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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Research article

Keywords: pneumoperitoneum, renal resistive index, interleukin-6, syndecan-1, sVEGFR-2, laparoscopic nephrectomy

Posted Date: October 25th, 2019

DOI: https://doi.org/10.21203/rs.2.195/v3

Version of Record: A version of this preprint was published at BMC Anesthesiology on February 4th, 2020. See the published version at https://doi.org/10.1186/s12871-020-0956-7.

Abstract

Background Laparoscopic nephrectomy is a preferred technique for living kidney donation. 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. Transperitoneal laparoscopic living donor nephrectomy represented a human model of mild increasing intraabdominal pressure. 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. Methods We conducted a prospective randomized study on 44 patients undergoing laparoscopic donor nephrectomy. Subjects were assigned to standard (12 mmHg) or low pressure (8 mmHg) groups. Baseline, intraoperative, and postoperative plasma interleukin-6, syndecan-1, and sVEGFR-2 were quantified by ELISA. Syndecan-1 and VEGFR-2 expression were assessed immunohistochemically in renal cortex tissue. Renal tubule and peritubular capillary ultrastructures were examined using electron microscopy. Perioperative hemodynamic changes, end-tidal CO2, serum creatinine, blood urea nitrogen, and urinary KIM-1 were recorded. Results The low pressure group showed lower intra- and postoperative heart rate, intraoperative plasma IL-6, sVEGFR-2 levels and plasma syndecan-1 than standard pressure group. Proximal tubule syndecan-1 expression was higher in the low pressure group. Proximal-distal tubules and peritubular capillary endothelium VEGFR-2 expression were lower in low pressure group. 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. Conclusion 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. Trial registration ClinicalTrial.gov NCT:03219398, July 17th 2017. Keywords: pneumoperitoneum, renal resistive index, interleukin-6, syndecan-1, sVEGFR-2, laparoscopic nephrectomy

Background

Minimally invasive surgery is increasingly performed in many institutions. The increased intra-abdominal pressure (IAP) that occurs as a result of pneumoperitoneum insufflation may have an unfavorable effect on the kidney and other distant organs. Minimally invasive laparoscopic nephrectomy is a less-invasive technique for living donor allograft kidney procurement and has become a preference to promote early postoperative recovery.[1,2] As these laparoscopic techniques advance, more living donors are undergoing surgery to save others. As such, the postoperative condition of these donors becomes a priority. It is important to ensure safety and minimize surgical risk in both the kidney recipient and donor.[3]

Increased IAP is frequently present in surgical or critically ill patients and becomes an independent predictor of morbidity and mortality. The mean IAP in a healthy patient while supine is 1.8 mmHg with a range between -1 to 6 mmHg.[4] The World Society of the Abdominal Compartment Syndrome (WSACS) defines the upper normal limit for IAP to be approximately 5-7 mmHg in adults.[5] 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.[6]

A prospective clinical study of living kidney donors undergoing transperitoneal laparoscopic nephrectomy with 12 mmHg IAP showed an increased inflammatory response and early signs of kidney injury when compared with patients undergoing open retroperitoneal nephrectomy.[3] Additionally, an animal study applying pneumoperitoneum to isolated perfused rat kidneys demonstrated early onset inflammation and renal apoptosis.[7] The decreased renal blood flow leads to tissue hypoperfusion that triggers an inflammatory response. After desufflation, reperfusion occurs when renal blood flow is normalized. This further stimulates the synthesis of inflammatory cytokines, which have been postulated to mediate the association between blood flow changes and endothelial and epithelial cell injury.[6] Vascular endothelial dysfunction and tubular cell injury in response to inflammatory cytokines play an important role in acute kidney injury (AKI).[7]

Syndecan-1 is a cell surface proteoglycan that consists of a heparan and chondroitin sulphate. Syndecan-1 is expressed on various epithelial and vascular endothelial cells and is involved in many cellular functions that promote cell proliferation and survival. The shedding of syndecan-1 may be an important proponent in the mechanism responsible for tubular epithelial injury in ischemic and inflammatory conditions. Elevated serum syndecan-1 has predicted AKI and mortality in patients with acute heart failure and in pediatric patients undergoing cardiac surgery.[8,9] Higher tubular epithelial syndecan-1 expression promotes tubular cell survival and repair, factors that are correlated with prolonged allograft survival in kidney transplant patients.[10]

Activation of vascular endothelial growth factor (VEGF) binding to VEGF receptor-2 (VEGFR-2) has an important role in maintaining angiogenesis and microvasculature permeability.[9] Overstimulation of VEGF-VEGFR-2 induces renal tubulointerstitial injury through altered endothelial proliferation, abnormal angiogenesis, and extracellular matrix deposition and is enhanced in the presence of endothelial nitric oxide deficiency.[10] These findings indicate that the inhibition of syndecan-1 shedding and VEGF-VEGFR-2 stimulation are novel targets in preventing or managing AKI, since serum blood urea nitrogen (BUN), creatinine, and urine output are delayed signs of deteriorating kidney function.[8]

The primary outcome was detecting the plasma level and tubular expression of syndecan-1. The secondary outcomes were VEGFR-2 and soluble VEGFR-2 (sVEGFR-2) expression in renal tubuloendothelial cells, plasma interleukin-6 (IL-6), and urinary KIM-1 content.

Methods

Ethical Considerations

A prospective single-blind clinical study on patients undergoing transperitoneal laparoscopic living donor nephrectomy was conducted at the university teaching hospital after receiving approval from the medical ethics committee (protocol no. 17-06-0619, approval date: June 19th, 2017). This study was registered on ClinicalTrial.gov (NCT:03219398).

Patient Enrolment

We enroled 44 patients between July 2017 and February 2018. All patients provided written informed consent prior to participation. The inclusion criteria were age between 18 and 65 years, American Society of Anesthesiologist (ASA) physical status classification I-II, and a body mass index (BMI) of 18-25 kg/m^2. Exclusion criteria were hemodynamic instability defined as the changes of mean arterial pressure or cardiac index > 25% below or above baseline despite intervention treatment, and conversion of laparoscopy to open nephrectomy. Patients were allocated using blocked randomization (Then, using a list of random numbers in sealed envelopes, patients were divided into 12 mmHg (standard pressure) or 8 mmHg (low pressure) pneumoperitoneum groups. Both the patients and principal investigator were blinded to group allocation. The principal investigator received the randomization codes after all measurements and calculations of all patients had been entered into the results database.

Anesthesia and Pneumoperitoneum

All patients underwent electrocardiography and monitoring of heart rate, non-invasive blood pressure, pulse oxygen saturation, end-tidal carbon dioxide (IntelliVue MP70 Philips Healthcare, Netherlands), and cardiac output relates to body surface area (BSA) using bioimpedance cardiometry (ICON^{TM}, Osypka Germany). After midazolam premedication, standardized anesthesia was induced with 1-2 mg/kg i.v propofol and 1 µg/kg i.v fentanyl. Intubation was facilitated with 0.5 mg/kg i.v atracurium. General anesthesia maintenance was performed using sevoflurane with an end-tidal sevoflurane target of 1.5-2% (Aisys C2, GE Healthcare, Illinois, USA) to maintain a bispectral index value between 40 and 50 (BIS^{TM}, Covidien, Minneapolis, USA). Maintenance with 0.005 mg/kg/min i.v atracurium and 2 µg/kg/hour fentanyl was conducted to achieve train of four between 0.15 and 0.25 (TOF-Watch, Organon, Ireland).

All patients received bilateral ultrasound-guided transmuscular QLB (anterior QLB or QLB3) that was performed by two anesthetist consultants (DA, RBS). Patients were in the supine position with the site to be blocked slightly facing upward. This position was facilitated by a pillow underneath the patient and table tilting. After ensuring skin asepsis of the area, a 2.0–5.5 MHz convex transducer (4C-RS, Logic e, GE Healthcare U.S.A; C5-1E, DC-70, Mindray, Shenzen, China) covered with sterile drapes was attached to the inferior area of the lumbar (Petit's triangle that consisted of the iliac crest in the inferior region, the latissimus dorsi muscle in the posterior region, and the external abdominal oblique muscle in the anterior region). The Shamrock sign appeared on the ultrasound, and a 21G 100-mm peripheral block needle (Stimuplex®, BBraun, Mesulngen, Germany) was inserted in-plane with the USG probe passing in an

anterior to posterior direction through the quadratus lumborum (QL) muscle and reaching the border between the QL and psoas major muscle. After confirming negative blood aspiration, 1 mL normal saline was injected to obtain a hydrodissection sign to verify the needle tip, and 0.4 mL/kg of bupivacaine 0.25% with a maximum of 20 mL was injected on each side.

Under general anesthesia, the patient was positioned in the lateral decubitus position. The research assistant then opened the sealed envelope and allocated the patient into the standard or low pressure group based on inclusion number. After introducing the Hasson trocar, pneumoperitoneum was established by carbon dioxide (CO_2) insufflation. The patients received 8 or 12 mmHg pneumoperitoneum pressure (Olympus, Tokyo Japan) depending on their randomization. The surgeon inserted an endoscopic 30° video and introduced two 5-mm and 10- or 12-mm laparoscopic trocars under direct vision. Details of port placement and surgical space conditions during 8 and 12 mmHg pressure pneumoperitoneum can be viewed in Additional file 1 and 2. In this study, all patients underwent left kidney procurement. The kidney was extracted through the Pfannenstiel incision using an endobag and was immediately flushed with a cold preservative solution (Custodiol® HTK). At the end of surgery, the pneumoperitoneum was desufflated and the incision was closed. All patients received bilateral QL block using 0.25% bupivacaine before extubation. The patients received a reversal of muscle relaxant if necessary and were extubated. In this study, all anesthesia and surgery was performed by the same consultant team with comparable distributions.

Sample Collection and Analysis

Intrarenal Doppler using a 3.5–5 MHz ultrasound transducer (Logic 7-GE, USA) was used to measure interlobar arterial peak systolic and end diastolic velocities, and the resistive index (RI) was calculated by peak systolic velocity minus end diastolic velocity and divided by peak systolic velocity. RI measurements were performed on the left kidney before anesthesia induction (baseline), intraoperatively at 2 hours of pneumoperitoneum, and on the remaining right kidney 2 hours after gas desufflation.

Brachial vein venous blood samples and urine samples were collected at the same time of RI measurements. All samples were stored at -80^{o} C until analysis, and each sample was run in duplicate. Plasma IL-6, syndecan-1, and sVEGFR-2 were analyzed by ELISA (Human IL-6, Quantikine®, R&D, Minneapolis USA, Human CD138/Syndecan-1, Diaclone, France, and Human VEGF R2/KDR Quantikine® R&D) following manufacturer's instructions. KIM-1 was determined from a 10 µL urine specimen and was measured by ELISA (Human Urinary KIM-1, Quantikine®, R&D). Perioperative hemodynamic profiles were represented by heart rate, systolic pressure, diastolic pressure, mean arterial pressure, and cardiac output and were recorded at the same times as blood sample collection. Pre-postoperative serum creatinine and BUN were also recorded.

