2 Expression in Living-donor Nephrectomy: A Randomized Controlled Study

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Editorial Department of BMC Anesthesiology

Dear Editors,

Please consider our revised manuscript, entitled “Effects of Low Versus Standard Pressure Pneumoperitoneum on Renal Syndecan-1 Shedding and VEGF Receptor-2 Expression in Living-donor Nephrectomy: A Randomized Controlled Study” for publication in BMC Anesthesiology. We appreciate the interest that the editors and reviewers (Anthony Bonavia, MD; Ahmed Abdalla Mohamed, MD; and Hassan Mohamed Hassan Sayed Ahmed, MD, EDIAC, FCAI, PhD) have taken in our manuscript and the constructive reviews they have given to improve our manuscript. We have addressed the major and minor concerns of the reviewers.

We have also included a point-by-point response to the reviewers in addition to the changes described above in the manuscript. We have highlight and add more discussion as suggested by the reviewers.

Thank you again for consideration of our revised manuscript.
POINTS OF REVISION:

Comment:
We thank the editors for their conscientious review in keeping our manuscript to adhere with the journal’s editorial policies and for the reviewers for their constructive criticism and suggestions. We attached referred manuscript page and line numbers following our responses to the reviewers.

Reviewer #1

Anthony Bonavia, MD

The authors are to be commended for the significant improvement in the quality and clarity of this manuscript, and for addressing many of the reviewer concerns. The grammar is significantly improved but there is still the need for additional grammatical improvement here and there.

We would like to thank Dr Bonavia for his valuable comments and suggestions. We made several grammar revisions:

- (Page 3, line 63) “Laparoscopic nephrectomy is a preferred technique for living kidney donation.”

- (Page 3 line 63–68) “However, positive-pressure pneumoperitoneum may have an unfavorable effect on the remaining kidney and other distant organs due to inflamed vascular endothelium and renal tubular cell injury in response to increased systemic inflammation. Early detection of vascular endothelial and renal tubular response is needed to prevent further kidney injury due to increased intraabdominal pressure induced by pneumoperitoneum.”

- (Page 3 line 70–75) “This study aimed to assess the effect of increased intraabdominal pressure on vascular endothelium and renal tubular cells by comparing the effects of low and standard pressure pneumoperitoneum on vascular endothelial growth factor receptor-2 (VEGFR-2) expression and the shedding of syndecan-1, as these are the early vascular
endothelial and renal tubular proinflammatory markers due to a systemic inflammatory response.”

- (Page 4 line 79–80) “Renal tubule and peritubular capillary ultrastructures were examined using electron microscopy.”

- (Page 4 line 88–91) “The low pressure group showed renal tubule and peritubular capillary ultrastructure with intact cell membranes, clear cell boundaries, and intact brush borders, while standard pressure group showed swollen nuclei, tenuous cell membrane, distant boundaries, vacuolizations, and detached brush borders.”

- (Page 4 line 93–96) “The low pressure pneumoperitoneum attenuated the inflammatory response and resulted in reduction of syndecan-1 shedding and VEGFR-2 expression as the renal tubular and vascular endothelial proinflammatory markers to injury due to a systemic inflammation in laparoscopic nephrectomy.”

- (Page 7 line 151–160) “We hypothesized that short-term increases in intraabdominal pressure could alter renal perfusion and induce a systemic inflammatory response that leads to tubular cell injury. In the current investigation, we aimed to evaluate the effect of low pressure pneumoperitoneum on vascular endothelium and renal tubular cells markers induced by a systemic inflammatory response during transperitoneal laparoscopic living donor nephrectomy. We further hypothesized that using a lower pressure pneumoperitoneum could reduce these effects. Here, we compared the effects of low and standard pressure pneumoperitoneum on shedding of syndecan-1 and activated vascular endothelial growth factor receptor-2 (VEGFR-2) expression, as the early vascular endothelial and renal tubular proinflammatory markers in response to the presence of systemic inflammatory cytokines.”

