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Impact of pneumoperitoneum on intra-abdominal microcirculation blood flow: an experimental randomized controlled study of two insufflator models during transanal total mesorectal excision

An experimental randomized multi-arm trial with parallel treatment design

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Abstract

Objective To compare changes in microcirculation blood flow (MCBF) between pulsatile and continuous flow insufflation.

Summary background data Transanal total mesorectal excision (TaTME) was developed to improve the quality of the resection in rectal cancer surgery. The AirSeal IFS® insufflator facilitates the pelvic dissection, although evidence on the effects that continuous flow insufflation has on MCBF is scarce.

Methods Thirty-two pigs were randomly assigned to undergo a two-team TaTME procedure with continuous ($n=16$) or pulsatile insufflation ($n=16$). Each group was stratified according to two different pressure levels in both the abdominal and the transanal fields, 10 mmHg or 14 mmHg. A generalized estimating equations (GEE) model was used.

Results At an intra-abdominal pressure (IAP) of 10 mmHg, continuous insufflation was associated with a significantly lower MCBF reduction in colon mucosa [13% (IQR 11;14) vs. 21% (IQR 17;24) at 60 min], colon serosa [14% (IQR 9.2;18) vs. 25% (IQR 22;30) at 60 min], jejunal mucosa [13% (IQR 11;14) vs. 20% (IQR 20;22) at 60 min], renal cortex [18% (IQR 15;20) vs. 26% (IQR 26;29) at 60 min], and renal medulla [15% (IQR 11;20) vs. 20% (IQR 19;21) at 90 min]. At an IAP of 14 mmHg, MCBF in colon mucosa decreased 23% (IQR 14;27) in the continuous group and 28% (IQR 26;31) in the pulsatile group ($p=0.034$).

Conclusion TaTME using continuous flow insufflation was associated with a lower MCBF reduction in colon mucosa and serosa, jejunal mucosa, renal cortex, and renal medulla compared to pulsatile insufflation.

Keywords Transanal total mesorectal excision · Randomized controlled trial · Continuous insufflation · Colored microspheres

The transanal total mesorectal excision (TaTME) assisted by laparoscopy was developed for the treatment of rectal cancer [1]. TaTME can be performed either via one-team or two-teams approach, the latest decreasing operative time [2]. This technique allows for an easier pelvic dissection and has been accepted by the surgical community due to its potential of increasing the quality of the specimen [1].

Laparoscopy is based on the insufflation of the peritoneal cavity in order to enlarge the working area, visualize and manipulate the organs. However, this pneumoperitoneum is associated with variations in systemic hemodynamics, pulmonary mechanics, and a decrease of intra-abdominal organs' microcirculation blood flow (MCBF) [3, 4]. The primary mechanism is the compression of the vessels due to increased intra-abdominal pressure (IAP) with a cranial shift of the diaphragm. Conventional CO_2 insufflators are based on autoregulated pulsatile flow fluctuation, and animal and human studies have demonstrated that laparoscopic

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insufflation with pulsatile flow affects vessel tone regulation, triggering hypoperfusion and reperfusion injury, inducing oxidative stress, cellular injury, and organ dysfunction [5–10].

The transanal approach of the TaTME can be technically demanding, and the difficulty is even higher with conventional insufflators because of the throb of the surgical field and poor visualization due to smoke. New technological developments recently appeared, such as a new valve-free CO_2 insufflator (AirSeal IFS®, SurgiQuest, Conmed Corporation, Milford, CT, USA), with a continuous flow and immediate response to minimal IAP variations, avoiding the billowing effect and with a constant smoke evacuation [11]. The AirSeal IFS® consists of three devices: the Intelligent Flow System (IFS), the AirSeal trocar, and the AirSeal Mode Evacuation (ASM-Evac) Tri-lumen Filter Tube Set. The AirSeal valve-free trocar includes CO_2 nozzles that act as pressure gas barriers and preserve the set IAP pressure, in contrast to the trapdoor valves of conventional trocars. The ASM-Evac Tri-lumen Filter consists on one lumen for CO_2 influx, one lumen for CO_2 outflux to the IFS, and a third lumen for concurrent uninterrupted pressure assessment. Once the fixed pressure is reached, the CO_2 flow is spontaneously diminished to 3 L/min, while preserving the fixed pressure. Nepple et al. proved that pneumoperitoneum generated by the AirSeal IFS® was more stable than with the conventional insufflators, even under suction maneuvers [12].

Among the many factors that have been proposed to contribute to hypoperfusion and oxidative stress, the pressure level and the duration of the pneumoperitoneum seem to be the most significant [13]. This study aimed to assess the role of a third variable: the type of gas flow, pulsatile versus continuous, through the use of the conventional or the AirSeal IFS® insufflators. We hypothesized that the continuous flow, by avoiding erratic fluctuations in IAP, would have less effect on vessel tone regulation and thus less impact on organ hypoperfusion. Therefore, the primary objective was to compare intra-abdominal MCBF between the two insufflation systems in pigs undergoing a two-team TaTME procedure. A secondary objective was to investigate changes in systemic hemodynamics, gas exchange, and endothelium-derived mediators.