Immunohistochemistry and Renal Ultrastructure Examination

Cold ischemic time was defined as the interval between kidney immersion in ice and intravascular perfusion with cold preservative solution. One renal biopsy was performed at the end of this cold ischemic time. Tissues were immersed in Dubosq solution for 30 minutes and fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned. For syndecan-1 immunostaining, 4 mm sections were stained using periodic acid Schiff. Sections were incubated with Anti-Syndecan-1 primary antibody (B-A38, ab714, Abcam, USA) overnight at 4°C. For VEGFR-2 immunostaining, sections were incubated with Anti-VEGFR-2/KDR primary antibody (SP123, ab115805, Abcam) overnight at 4°C. After washing, sections were incubated with horseradish peroxidase conjugated secondary antibody for 30 minutes at room temperature. The slides were then washed and incubated with 3,3-Diaminobenzidine (DAB)-peroxidase substrate solution for 20 seconds.

Protein expression of syndecan-1 and VEGFR-2 was determined by immunohistochemistry, observed under a light microscope (Leica DM500) and photographed with a digital camera (Leica ICC50 HD, Germany). On each slide, 20 different fields (x400 magnification) were selected. Syndecan-1 expressions in the proximal and distal tubular epithelial cells were assessed, and semiquantitative analysis was performed using HER-2 score and H-Score. Five hundred proximal and distal tubular cells were assessed on each slide. Tagging and evaluation of intensity (0 – 3+) of these 500 cells were based on HER-2 criteria (0: no staining; 1+: weak and incomplete membrane staining in less than 10% of the cells; 2+: weak complete staining of the membrane in more than 10% of the cells; 3+: strong complete homogenous membrane staining in more than 30% of the cells)[11] with the help of the ImageJ software. This scoring was converted into percentages and entered into the histological score (H-score) formula. The resulting value equates to between 0-300:

VEGFR-2 expression in arterial endothelial cells, peritubular and glomerular capillaries, podocyte cells, and proximal and distal tubular epithelial cells was assessed. Semiquantitative analysis was performed by scoring the percentage of positive VEGFR-2 expression in 25 peritubular arteries and 50 peritubular capillaries in each sample. VEGFR-2 expression in proximal and distal tubular epithelial cells was assessed using HER-2 score and H-Score, as described above. All scoring was performed by three observers who were blinded to sample randomization.

Electron microscopy (EM) was performed to examine the ultrastructure of proximal tubules, distal tubules, peritubular capillaries, and arteries. After perfusion fixation with 4% paraformaldehyde, kidney tissue was fixed in 2.5% glutaraldehyde and postfixed with 2% osmium tetroxide in 2,5% $K_3Fe(CN)_6$ and 3% sucrose. The samples were dehydrated in graded ethanol, embedded in Spurr resin, and vacuumed. Ultrathin sections were stained with 2% uranyl acetate with triple lead citrate and examined by EM (JEOL 1010, Tokyo, Japan) at 80 kV.

Statistical Analysis

Sample calculations were performed based on a preliminary study containing 5 patients in each group (total of 10 patients) assessing effects of reductions in plasma syndecan-1 and sVEGFR-2 levels and

previous study.[12] Power analysis (a = 0.05, b = 0.20) with a 20% reduction in plasma syndecan-1 (SD ± 47) and sVEGFR-2 (SD ± 2062.32) was used to determine the sample size of 20 patients per group. A total sample size of 44 subjects was considered sufficient to allow for a 10% dropout.

A Chi-squared test was used for categorical variables. Parametric data were presented as the mean ± standard deviation or median (interquartile range) and were compared using unpaired t-test or Mann-Whitney test. Repeated analysis of variance followed by post hoc analysis was also performed. Transformed data were analyzed and presented as geometric means and 95% confidence interval (minimum–maximum) using a general linear model. All analysis was performed using SPSS 20.0 software. P-value < 0.05 was considered statistically significant.

Results

Patients were recruited between August 2017 and February 2018. The CONSORT flow diagram is presented in Figure 1. After exclusion of 2 patients, 44 patients were enrolled and analyzed.

All baseline and perioperative characteristics are presented in Table 1. More men (56%) than women (44%) were recruited, age range was 30–35 years, BMI ranged between 22–25 kg/m^2, and BUN and creatinine levels were 19.82–26.18 and 0.8–1.18 mg/dL, respectively. Perioperative subject data showed comparable pre- and postoperative urine output, total warm and cold ischemic time, and surgery and anesthesia duration between groups.

Table 1. Patients characteristics and perioperative data.

Characteristics	12 mmHg group (n = 22)	8 mmHg group (n = 22)
Sex		
Male (%)	45.5	68.2
Female (%)	54.5	31.8
Age	35.14 ± 19.41	30.50 ± 20.25
Weight (kg)	63.67 ± 9.02	60.19 ± 12.31
Height (cm)	159.80 ± 6,97	164.05 ± 8.27
Body Mass Index (BMI)	25.75 ± 5.37	22.23 ± 3.26
Pre-operative		
BUN (mg/dL)	19.82 ± 5.55	23.32 ± 5.64
Creatinine (mg/dL)	0.80 ± 0.5	0.87 ± 0.20
Post-operative		
BUN (mg/dL)	26.18 ± 4.76	28.05 ± 6.67
Creatinine (mg/dL)	1.10 ± 0.45	1.18 ± 0.31
Post-operative urine output (ml/kg/hour)	1.04 (0.70–3.30)	1.26 (0.70–1.98)
Duration of pneumoperitoneum (minute)	250 (190 – 245)	253 (189 – 330)
Duration of surgery (minute)	273 (258–288)	270 (210–360)
Duration of anesthesia (minute)	295 (245–385)	300 (230–390)
First warm ischemic time (minute)	3.50 (2.50–3.40)	3.51 (2.51–3.42)
Cold ischemic time (minute)	25.40 (23.10–30.40)	25.40 (23.12–30.41)

Categorical variable presented in n (%).

Numeric variable presented with mean (± standard deviation) or median (minimum–maximum).

Table 2 shows that hemodynamic cardiac index (CI), stroke volume index (SVI), mean arterial pressure (MAP), and end-tidal CO_2 are not significantly different between the 12 mmHg and 8 mmHg groups. However, heart rate (HR) in the 12 mmHg group was significantly higher than that in the 8 mmHg group ($p < 0.001$).

[Table 2. Intraoperative hemodynamic parameters and *end-tidal* CO_2.**]**

Parameters	Mean (CI 95%)		*p*	Mean Difference	*p*
	12 mmHg	8 mmHg		CI 95%	
1. Cardiac index (L/minute/m^2)					
a. baseline	2.84	2,76	0.740	1.028	
	(2.49–3.23)	(2.48–3.07)		(0.872–1.211)	
b. at 2 hours of pneumoperitoneum	3.20	3.35	0.628	0.957	0.593
	(2.78–3.70)	(2.98–3.77)		(0.800–1.146)	
c. 2 hours after desufflation	3.24	3.39	0.589	0.957	
	(2.85–3.69)	(3.08–3.73)		(0.818–1.119)	
2. Stroke volume index (mL/m^2)					
a. baseline	36.84	34.70	0.480	0.690	
	(32.77–41.67)	(31.05–38.47)		(0.240–1.980)	
b. at 2 hours of pneumoperitoneum	33.70	37.20	0.057	0.33	0.499
	(28.98–38.550	(32.34– 42.06)		(0.10–1.03)	
c. 2 hours after desufflation	32.12	37.64	0.07	0.35	
	(28.57–35.77)	(32.6–42.77)		(0.11–1.10)	
3. End-tidal CO_2 (mmHg)					
a. baseline	35.59	34.86	0.494	0.727	
	(33.89–37.29)	(33.47–36.25)		(-1.41–2.86)	
b. at 2 hours of pneumoperitoneum	37.77	38.00	0.800	-0.23	0.339
	(36.36–39.18)	(36.79–39.21)		(-2.03–1.57)	
c. 2 hours after desufflation	37,14	38.32	0.248	-1.182	
	(35.46–38.81)	(37.06–39.58)		(-3.22–0.85)	
4. Mean arterial pressure (mmHg)					

Parameters	Mean (CI 95%)		*p*	Mean Difference	*p*
	12 mmHg	8 mmHg		CI 95%	
a. baseline	77.92	78.30	0.917	-0.38	
	(72.75–83.10)	(72.85–83.76)		(-7.68–6.92)	
b. at 2 hours of pneumoperitoneum	80.05	82.31	0.467	-2.26	0.499
	(75.72–84.37)	(77.59–87.15)		(-8.47–3.95)	
c. 2 hours after desufflation	85.77	83.06	0.409	2.73	
	(80.48–91.07)	(78.79–87.31)		(-3.87–9.32)	
5. Heart rate (beats/minute)					
a. baseline	76.82	65.69	0.05	1.17	
	(72.34–81.56)	(61.56–70.10)		(0.07–1.27)	
b. at 2 hours of pneumoperitoneum	86.98	74.10	< 0,001	1.18	0.033
	(82.62–91.56)	(69.41–79.10)		(1.08–1.27)	
c. 2 hours after desufflation	95.39	77.66	< 0,001	1.23	
	(89.62–101.53)	(72.23–83.52)		(1.12–1.35)	

Data are presented as geometric mean and confidence interval 95% (minimum–maximum), $p<0.05$ is significant.

The two groups were compared with unpaired t-test and a general linear model.

Figure 2 shows a between-group comparison of changes in renal RI values, plasma IL-6, syndecan-1, sVEGFR-2, and urinary KIM-1 at baseline, 2 hours of pneumoperitoneum and 2 hours after desufflation. In both groups, RI was significantly increased during pneumoperitoneum and 2 hours after desufflation when compared to baseline (p <0.001). Perioperatively, RI was not significantly different between pressure groups (p = 0.746). Compared to baseline values, plasma IL-6 levels were significantly increased during 2 hours of pneumoperitoneum (almost 4-8 times greater), and increased 5–8 times more 2 hours after desufflation (p < 0.001). When compared to those in the 12 mmHg group, plasma IL-6 levels in the 8 mmHg group were significantly lower during pneumoperitoneum (4.58 vs 8.50 pg/mL) and 2 hours after

desufflation (36.18 vs 44.89 pg/mL) (p = 0.003). When compared to baseline values, plasma syndecan-1 levels were significantly increased during 2 hours of pneumoperitoneum, and increased 2 times further 2 hours after desufflation (p < 0.001). Plasma syndecan-1 levels during pneumoperitoneum (11.99 vs. 13.09 ng/mL) and postoperatively (30.07 vs 32.03 ng/mL) were consistently lower in the 8 mmHg group than in the 12 mmHg group. When compared to that in the 12 mmHg group, plasma sVEGFR-2 was significantly lower in the 8 mmHg group during pneumoperitoneum (8105.99 vs. 6841.05 pg/mL; p = 0.032) and 2 hours after desufflation (8452.25 vs. 7263.92 pg/mL; p = 0.044). When compared to baseline values, urinary KIM-1 was significantly higher during pneumoperitoneum and markedly decreased 2 hours after desufflation in both groups (p < 0.001).

(**A**) Renal resistive index (RI). (**B**) Interleukin-6 (IL-6). (**C**) Syndecan-1. (**D**) Soluble VEGFR-2. (**E**) KIM-1.

