

Analysis of cumulative fluid balance impact on the stability of gastrointestinal tract anastomoses

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Abstract

Background Anastomotic leakage is a serious post-operative complication after rectal resection. The main objective of this study was to gauge the impact of cumulative fluid balance on microcirculatory changes at anastomotic sites in the wall of the large bowel.

Methods The study was performed using 14 female domestic pigs. Rectal resection was performed on all of them. During the operation and post-operative period they received IV crystalloids at constant rates 5,10 and 15ml/h. Large bowel micro-perfusion was observed at several points during the experiment using LDF. Each subject was observed for six hours after surgery. The measured LDF values for each group was statistically analyzed using Levene's test and Welch's ANOVA.

Results The primary findings of this study found that the group mean values agreed at baseline; i.e., prior to intervention, as well as at the end of the study ($p > 0.05$). The same result was also confirmed for the second measurement, and the next to last measurement ($p > 0.05$). Conversely, differences in group mean values were demonstrated at the 2 intermediate time points ($p < 0.05$).

Conclusions The original hypothesis of a blood perfusion disorder developing in the large bowel region after administration of large fluid volumes was not confirmed. However, a 10 fold decrease in blood supply to the large bowel was observed after inferior mesenteric artery ligation. Secondly, LDF was found to have high accuracy in measuring tissue microcirculation and has the potential to be used in clinical practice.

Background

The incidence of anastomotic leakage (AL) following rectal resection ranges from 2–22% [1], the highest incidences of which occur in the distal and middle third of the rectum (range 5–22%). There are many causes of dehiscence, the majority of which stem from overlapping factors. The primary etiological factor for development of AL is ischemia in the anastomotic region. Well known risk factors for dehiscence include male gender, smoking, diabetes, obesity, malnutrition, chronic obstructive pulmonary disease, use of immunosuppressants, radiotherapy, and resection of the distal third of the rectum [2,3]. The impact of infusion volume on anastomotic stability during perioperative and postoperative periods is less studied. Anastomotic oedema and microcirculatory disorders in the anastomotic region can be expected during administration of large volumes of crystalloids or rapidly administered crystalloids [4]. Currently, laser Doppler flowmetry (LDF) is the most reliable and accurate technique available for monitoring microcirculation in the region of the large bowel or rectum.

The objective was to verify the impact of cumulative fluid balance on microcirculatory changes at anastomotic sites in the wall of the large bowel in animal model. A further objective was to explore the potential use of LDF in human clinical practice during perioperative and postoperative periods.

Methods

The study was approved by the Institutional Animal Care and Use Committees (Committees for IAC & U) of the University of Defence Faculty of Military Health Sciences and the Charles University Faculty of Medicine in Hradec Králové, Czech Republic. All procedures were conducted in accordance with Act No 246/1992 Coll. on the Protection of Animals Against Cruelty. The study used 15 female domestic pigs weighing 31–42 kg each. All experimental animals were owned by the University of Defence Faculty of Military Health Sciences and the Charles University Faculty of Medicine in Hradec Králové, Czech Republic, which issued written informed consent to use the animals in this study. The manuscript adheres to the ARRIVE guidelines for the reporting of animal experiments.

After a 24 hour fast with free access to water, general anaesthesia was induced using an intramuscular administration of 20 mg/kg of ketamine (Narkamon, Zentiva Group, a.s., Prague, Czech Republic), 4 mg/kg of azaperone (Stresnil, Janssen Pharmaceutica, Beerse, Belgium), and 0.05 mg/kg of atropine (Atropin, Hoechst-Biotika, Martin, Slovakia), followed by cannula insertion into the peripheral vein to ensure venous access. The pigs were orotracheally intubated with a Müller long blade laryngoscope and the lungs were ventilated with a Servo 900C ventilator (Siemens-Elma AB, Solna, Sweden) at a tidal volume of 10–15 ml/kg and with an inspired oxygen fraction (F_{iO_2}) of 40%.