Methods

Animal model

The Institutional Review Board of the Hospital Clinic approved this randomized multi-arm parallel trial for the Care and Use of Laboratory Animals. The study was conducted following the Principles of Laboratory Animal Care. The University of Barcelona Committee on Ethics in Animal Experimentation and the Catalan Department of the Environment Commission on Animal Experimentation granted ethical approval for the study (Reg. 0006S/11367/2015). Thirty-two healthy female Yorkshire pigs weighing 25–28 kg were studied between October 2015 and March 2016. All animals fasted 12 h before surgery, with free access to water. They were sedated with 4 mg/kg ketamine + 2 mg/kg xylazine + 0.1 mg/kg midazolam and anesthetized by intravenous sodium thiopental (10 mg/kg) and fentanyl (50 µg), before proceeding to endotracheal intubation. Lung-protective mechanical ventilation strategy (tidal volume 8 mL/kg, plateau airway pressure ≤ 30 cmH_2O, positive end-expiratory pressure 4 cmH_2O, and FiO_2 0.4 in air) was established. Appropriate respiratory rate changes were allowed to maintain end-tidal CO_2 ($PetCO_2$) between 30 and 40 mmHg. Anesthesia was maintained with desflurane (MAC ~ 2.5). Cisatracurium (0.75 mg/Kg/h) and continuous infusion of fentanyl (10 µg/kg/h) were administered for muscle relaxation and analgesia, respectively. Fluid therapy was based on 5 mL/kg/h of isotonic saline; a bolus of 1 mL/kg of 6% hydroxyethyl starch was administered, when needed, to maintain constant cardiac output avoiding flow-dependent changes in mediator release and vascular resistance. Core temperature was monitored (PiCCO™; Pulsion Medical Systems, AG, Munich, Germany) and maintained between 36.5 and 38 °C. Once anesthetized, the animals were placed and maintained in supine position.

Animal instrumentation

Under aseptic conditions, a 5-French PICCO catheter and a 7-French bilumen catheter were introduced into the right common carotid artery and in the right jugular vein respectively, to obtain hemodynamic parameters and blood sampling. For MCBF determination, colored microspheres (Dye Trak, Triton Technology, San Diego, CA, USA) were injected by a catheter that was inserted through the left carotid artery to the left ventricle guided by morphology and ventricular pressure curve. To obtain arterial reference sampling of microspheres, a catheter was placed in the right femoral artery.

Randomization and experimental procedure

After instrumentations, animals were stabilized for 30 min (stabilization was defined as MAP ≥ 70 mmHg and HR ≤ 80 b/min and normal arterial blood gases). Randomization to one of the two types of CO_2 insufflation used in both the abdominal and the transanal fields [continuous vs. pulsatile, the latter being performed with the UHI-4 device (Olympus, Hamburg, Germany)] was done using a random-number table. The table contained five-digit numbers arranged in

rows and columns. Sequentially numbered sealed envelopes were used by a surgeon not directly involved in the study, opening them the night before each intervention and informing the principal investigator (FBL). Therefore, a random allocation sequence was developed, and animals were randomized into two experimental groups ($n = 16$ for each one). Each group was stratified according to two different levels of pressure in both fields, 10 mmHg ($n = 8$) or 14 mmHg ($n = 8$). IAP was monitored continuously through the laparoscope, together with digital monitorization through an intra-abdominal catheter (Angiocath, BD Medical Systems, Sandy, UT) connected to a pressure transducer (Edwards Lifesciences, Irvine, CA).

Primary objective: intra-abdominal microcirculation blood flow

Colored microspheres with a 15-µm diameter were used. The delivery of microspheres depends on the tissue perfusion at the time of microspheres injection, and tissue concentration is therefore proportional to the flow *per unit volume or mass of tissue* at the level of the capillaries. Due to the movement of microspheres out of the capillaries into the interstitial space, retention of microspheres is excellent. The specific description of the technique has been detailed elsewhere [14].

The microspheres were administered at three time points: (1) baseline, after stabilization and before insufflation (T1); (2) 60 min after starting insufflation (T2); (3) 90 min after starting insufflation (T3). Following the last microsphere injection, the animals were euthanized with a deep overdose of sodium thiopental and intravenous potassium chloride. After certifying a correct position of the catheter above the aortic valves, tissue samples were carefully harvested from the organs of interest: ascending colon mucosa (CM) and serosa (CS), mesentery of the colon (MC); jejunum mucosa (JM) and serosa (JS), mesentery (M); renal cortex (RC) and renal medulla (RM). The trapped microspheres in each tissue and blood sample were quantified using flow cytometry [14].

Secondary objectives: systemic hemodynamics, blood gases, and endothelium-derived mediators

Hemodynamic variables included cardiac index (CI), which was monitored by thermodilution technique in triplicate, and continuously by pulse contour analysis; heart rate (HR); mean arterial pressure (MAP); global end-diastolic index (GEDI, a reliable preload index), through the PICCO system [15]; and systemic vascular resistance index (SVRI), which were continuously recorded using computerized interface data (PICCO).