All data are presented as mean ± standard deviation. Continuous data was analyzed using repeated ANOVA. Between-group comparisons were analyzed using unpaired t-test and a general linear model; * p < 0.001, ** p < 0.05.

The 8 mmHg pressure group showed greater expression of syndecan-1 in the proximal tubules than the 12 mmHg pressure group. There was no difference in syndecan-1 expression in the distal tubules between groups. In both groups, syndecan-1 expression was negative in both glomerular and peritubular capillaries. The H-score of proximal tubule syndecan-1 expression was significantly higher in the 8 mmHg group (223.48 vs. 209.36; p = 0.030) than in the 12 mmHg group, while distal tubule expression was comparable (111.32 vs .108.40; p = 0.757) between groups (Figure 3).

(**A**)(**D**)(**G**) Negative control. **B**) Reduced intensity of proximal tubule syndecan-1 expression in the 12 mmHg group. (**C**) Proximal tubule syndecan-1 expression is stronger in the 8 mmHg group than in the 12 mmHg group. (**E**) Syndecan-1 expression between the distal tubule of the 12 mmHg group and (**F**) the 8 mmHg group was not different. (**H**)(**I**) Syndecan-1 expression is negative in the glomerular and peritubular capillaries of both pressure groups. Original magnification was x400, and red dashed boxes show a higher magnification. Red arrows indicate positive syndecan-1 expression, yellow arrows indicate negative syndecan-1 expression. (**J**) The H-score of proximal tubule syndecan-1 expression is higher in the 8 mmHg group than the 12 mmHg group (p = 0.030), and not significantly different between groups in the distal tubules (p = 0.757). Data are presented as mean ± standard deviation. The two groups were compared via unpaired t-test; * p < 0.05.

The expression of VEGFR-2 in the proximal and distal tubules was lower in the 8 mmHg group than the 12 mmHg group. The H-score of proximal and distal tubule VEGFR-2 expression was significantly lower in the 8 mmHg group than in the 12 mmHg group (p = 0.030 and 0.024, respectively). Peritubular capillary VEGFR-2 expression was lower in the 8 mmHg group. Peritubular capillary VEGFR-2 histological score

comparisons showed a lower percentage of strong expression cells and a lower histological score in the in 8 mmHg group ($p < 0.001$) than in the 12 mmHg group. Peritubular arterial endothelial cell VEGFR-2 expression was similar between groups (Figure 4).

(**A**)(**D**) Negative control. (**B**) Increased proximal tubule VEGFR-2 expression in the 12 mmHg group. (**C**) Proximal tubule VEGFR-2 expression is lower in the 8 mmHg group than in the 12 mmHg group. (**E**) Increased distal tubule VEGFR-2 expression in the 12 mmHg group. (**F**) Distal tubule VEGFR-2 expression is lower in the 8 mmHg group than in the 12 mmHg group. Original magnification was x400, and red dashed boxes show a higher magnification. Red arrows indicate positive VEGFR-2 expression. (**G**) The H-score of proximal and distal tubule VEGFR-2 expression are higher in the 12 mmHg group than in the 8 mmHg group (p = 0.005 and 0.024, respectively). Data are presented as mean ± standard deviation. Pressure groups were compared via unpaired t-test; * $p < 0.05$.

(**A**) Negative control. (**B**) Strong peritubular capillary and artery VEGFR-2 expression in the 12 mmHg group. (**C**) Peritubular capillary and artery VEGFR-2 expression is lower in the 8 mmHg group than in the 12 mmHg group. Original magnification was x400, and red dashed boxes show a higher magnification. Red arrows indicate positive syndecan-1 expression in the peritubular capillary endothelium, and yellow arrows indicate positive syndecan-1 expression in the peritubular artery endothelium. (**D)** VEGFR-2 peritubular capillary expression score is higher in the 12 mmHg group. (**E)** Artery VEGFR-2 expression score is not different between groups. Score value represents the percentage of positive VEGFR-2 expression in 25 peritubular arteries and 50 peritubular capillaries in each sample. Data were analyzed using Chi-square test for trends or Mann-Whitney test; * p < 0.001, ** p < 0.05.

Electron microscopy studies were performed to determine the early changes in tubular epithelial cells, peritubular capillaries, and glomerulus ultrastructure. Proximal tubule, distal tubule, and peritubular capillary endothelial cell ultrastructure morphology is shown in Figure 5. The 8 mmHg pressure group had better proximal and distal tubule ultrastructure morphology that showed intact cell membranes with clear cell boundaries, and intact brush borders compared to the 12 mmHg group. The 12 mmHg group showed swollen nuclei, a tenuous cell membrane, a distant boundary between cells, many vacuolizations, and the brush border was detached from the cell body. This indicates greater injury than in the 8 mmHg group. Vacuolization was not seen as much in the distal tubule of the 8 mmHg group as it was in the 12 mmHg group. The peritubular capillary in the 8 mmHg group showed an intact endothelial cell nucleus, endothelial layer, and basement membrane. Comparatively, the 12 mmHg group showed a swollen endothelial cell nucleus, an edematous endothelial layer, and basement membrane disruption in the peritubular capillary.

(**A**) Proximal tubular epithelial cells in the 12 mmHg group. Arrows show tenuous epithelial membranes and detached brush borders. (**B**) Proximal tubular epithelial cells in the 8 mmHg group. Arrows indicate a tight epithelial membrane and intact brush border. (**C**) Distal tubular epithelial cells in the 12 mmHg group show vacuolizations and a diffuse nuclear border. (**D**) Distal tubular epithelial cells in the 8 mmHg group show an intact nucleus and no vacuolization. (**E**) The peritubular capillary in the 12 mmHg group shows a swollen nucleus and edematous endothelial layer. The arrow shows a disrupted basement membrane. (**F**) The peritubular capillary endothelium in the 8 mmHg group shows an intact nucleus and endothelial layer. The arrow shows an intact basement membrane. The red box represents the details of images (**E**) and (**F**), and can be seen at a larger scale in (**G**) and (**H**); scale bar = 2 mm. N = nucleus, P = Podocyte, FP = Foot podocyte, BB = Brush Border, V = vacuole, BM = basement membrane, e = endothelium.

Follow up appointments were conducted with all patients within one year after laparoscopic nephrectomy. The one year follow up levels of BUN (25.46 ± 1.33 vs 29.33 ± 1.37, p = 0.185) and blood creatinine (1.08 ± 1.30 vs 1.25 ± 1.26, p = 0.089) were not significantly different between pressure groups. In the 8 mmHg group, one patient had a period of bloody urine after surgery, and one patient had minor complaints of surgical site discomfort during activity. In the 12 mmHg group, two patients had minor complaints of surgical site discomfort during activity. The remaining 40 patients had no surgical complaints.

Discussion

While several studies have demonstrated the negative effects of positive-pressure pneumoperitoneum on cardiovascular and organ perfusion, many institutions still continue to use standard pressure pneumoperitoneum at 12–14 mmHg due to its surgical space convenience. Unfavorable consequences are not expected during most elective laparoscopic operations in healthy or low-risk individuals. However, increased intraabdominal pressure has a significant clinical impact on high-risk patients including the elderly population, cardiac dysfunction patients or critically ill patients.[6,13]

Our study results consistent with previous study results indicating that CI, SVI, MAP, and end-tidal CO_2 levels were not significantly different between low and standard pressure groups.[14–16] Despite transient reductions in CI and SVI at the beginning of insufflation, the levels recovered after gas insufflation.[14] We did not expect any between-group differences in these parameters to confound hemodynamic effects, volume status, and CO_2 outcomes. In our study, the heart rate in the low pressure group trended significantly lower than the standard pressure group. This difference has not been reported in previous studies.[14,16] One effect of low pressure pneumoperitoneum was reduced postoperative pain due to lower visceral pain secondary to peritoneal stretch receptors.[14] However, we excluded pain or hypercarbia effects from the outcome since all subjects received fentanyl i.v. maintenance and QL block during surgery for intra and postoperative pain management. Additionally, normal end-tidal CO_2 value, level of BIS and TOF were maintained at comparable levels during pneumoperitoneum and surgery in both

groups. We assumed the higher heart rate in the standard pressure group was a response to the higher inflammatory response due to higher pneumoperitoneum pressure.

Kidney function is very sensitive to increases in intraabdominal pressure. 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.[7,17] An animal study showed that CO_2 pneumoperitoneum of 12–18 mmHg induced renal cell apoptosis in the outer medulla and cortex.[17] In humans, increased intra-abdominal pressure caused hypoperfusion in the abdominal or splanchnic regions with or without hypotension. Research on animals showed that 12 mmHg pneumoperitoneum resulted in hypoperfusion that induced the release of inflammatory cytokines and neutrophil migration.[7,18] Advanced venous congestion and decreased renal blood flow leads to tissue hypoperfusion or ischemia that triggers an inflammatory response. After desufflation, reperfusion occurs when renal blood flow is normalized. This leads to oxidative stress that stimulates the synthesis of inflammatory cytokines, which have been postulated to mediate the association between blood flow changes and endothelial-epithelial injury.[6,19]

Increased intraabdominal pressure causes mechanical compression of the inferior vena cava, renal vasculature, and parenchyma.[20,21] It increases sympathetic activity, which is regulated through CO_2-mediated baroreceptors, and can lead to renal cortical vasoconstriction and its sequelae.[18,19] Kidney autoregulation is influenced by vascular (myogenic) and tubuloglomerular feedback (TGF). Vascular factors affect autoregulation of renal perfusion through blood flow and pressure on blood vessels, which depend on cardiac output and blood pressure (as long as it is on the threshold of autoregulation).[22] Under normal conditions, blood flow is laminar, which gives constant pressure to the blood vessel walls. Changes in blood flow cause shear stress due to turbulent or oscillatory flow. Shear stress stimulates the pro-inflammatory transcription factor NF-κB signalling pathway and the synthesis of nitric oxide through VEGFR-2 activation, which is present on the surface of endothelial cells.[23–26] Furthermore, stimulation of inflammatory responses plays a role in endothelial and epithelial cell activation, injury, repair, and apoptosis. Shear stress in the form of a constant or uniform laminar flow has a protective effect on the endothelium. Low or turbulent shear stress caused by impaired blood flow will stimulate the inflammatory response, increasing the expression of endothelial adhesion molecules and their interactions with neutrophils and monocytes in the endothelium.[23]

During pneumoperitoneum insufflation, an increase in RI indicates that increased intra-abdominal pressure causes a decrease in interlobar arterial blood flow, stimulating a systemic inflammatory response that triggers the release of IL-6.[23] Our study showed a higher release of IL-6 during the increasing intra-abdominal pressure in the standard pressure group than in the low pressure group. Pneumoperitoneum insufflation using CO_2 gas is not ideal, as the high solubility of CO_2 gas makes it readily absorbable by tissue, resulting in sympathetic stimulation such as tachycardia. Although CO_2 and surgical techniques can contribute to the release of pro-inflammatory cytokines,[27] our study showed that an acute, slight increase in intraabdominal pressure results in significantly increased IL-6 levels. Furthermore, using a low pressure pneumoperitoneum can attenuate this response.

