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Endothelial dysfunction in the early postoperative period after major colon cancer surgery

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Abstract

Background. Evidence suggests that endothelial dysfunction in the early postoperative period promotes myocardial injury after non-cardiac surgery. The aim of this study was to investigate the impact of colon cancer surgery on endothelial function and the association with the l-arginine-nitric oxide pathway postoperatively.

Methods. Patients undergoing elective colon cancer surgery (n = 31) were included in this prospective observational cohort study. Endothelial function, as measured using the reactive hyperaemia index (RHI), was assessed non-invasively using digital pulse tonometry. RHI and plasma concentrations of L-arginine, asymmetric dimethylarginine (ADMA), dihydrobiopterin and biopterin metabolites, tetrahydrobiopterin (BH4) and total biopterin were measured before surgery, at four h after surgery and at postoperative day one and two. Cardiac troponin I was measured before surgery and once daily on postoperative days one to four.

Results. Preoperative RHI was 1.86 (1.64 – 2.11) and decreased significantly during the observation period (linear mixed effects model of serial measurements, $P = 0.015$). Both L-arginine ($P < 0.001$) and ADMA ($P = 0.024$) decreased during the postoperative period. All biopterin metabolites were significantly decreased after surgery. A significant positive correlation was found between logAUC(l-arginine/ADMA) and logAUC(RHI) ($P = 0.015$) and between logAUC(L-arginine/ADMA) and logAUC(BH4) ($P = 0.015$). None of the patients had cardiac troponin I elevations.

Conclusions. RHI was attenuated in the first days after colon cancer surgery indicating acute endothelial dysfunction. Endothelial dysfunction correlated with disturbances in the L-arginine – nitric oxide pathway. Our findings provide a rationale for investigating the hypothesized association between acute endothelial dysfunction and cardiovascular complications after non-cardiac surgery.

Clinical trial registration. NCT02344771.

Key words: cardiovascular optimisation; myocardial injury; non-cardiac surgery; perioperative complications

Myocardial infarction is the most frequent perioperative cardiovascular complication after non-cardiac surgery.[1] Myocardial injury that does not fulfil the universal definition of myocardial infarction is present in 8% of patients in the perioperative period and is associated with 30-days mortality.[2] Myocardial oxygen supply-demand mismatch is believed to be central in the

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Editor's Key Points

- The endothelium regulates vascular tone, modulates inflammation and has anti-thrombotic and anti-atherosclerotic properties.
- Endothelial dysfunction is commonly associated with cardiovascular disease.
- This study found that endothelial dysfunction was associated with disturbances in the L-arginine nitric oxide pathway.
- Interventions that could protect endothelial function may result in reductions in perioperative cardiovascular risk.

development of myocardial injury.[1] In the perioperative period, the cardiovascular system is challenged as a result of surgical stress and trauma, anaesthesia, pain, hypoxemia and hypovolaemia. These factors are likely to contribute to the development of oxygen supply-demand mismatch.[1] Moreover, the vascular integrity and homeostasis may be compromised postoperatively because of endothelial dysfunction.[3–5]

The endothelium regulates vascular tone, modulates inflammation and has anti-thrombotic and anti-atherosclerotic properties.[6] Endothelial dysfunction is largely characterized by decreased vascular bioavailability of nitric oxide (NO).[6] Vascular NO is produced by the endothelial NO synthase (eNOS), which generates NO from L-arginine and O_2 in response to receptor dependent agonists (bradykinin, acetylcholine, adenosine triphosphate) and shear stress of the vessel wall.[6] Studies have shown that NO bioavailability is highly sensitive to increased oxidative stress,[6] as both direct reaction between NO and reactive oxygen species and indirect redox-induced uncoupling of eNOS function may contribute to a rapidly declining NO concentration.[7]

Endothelial function can be estimated by measuring the peripheral vasodilation in response to a stimulus that increases the vascular NO production.[8] Digital pulse amplitude tonometry is a non-invasive and operator-independent method, that measures the peripheral vasodilation in response to reactive hyperaemia.[8] The endothelial function is expressed as a reactive hyperaemia index (RHI), where a low RHI indicates worse endothelial function.[8] For patients undergoing non-cardiac surgery, preoperative endothelial dysfunction predicted myocardial injury within the first three postoperative days, with an odds ratio of 10.1 (95% confidence interval 3.3-30.9),[9] and an impaired preoperative endothelial function predicted major adverse cardiac events within 30 days of vascular surgery.[10] Moreover, sudden changes of the endothelial function have been observed in patients suffering from acute brain dysfunction,[11] aneurysmal subarachnoid haemorrhage[12] and acute myocardial infarction.[13] Thus, sudden postoperative endothelial dysfunction may influence the risk of postoperative myocardial injury. The effect of surgery on postoperative endothelial function measured by digital pulse amplitude tonometry has not been investigated before.