Blood gases and pH corrected for temperature were measured with a blood gas analyzer (Rapidjoint 405, Bayer Health Care Systems) calibrated daily with tonometer whole human blood. The pulmonary gas exchange was evaluated by calculation of the alveolar/arterial oxygen difference as $PA\text{-}aO_2 = (713 \times FiO_2 - PaCO_2/0.8) - PaO_2$. Also, the collapse of lung tissue (atelectasis) was estimated by an increased $Pa\text{-}ET_{CO_2}$ gradient [16].

At the three time points, the measured endothelium-derived mediators were:

Whole blood nitrite-to-nitrate: vascular function is affected by both perpendicular transmural pressure (myogenic response) and parallel frictional force (shear stress) [17]. Nitric oxide (NO) release from the endothelium may act as a brake to limit arteriolar vasoconstriction [18]. Nitrite (NO_2^-) and nitrate (NO_3^-) anions are part of the endogenous NO metabolism, representing an indirect measurement of the activation of nitric oxide synthase and NO release during pneumoperitoneum. Therefore, samples (10 ml) containing NO_2^- and NO_3^- were quantitatively reduced to NOx in a solution containing vanadium (III) chloride. Next, NOx was quantified by a chemiluminescence detector after reaction with ozone in a NO analyzer (NOA 280i, Sievers, GE Instruments, CO, USA).

Plasma renin activity (PRA): the oliguria caused by IAP is due to compression of the renal vessels and parenchyma, but also the β-adrenergic stimulation and activation of the renin–angiotensin–aldosterone system, resulting in renal cortical vasoconstriction [19]. PRA, an estimation of plasma renin form released in response to β-adrenergic stimulation, measures the rate of formation of active peptide angiotensin II which is a potent endogenous vasoconstrictor that may enhance microcirculation vasoconstriction, and was quantified by RIA technique (GammaCoat Plasma Renin Activity, DiaSorin Inc., MN, USA).

Surgical technique

Two teams working simultaneously performed the surgical procedure. The transabdominal part was initiated with insufflation of CO_2 through a Veress needle and insertion of four laparoscopic ports. The goal of this team was to divide inferior mesenteric vessels and dissect the descending colon.

The transanal team started with the introduction of the transanal platform (Gelpoint path, Applied Medical, Inc., Rancho Santa Margarita, CA). Pneumorectum was initiated and the rectal lumen was closed with a purse-string suture. After the rectotomy, the mesorectal fascia was reached and the dissection continued upwards. When the two teams met ("rendez-vous"), both continued working together until the rectum and sigmoid were entirely free. Then the surgical procedure was finished, without specimen retrieval or anastomosis performance. As the effects of IAP on microcirculation are time dependent, all surgeries were terminated at 90 min (T3). In case of finishing the task before,

pneumoperitoneum was maintained until reaching the final time point (T3).

Statistical analysis

Descriptive results were presented as the median with interquartile range [IQR: 25th, 75th percentiles]. A generalized estimating equations (GEE) model, with an estimation of within-subject correlation from AR(1) approach to account for the repeated measurements, was used. This methodology considers for statistical analyses all follow-up of each experimental animal, not time-to-time independently. We performed three different models to evaluate the effect of the use of continuous or pulsatile insufflation, IAP (10 mmHg or 14 mmHg) and their combination. All models included one of these independent factors, time, and the interaction of factor by time. These analyses were performed by a non-parametrical approach through a rank-transformation of the dependent variables.

A two-sided Type I Error equal to 0.05 was used in all statistical analyses. All calculations were performed with SPSS version 25 (IBM) software. Due to the experimental proof of concept of this study, no formal sample size calculation was performed, and no methods for multiplicity were done.

Researchers who evaluated biochemical variables and organ blood flow were blind to the knowledge of the groups of samples.

Results

During the study period, a total of 32 animals were randomly assigned to either TaTME with continuous or pulsatile CO_2 insufflation. All animals survived until the end of the experiment. However, one case in the continuous-14 group was excluded from the final analyses due to a bilateral empyema that was discovered when the correct positioning of the catheter above the aortic valves was certified. Moreover, in two pigs the biopsies could not be analyzed due to technical problems with the spectrophotometer and were finally excluded from the MCBF analysis.

The TaTME procedure was completed in all cases with no intraoperative events. The median duration until the complete release of the specimen was 76 min (IQR 73;80) in the set of pulsatile groups, compared with 71 min (IQR 64.2;72) in the set of continuous groups ($p = 0.001$).

Microcirculation blood flow

Table 1 shows MCBF in the median percentage of change, while absolute median outcomes are presented in Supplementary Table 1. Throughout the study, a significant reduction of MCBF from baseline was found in every organ. At an IAP of 10 mmHg, continuous insufflation was associated with a significantly lower MCBF reduction in colon mucosa, colon serosa, jejunal mucosa, renal cortex, and renal medulla. At an IAP of 14 mmHg, MCBF in colon mucosa decreased 23% (IQR 14;27) in the continuous group and 28% (IQR 26;31) in the pulsatile group (p = 0.034) (Figs. 1, 2, 3, 4, 5).