Studies on the impact of low versus standard pressure pneumoperitoneum have shown various results. A laparoscopic cholecystectomy study performed with low and standard pressures showed no differences in the increase of IL-6, IL-8, and IL-10. Our study results mirrored another laparoscopy study that found significantly higher IL-1, IL-6, and CRP levels in the standard-pressure pneumoperitoneum group than in the low pressure pneumoperitoneum group.[19] Yap et al. found that laparoscopic donor nephrectomy resulted in nearly 50% of their subjects showing an increase in tumor necrosis factor-alpha (TNF-α) excretion at both 5 and 24 hours and increased urine neutrophil gelatinase-associated lipocalin (NGAL) after donor nephrectomy without any significant differences were observed in cardiopulmonary parameters. These results also suggested that elevated cytokine content may be due in part to increased endogenous production. The results of this study validate the previously published study demonstrating that animal models of AKI nephrectomy resulted in increased TNF-α, IL-6, and monocyte chemoattractant protein-1 expression. IL-6 has been shown to induce neutrophil infiltration with increased macrophage infiltration.[28] An animal study showed extrarenal IL-6 production from the liver after unilateral nephrectomy.[29] As urine output and serum creatinine were within the normal limit before and after the procedure, our results suggest that the increased plasma IL-6 was due to increased endogenous production and not decreased renal excretion.

As hypothesized, we found that increasing plasma syndecan-1 corresponded to elevated plasma IL-6. IL-6 is a proinflammatory cytokine that causes syndecan-1 activation and shedding from the endothelial surface of blood vessels into the bloodstream. In accordance with the degree of inflammation that occurs, the shedding of syndecan-1 increased in both levels of pneumoperitoneum pressure compared to the baseline conditions. However, the increasing plasma syndecan-1 level was lower and proximal tubular cell syndecan-1 expression was higher in the low pressure group than in the standard pressure group. There is a significant increase in syndecan-1 glycocalyx product degradation after major surgery in humans and animals.[30] The duration of laparoscopic nephrectomy is longer than open nephrectomy, and the addition of high pressure pneumoperitoneum use leads to longer and more profound warm ischemia that contributes to syndecan-1 shedding.[31]

The renal tubular epithelium can not only be passively injured but can also produce an active response to inflammation. The release of proinflammatory and chemotactic cytokines activates T-cells and their co-stimulating molecules. Proximal tubular cells respond to T-cell ligands through cell surface receptor activation.[19] The increasing syndecan-1 expression and its shedding into the blood are considered an adaptive response to repair and early cell injury.[32] Syndecan-1 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 human kidney transplantation. In early renal injury, tubular epithelial cells increase syndecan-1 regulation to repair injured cells. In response to a mild inflammatory condition, increasing tubular syndecan-1 expression results in better re-epithelialization in allografts, and correlates with less proteinuria and tubular atrophy, lower serum creatinine, and lower risk of delayed graft function. Syndecan-1 becomes a tubular marker that correlates with kidney graft function and survival.[33] In further injury, epithelial cells will increasingly lose syndecan-1 due to their decreasing ability

to proliferate and regenerate this factor. The sustained elevating plasma syndecan-1 and low syndecan-1 expression correlate with the degree of kidney tubular function loss.[34] 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]

Vascular endothelial growth factor-A (VEGF-A) is a strong angiogenic cytokine that has a role in maintaining the microvascular system and increasing vascular permeability. One regulator of VEGF-A is VEGFR-2, which is expressed during ischemic or inflammatory conditions.[36,37] When inflammation occurs, 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 the endothelial injury.[34,38] 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.[34] In a normal human kidney, VEGFR-2 is expressed on glomerular endothelial cells and peritubular capillaries, as well as tubular epithelial cells at 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.[36,39]

The synthesis and activation of VEGFR-2 in baseline conditions occurs but is very mild. Our study found an increase in the synthesis and higher activation of VEGFR-2 in the standard pressure than the low pressure group. The level of plasma sVEGFR-2 was significantly higher when standard pressure was used. In comparison, the low pressure pneumoperitoneum attenuated the inflammatory response and produced lower sVEGFR-2 levels. Activation of VEGFR-2 as the marker of vascular endothelial permeability depends on the extent of inflammation and results in an increase in endothelial permeability and increased levels of plasma sVEGFR-2. Plasma soluble VEGFR-2 is the result of an increase in alternative splicing of mRNA or as a proteolytic product of membrane-bound VEGFR-2 released into the bloodstream. During ischemia-reperfusion injury, VEGFR-2 mRNA expression and sVEGFR-2 increase as a response of VEGFR-2 receptors. Increased VEGFR-2 expression is a direct effect of VEGF released by ischemic tubular epithelial cells to the adjacent endothelial cells to maintain capillary blood supply and promote tubular cell survival and recovery. As a comparison, previous studies have shown that at laparoscopic sites, there is an increase in protective VGEF-mRNA expression as a response to injured tissue repair.[36,40,41]

Our observed the increase in tubular epithelial cell VEGFR-2 expression may suggest that inflammatory responses occurring in circulation reach the extracellular matrix and renal tubules. Tubular epithelial cell VEGFR-2 expression was higher in the standard than in the low pressure group. The low pressure group produced less injury to the kidney due to less inflammation and less stimulation of VEGFR-2 in the renal endothelial and tubular epithelial cells. A previous study showed that overstimulation of VEGFR-2 occurring before unilateral nephrectomy induced endothelial proliferation, abnormal angiogenesis, extracellular matrix deposition, and acute tubulointerstitial injury in experimental animals.[39] There is a hypothesis that syndecan-1 acts as a VEGFR-2 co-receptor and has a role in modulating VEGF-VEGFR-2

signals for endothelial cell proliferation and survival. It has been proposed that syndecan-1 and VEGFR-2 act as new markers for AKI and its treatment.[34,42]

From electron microscopy examination, the low pressure group showed intact tubular cell membranes with clear cell boundaries and intact brush borders. These morphologies were healthier when compared to the standard pressure group, which showed greater injury, tenuous tubular cell membranes, brush borders detached from the cell body, and more vacuolization. The extracellular matrix peritubular endothelial cell was also more edematous in the standard pressure pneumoperitoneum group. These results support the preference of low pressure pneumoperitoneum, which also results in a lower degree of ischemia and tissue inflammation, and reduced endothelial and tubular epithelial cell injury. Perioperative ischemia and reperfusion cause injury to donor kidney epithelial cells that can continue to induce a response from the vascular endothelium.[31,33] Animals treated with various CO_2 pneumoperitoneum pressure gradients indicate that increased intraabdominal pressure causes reperfusion ischemic injury leading to cell apoptosis.[17] Damage or loss of tubular epithelial cells is the main histological finding of tissue damage that occurs in renal ischemia-reperfusion injury. In humans, acute tubular necrosis is observed in 44% of open nephrectomy and 45% of laparoscopic patients. In patients undergoing laparoscopy nephrectomy, 54% of renal biopsy specimens taken showed subcapsular cortical injury. These injuries indicate that pneumoperitoneum and mechanical injury during laparoscopic surgical manipulation causes acute tubular necrosis accompanied by peritubular capillary congestion.[41]

In our study, urinary KIM-1 level was lower during low pressure pneumoperitoneum than standard pressure pneumoperitoneum. The increasing urinary KIM-1 during pneumoperitoneum expressed the proximal tubule cell stress injury accompanied by the formation of debris and apoptotic cells. This process causes an increase in KIM-1 molecule synthesis that will be released into the lumen of the tubule and detection in the urine.[43] 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 laparoscopy procedure.

We found syndecan-1 to be expressed in proximal and distal tubular epithelial cells, with negative syndecan-1 expression within the glomerular or peritubular vasculature. This result was similar to that of Adepu et al., who found syndecan-1 in the basolateral layer in proximal tubular epithelial cells in human kidney biopsy samples and hypothesized that the increase in plasma syndecan-1 levels was partly derived from an extravascular source such as the renal tubular epithelial cells.[33] Our study on living donor patients showed contradictory results to a previous animal study that showed the presence of syndecan-1 protein in the glomerulus and peritubular capillaries.[34] Syndecan-1 may not have been detected in the glomerular endothelium because the dominant proteoglycan expression in glomerular endothelial cells are syndecan-4, perlecan, and glypican according to research on human glomerular endothelial culture cells in vitro. Other studies also show the dominant proteoglycan layer in IgA nephropathy to be of perlecan and biglycan.[35,37] Urinary syndecan-1 can be used as another alternative to detect extravascular shedding of the glycocalyx layer.

Our study showed that laparoscopic donor nephrectomy resulted in increased plasma IL-6, syndecan-1, and sVEGFR-2 during pneumoperitoneum and 2 hours after gas desufflation. IL-6 as a mediator of the extrarenal effects of AKI is a clinically important finding, since it may lead to the use of cytokine-binding proteins and other anti-inflammatory agents to improve outcome beyond what current supportive renal measures can offer. Despite the absence of syndecan-1 from the glomerular and peritubular endothelial glycocalyx, it was found in the membrane of the proximal and distal tubules and was important for renal tubular cell survival during inflammation. VEGFR-2 can be a sensitive marker to detect endothelial injury due to perfusion disturbance and inflammation. Both increasing plasma syndecan-1 and sVEGFR-2 levels, rather than plasma creatinine, BUN or urine output, can be interpreted as an early warning of the underlying injury.

Endothelial and renal tubular responses are the earliest signs of hypoperfusion and inflammation due to increased intra-abdominal pressure.[3,7,18] From our study results, although the 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. The usage of low pressure pneumoperitoneum can attenuate this systemic inflammatory and vascular response. Inhibiting syndecan-1 shedding and the release of VEGFR-2 are believed to have renal-protective roles.[31,34,39,42] 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. It is also important to evaluate the risks and benefits between low and standard pressure in operator's point of view related to operative comfort such as space for dissection, and vision while using suction.

Conclusions

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. As such, we should consider using lower pneumoperitoneum pressure during laparoscopic nephrectomy. Lowering pneumoperitoneum pressure is a logical modification to implicate in an effort to reduce endothelium and renal tubular epithelium injury.

Abbreviations

IAP, intra-abdominal pressure; AKI, acute kidney injury; VEGF, vascular endothelial growth factor; sVEGFR-2, soluble vascular endothelial growth factor receptor-2; KIM-2, kidney injury molecule-1; IL-6, interleukin-6; QL, quadratus lumborum; RI, resistive index; BM, basement membrane.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the medical ethics committee of the Faculty of Medicine, Universitas Indonesia (protocol no. 17-06-0619, approval date: June 19th, 2017), and was registered on ClinicalTrial.gov (NCT:03219398). Written consent was obtained from each participant.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interest.

Funding

Funding was provided by grants from Tugas Akhir Doktor Universitas Indonesia (1303/UN2.R3.1/HKP.05.00/2018).

Authors' contributions

Study design: DA and CAM. Study conduction: DA and CAM. Data analysis: DA, AL, NCS, NIM, ASM. Manuscript preparation: DA, AL, SS. All authors contributed to the development of interim and final drafts and read and approved the final manuscript.