We conducted a prospective cohort study on patients undergoing major colon cancer surgery in order to investigate the impact of surgery on the RHI response and its relation to the NO pathway in the early postoperative period.

Methods

The study was approved by the Regional Ethic Committee of Region Zealand (SJ-429) and the Danish Data Protection Agency (REG-116-2014). The study was reported on clinicaltrials.gov (NCT-02344771). All included patients signed a written informed consent before participation. The study was a prospective cohort study and was performed between March and June 2015 at Zealand University Hospital. Patients referred to elective colon cancer surgery at the Department of Surgery were consecutively screened and included in the study. Exclusion criteria: not capable of giving informed consent after oral and written information, previously included in the study, surgery within seven days of the study. Patients were consecutively screened for inclusion. After withdrawal and exclusion, 31 patients completed the study. The attending surgeon and anaesthetist did a routine preoperative evaluation of the patient. In addition, data were collected on cardiovascular risk factors and the revised cardiac risk (Lee) index was calculated.[14] Colon cancer was staged according to the Union Internationale Contre le Cancer staging system. We classified postoperative surgical complications according to the Clavien-Dindo Classification.[15]

Endothelial function

The primary outcome was endothelial function assessed by non-invasive digital pulse amplitude tonometry by the use of an EndoPat2000 (Itamar Medical Ltd., Caesarea, Israel). We measured the endothelial function preoperatively, four h postoperatively and on days one and two postoperatively. All measurements were performed at the same h (plus or minus two h). An EndoPat endothelial function measurement consists of three phases: baseline, occlusion, and hyperaemia.[6] An EndoPat probe is placed on the index finger of each hand. Recordings are done simultaneously from both fingers throughout the study to adjust for any systemic effects.[6] After baseline data acquisition, a bp cuff is inflated on one arm to 200 mm Hg (suprasystolic pressure) for five min. During the occlusion period, signals are absent from the hyperaemic finger but continue from the control finger. After cuff release, pulse amplitude increases in the hyperaemic finger. The EndoPat system collects data digitally and performs automatic calculations of the post-occlusion (hyperaemia)/pre-occlusion (baseline) ratio, the reactive hyperaemia index (RHI).

Laboratory assays

NO bioavailability was indirectly measured by plasma L-arginine, plasma asymmetric dimethylarginine (ADMA), plasma tetrahydrobiopterin (BH4), plasma dihydrobiopterin (BH2) including biopterin metabolites and total plasma biopterin concentration preoperatively, four h postoperatively and on days one and two postoperatively. Blood samples were drawn into EDTA tubes just before the endothelial function measurement. For analysis of biopterins, 1ml blood was drawn into a vacutainer containing 1 mg of dithioerythritol dissolved in 25μL H_2O. The sample was gently mixed to avoid haemolysis and centrifuged at 2000g for five min. Plasma was immediately snap-frozen and stored at -80 °C until analysis. Biopterins were analysed using HPLC with fluorescence detection as described previously.[16] For arginine and ADMA, blood samples were centrifuged at 3000g for 10 min and the plasma snap-frozen and stored at -80 °C until analysis. Quantification of L-arginine and ADMA was achieved by HPLC with fluorescence detection using

a previously published method[17] modified as follows: Plasma samples were protein-precipitated with trichloroacetic acid and the supernatant was subsequently neutralized before isocratic elution of L-arginine and ADMA on a Phenomenex Gemini C18 column (150 x 4.6 mm; 5 um). The mobile phase consisted of 50 mM potassium phosphate (pH 6.5) containing 14% (v/v) acetonitrile pumped with a flow rate of 1.2 ml min^{-1}. L-Arginine and ADMA were detected at excitation and emission wavelengths of 340 and 455 nm, respectively.

Cardiac troponin I was measured preoperatively and on a daily basis until postoperative day four. Myocardial injury was defined as peak plasma cardiac troponin I ≥ 45 ng $litre^{-1}$ (99^{th} percentile URL, 10% CV at 40 ng $litre^{-1}$, Siemens Healthcare Dimension Vista).