During the surgical part of the experiment, anaesthesia and analgesia were maintained with continuous intravenous (IV) infusions of midazolam at 0.3 mg/kg/h and fentanyl at 30 µg/kg/h. Postoperatively, the dosage of fentanyl was reduced to 5 µg/kg/h and neuromuscular blockade was induced by continuous IV infusion of pancuronium bromide at 0.2 mg/kg/h. The right femoral artery was cannulated for blood sampling and invasive blood pressure monitoring. The right jugular vein was cannulated for pulmonary artery catheterization and insertion of a central venous catheter (Pressure transducer, Gabarith PMSET 1DT-XX, Becton Dickenson, Singapore; Datex-Ohmeda S/5 monitoring system, Instrumentarium Corp, Helsinki, Finland) [Fig1]. Electrodes were attached to measure electrocardiogram signals, oxygen saturation, and rectal body temperature. Cystostomy and urinary catheterization were performed perioperatively through a middle laparotomy.

Preoperative blood samples were taken from all pigs to determine serum lactate concentrations, pH, base excess, and PaO₂. In addition, a Swan-Ganz catheter was used to measure cardiac output.

The initial step in the procedure involved irrigating the rectum with an antiseptic Betadine solution (10% Povidone-iodine, Egis Pharmaceuticals Ltd., Budapest, Hungary) and evacuating residual stool from the rectum.

The abdominal cavity was accessed through a lower middle laparotomy, and a laser Doppler electrode was attached to the distal region of the sigmoid colon. The electrodes were then attached to the rectal wall using 4–0 monofilament sutures (Premilene, B. Braun Melsungen AG, Melsungen, Germany) [Fig2]. The electrode was then connected to a tissue perfusion monitor (Moor Instruments Ltd, Devon, UK) and tissue microperfusion values (baseline) were read and recorded. The inferior mesenteric artery (IMA) and inferior mesenteric vein (IMV)[Fig3] were then ligated, and tissue microperfusion values were recorded

(starting 30 min after ligation); values were recorded at 30 min intervals until 120 min, and then at 60 min intervals until 240 min of the experiment.

The rectum and mesorectum were mobilized enbloc down to the pelvic floor, at the level where the rectum was ligated, using a Contour® curved stapler (Johnson & Johnson Inc, Cornelia, GA, USA). The rectal stump was irrigated with an antiseptic Betadine solution, during which the staple line was monitored for leakage. The ligated rectum was then extracted, followed by ligation of the large bowel in the distal region of the sigmoid colon. A circular stapler cap was inserted into the ligated lumen of the large bowel and the lumen was closed with a circular, monofilament, suture.

An LDF electrode was attached 1 cm proximal to the expected anastomosis site. An end-to-end stapled anastomosis was performed using a 25 mm circular stapler (Johnson & Johnson Inc., Cornelia, GA, USA). Following anastomosis, LDF values were recorded. Saline was instilled in the abdominal cavity and air was introduced into the rectum to test for anastomotic air leakage and verify anastomotic integrity. After saline aspiration, a laparotomy was used to remove the electrodes and the laparotomy was closed with a continuous suture.

Throughout the entire operation and post-operative period, the pigs received IV crystalloids (Infusio Hartmanni, Medicamenta, Vysoké Mýto, Czech Republic) at a constant rate of 5, 10, or 20 ml/kg/h depending on group. After 6 hours, the electrodes were removed and the animals were euthanized by the T61 Euthanasia Solution (Merck Animal Health, Canada). Each mL of T-61 contains 200 mg embutramide which produces a strong narcotic action and concurrently paralyses the respiratory centre, 50 mg mebezonium iodide which produces a curariform paralytic action on striated skeletal and respiratory muscles and rapidly induces circulatory collapse and 5 mg tetracaine hydrochloride, in aqueous solution.

Results

A total of 15 pigs were used in the study. The pigs were divided into 3 groups, with 5 animals per group. The groups were divided according to the following infusion regimes: 5 ml/kg/h, 10 ml/kg/h, and 20 ml/kg/h. The experiment was successfully completed on all 15 animals. Throughout the course of the experiment, the animals were monitored, and their status was recorded. Oxygen saturation, cardiac events, body temperature, invasive blood pressure measurements, central venous pressure, pulmonary artery pressure, and diuresis were measured. Large bowel perfusion results were recorded at baseline, 30 minutes after IMA ligation, then every 30 min up to 120 min, and then every 60 min up to 240 min (a total of 6 experimental data points, plus a baseline, for each animal). No statistical differences in circulation relative to the administered fluid volume were observed between the groups. A significant decrease in blood supply was observed in all animals after IMA ligation. No improvement in circulation was observed in any of the groups [Tab 1]. Thus, the original hypothesis regarding a large bowel perfusion disorder developing after administration of large fluid volumes was not confirmed.