Acknowledgements

The authors would like to thank Meilania Sardjana, MD from Universitas Indonesia, Faculty of Medicine, Department of Anatomical Pathology; Alida Harahap, MD PhD from Eijkman Institute of Molecular Biology, Jakarta; Kuntjoro Harimurti, MD PhD from Universitas Indonesia, Faculty of Medicine, Department of Internal Medicine; Isabella Kunia Liem, MD, M. Biomed, PhD from Universitas Indonesia, Faculty of Medicine, Department of Anatomy.

References

1. Smit M, Hofker HS, Leuvenink H, Krikke C, Jongman RM, Zijlstra JG, van Meurs M. A human model of intra-abdominal hypertension: even slightly elevated pressure lead to increased acute systemic inflammation and signs of acute kidney injury. Crit Care. 2013;17:425–6. doi:1186/cc12568.

2. Cobb WS, Burns JM, Kercher KW, Matthews BD, Norton HJ, Henniford BT. Normal Intraabdominal Pressure in Healthy Adults. J.Surg.Res. 2005;129(2):231–5. doi:10.1016/j.jss.2005.06.015.
3. Kirkpatrick AW, Roberts DJ, De Waele J, Jaeschke R, Malbrain ML, De Keulenaer B, et al. Intra-abdominal hypertension and the abdominal compartment syndrome: updated consensus definitions and clinical practice guidelines from the World Society of the Abdominal Compartment Syndrome. Intensive Care Med. 2013;39(7):1190–1206. doi:10.1007/s00134-013-2906-z.
4. Sodha S, Nazarian S, Adshed JM, Vasdev N, Mohan-S G. Effect of Pneumoperitoneum on Renal Function and Physiology in Patient Undergoing Robotic Renal Surgery. Curr Urol. 2015;9:1–4.
5. Wiesenthal JD, Fazio LM, Perks AE, et al. Effect of pneumoperitoneum on renal tissue oxygenation and blood flow in a rat model. 2011;77(6):1508.e9–e15. doi: 10.1016/j.urology.2011.02.022.
6. Neves FM, Meneses GC, Sousa NE, et al. Syndecan-1 in acute decompensated heart failure–association with renal function and mortality. Circulation journal: official journal of the Japanese Circulation Society. 2015;79(7):1511-9. doi: 1253/circj.cj-14-1195.
7. de Melo Bezerra Cavalcante CT, Castelo Branco KM, Pinto Junior VC, et al. Syndecan-1 improves severe acute kidney injury prediction after pediatric cardiac surgery. The Journal of thoracic and cardiovascular surgery. 2016;152(1):178–e172. doi: 10.1016/j.jtcvs.2016.03.079.
8. Celie J, Katta KK, Adepu S, Melenhorst W, Reijmers R, Slot EM. Tubular epithelial Syndecan-1 maintains renal function in murine ischemia/reperfusion and human transplantation. Kidney Inter. 2012;81:651–61. doi: 1038/ki.2011.425.
9. Varga Z, Noske A, Ramach C, Padberg B, Moch H. Assessment of HER2 status in breast cancer: overall positivity rate and accuracy by fluorescence in situ hybridization and immunohistochemistry in a single institution over 12 years: a quality control study. BMC Cancer. 2013 Dec 30; 13:615. doi: 1186/1471-2407-13-615.
10. De Souza DB, Costa WS, Cardoso LEM, Benchimol M, Pereira-Sampaio MA, Sampaio FJB. Does prolonged pneumoperitoneum affect the kidney? Int Braz J Urol. 2013;39:30– doi: 10.1590/S1677-5538.IBJU.2013.01.05
11. Bickel A, Loberant N, Bersudsky M, Goldfeld M, Ivry S, Herskovits M, Eitan A. Overcoming reduced hepatic and renal perfusion caused by positive-pressure pneumoperitoneum. Arch Surg. 2007;142:119–124. doi:1001/archsurg.142.2.119.
12. Wallace DH, Serpell MG, Baxter JN, O'Dwyer PJ. Randomized trial of different insufflation pressures for laparoscopic cholecystectomy. Br J Surg. 1997;84(4):455– doi: 10.1046/j.1365-2168.1997.02628.x
13. Sefr R, Puszkailer K, Jagos F. Randomized trial of different intraabdominal pressures and acid-base balance alterations during laparoscopic cholecystectomy. Surg Endosc. 2003;17(6):947–doi: 10.1007/s00464-002-9046-9.
14. Joshipura VP, Haribhakti SP, Patel NR, Naik RP, Soni HN, Patel B, et al. A prospective randomized, controlled study comparing low pressure versus high pressure pneumoperitoneum during

laparoscopic cholecystectomy. Surg Laparosc Endosc Percutan Tech. 2009;19(3):234– doi: 10.1097/SLE.0b013e3181a97012.

15. Malbrain ML, Vidts W, Ravyts M, De Laet I, De Waele J. Acute intestinal distress syndrome: the importance of intra-abdominal pressure. Minerva Anestesiol. 2008;74:657–
16. Khoury W, Jakowlev K, Fein A, Orenstein H, Nakache R, Weinbroum AA. Renal apoptosis following carbon dioxide pneumoperitoneum in a rat model. J Urol. 2008;180:1554–8. doi: 10.1016/j.juro.2008.06.008.
17. Schietroma M, Pessia B, Stifini D, Lancione L, Carlei F, Cecilia EM, et al. Effects of low and standard intra-abdominal pressure on systemic inflammation and immune response in laparoscopic adrenalectomy: A prospective randomized study. J Minim Access Surg. 2016;12(2):109–17.
18. Post EH, Vincent JL. Renal autoregulation and blood pressure management in circulatory shock. 2018;22:8. doi: 1186/s13054-018-1962-8.
19. Bryan TM, Ducles H, Feng S, Hsiao ST, Kim HJ, Serbanovic-canic J, et al. Mechanoresponsive networks controlling vascular inflammation. Arterios Thromb Vasc Biol. 2014;34:2199–205. doi: 1161/ATVBAHA.114.303424.
20. Jin ZG, Ueba H, Tanimoto T, Lungu AO, Framme MD, Berk BC. Ligand-independent activation of vascular endothelial growth factor receptor 2 by fluid shear stress regulates activation of endothelial nitric oxide synthase. Circ Res. 2003;93(4):354– doi: 10.1161/01.RES.0000089257.94002.96.
21. Dela Paz NG, Melchior B, Frangos JA. Early VEGFR2 activation in response to flow is VEGF-dependent and mediated by MMP activity. Biochem Biophys Res Commun. 2013;434(3):641– doi: 10.1016/j.bbrc.2013.03.134.
22. Bovens SM, Foin N, O'Clery N, Van Der Heiden K, Cuhlmann S, Carlsen H, Barahona M, Evans PC, Krams R. Shear stress and nitric oxide transport affect NFkB dynamics in endothelial cells. Eur Heart J. 2013;34(suppl1):P582. doi: 10.1093/eurheartj/eht307.P582.
23. Atkinson TM, Giraud GD, Togioka BM, Jones DB, Cigarroa JE. Cardiovascular and Ventilatory Consequences of Laparoscopy Surgery. Circulation. 2017;135:700– doi:10.1161/circulationaha.116.023262.
24. Yap S, Park SW, Egan B, Lee HT. Cytokine elevation and transaminitis after laparoscopic donor nephrectomy. Am J Physiol Renal Physiol. 2012;302: F1104– doi: 10.1152/ajprenal.00543.2011.
25. Park SW, Chen SW, Kim M, Brown KM, Kolls JK, D'Agati VD, Lee HT. Cytokines induce small intestine and liver injury after renal ischemia or nephrectomy. Lab Invest. 2011;91:63–84. doi: 10.1038/labinvest.2010.151.
26. Steppan J, Hofer S, Funke B, Brenner T, Henrich M, Martin E, et al. Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalyx. J Surg Res. 2001;165:136– doi:10.1016/j.jss.2009.04.034.
27. Shimizu T, Tanabe K, Ishida H, Toma H, Yamagushi Y. Histopathological evaluation of biopsy specimens of donor kidney procured by laparoscopic donor nephrectomy. Clin Transplant. 2004;18(11):24– doi: 10.1111/j.1399-0012.2004.00243.x.

28. Kolálová H, Ambruzová B, Šindlerová LS, Klinke A, Lukáš Kubala L. Modulation of Endothelial Glycocalyx Structure under Inflammatory Conditions. Mediators of Inflammation. 2014; doi: 1155/2014/694312.
29. Adepu S, Rosman CW, Dam W, van Dijk MC, Navis G, van Goor H, Bakker SJ, van den Born J. Incipient renal transplant dysfunction associates with tubular Syndecan-1 expression and shedding. Am J Physiol Renal Physiol. 2015;309:F137–45. doi: 1152/ajprenal.00127.2015.
30. Jing Z, Wei-Jie Y, Yi-Feng ZG, Jing H. Downregulation of Syndecan-1 induce glomerular endothelial cell dysfunction through modulating internalization of VEGFR-2. Cellular signalling. 2016 Apr;28(8):826– doi: 10.1016/j.cellsig.2016.04.001.
31. Bjornson A, Moses J, Ingemansson A, Haraldsson B, Sorensson J. Primary human glomerular endothelial cells produce proteoglycans, and puromysin affects their posttranslational modification. Am J Renal Physiol. 2005;288:F748– doi: 10.1152/ajprenal.00202.2004.
32. Kanellis J, Paizis K, Cox AJ, Stacker SA, Richard EG, Mark EC, David AP. Renal ischemia-reperfusion increases endothelial VEGFR-2 without increasing VEGF or VEGFR-1 expression. Kidney International. 2002;61:1696–706. doi: 1046/j.1523-1755.2002.00329.x
33. Ebefors K, Granqvist A, Ingelsten M, Molne J, Haraldsson B, Nystrom J. Role of glomerular proteoglycan in IgA nephropathy. PloS ONE. 2011;6(4):e18575. doi: http://10.1371/journal.pone.0018575.
34. Sison K, Eremina V, Baelde H, Min W, Hirashima M, Fantus IG, et al. Glomerular structure and function require paracrine not autocrine VEGF-VEGFR-2 signaling. J. Am. Soc. Nephrol. 2012;12: 1691– doi: 10.1681/ASN.2010030295.
35. Sato W, Tanabe K, Kosugi T, Hudkins K, Lanaspa MA, Zhang L, et al. Selective stimulation of VEGFR2 accelerates progressive renal disease. Am J Pathol. 2011;179:155– doi: 10.1016/j.ajpath.2011.03.024.
36. Nekouei S, Ahmadnia H, Abedi M, Alamolhodaee MH, Abedi MS. Resistive index of the remaining kidney in allograft kidney donors. Exp Clin Transplant. 2012;5:454– doi: 10.6002/ect.2012.0054.
37. Machado C, Malheiros DM, Adamy A, Santos LS, Silva Filho AF, Nahas WC, et al. Protective response in renal transplantation: no clinical or molecular differences between open and laparoscopic donor nephrectomy. Clinics. 2013;68(4):483– doi: 10.6061/clinics/2013(04)08.
38. Lu Z, Song N, Shen B, Xu X, Fang Y, Shi Y, et al. Syndecan-1 Shedding Inhibition to protect against ischemic acute kidney injury through HGF target signaling pathway. Transplantation. 2018;102(7):e331– doi: 10.1097/tp.0000000000002170.
39. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest. 2011;121(11):4210–21. doi: 1172/JCI45161.