Sample size

Endothelial function was measured repeatedly with EndoPat in a population of patients with subarachnoid haemorrhage.[12] RHI increased during the hospitalization from 1.67 (0.46) (mean (standard deviation)) to a steady state of 1.88 (0.51) – an increase of 11%. We hypothesized that the RHI in our study would decline 15% (preoperative *vs* postoperative). Based on a sample size calculation with a type I error 5% and power 80% a minimum of 30 patients should be included in the study.

Statistics

Statistical analyses were done with SAS version 9.4 using parametric statistics. Continuous data were expressed as mean (95% confidence interval) or median (25^{th} and 75^{th} percentiles) and categorical data as units (%). Longitudinal measurements of variables were analysed with linear mixed effects models that accounted for within subject correlation of serial measurements. Area under the curve (AUC) by subject for longitudinal measurements of variables was calculated with GraphPad Prism version 6. The AUC by subject was used in linear regression analysis to correlate the L-arginine/ADMA ratio and BH4 with RHI and BH4 with the L-arginine/ADMA ratio. Data distribution and equality of variances were checked with histograms and residual plots. If necessary, data were logarithm transformed. Analyses were considered significant when $P < 0.05$.

Results

Study subjects

Patient characteristics and clinical details are shown in Table 1. The patients did not receive premedication. All 31 patients underwent general anaesthesia. Anaesthesia was induced and maintained with propofol and remifentanil. Neuromuscular block was achieved with rocuronium. The attending anaesthetist decided on additional perioperative medication. Preoperative chemotherapy was administered to three patients and three patients had liver metastases. All patients were operated by minimally invasive surgical technique (laparoscopic or robot-assisted laparoscopic approach), but two operations were converted from laparoscopic to open technique. Sigmoid resection was performed on 11 patients while 19 patients had a right colon resection and one patient had a sub total colectomy. Three patients had a temporary stoma. Based on the postoperative histological analysis, one patient was diagnosed with a benign stenosis as a result of diverticulitis and not colon cancer. The median postoperative length of stay was two (IQR 2 – 5) days. Three patients suffered an anastomotic leakage, two patients suffered a postoperative ileus and two patients suffered postoperative hypovolemic shock. One patient died during hospitalization as a result of anastomotic leakage and six patients were readmitted because of surgical complications (Table 1). None of the patients had a postoperative troponin I ≥ 45 ng $litre^{-1}$ or adverse cardiovascular events.

Table 1. Patient characteristics. Data are expressed as mean (95% confidence interval) or n (%). RCRI, revised cardiac risk index; UICC stage, Union Internationale Contre le Cancer. Q1, 25^{th} percentile; Q3, 75^{th} percentile

Baseline characteristics	
Age (yr)	67 (63 - 71)
Sex	
male	18 (58)
female	13 (42)
BMI	26.6 (24.8 - 28.3)
Cardiovascular risk factors	
Smoking	
No	10 (32)
Yes (active/former)	5 (16)/16 (52)
Diabetes	3 (10)
Ischaemic Heart Disease	3 (10)
Heart Failure	2 (6)
Atrial Fibrillation	2 (6)
Hypercholesterolemia	7 (23)
Acetylsalicylic acid	6 (19)
B-blockers	9 (29)
Statins	7 (23)
Risk prediction indices	
RCRI 1/2/3	24 (78)/6 (19)/1 (3)
ASA 1/2/3	8 (26)/21 (68)/2 (6)
Surgical characteristics	
Duration of Anaesthesia (min)	231 (214 - 249)
Duration of Surgery (min)	162 (144 - 182)
Blood loss (ml)	30 (16 - 53)
UICC stage	
0	3 (10)
I	7 (23)
II	11 (35)
III	6 (19)
IV	3 (10)
Clinical outcomes	
Clavien-Dindo Classification	
0	16 (52)
1	3 (10)
2	9 (29)
3B	1 (3)
4A	1 (3)
5	1 (3)
Postoperative length of stay (median, Q1-Q3) (days)	2 (2 - 5)