- (Page 8 line 176–180) “Exclusion criteria were hemodynamic instability defined as the changes of mean arterial pressure or cardiac index &gt; 25% below or above baseline despite intervention treatment, and conversion of laparoscopy to open nephrectomy. Patients were allocated using blocked randomization (https://www.sealedenvelope.com/simple-randomiser/v1/lists) with a block size of 4.”

- (Page 19 line 451–452) “Unfavorable consequences are not expected during most elective laparoscopic operations in healthy or low-risk individuals.”

- (Page 20 line 462–464) “One effect of low pressure pneumoperitoneum was reduced postoperative pain due to lower visceral pain secondary to peritoneal stretch receptors.”

- (Page 20 line 472–474) “Even a slight pressure increase of 10 mmHg has shown to affect the kidney, and pressures as high as 20 mmHg have disrupted the kidney function.”
"Syndecan-1 expression in the proximal renal tubules is related to the degree of proteinuria in various kidney diseases, therefore the plasma syndecan-1 could become an early sign of renal tubular injury.35"

"From our study results, although duration of pneumoperitoneum was relatively short, the inflammatory reaction and presence of endothelial and renal tubular markers to inflammation were higher, especially when standard pressure, and not low pressure pneumoperitoneum was used."

"Syndecan-1 and VEGFR-2 are the early markers of renal tubular and vascular endothelial response due to a systemic inflammation, however, the inhibition of syndecan-1 shedding and sVEGFR-2 response to endothelial injury in preventing or reducing kidney injury demands further experimental and clinical studies."

"Our findings demonstrate that using a low pressure pneumoperitoneum attenuated the inflammatory response, measured by quantifying plasma IL-6. This may have caused the observed reductions in syndecan-1 shedding and VEGFR-2 expression; the renal tubular and vascular endothelial proinflammatory markers of injury in response to the presence of systemic inflammatory cytokines."

Major comments:

My main issue with this manuscript is that there appear to be 2 main hypotheses. The first is that "low pressure pneumoperitoneum is superior to standard pressure pneumoperitoneum in terms of inflammatory markers syndecan-1 and VEGFR-2" and the second is that "syndecan-1 and VEGFR-2 are validated markers of renal tubular injury". While this study may validate the first hypothesis, it is insufficient to prove the second. There are still several instances in the manuscript in which the distinction between hypotheses is still unclear. For example: "This study aimed to assess the effect of low intraabdominal pressure on kidney injury prevention." While it is important to identify the renal source of the biomarkers selected in this study in order to justify their reason for looking at kidney immunohistochemistry, the two hypotheses should be kept distinct.

Clarification:

We thank the reviewer for this points that the study is insufficient to prove the second hypothesis. We have revised and rewritten several statements in our manuscript based on the reviewer suggestions to emphasize the validation of the first hypothesis and keep the second hypothesis in distinct.

In Abstract:
“However, positive-pressure pneumoperitoneum may have an unfavorable effect on the remaining kidney and other distant organs due to inflamed vascular endothelium and renal tubular cell injury in response to increased systemic inflammation. Early detection of vascular endothelial and renal tubular response is needed to prevent further kidney injury due to increased intraabdominal pressure induced by pneumoperitoneum.”

“This study aimed to assess the effect of increased intraabdominal pressure on vascular endothelium and renal tubular cells by comparing the effects of low and standard pressure pneumoperitoneum on vascular endothelial growth factor receptor-2 (VEGFR-2) expression and the shedding of syndecan-1 as the early markers to a systemic inflammation.”

“The low pressure pneumoperitoneum attenuated the inflammatory response and resulted in reduction of syndecan-1 shedding and VEGFR-2 expression as the renal tubular and vascular endothelial proinflammatory markers to injury due to a systemic inflammation in laparoscopic nephrectomy.”