Additional Files

Additional file 1: The port placement and surgical space condition during 12 mmHg pressure pneumoperitoneum. (MPEG-4; 19,986 kB)

Additional file 2: The port placement and surgical space condition during 8 mmHg pressure pneumoperitoneum. (MPEG-4; 20,003 kB)

Figures

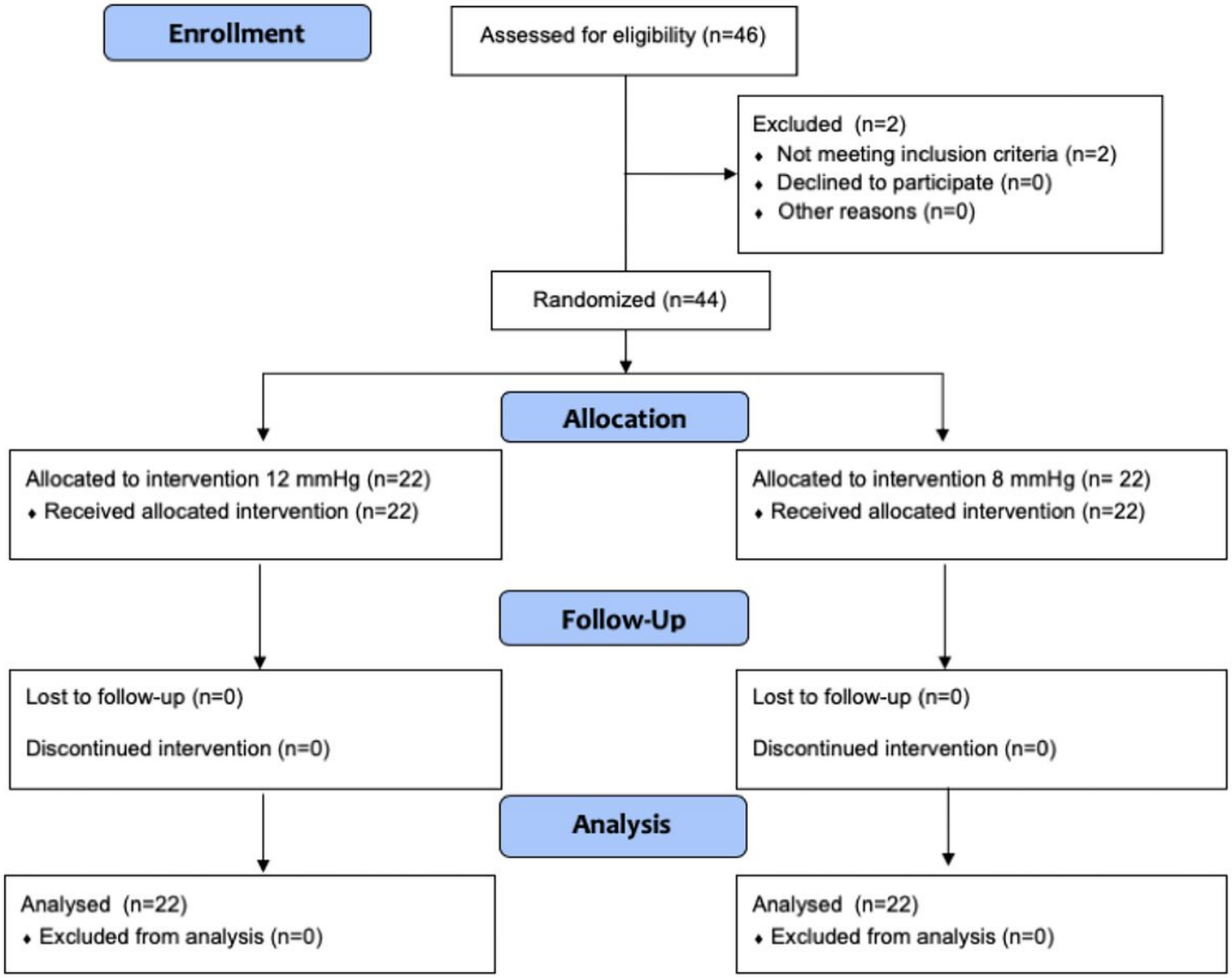

Figure 1

CONSORT flow diagram.

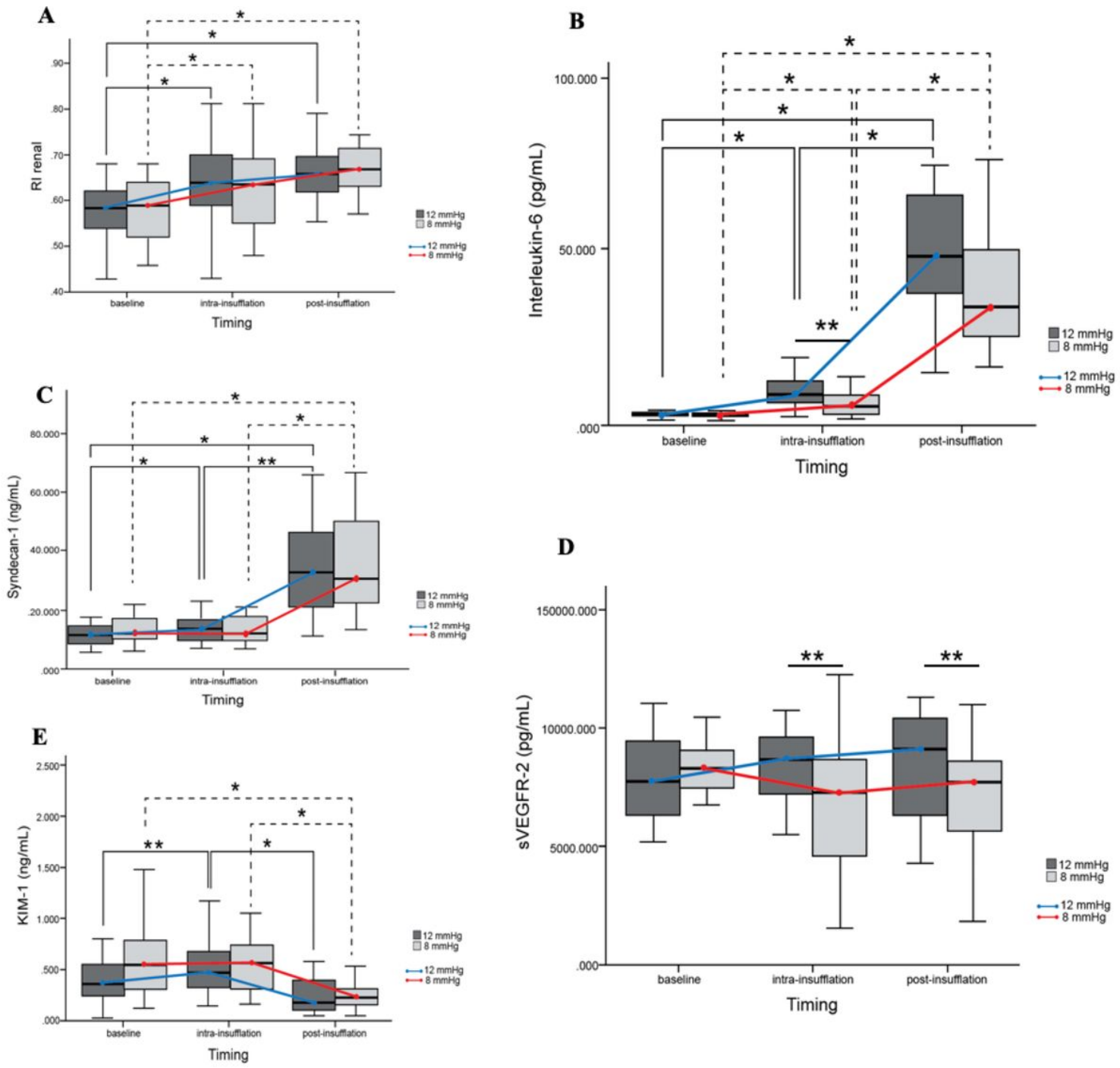

Figure 2

Comparison of renal RI, plasma IL-6, syndecan-1, sVEGFR-2, and urinary KIM-1 between 12 mmHg and 8 mmHg groups. (A) Renal resistive index (RI). (B) Interleukin-6 (IL-6). (C) Syndecan-1. (D) Soluble VEGFR-2. (E) KIM-1. All data are presented as mean ± standard deviation. Continuous data was analysed using repeated ANOVA. Between-group comparisons were analysed using unpaired t-test and a general linear model; * $p < 0.001$, ** $p < 0.05$.