Endothelial function and nitric oxide bioavailability

All but one patient had a minimum of three EndoPat measurements within postoperative day two. Reasons for not being measured all four times were early discharge or decline because of postoperative nausea. The patients had a normal preoperative endothelial function with an RHI at 1.86 (95% CI 1.64 – 2.11). RHI was significantly reduced from the preoperative baseline to postoperative day two (Table 2). L-arginine, ADMA and the L-arginine/ADMA ratio were significantly reduced during the early

Table 2. Serial measurements of reactive hyperemia index, biopterins, L-arginine and asymmetric dimethylarginine. Values as mean (95% confidence interval). RHI, reactive hyperemia index; ADMA, Asymmetric Dimethylarginine; BH4, tetrahydrobiopterin; BH2, dihydrobiopterin, POD, postoperative day

	Preoperative	4 h	POD1	POD2	P-value
RHI	1.86 (1.64 - 2.11)	1.47 (1.08 - 1.98)	1.51 (1.16 - 1.95)	1.65 (1.25 - 2.17)	0.015
L-Arginine (μmol/l)	77.25 (70.61 - 84.51)	50.98 (40.92 - 63.51)	47.00 (38.11- 58.56)	47.81 (38.68 - 59.08)	< 0.001
ADMA (μmol/l)	0.35 (0.32 - 0.39)	0.33 (0.27 - 0.40)	0.29 (0.23 - 0.37)	0.30 (0.24 - 0.37)	0.024
L-Arginine/ADMA	218.00 (197.10 - 241.12)	157.74 (128.03 - 194.35)	162.13 (128.98 - 203.81)	163.10 (128.90 - 206.37)	< 0.001
BH4 (nmol/l)	8.16 (7.21 - 9.23)	6.29 (5.03 - 7.87)	8.11 (6.67 - 9.86)	7.79 (6.15 - 9.88)	< 0.001
BH2 and biopterin metabolites (nmol/l)	4.30 (3.84 - 4.81)	2.81 (2.24 - 3.54)	3.57 (2.24 - 3.54)	3.29 (2.51 - 4.33)	< 0.001
Total biopterin concentration (nmol/l)	12.65 (11.46 - 13.97)	9.17 (7.55 - 11.14)	11.79 (9.88 - 14.07)	11.24 (9.26 - 13.64)	< 0.001
BH2/BH4	0.53 (0.46 - 1.37)	0.44 (0.34 - 1.27)	0.44 (0.34 - 1.26)	0.43 (0.31 - 1.24)	0.035

postoperative period (Table 2). Likewise, BH4, BH2 including biopterin metabolites and the total biopterin concentration were significantly reduced over time. We did explorative analyses on the association between RHI, the L-arginine/ADMA ratio and BH4. Figure 1 shows scatter plots with regression lines. In a linear regression model, a significant positive correlation was found between logAUC(L-arginine/ADMA) and logAUC(RHI), $P = 0.015$, Pearson correlation coefficient = 0.43, and between logAUC(L-arginine/ADMA) and logAUC(BH4), $P = 0.015$, Pearson correlation coefficient = 0.43. No significant correlation was found between BH4 and RHI, $P > 0.05$, Pearson correlation coefficient = 0.19.

Discussion

We found that endothelial function measured non-invasively by digital pulse amplitude tonometry and biomarkers of the NO pathway, were significantly reduced in the early postoperative period after major colon cancer surgery. Preoperatively, RHI was within the normal range but decreased postoperatively to a level indicating endothelial dysfunction.

Clinical studies have previously used ultrasound assessment of brachial artery flow-mediated dilation (FMD) to study the impact of surgery on peripheral endothelial function during reactive hyperaemia.[3–5] One of these studies did serial postoperative FMD measurements after total knee replacement surgery.[3] FMD was progressively reduced until 24 h after surgery, after which FMD gradually improved and reached the preoperative value at postoperative day seven.[3] When studying RHI, we observed similarly U-shaped dynamics with a sudden decrease during the first 24 h after surgery followed by a slow gradual increase during the following postoperative days. Interestingly, myocardial injury and postoperative myocardial infarction are most likely to occur within 48 h of surgery. As no patients in our study had troponin elevations or other cardiovascular events after surgery, the association between postoperative endothelial dysfunction and cardiovascular events including troponin elevation could not be investigated further.