In Background:

“We hypothesized that short-term increases in intraabdominal pressure could alter renal perfusion and induce a systemic inflammatory response that leads to tubular cell injury. In the current investigation, we aimed to evaluate the effect of low pressure pneumoperitoneum on vascular endothelium and renal tubular cells markers induced by a systemic inflammatory response during transperitoneal laparoscopic living donor nephrectomy. We further hypothesized that using a lower pressure pneumoperitoneum could reduce these effects. Here, we compared the effects of low and standard pressure pneumoperitoneum on shedding of syndecan-1 and activated vascular endothelial growth factor receptor-2 (VEGFR-2) expression, as the early vascular endothelial and renal tubular proinflammatory markers in response to the presence of systemic inflammatory cytokines.”

In Discussion;

“From our study results, although duration of pneumoperitoneum was relatively short, the inflammatory reaction and presence of endothelial and renal tubular markers to inflammation were higher, especially when standard pressure, and not low pressure pneumoperitoneum was used.”

“Syndecan-1 and VEGFR-2 are the early markers of renal tubular and vascular endothelial response due to a systemic inflammation, however, the inhibition of syndecan-1 shedding and sVEGFR-2 response to prevent or reduce kidney injury demands further experimental and clinical studies.”
In Conclusion:

- (Page 29, line 689–693)“Our findings demonstrate that using a low pressure pneumoperitoneum attenuated the inflammatory response, measured by quantifying plasma IL-6. This may have caused the observed reductions in syndecan-1 shedding and VEGFR-2 expression; the renal tubular and vascular endothelial proinflammatory markers of injury in response to the presence of systemic inflammatory cytokines.”

- I am very curious as to why the authors went through the trouble to do a randomized trial, using these biomarkers as their primary outcome. Why wouldn't the authors use nGAL, TIMP-2 or other biomarkers that are more established in the literature? The supporting literature for syndecan-1 and VEGF are so sparse.

Clarification:

We agree with the reviewer suggestion about the preference of using more established biomarker such as NGAL or TIMP-2. We the authors have discussed this point during the proposal meeting before the study was conducted, and finally decided that our study used KIM-1 as biomarker of kidney injury.

In our study, urinary KIM-1 level was lower during low pressure pneumoperitoneum than standard pressure pneumoperitoneum. The reversible tubular injury that was represented by KIM-1 returning to baseline levels 2 hours after desufflation that may be due to the short length of the pneumoperitoneum duration during laparoscopic procedure. (Page 26, line 630–637)

KIM-1 was chosen based on the study that showed the ectodomain of KIM-1 is shed from proximal tubular kidney epithelial cells and KIM-1 mRNA levels are elevated more than any other known gene in human after initiation of kidney injury. In kidney, KIM-1 baseline level was very low and increase up to 100-fold after kidney injury. KIM-1 expression did not change in any of the other organs demonstrating the specificity of KIM-1 for kidney injury. Based on 17 studies, the increase in urinary Kim-1 was compared to histopathology, which is considered the “gold standard” for assessing preclinical renal injury. The AUC and the sensitivity of KIM-1 was nearly 1, irrespective of the mechanism of kidney injury and correlated highly with histopathologic changes even for low-grade injuries (grade 1). A study by Han et al. demonstrated marked expression of KIM-1 in kidney biopsy specimens from six patients with acute tubular necrosis, and found elevated urinary levels of KIM-1 after an initial ischemic renal insult, prior to the appearance of casts in the urine (Vaidya VS, Ozer JS, Frank D, Collings FB, Ramirez V, Troth S, Muniappa N. Kidney Injury Molecule-1 Outperforms Traditional Biomarkers of Kidney Injury in Multi-site Preclinical Biomarker Qualification Studies. Nat Biotechnol. 2010 May ; 28(5): 478–485)