Systemic hemodynamics, blood gases, endothelium-derived mediators

The secondary outcomes are shown in Table 2 (median percentage of change) and Supplementary Tables 2 and 3 (absolute medians). HR significantly increased only in the pulsatile-14 group at 60 min ($p < 0.001$) compared to baseline. At an IAP of 14 mmHg and 60 min, HR decreased 5.4% (IQR − 18;20.6) in the continuous group and increased 18.3% (IQR 12.6;31.7) in the pulsatile group, $p = 0.027$ (Supplementary Fig. 1).

Pa-ET_{CO_2} gradient increased at all times when the pulsatile insufflator was used, although no significant differences were found between groups. At an IAP of 10 mmHg, NOx remained stable in the pulsatile group, while it significantly decreased in the continuous group ($p < 0.001$), compared to baseline. At 14 mmHg and 90 min, the decrease in NOx concentration was greater in the continuous group: 13% (IQR 1.9;16) versus 1.1% (IQR − 6.3;9.6); $p = 0.013$ (Supplementary Fig. 2). PRA concentration showed a non-significant decrease in the continuous-10 group at an IAP of 10 mmHg ($p = 0.814$), and a non-significant increase in both groups at an IAP of 14 mmHg (continuous $p = 0.107$, pulsatile $p = 0.280$), compared to baseline values.

Discussion

A lower impact over MCBF on colon mucosa, colon serosa, jejunal mucosa, renal cortex, and renal medulla was found when continuous flow insufflation was used during TaTME. Therefore, continuous insufflation not only seems to facilitate the pelvic dissection from a purely surgical point of view, but it might potentially prevent the development of hypoperfusion and reperfusion injury in selected organs.

The effects of pneumoperitoneum during laparoscopic surgery have been well reported and might have significant consequences for the patient, resulting in morbidity and even mortality [6, 7, 20, 21]. These adverse effects can be both regional and systemic. Mechanical compression and humoral-mediated vasoconstriction are suggested to be responsible for the regional effect [20]. Moreover, the increase of IAP might result in hypoperfusion, oxidative stress, and organ dysfunction [10]. To this cascade of events, variations on systemic hemodynamics must be

Table 1 Microcirculation blood flow of intra-abdominal organs

	IAP 10 mmHg		IAP 14 mmHg	
	60 min	90 min	60 min	90 min
CM				
Cont	−13 (−14;−11)*ᵗ	−15 (−16;−14)*ᵗ	−23 (−27;−14)*ᵗ	−26 (−31;−19)*
Puls	−21 (−24;−17)*ᵗ	−20 (−24;−16)*ᵗ	−28 (−31;−26)*ᵗ	−31 (−32;−25)*
CS				
Cont	−14 (−18;−9.2)*ᵗ	−14 (−18;−11)*ᵗ	−27 (−29;−14)*	−31 (−32;−15)*
Puls	−25 (−30;−22)*ᵗ	−26 (−32;−24)*ᵗ	−33 (−38;−25)*	−35 (−39;−25)*
MC				
Cont	−19 (−21;−15)*	−21 (−25;−17)*	−25 (−26;−23)*	−29 (−33;−20)*
Puls	−20 (−25;−15)*	−18 (−19;−17)*	−32 (−37;−28)*	−35 (−40;−33)*
JM				
Cont	−13 (−14;−11)*ᵗ	−13 (−18;−8.1)*ᵗ	−21 (−28;−16)*	−24 (−27;−21)*
Puls	−20 (−22;−20)*ᵗ	−24 (−25;−23)*ᵗ	−31 (−33;−22)*	−32 (−34;−26)*
JS				
Cont	−17 (−20;−15)*	−19 (−21;−16)*	−26 (−30;−24)*	−30 (−35;−23)*
Puls	−22 (−24;−15)*	−25 (−27;−20)*	−31 (−36;−26)*	−34 (−37;−30)*
M				
Cont	−14 (−15;−10)*	−13 (−23;−9.3)*	−29 (−40;−23)*	−30 (−43;−28)*
Puls	−17 (−21;−12)*	−19 (−20;−15)*	−31 (−33;−28)*	−33 (−34;−25)*
RC				
Cont	−18 (−20;−15)*ᵗ	−18 (−22;−15)*ᵗ	−31 (−33;−30)*	−32 (−36;−29)*
Puls	−26 (−29;−26)*ᵗ	−32 (−34;−29)*ᵗ	−33 (−39;−30)*	−35 (−41;−33)*
RM				
Cont	−13 (−17;−12)*	−15 (−20;−11)*ᵗ	−25 (−28;−18)*	−25 (−28;−22)*
Puls	−18 (−21;−17)*	−20 (−21;−19)*ᵗ	−24 (−27;−23)*	−26 (−30;−24)*