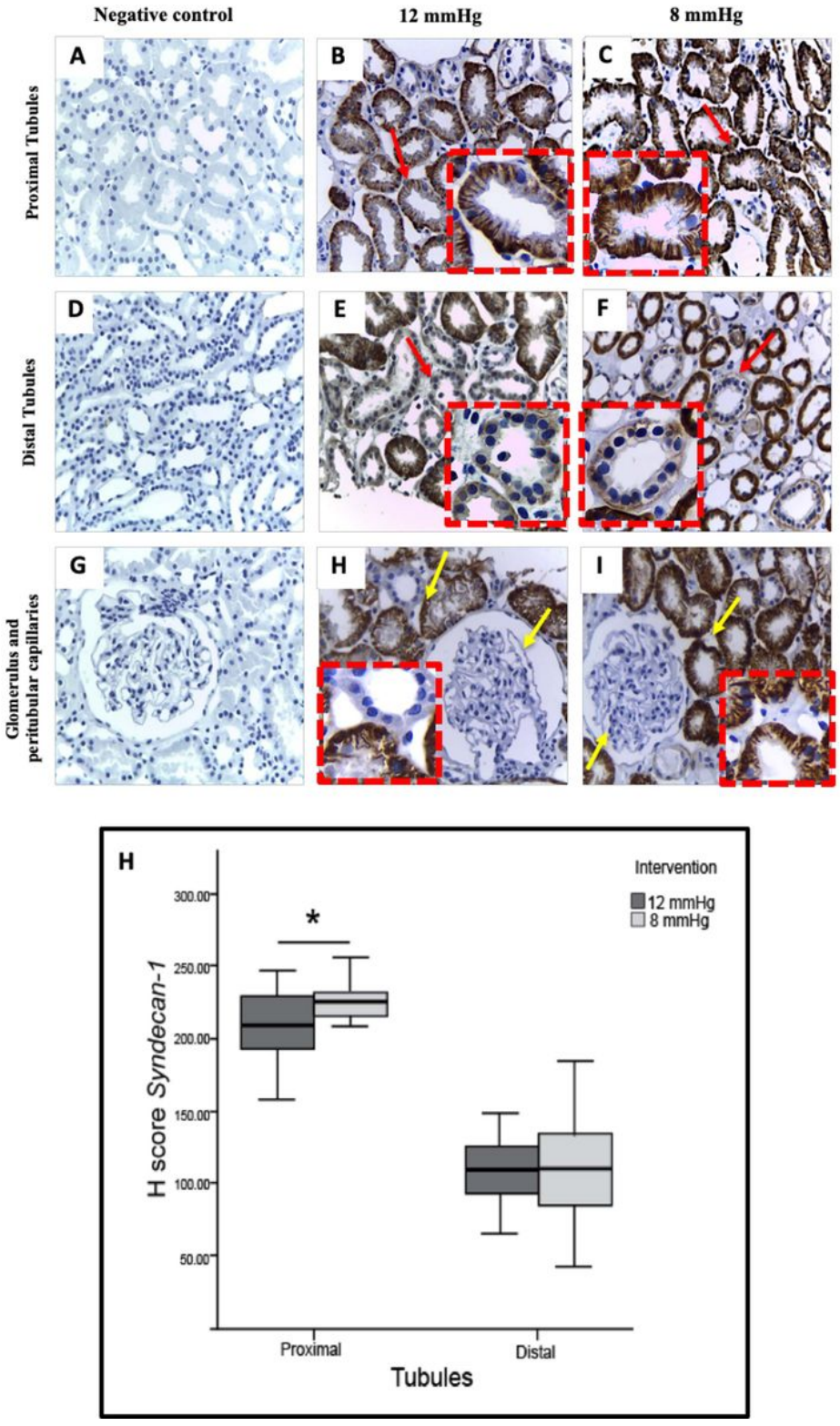

Figure 3

Syndecan-1 expression of tubular epithelial cells in 12 mmHg and 8 mmHg groups. (A)(D)(G) Negative control. B) Reduced intensity of proximal tubule syndecan-1 expression in the 12 mmHg group. (C) Proximal tubule syndecan-1 expression is stronger in the 8 mmHg group than in the 12 mmHg group. (E) Syndecan-1 expression between the distal tubule of the 12 mmHg group and (F) the 8 mmHg group was not different. (H)(I) Syndecan-1 expression is negative in the glomerular and peritubular capillaries of

both pressure groups. Original magnification was x400, and red dashed boxes show a higher magnification. Red arrows indicate positive syndecan-1 expression, yellow arrows indicate negative syndecan-1 expression. (J) The H-score of proximal tubule syndecan-1 expression is higher in the 8 mmHg group than the 12 mmHg group (p = 0.030), and not significantly different between groups in the distal tubules (p = 0.757). Data are presented as mean ± standard deviation. The two groups were compared via unpaired t-test; * p < 0.05.

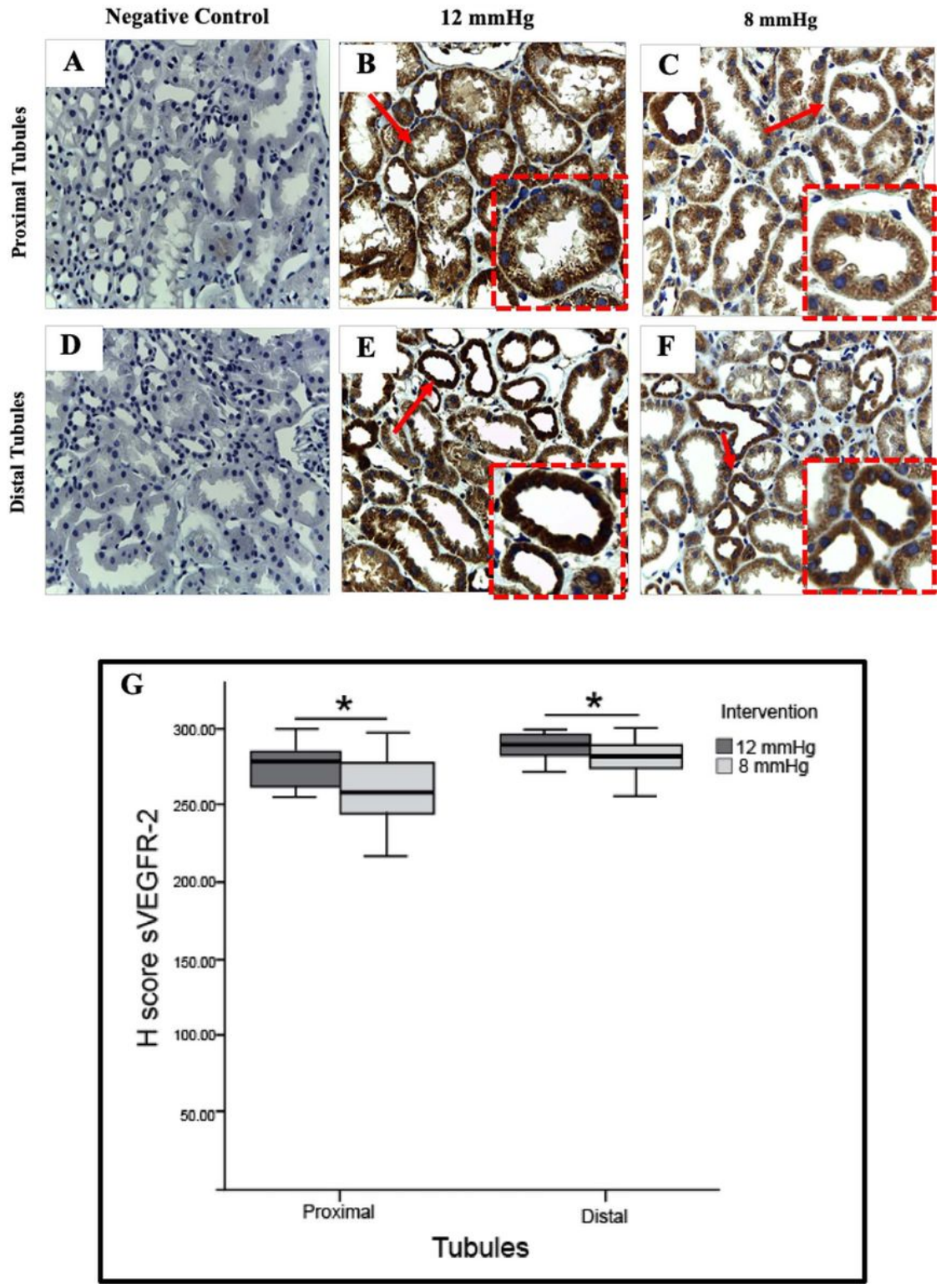

Figure 4

Tubular epithelial VEGFR-2 expression in the 12 mmHg and 8 mmHg groups. (A)(D) Negative control. (B) Increased proximal tubule VEGFR-2 expression in the 12 mmHg group. (C) Proximal tubule VEGFR-2 expression is lower in the 8 mmHg group than in the 12 mmHg group. (E) Increased distal tubule VEGFR-2 expression in the 12 mmHg group. (F) Distal tubule VEGFR-2 expression is lower in the 8 mmHg group than in the 12 mmHg group. Original magnification was x400, and red dashed boxes show a higher magnification. Red arrows indicate positive VEGFR-2 expression. (G) The H-score of proximal and distal tubule VEGFR-2 expression is higher in the 12 mmHg group than in the 8 mmHg group ($p = 0.005$ and 0.024, respectively). Data are presented as mean ± standard deviation. Pressure groups were compared via unpaired t-test; * $p < 0.05$.

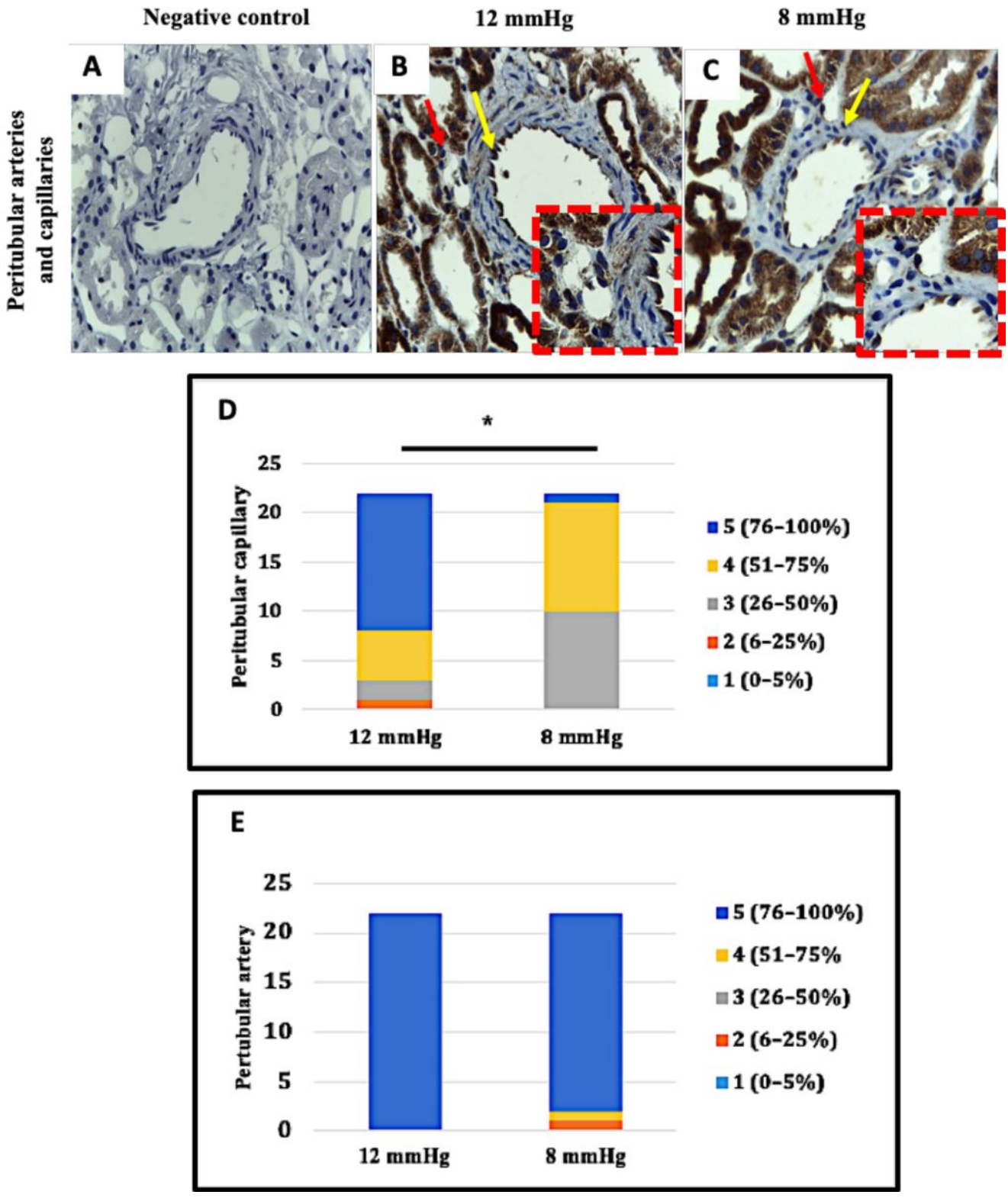

Figure 5

Peritubular vascular endothelial cell VEGFR-2 expression in 12 mmHg and 8 mmHg pneumoperitoneum pressure groups. (A) Negative control. (B) Strong peritubular capillary and artery VEGFR-2 expression in the 12 mmHg group. (C) Peritubular capillary and artery VEGFR-2 expression is lower in the 8 mmHg group than in the 12 mmHg group. Original magnification was x400, and red dashed boxes show a higher magnification. Red arrows indicate positive syndecan-1 expression in the peritubular capillary

endothelium, and yellow arrows indicate positive syndecan-1 expression in the peritubular artery endothelium. (D) VEGFR-2 peritubular capillary expression score is higher in the 12 mmHg group. (E) Artery VEGFR-2 expression score is not different between groups. Score value represents the percentage of positive VEGFR-2 expression in 25 peritubular arteries and 50 peritubular capillaries in each sample. Data were analysed using Chi-square test for trends or Mann-Whitney test; * $p < 0.001$, ** $p < 0.05$.