A previous study of patients undergoing non-cardiovascular surgery, showed that the postoperative reduction in FMD was significantly larger at two h, one and seven days in patients undergoing laparotomy compared with patients undergoing laparoscopic surgery,[4] suggesting that the degree of surgical stress and trauma influences endothelial function.[4] Other factors that have been shown to influence on FMD are length and type of anaesthesia.[5] A randomized study of 59 patients undergoing non-cardiac surgery, found that patients allocated to nitrous oxide-based anaesthesia led to a reduced FMD 24 h after surgery, which was not the case for patients allocated to the nitrous oxide-free group anaesthetic.[5] However, the postoperative L-arginine/ADMA ratio did not differ between the groups and, as in our study, the ratio was significantly reduced 24 h after surgery.[5]

Endothelial NO is mainly produced from L-arginine by eNOS, while ADMA is an endogenous NOS inhibitor.[18] As ADMA competes with L-arginine for NOS binding, the NO production might be expressed as the ratio between substrate and inhibitor: the L-arginine/ADMA ratio.[18] In the acute clinical setting, decreased plasma NO bioavailability has been shown to correlate with haemodynamic instability and mortality after acute myocardial infarction[19] and clinical scores of disease severity in the intensive care unit.[20] In addition, plasma ADMA concentrations are increased in patients with unstable angina,[21] acute heart failure[19] and peripheral vascular disease.[22] An observational study in 2096 adults, showed that the ADMA concentration was inversely correlated with endothelial function measures by FMD.[23] Surprisingly, we observed a significant decrease in ADMA in the early postoperative period. Although speculative, this could be explained by a compensatory increase of dimethylarginine dimethylaminohydrolase, that removes ADMA in a metabolic conversion. A study on patients with subarachnoid haemorrhage observed an initial significant decrease in ADMA 49-72 h after the insult, followed by a delayed increase 97-120 h after the insult.[24] A similar study observed a sudden decrease in RHI day zero to two while ADMA was stable until a significant increase at day six to eight. Thus, an increase in ADMA may occur in the later postoperative period not monitored in the present study.[12]

Several techniques have been developed to measure peripheral and coronary endothelial dysfunction.[25] The advantage of the EndoPat is that the method is operator independent, adjusts for systemic effects by recording simultaneously from both fingers and a computer algorithm calculates the RHI automatically, based on the recorded digital pulse volume amplitude, which largely reduces the interoperator variability.[25] Moreover, the method has a high reproducibility and no carry-over effect after repeated measurements.[8 26] Clinical studies have shown a significant association between RHI and coronary endothelial

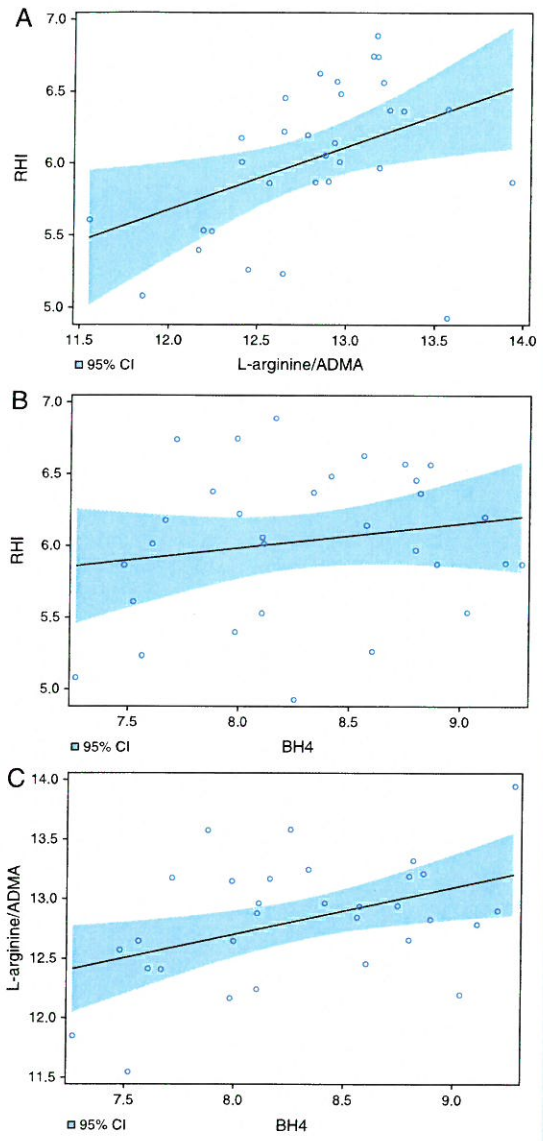

Fig 1. Scatter plots with regression lines. All variables are expressed as the logarithm to the area under the curve. **A.** L-arginine/ADMA and RHI, $P = 0.015$, Pearson correlation coefficient = 0.43. **B.** BH4 and RHI, $P > 0.05$, Pearson correlation coefficient = 0.19. **C.** BH4 and L-arginine/ADMA, $P = 0.015$, Pearson correlation coefficient = 0.43.
ADMA, asymmetric dimethylarginine; 95% CI, 95% confidence interval