Data are presented as median percentage of change (IQR)

CM colon mucosa, *CS* colon serosa, *MC* mesentery of the colon, *JM* jejunum mucosa, *JS* jejunum serosa, *M* mesentery, *RC* renal cortex, *RM* renal medulla, *Cont* continuous insufflation, *Puls* pulsatile insufflation

*Difference from baseline values with $p < 0.05$

ᵗDifference between groups with $p < 0.05$

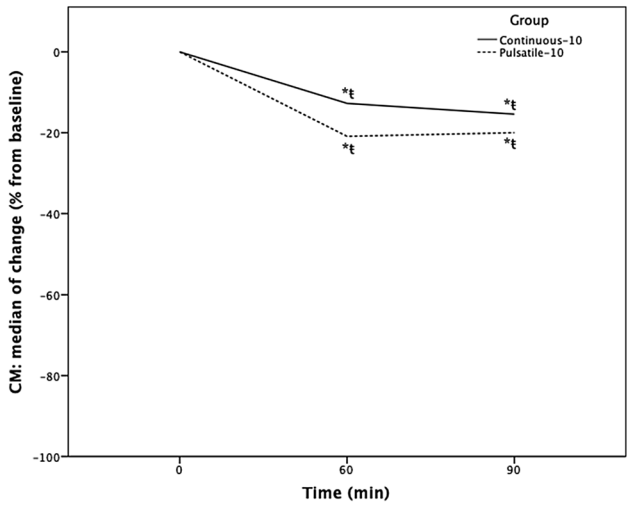

Fig. 1 Microcirculation blood flow at colon mucosa at an IAP of 10 mmHg

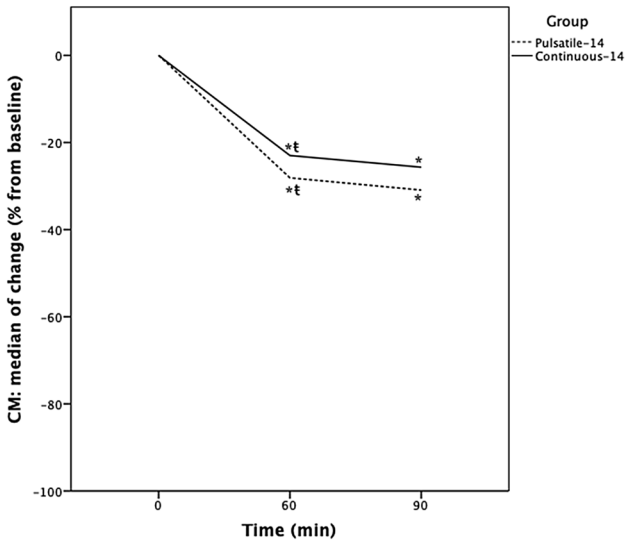

Fig. 2 Microcirculation blood flow at colon mucosa at an IAP of 14 mmHg

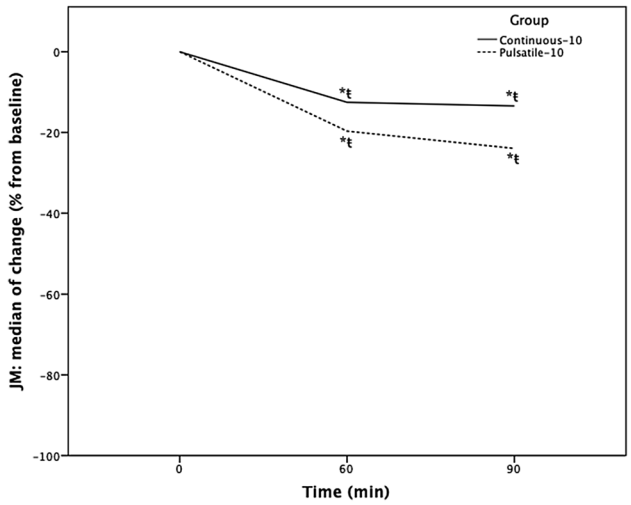

Fig. 3 Microcirculation blood flow at jejunal mucosa at an IAP of 10 mmHg

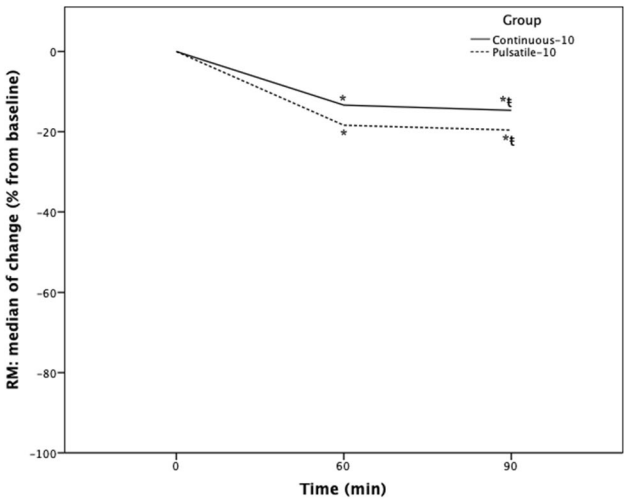

Fig. 5 Microcirculation blood flow at renal medulla at an IAP of 10 mmHg

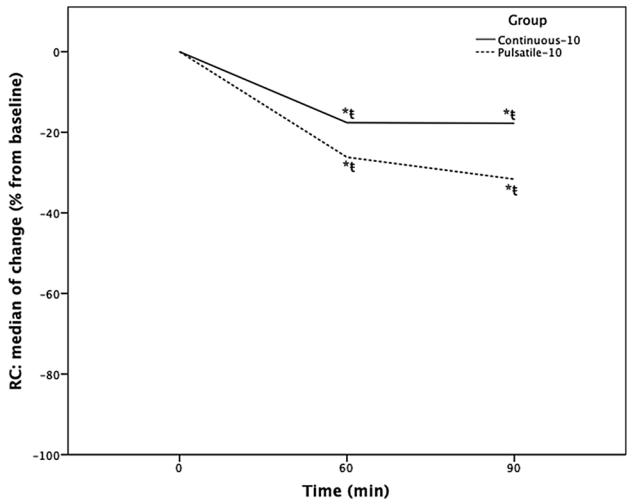

Fig. 4 Microcirculation blood flow at renal cortex at an IAP of 10 mmHg

added, and the effect of the cranial shift of the diaphragm, which directly affects pulmonary mechanics. Besides, our group previously reported that shear stress might have a significant impact on MCBF and its redistribution, despite a lower mean IAP level [22]. The shear stress and its essential role in acute vessel tone regulation depend on the hemodynamic frictional force on the surface of the endothelium. Fluctuations in pneumoperitoneum, more common with pulsatile insufflation, are believed to create increase shear stress gradients and induce greater vessel adaptations in response to acute MCBF variations. On the other hand, the constant pressure of the pneumoperitoneum during continuous insufflation theoretically may have a minor impact on hemodynamic shear stress, blood flow, and endothelial function compared to pulsatile insufflation. Also, continuous insufflation systems have been associated with lower CO_2 employment, absorption, and elimination [23].