Statistical analysis

In all 3 groups (5 ml/kg/h, 10 ml/kg/h, and 20 ml/kg/h), the null hypothesis was tested; i.e., the mean values did not differ from the alternative hypothesis. This was tested using ANOVAs that were conducted separately for each of the 6 measurements; i.e., baseline, 30 min, 60 min, 120 min, 180 min, and 240 min.

The homogeneity of scatter was always tested first with Levene's test for homogeneity of variances. A statistically significant difference in homogeneity scatter was found only for the baseline measurement. The conformity of this mean value was further tested using Welch's ANOVA. The null hypothesis, for scatter homogeneity, was not rejected for the 5 other time points; therefore, a standard ANOVA was performed using Tukey's post hoc test. The results are summarized in Table 1. The systemic hemodynamic data and fluid intake volumes are presented as mean \pm standard deviation (SD). Non-normally distributed microcirculatory data are presented as the mean (95% confidence interval (CI) around the mean). Baseline and consecutive data were compared with a variance analysis (ANOVA) and the Kruskal-Wallis test for repeated measurements; the unpaired Student's t-test was used to analyse differences between treatment groups. P values less than 0.05 were considered statistically significant. All statistical analyses were performed with Sigma-Stat 2.0 (Jandel Scientific, San Rafael, CA, USA).

The primary findings of this study found that the mean values agreed at baseline; i.e., prior to intervention, as well as at the end of the study, i.e., 240 min ($p > 0.05$). The same result was also confirmed for the second measurement at 30 min, and the next to last measurement at 180 min ($p > 0.05$). Conversely, differences in group mean values were demonstrated at the 2 intermediate time points of 60 min and 120 min ($p < 0.05$).

Statistical analyses were performed using SPSS Statistics 22.0 (SPSS Inc., Chicago, IL, USA). The dependent variable was a continuous variable Perfusion Index (PI). A significance level of 0.05 was always chosen to analyse dependence. (Tab.2 One-way ANOVA results)

Discussion

AL in the region of the large bowel and rectum has multiple causes and contributes significantly to post-operative lethality and morbidity. It also significantly affects patients in that it can lead to functional disturbances or the need for a permanent colostomy. It is important to assess all risk factors and eliminate or minimize them as much as possible. The primary etiological factor for AL is ischemia in the anastomotic region, which may arise from nutritional disturbances in the surgically affected regions of the large bowel and rectum, or as a result of ischemia in atherosclerotic patients, which can also involve disorders of splanchnic tissues. Ischemia can also develop due to capillary thrombosis in the anastomotic region resulting from increased thrombophilia. Prolonged hypotension or hypovolemia, associated with greater blood loss, may also lead to circulatory disorders in the anastomotic region.

The impact of infusion volume and infusion rates on anastomotic stability in the perioperative and postoperative periods has not yet been sufficiently investigated [4]. Oedema and, thus, microcirculatory disorders in the anastomotic region can be expected during administration of large crystalloid volumes or rapid crystalloid infusion. There are several methods for monitoring tissue perfusion in the preoperative,

perioperative, and postoperative periods that have been used experimentally, including: macro-hemodynamic indicators such as pulse-induced contour cardiac output (PiCCO), and lithium dilution cardiac output (LiDCO) measurements; additionally, tissue perfusion laboratory markers can be monitored. LDF appears to be the most reliable method for monitoring the microcirculation in the large bowel or rectum. Other methods include sidestream dark field imaging, which is based on orthogonal polarization spectral imaging, and intestinal microdialysis [5–9]. Larger studies, as well as comparisons of individual methods in larger samples, have yet to be conducted.

LDF

LDF is a non-invasive diagnostic method for measuring tissue blood flow, which is based on measuring the Doppler effect that occurs when moving red blood cells shift the frequency of incident light. LDF can record the flow, speed and concentration of moving red blood cells. These values are extracted from the power spectrum via photographic flow fluctuations that are produced when light coming from the diode illuminator is reflected onto a photodetector.