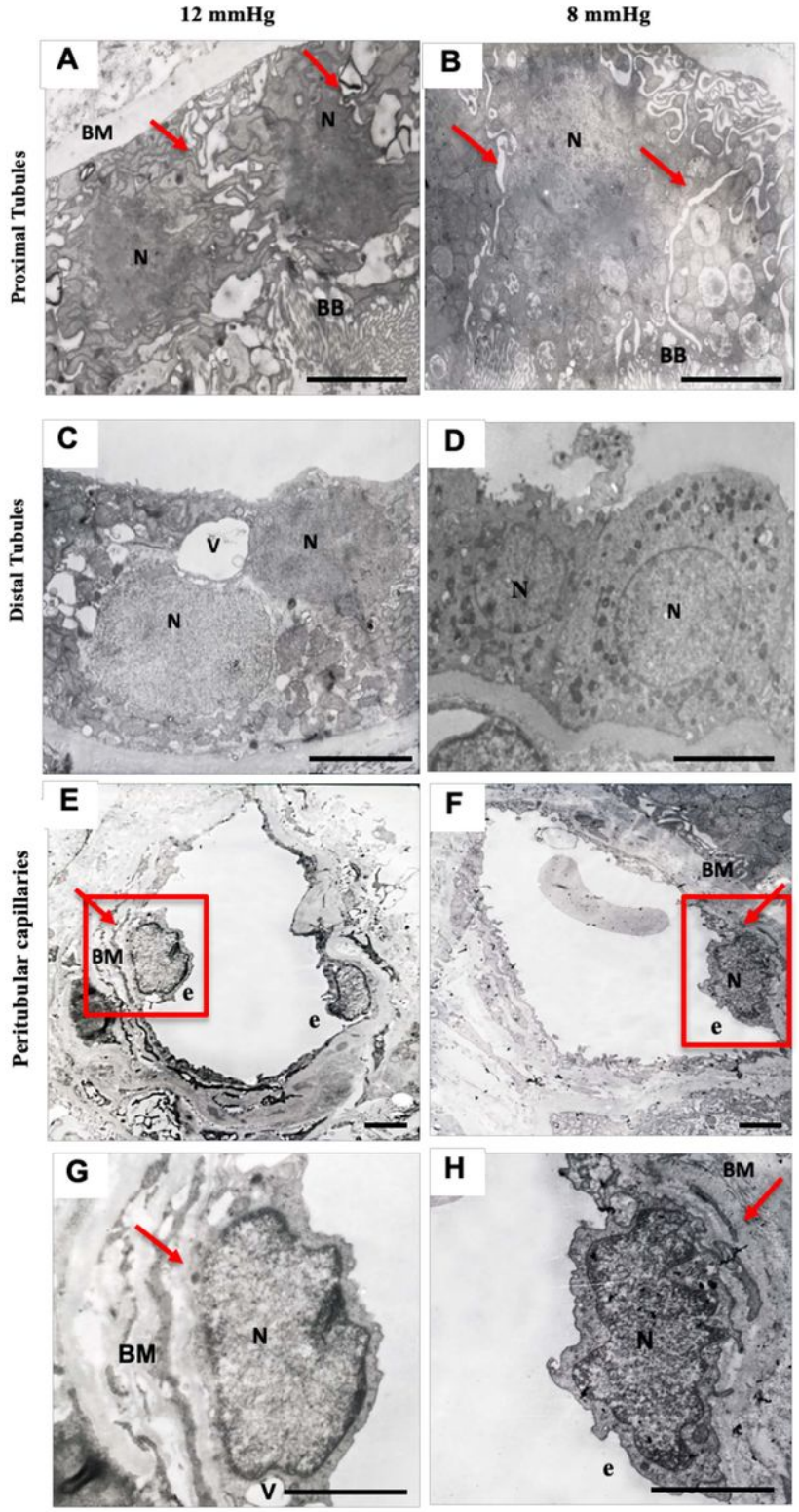

Figure 6

Renal tubule and peritubular capillary ultrastructure in the 12 mmHg and 8 mmHg pneumoperitoneum pressure groups. (A) Proximal tubular epithelial cells in the 12 mmHg group. Arrows show tenuous epithelial membranes and detached brush borders. (B) Proximal tubular epithelial cells in the 8 mmHg group. Arrows indicate a tight epithelial membrane and intact brush border. (C) Distal tubular epithelial cells in the 12 mmHg group show vacuolisations and a diffuse nuclear border. (D) Distal tubular epithelial cells in the 8 mmHg group show an intact nucleus and no vacuolisation. (E) The peritubular capillary in the 12 mmHg group shows a swollen nucleus and oedematous endothelial layer. The arrow shows a disrupted basement membrane. (F) The peritubular capillary endothelium in the 8 mmHg group shows an intact nucleus and endothelial layer. The arrow shows an intact basement membrane. The red box represents the details of images (E) and (F), and can be seen at a larger scale in (G) and (H); scale bar = 2 m. N = nucleus, P = Podocyte, FP = Foot podocyte, BB = Brush Border, V = vacuole, BM = basement membrane, e = endothelium.

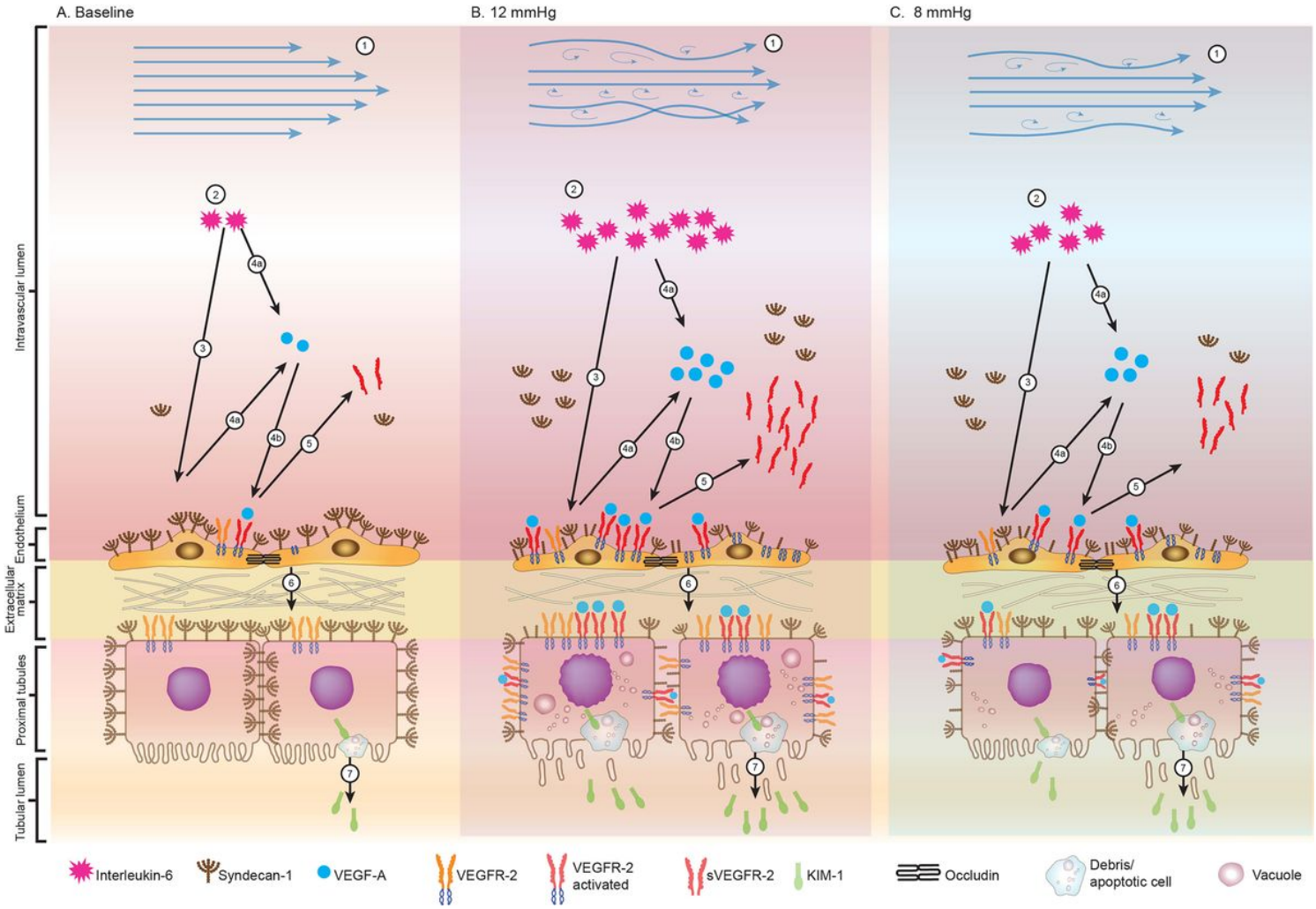

Figure 7

The proposed mechanism of endothelial cell and kidney tubule injury that occurs in the standard and low-pressure pneumoperitoneum. 1. Standard pressure (12 mmHg group) decreases interlobar artery blood flow and results in more changes from laminar flow to turbulent flow [B] than low pressure (8 mmHg group) [C]. 2. The inflammatory response in the 12 mmHg group produces higher IL-6 levels than the 8

mmHg group. 3. Interleukin-6 causes more syndecan-1 activation and shedding from the endothelial surface into the bloodstream in the 12 mmHg group than in the 8 mmHg group. 4.(a) Interleukin-6 and syndecan-1 stimulate VEGF-A synthesis and (b) binding to VEGFR-2 on the endothelial surface. 5. Activation of VEGFR-2 increases sVEGFR-2 levels more so in the 12 mmHg group than in the 8 mmHg group. 6. The expression of VEGFR-2 in tubular epithelial cells is higher in the 12 mmHg group, and the expression of syndecan-1 is lower in the 12 mmHg group than in the 8 mmHg group. 7. Due to inflammation, tubular epithelial cell injury stimulates the synthesis of KIM-1 molecules that will be released into the tubular lumen (urine).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1Portplacementsurgicalspace12mmHg.mp4
- Additionalfile2Portplacementsurgicalspace8mmHg.mp4