In the literature, several studies have analyzed the safety of continuous flow insufflation during laparoscopy. Sroussi et al. reported safe gynecological surgeries with low IAP (7 mmHg), with lower maximal values of ET_{CO_2}, systolic blood pressure, and peak airway compared to pulsatile insufflation [24]. Annino et al. studied the effect of the valve-free insufflator during robotic partial nephrectomy, obtaining improved outcomes in operative time and warm ischemia time [25]. The published data suggest that working with this novel insufflation system might decrease the negative effects in arteriolar blood flow, which could potentially improve patients' outcomes. However, results from controlled clinical trials are pending [26].

Our group has extensive experience with experimental gastrointestinal microcirculation studies [20, 22, 27]. However, to our knowledge, this is the first study focused on analyzing the impact of pneumoperitoneum on MCBF with continuous flow insufflation. In this RCT, pneumoperitoneum caused an expected decrease in bowel mucosal and serosal blood flow. Moreover, a lower decrease of the mesenteric capillary beds flow in bowel biopsies was observed with the continuous insufflation, especially at 10 mmHg of IAP. It is clear that elevated IAP decreases mesenteric blood flow, which could result in intestinal damage [28]. During laparoscopic procedures, IAP should be maintained under 15 mmHg, and if the lower adverse effects of IAP obtained with the continuous insufflation improve postoperative organ function justifies further investigation.

Table 2 Systemic hemodynamics, gas exchange measurements, whole blood nitrite-to-nitrate concentration and plasma renin activity