function and severity of coronary artery disease.[27 28] Likewise, RHI has been shown to be an independent predictor of cardiovascular events in patients at risk of cardiovascular diseases.[29] Few studies have examined RHI in an acute clinical setting. A study on 48 patients diagnosed with an aneurysmal subarachnoid haemorrhage, observed an increase in RHI from 1.67 (standard deviation 0.46) on days zero to two after the event to 1.87 (standard deviation 0.34) on days three to five after the event.[12] The RHI measurements and increase were similar to our findings. In addition, the study found an association between low RHI in week two after the event and 30 days mortality, where a decrease in RHI by 0.5 corresponded to a hazard ratio of 3.3 (95% CI 1.0-11). A study on patients suffering from acute brain dysfunction in the intensive care unit, associated RHI from enrolment to follow-up with survival and a greater increase in RHI was associated with 1.9 more delirium/coma-free days (95% CI 0.44-3.34, $P = 0.02$).[11] The studies suggests that the increase in RHI over time may predict clinical outcomes in the acute setting.

Endothelial dysfunction is characterized by a reduced NO bioavailability. The link between NO and the digital pulse volume amplitude during reactive hyperaemia, has been studied in healthy subjects infused with a NOS inhibitor that reduced the digital pulse volume amplitude.[30] Likewise, we showed a positive significant correlation between logAUC(L-arginine/ADMA) and logAUC(RHI) supporting the use of RHI as a marker of endothelial NO bioavailability.

BH4 is a cofactor for NOS and modulates the enzymatic activity.[31] Experimental studies have shown that low BH4 concentrations reduce NO production by uncoupling of the endothelial NO synthase.[31] Instead of producing NO from L-arginine and oxygen, NOS switch to superoxide production thus increases oxidative stress.[32] Superoxide further reacts with NO to form the reactive peroxinitrite, which contributes to further vascular oxidative stress and further reduces NO bioavailability.[32] We found a significant positive correlation between BH4 and the L-arginine/ADMA ratio, which additionally supports the role of BH4 in NO production. However, no significant correlation was found between BH4 and RHI.

The bioavailability of BH4, the reduced and active form of biopterin, is likely as a result of a complex equilibrium between de novo synthesis, oxidative loss and recycling pathways.[31] We observed a significant reduction in plasma BH4 in the early postoperative period, which could partly be explained by BH4 oxidation to the inactive BH2 and other oxidized biopterin species, as a result of an increased level of reactive oxygen species after surgery. However, the significant decrease in BH2 and total biopterin concentrations, suggest that additional mechanisms are in play such as reduced biopterin synthesis or increased clearance of BH2.

The study has strengths and limitations. We included a homogenous population that underwent a standardized operation. The study was prospective with a sample size calculation and predetermined outcomes. We sought to investigate several aspects of the postoperative endothelial function, by studying both the RHI and biomarkers of the L-arginine-NO pathway. The study was a single-centre trial and given the small sample size we could not adjust for any confounding variables. The interpretation of the biomarker pattern should be viewed with caution, as only a few but central biomarkers of the L-arginine-NO pathway were measured. Initially, we planned to follow the patients with blood samples and EndoPat measurements until postoperative day four. However, this was not accomplished as the majority of patients were discharged at postoperative day two.

In conclusion, we found that the RHI response is impaired in the first h and days after major colon cancer surgery. The study suggests that this endothelial dysfunction is closely coupled to disturbances in the L-arginine - NO pathway. The sudden postoperative endothelial dysfunction could potentially contribute to the development of major cardiovascular events after non-cardiac surgery.

Authors' contributions

Study design/planning: S.E., J.R., I.G.
Study conduct: S.E., M.H.H-L.
Data analysis: S.E., J.L., A.M.V.S-P.
Writing paper: S.E.
Revising paper: all authors

Declaration of interest

None declared.

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