With many respects to the reviewer, we agree NGAL as an established biomarker of early AKI especially in patients with previous normal baseline renal function. Urinary NGAL increases due to an impaired reabsorption of the filtered load in the proximal tubule, increased synthesis from stressed tubular cells in the distal nephron and release by infiltrating neutrophils. Neutrophils
mainly release the dimeric form (and to some extent the monomeric form), whereas tubular cells mainly produce the monomeric form, and to some extent NGAL conjugated with MMP-9 (heterodimeric NGAL). Systemic inflammation induces NGAL synthesis by extrarenal tissues and the release of NGAL from neutrophils that mainly release the dimeric form. The monomeric, tubular cell-specific signal, have been less successful since commercial NGAL assays such as the BioPorto (Gentofte, Denmark) and R&D Systems (Minneapolis, Minn., USA) are unable to specifically measure monomeric NGAL secreted by stressed or injured tubular cells. (Mårtensson J, Bellomo R. The Rise and Fall of NGAL in Acute Kidney Injury. Blood Purif 2014;37:304–310).

TIMP2/IGFBP7 as another established biomarker of early AKI rise up when the renal “stress response” occurred. Immunohistochemistry showed progressive TIMP2/IGFBP7 losses from injured proximal tubule cells and undergo increased filtration, decreased tubule reabsorption, and proximal tubule cell TIMP2/IGFBP7 urinary leakage seem to be the most likely mechanisms. However, the study in cardiac surgery patients showed most measured [TIMP-2]•[IGFBP7] level within 4 h post-surgery. Studies have reported a variety of results in this regard. In several studies, TIMP-2/IGFBP7 elevations was detected at 4 h; in another study, elevations were not detected until 6-24 hours after surgery. A recent study, shows bimodal elevations of TIMP-2/IGFBP7 with the first peak occurring intraoperatively and the second 6 h after ICU admission in patients who developed stages 2/3 AKI. The authors postulate that the first peak indicates kidney stress caused during the surgery, while the second peak may indicate kidney stress caused during early postoperative care. Measurement at both times resulted in the best predictive ability, as would be expected for two independent episodes of stress. Overall, TIMP-2/IGFBP7 is considered accurate in identifying patients at risk for AKI. However, only two studies, have attempted to evaluate whether use of the test alters the clinical course of AKI. Performing the test at a few different postoperative timepoints is helpful to identify the most appropriate testing time for a particular program given inherent differences in care across institutions. The future research is warranted to better understand how treatment protocols based on TIMP-2/IGFBP7 results can improve outcomes. (Guzzi LM, Bergler T, Binnall B, Engelman DT, Forni L, Germain MJ, et al. Clinical use of [TIMP-2]•[IGFBP7] biomarker testing to assess risk of acute kidney injury in critical care: guidance from an expert panel. Critical Care. 2019: 23:225)

We agree with the reviewer that the supporting literature for syndecan-1 and VEGF are so sparse. The usage of low pressure pneumoperitoneum can attenuate this systemic inflammatory and vascular response. We considered to evaluate Syndecan-1 in our study based on plays a role in the process of re-epithelialization during inflammation, and is involved in promoting renal tubular epithelial cell survival in animal models of ischemia/reperfusion and in human kidney transplantation. In early renal injury, tubular epithelial cells increase syndecan-1 regulation to repair injured cells. VEGFR-2 is expressed during ischemic or inflammatory conditions which IL-6 and activated syndecan-1 in the endothelial cells stimulate the synthesis of VEGF-A molecules and its binding to VEGFR-2 on the endothelial surface. This increases VEGFR-2 phosphorylation in order to repair endothelial injury. Plasma syndecan-1 levels are hypothesized to correlate with plasma soluble VEGF-A as a marker of endothelial damage and plasma creatinine and urea as a marker of kidney function. In a normal human kidney, VEGFR-2 is expressed on glomerular endothelial cells and peritubular capillaries, as well as tubular epithelial
cells in a low degree. Regulation of protein expression through the VEGFR-2 receptor is important for the survival of kidney endothelial cell tissue after ischemic injury.

Inhibiting syndecan-1 shedding and the release of VEGFR-2 are believed to have renal-protective roles. Syndecan-1 and VEGFR-2 are the potential early markers of renal tubular and vascular endothelial response due to a systemic inflammation, however, the inhibition of syndecan-1 shedding and sVEGFR-2 response to endothelial injury in preventing and reducing kidney injury still demand further experimental and clinical studies.