	IAP 10 mmHg		IAP 14 mmHg	
	60 min	90 min	60 min	90 min
HR				
Cont	−1.9 (−3.9;3.98)	5.06 (−3.7;10)	−5.4 (−18;20.6)[t]	−5.4 (−26;17.6)
Puls	−3.1 (−15;−0.74)*	0.62 (−8;7.46)	18.3 (12.6;31.7)*[t]	10.3 (−3;23)
MAP				
Cont	9.18 (−1.2;15.1)	1.33 (0.00;8.1)	7 (0.93;33.8)*	4 (−9.1;17.6)
Puls	0.77 (−5.9;12.9)	0.67 (−13;10.3)	2.19 (−10;12.4)	−5.5 (−18;4.75)
SVRI				
Cont	−5.0 (−10;10.1)	2.09 (−13;13.5)	10.5 (−19;20.1)	−2.9 (−20;17.6)
Puls	−4.9 (−14;14.8)	−0.43 (−30;7.78)	2.9 (1.32;23.8)	10.4 (−3.4;21.2)*
CI				
Cont	−6.2 (−11;15.9)	−4.5 (−8.7;10.4)	−4.9 (−10;7.88)	−0.15 (−7.9;12−3)
Puls	1.42 (−7.6;7.11)	1.44 (−9.3;10.4)	−6.6 (−8.7;−0.81)*	−14 (−16;−5.1)*
GEDI				
Cont	−1.3 (−6.4;3.22)	−2.7 (−8.1;1.01)	−0.0 (−9.2;6.21)	−0.17 (−6.3;2.89)
Puls	−0.9 (−11;10.4)	−2.6 (−10;4.25)	−1.6 (−5.3;5.15)	−0.18 (−7.1;8.44)
PaO_2				
Cont	−16 (−21;−9.8)*	−9.1 (−21;−3.9)*	−10 (−20;1.05)*	4.21 (−16;9.65)
Puls	−17 (−26;−9.6)*	−12 (−26;−6.6)*	−12 (−20;−1.9)*	−10 (−19;4.09)*
$PaCO_2$				
Cont	14.3 (2.48;22.2)*	18 (8.39;28.9)*	14.3 (10.1;41.1)*	20.4 (13.8;37.3)*
Puls	16.2 (2.14;81.9)*	18.2 (7.64;84.5)*	15.4 (4.36;17.2)*	16.7 (−4.8;20.7)*
$Pa\text{-}ET_{CO_2}$				
Cont	5.72 (2.7;7.41)*	2.7 (−1.5;6.08)	0.00 (−5.6;8.82)	0.00 (−2.8;11.8)
Puls	6.61 (0.00;12.5)*	2.94 (1.47;6.92)*	6.25 (2.86;8.89)*	4.44 (0.00;14.6)*
$P_{A\text{-}a}O_2$				
Cont	0.27 (0.16;0.52)*	0.1 (0.06;0.32)*	0.11 (−0.04;0.59)*	−0.08 (−0.2;0.37)
Puls	0.37 (0.15;0.66)*	0.13 (0.1;0.51)*	0.34 (−0.01;0.77)*	0.31 (−0.07;0.61)*
NOx				
Cont	−8.5 (−21;−2.3)*	−9.2 (−32;−0.78)*	−15 (−19;−7.8)*	−13 (−16;−1.9)*[t]
Puls	−4.8 (−13;3.9)	−4.3 (−19;11.2)	−5.9 (−8.7;−2.1)*	−1.1 (−6.3;9.68)[t]
PRA				
Cont	−31 (−67;5.56)	−19 (−56;114)	40 (0.00;71.4)	114 (−10;286)
Puls	−15 (−48;105)	0.00 (−46;109)	110 (−27;234)	33.7 (−39;484)

Data are presented as median percentage of change (IQR)

HR heart rate, *MAP* mean arterial pressure, *SVRI* systemic vascular resistance index, *CI* cardiac index, *GEDVI* end global diastolic ventricular index, *PaO_2* arterial oxygen tension, *$PaCO_2$* arterial carbon dioxide tension, *PA-a O_2* alveolar/arterial tension difference for oxygen, *Pa-$EtCO_2$* arterial to end-tidal CO_2 gradient, *NOx* nitrite/nitrate, *PRA* plasma renin activity, *Cont* continuous insufflation, *Puls* pulsatile insufflation

*Difference from baseline values with $p < 0.05$

[t]Difference between groups with $p < 0.05$

The question is whether the decreased mesenteric blood flow caused by pneumoperitoneum produces relevant intestinal ischemia in healthy subjects, or only in high-risk patients. From the evidence-based knowledge, it seems that only patients with underlying vasculopathy or those critically ill are at risk [3]. Moreover, during TaTME, a colorectal anastomosis is usually performed. It is considered a high-risk anastomosis, especially in cases treated with neoadjuvant chemoradiotherapy. Tygat et al. found a direct relationship between applied CO_2 IAP and impaired intestinal healing after enterotomy closure in rats [29]. Laparoscopic surgery has proven benefits for the patient, but caution should be taken in those surgeries where high-risk intestinal anastomoses are performed. The documented reduction in the adverse effects of pneumoperitoneum in colon biopsies suggests that working with continuous flow

could also be potentially helpful in diminishing the undesirable effects of elevated IAP on anastomotic healing.

In the present study, continuous insufflation was associated with a lower MCBF reduction in renal cortex and renal medulla at an IAP of 10 mmHg. During pneumoperitoneum, there is a decrease in renal blood flow, which can be up to 40% depending on the IAP level [30]. Nguyen et al. compared laparoscopic and open gastric bypass in a randomized controlled trial and found that laparoscopy was associated with a decrease in urinary output of 64%, although postoperative renal function was not affected [31]. Once again, it seems that the impairment of both renal flow and function might have clinical effects only in patients with preoperatively deficient renal function.