There are 2 techniques used to measure perfusion in clinical practice: laser Doppler perfusion monitoring (LDPM) and laser Doppler perfusion imaging (LDPI). LDF is able to measure real-time tissue microcirculation and perfusion changes. The drawbacks of LDF measurements are associated with a larger probe diameter and the need to fix the probe to the tissue (in our case, the bowel wall) during measurements. A new type of probe that can also be used for laparoscopic procedures and is more easily used has already come into use. Another disadvantage is the lack of post-operative monitoring, since the LDF probe would have to be left *in situ* [10–13].

In everyday clinical practice, fluorescence methods using indocyanine green (ICG), such as Pinpoint (Novadaq, Mississauga, ON, Canada) intraoperative near infrared (NIR) fluorescence imaging of perfusion, or the Karl Storz NIR-ICG fluorescence imaging system (Karl Storz, Tuttlingen, Germany), are frequently encountered. The above mentioned systems are limited in that they: only evaluate final blood flow, do not monitor the dynamics of the process, and cannot be calibrated. Further potential issues include subjective image evaluation, limitations in viewing less accessible locations, or viewing an obscured anastomotic region (most often by fatty tissue). Nevertheless, in practice, their clinical application is easier [14–16].

A common drawback shared by all these methods, relative to anastomoses, is the absence of threshold values that can indicate if there is an adequate blood supply for timely healing of an anastomotic site, or whether another solution is required (e.g. anastomotic re-resection), or when an anastomosis should be ruled out altogether.

Regarding the values recorded after IMA ligation, it must be noted that there are differences between porcine and human vascular systems [17,18]. Pigs lack a developed arcade system; thus, they are prone to develop ischemia after high IMA ligation [19–23]. However, it should also be noted that all pigs used in our study were young, clinically healthy specimens with no sign of disease in the large bowel or rectum.

The surgical procedures were carried out with optimal preoperative preparation. Obviously, this differs significantly from conditions encountered with human patients, the majority of whom are of advanced age, frequently have multiple comorbidities with pathological afflictions of the walls of the large bowel or rectum and are often being treated subsequent to radiotherapy.

Conclusions

The original hypothesis of a blood perfusion disorder developing in the large bowel region after administration of large fluid volumes was not confirmed. Nonetheless, we did observe an up to 10-fold decrease in the blood supply of the large bowel after high IMA. LDF was found to be an accurate method for measuring tissue microcirculation and has the potential to be used in clinical practice.

List Of Abbreviations

LDF : Laser Doppler Flowmetry

AL : Anastomotic Leakage

FiO2 : Inspired Oxygen Fraction

IV: Intravenous

IMA : Inferior Mesenteric Artery

IMV : Inferior Mesenteric Vein

SD : Standard Deviation

CI : Confidence Interval

PiCCO : Pulse-induced Contour Cardiac Output

LIDCO : Lithium Dilution Cardiac Output

LDPM : Laser Doppler Perfusion Monitoring

LDPI : Laser Doppler Perfusion Imaging

ICG : Indocyanine Green

NIR : Near Infrared

Declarations

Statement on ethics approval

The study was approved by the Institutional Animal Care and Use Committees (Committees for IAC & U) of the University of Defence Faculty of Military Health Sciences and the Charles University Faculty of Medicine in Hradec Králové, Czech Republic. All procedures were conducted in accordance with Act No 246/1992 Coll. on the Protection of Animals Against Cruelty.

The written informed consent to use the animals in this study was issued by the University of Defence Faculty of Military Health Sciences and the Charles University Faculty of Medicine in Hradec Králové, Czech Republic, which was the owner of the animals.

The manuscript adheres to the ARRIVE guidelines for the reporting of animal experiments.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contributions

Julius Örhalmi performed the surgical procedure of the experiment, was involved to evaluation of the experiment, was involved in drafting and revising the manuscript, and gave a final approval of the version to be published.

Zdeněk Turek Performed the anaesthesia for the experimental animals, was involved to the recording of the measuring data, was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Filip Čečka designed the study, was involved in the analysis and interpretation of the data and revising the manuscript and gave final approval of the version to be published.