- "In our study, the heart rate in the low-pressure group trended significantly lower than the standard pressure group." - I find this difficult to understand, given that the CI and SV were unchanged. Isn't the cardiac output the product of the SV and the HR? How can the HR be significantly different if the CI and SV are unchanged?

Clarification:

The reviewer is right with this interpretation. Our explanation is that our result showed the significantly lower heart rate in the low pressure group, but the SV was non-significantly higher compared to standard pressure group. Therefore, the CI as the result of the SV and HR was non-significantly higher. The systemic vascular resistance could also influence the CI since the MAP was unchanged we can assume that the SVRI between the two group was not significantly different, therefore the CI was non-significantly higher in the low pressure group. However, we did not measure the SVRI since it needed the CVP value and it is not common to insert the CVC during laparoscopic donor nephrectomy unless there was an indication for it.

Our study results showed the IL-6 level in standard pressure group was higher than low pressure group. Inflammatory cytokines enhance the spontaneous beating rate of cardiac myocytes. The higher levels of interleukin-6 (IL-6) may be associated with a higher resting heart rate in a healthy population-based sample. A study by Tracy et al showed that Circulating IL-6 was strongly and independently correlated with resting heart rate. Circulating IL-6 is a possible biological mediator that may contribute to explain associated with high heart rate. (Tracy RP, Newman AB, Williamson JD, Harris TB, Cummings SR. Interleukin-6 and heart rate in a population-based sample: The health ABC study. Circulation. 2001;103:1348)

Minor comments:

- "Laparoscopic nephrectomy is a preferable technique for living kidney donation." needs supporting reference

Clarification:

(Page 5 line 103–107 and page 31 line 743–747) We added the supporting references for the statements in the background as suggested:


"The low-pressure group showed better morphological renal tubule and peritubular capillary ultrastructure"- what does 'better' mean? I would define with histological terms

Clarification:

We revised and defined ‘the better morphological renal tubule and peritubular capillary ultrastructure’ into histological terms:

(Page 4, line 88–91) “The low pressure group showed renal tubule and peritubular capillary ultrastructure with intact cell membranes, clear cell boundaries, and intact brush borders, while standard pressure group showed swollen nuclei, tenuous cell membrane, distant boundaries, vacuolizations, and detached brush borders.

"It is important to ensure safety and minimize surgical risk to allograft kidney function in both the kidney recipient and donor." - you can delete "to allograft kidney function" in this statement

Clarification:

(Page 5, line 109–110) We deleted the "to allograft kidney function" as suggested into: “It is important to ensure safety and minimize surgical risk in both the kidney recipient and donor.”

"The kidneys are at risk of injury induced by increased IAP. A direct correlation of increased IAP secondary to pneumoperitoneum is renal venous congestion and decreased renal blood flow due to compression of the renal vasculature and renal parenchyma"

- should be changed to "The kidneys are at risk of injury induced by increased IAP secondary to pneumoperitoneum-induced renal venous congestion and compression of the renal vasculature and parenchyma"

Clarification:

We thank the reviewer and changed the statement into:

(Page 5, line 116–118) "The kidneys are at risk of injury induced by increased IAP secondary to pneumoperitoneum-induced renal venous congestion and compression of the renal vasculature and parenchyma"
"Exclusion criteria were hemodynamic instability &gt; 25% of baseline despite intervention treatment" - what does this mean? 25% of what?

Clarification:

We revised the sentence to define the hemodynamic instability into:

(Page 8, line 176–178) "Exclusion criteria were hemodynamic instability defined as the changes of mean arterial pressure or cardiac index &gt; 25% below or above baseline despite intervention treatment, and conversion of laparoscopy to open nephrectomy."

"Patients were allocated using blocked randomization of 4." - what does this mean? what were the 4?