Even with the ventilation control by the anesthesiologist, which minimizes the amount of CO_2 during pneumoperitoneum, both CO_2 levels and IAP affect venous return, decrease cardiac output, and might increase HR, MAP and SVRI [3]. In this RCT, no differences were found in CI, MAP, and GEDI between groups, although the effect in HR was statistically significant at an IAP of 14 mmHg. This might be explained by a decreased neurogenic sympathetic activity in the continuous insufflation group, with theoretically lower vasoactive peptides and angiotensin levels. However, no increase in SVRI was observed, which goes against the neurogenic sympathetic activity theory. Andersson et al. questioned the effect of vasoactive peptides and catecholamines in SVRI during pneumoperitoneum and highlighted the role of myogenic activity in vascular resistance [32]. Though there were no differences in blood gases, the difference in Pa-ET_{CO_2} gradient could be related to the alveolar collapse of dependent pulmonary regions (atelectasis) induced by diaphragmatic movements and cyclic alveolar opening and closing resulting in units with low ventilation/perfusion ratio. In patients with pathological respiratory conditions and long-lasting surgical procedures, this could be cause of impaired gas exchange.

Several studies among patients treated by laparoscopy have shown a relationship between capillary vasoconstriction and endothelial NO release [33, 34]. In contrast to those studies, results from this RCT indicate that nitrite and nitrate anions remained somewhat stable in the pulsatile groups, while there was a decrease in the continuous insufflation groups. The may support the theory that pulsatile insufflation promotes pulsatile blood flow, which has been shown to increase NO production [35]. On the other hand, continuous insufflation may limit vasoconstriction and capillary pulsatility, although other vascular regulatory models such as metabolic and myogenic mechanisms need to be integrated for a complete understanding of the complex capillary network.

Altintas et al. reported an increase in renin levels in the renal vein after laparoscopy [36]. This, together with the previously commented myogenic response and the purely effect of IAP, triggers renal vasoconstriction. Despite an absence of statistical significance, the observed increase in PRA levels at 14 mmHg was even lower in comparison to the literature [37, 38]. Interestingly, a reduction in PRA levels was found in the continuous-10 group. One theoretical explanation is that, at a low IAP, the lesser reduction in renal MCBF obtained with the continuous insufflation might also carry a decreased impact of humoral factors such as the renin–angiotensin–aldosterone system.

The AirSeal IFS® insufflator was developed to facilitate the transanal dissection, since the combination of the IFS, the AirSeal trocar, and the ASM-Evac Tri-lumen Filter Tube Set allows swift insufflation with a steady pressure, even during suction or smoke evacuation. However, its direct effect on MCBF and hemodynamics required investigation [11, 39]. Moreover, since the AirSeal IFS® prevents the collapse of the working cavity, an expected shorter effective surgical time was found in the continuous groups. This decreased real-surgical time required for completing the task suggests that, in clinical practice, operative time could be shortened and therefore minimize the risk of reduced functional residual capacity and increased Pa-ET_{CO_2}, which promote atelectasis [40].

An important limitation of this study is the fact that the sample size is small, as is usual in this type of experimental studies. Moreover, the technical problems with the spectrophotometer forced us to exclude two piglets. However, following ethics in animal research, our purpose was to use the minimum number of animals required, rather than replace. Therefore, further studies are required to draw reliable conclusions. Another limitation is that all the animals were healthy, which hinders the translation of the outcomes to an often-vulnerable surgical population. Moreover, by microsphere technique protocol, the animals were euthanized at the end of the procedure. Consequently, it is not possible to make a comparison in postoperative organ function. The results of this study should be interpreted with caution since the clinical and physiological significance of the observed difference may have little impact on medical practice in healthy subjects. Nevertheless, the potential risks when dealing with unhealthy patients (with underlying vasculopathy, liver, pulmonary or cardiac diseases, chronic renal insufficiency, deficient nutritional state, or even patients with Inflammatory Bowel Disease) suggest that the pneumoperitoneum-related effects in this subset of patients may be of clinical importance.

In summary, this experimental RCT showed that TaTME using the continuous flow insufflation was associated with a lower MCBF decrease in colon mucosa, colon serosa, jejunal mucosa, renal cortex and renal medulla, compared to pulsatile insufflation. For this promising but challenging surgery, the valve-free with continuous flow insufflator facilitates the dissection and might also carry a decreased risk of

hypoperfusion and reperfusion injury. Even though there is a lack of evidence-based knowledge, this appears to be a substantial matter especially in patients with pre-existing impairment of intra-abdominal blood flow or limited reserve capacity.

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Compliance with ethical standards

Conflict of interest Apart from the funding previously mentioned, Dr. AM Lacy is a consultant for Medtronic, Conmed Corporation, Olympus Medical, Touchstone International Medical Science Co. Ltd., Applied Medical, and Johnson & Johnson. Dr. de Lacy, Dr. Taurà, Dr. Arroyave, Dr. Trépanier, Mr. Ríos, Dr. Bravo, Dr. Ibarzabal, Dr. Pena and Dr. Deulofeu have no other conflicts of interest or financial ties to disclose.

Ethical approval The Institutional Review Board of the Hospital Clinic approved this trial for the Care and Use of Laboratory Animals. The University of Barcelona Committee on Ethics in Animal Experimentation and the Catalan Department of the Environment Commission on Animal Experimentation granted ethical approval for the study (Reg. 0006S/11367/2015).

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