Jiří Páral - provided facilities for the study, provided experimental animals, was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Josef Dolejš provided statistical analysis of the data and was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Austin Paul Kallarackal cooperated in preparing of the manuscript, and as a native speaker corrected the English version of the manuscript, was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Milan Kaškap provided the grant support and was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Otakar Sotonaco cooperated on the surgical procedures, was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Ondřej Malý - cooperated on the surgical procedures, was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Marian Mynář - Performed the anaesthesia for the experimental animals, was involved to the recording of the measuring data, was involved in drafting and revising the manuscript, and gave a final approval of the version to be published

Vladimír Černý - designed the study, was involved in the analysis and interpretation of the data and revising the manuscript, and gave final approval of the version to be published

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Not applicable

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Tables

Due to technical limitations, the Table(s) are only available as a download in the supplemental files section.

Figures

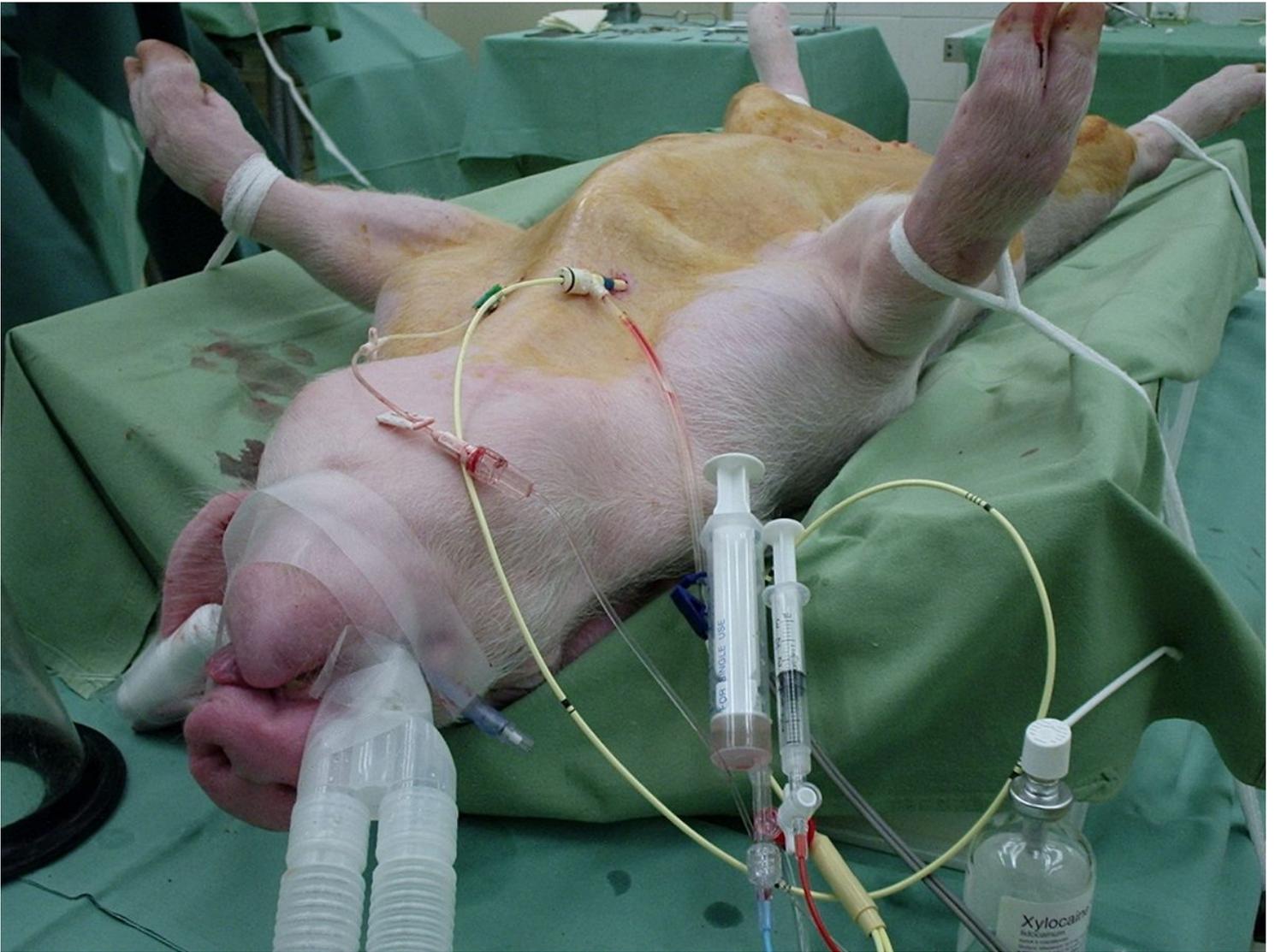


Figure 1

Monitored animal prepared for the experiment

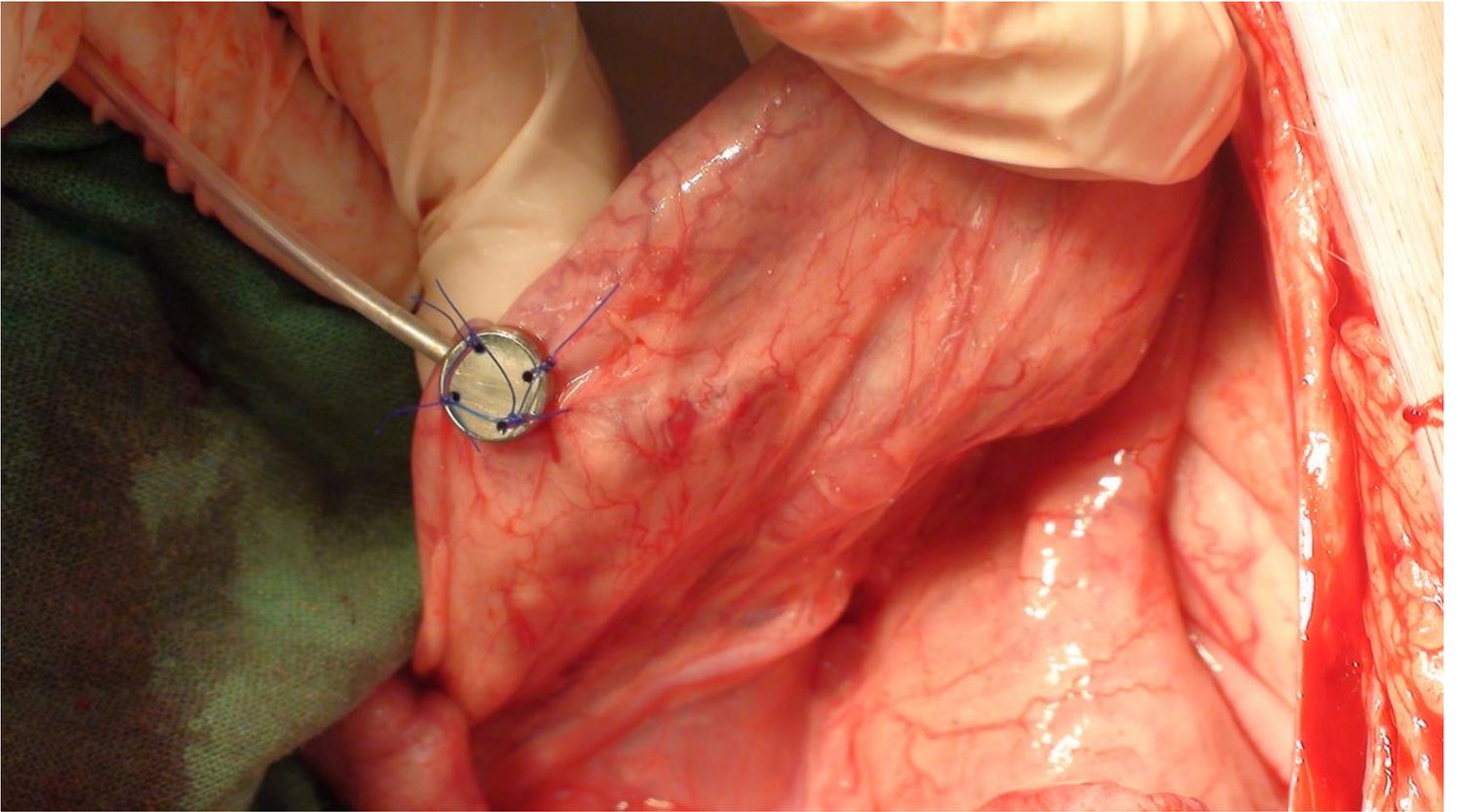


Figure 2

Fixed Laser Doppler Probe on the large bowel wall

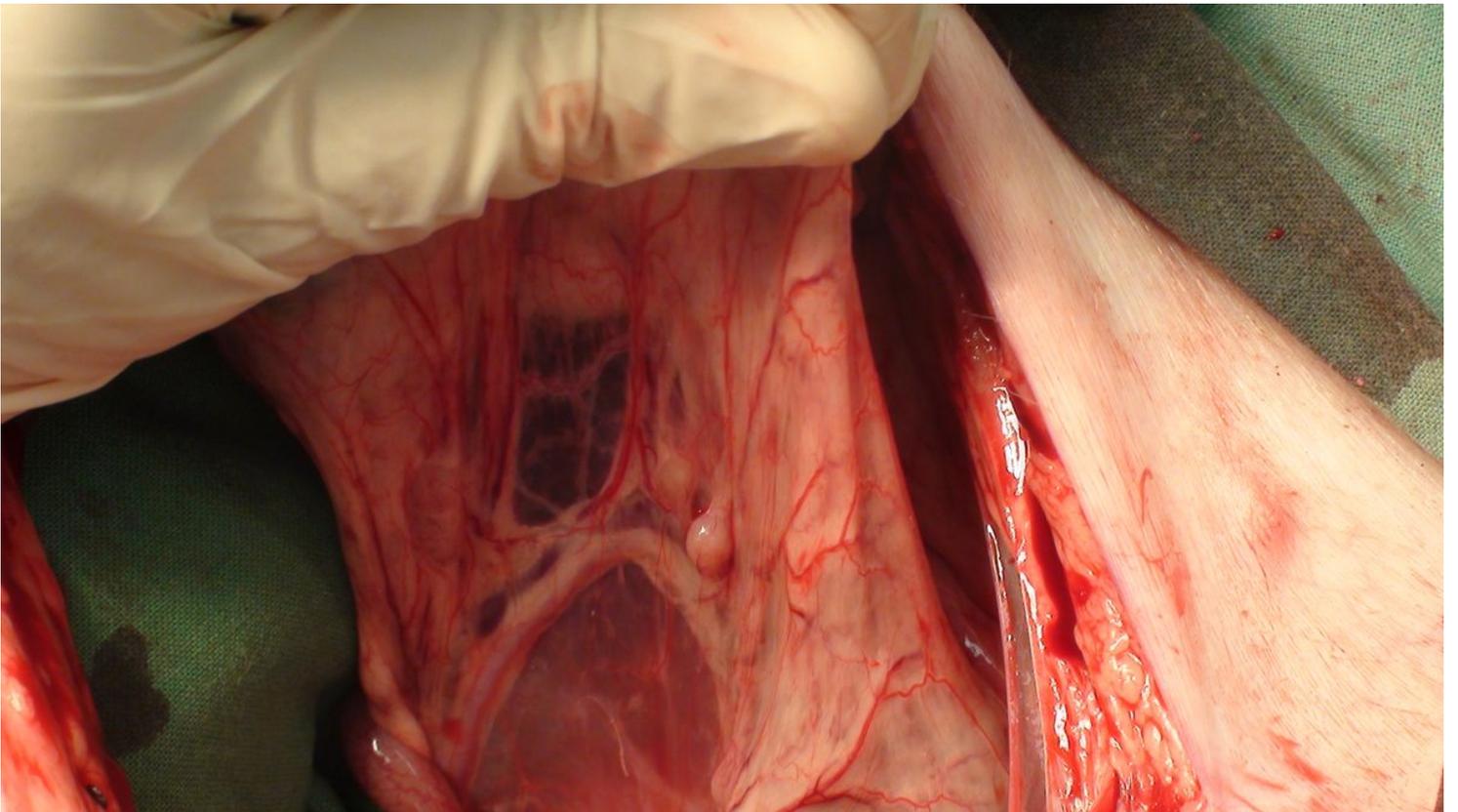


Figure 3

Inferior Mesenteric Artery and large bowel

Supplementary Files

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

- [Table1TissueperfusionTab..xlsx](#)
- [Table2Statisticalanalysis.xlsx](#)
- [NC3RsARRIVEGuidelinesChecklist2014.docx](#)