Clarification:

We revised the sentences into:

(Page 8, line 179–180) “Patients were allocated using blocked randomization (https://www.sealedenvelope.com/simple-randomiser/v1/lists) with a block size of 4.”

Block randomization is a commonly used technique in clinical trial design to reduce bias and achieve balance in the allocation of participants to treatment arms, especially when the sample size is small. This method increases the probability that each arm will contain an equal number of individuals by sequencing participant assignments by block. Yet still, the allocation process may be predictable, for example, when the investigator is not blind and the block size is fixed. A chance run of participants to a particular study group also may occur under a simple randomization scenario. This can lead to bias, for example, if the initial participants in the trial are healthier than the later ones. Blocked randomization offers a simple means to achieve balance between study arms and to reduce the opportunity for bias and confounding.

Allocation proceeds by randomly selecting one of the orderings and assigning the next block of participants to study groups according to the specified sequence. Note that repeat blocks may occur when the total sample size is greater than the block size times the number of possible orderings. Furthermore, the block size must be divisible by the number of study groups. A disadvantage of block randomization is that the allocation of participants may be predictable and result in selection bias when the study groups are unmasked. That is, the treatment assignment that has so far occurred least often in the block likely will be the next chosen. Selection bias may be reduced by using random block sizes and keeping the investigator blind to the size of each block.

For example, given a block size of 4, there are 6 block combinations to equally assign participants to a block. The enrolment of 44 participants, be randomly assigned to the two intervention arms. Below, a computer algorithm written is presented for performing a block
randomization with randomly selected block sizes of 4 combinations in a sealed envelopes for 44 subjects:

Block 1: 1. Standard pressure; 2. Standard pressure; 3. Low pressure; 4. Low pressure
Block 2: 5. Standard pressure; 6. Low pressure; 7. Standard pressure; 8. Low pressure

"Increased intra-abdominal pressure causes mechanical compression of the inferior vena cava, renal vasculature, and parenchyma." - needs reference

Clarification:

We added the 2 references for the statement on page 21, line 486–487 and page 33–34, line 806–810:


Reviewer #2
Ahmed Abdalla Mohamed, MD

Thank you very much for your appreciations and valuable reviews to improve our manuscript.

Reviewer #3
Hassan Mohamed Hassan Sayed Ahmed, MD, EDIAC, FCAI, PhD

I would like to thank the authors for their detailed responses, their corrections and explanations enhanced the manuscript and give it a higher chance for publication. This manuscript is really
valuable but need some adjustments in the discussion section, I believe that the discussion section is too long and needed to be shorter.

Clarification:

Thank you very much for your appreciations. We couldn’t have done it without your constructive reviews and comments. We have tried to make the discussion more concise together with suggestions from reviewer 1, hopefully they meet your criteria.

- (Page 19 line 451–452) “Unfavorable consequences are not expected during most elective laparoscopic operations in healthy or low-risk individuals.”

- (Page 20 line 462–464) “One effect of low pressure pneumoperitoneum was reduced postoperative pain due to lower visceral pain secondary to peritoneal stretch receptors.”

- (Page 20 line 472–474) “Even a slight pressure increase of 10 mmHg has shown to affect the kidney, and pressures as high as 20 mmHg have disrupted the kidney function.”

- (Page 24 line 561–563) “Syndecan-1 expression in the proximal renal tubules is related to the degree of proteinuria in various kidney diseases, therefore the plasma syndecan-1 could become an early sign of renal tubular injury.”

- (Page 28 line 675–678) “From our study results, although duration of pneumoperitoneum was relatively short, the inflammatory reaction and presence of endothelial and renal tubular markers to inflammation were higher, especially when standard pressure, and not low pressure pneumoperitoneum was used.”

- (Page 28 line 681–684) “Syndecan-1 and VEGFR-2 are the early markers of renal tubular and vascular endothelial response due to a systemic inflammation, however, the inhibition of syndecan-1 shedding and sVEGFR-2 response to endothelial injury in preventing or reducing kidney injury demands further experimental and clinical studies.”