Effect of remote ischemic preconditioning on fibrin formation and metabolism in patients undergoing hip fracture surgery: a randomized clinical trial

Kirsten L. Wahlstrøm[a], Sarah Ekeloef[a], Johannes J. Sidelmann[b], Ismail Gögenur[a] and Anna-Marie B. Münster[b,c]

Remote ischemic preconditioning (RIPC) prior to surgery has recently been shown to reduce the risk of myocardial injury and myocardial infarction after hip fracture surgery. This study investigated whether RIPC initiated antithrombotic mechanisms in patients undergoing hip fracture surgery. This trial was a predefined sub-study of a multicentre randomized clinical trial. Adult patients with cardiovascular risk factors undergoing hip fracture surgery between September 2015 and September 2017 were randomized 1:1 to RIPC or control. RIPC was initiated before surgery with a tourniquet applied to the upper arm and it consisted of four cycles of 5 min of forearm ischemia followed by five minutes of reperfusion. The outcomes such as surgery-induced changes in thrombin generation, fibrinogen/fibrin turnover, tissue plasminogen activator, plasminogen activator inhibitor-1 and fibrin structure measurements were determined preoperatively (prior to RIPC) and 2 h postoperatively. One hundred and thirty-seven patients were randomized to RIPC ($n = 65$) or control ($n = 72$). There were no significant changes in thrombin generation, fibrinogen/fibrin turnover or fibrin structure measurements determined pre and postoperatively between patients in the RIPC and control groups. Subgroup analyses on patients not on anticoagulant therapy ($n = 103$), patients receiving warfarin ($n = 17$) and patients receiving direct oral anticoagulant therapy ($n = 18$) showed no significant changes between the RIPC-patients and controls. RIPC did not affect changes in thrombin generation, fibrin turnover or fibrin structure in adult patients undergoing hip fracture surgery suggesting that the cardiovascular effect of RIPC in hip fracture surgery is not related to alterations in fibrinogen/fibrin metabolism. *Blood Coagul Fibrinolysis* 33:25–33 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

[a]Centre for Surgical Science, Department of Surgery, Zealand University Hospital, Køge, [b]Unit for Thrombosis Research, Department of Regional Health Research, University of Southern Denmark and Department of Clinical Biochemistry, University Hospital of Southern Denmark, and [c]Department of Clinical Biochemistry, Regional Hospital West Jutland, Holstebro, Denmark

Correspondence to Kirsten L. Wahlstrøm, MD, PhD-student, Centre for Surgical Science, Department of Surgery, Zealand University Hospital, Lykkebækvej 1, 4600 Køge, Denmark
Tel: +45 20246286; e-mail: kwah@regionsjaelland.dk

Introduction

Remote ischemic preconditioning (RIPC) has shown promising results in protecting tissues and vital organs from injury during noncardiac surgery [1]. RIPC is most often induced by brief cycles of extremity ischemia and reperfusion controlled using an inflatable tourniquet. Although the effect of RIPC has been investigated for more than 25 years in both experimental and clinical studies in medical and surgical settings [2], the mechanisms responsible for the protective effect of RIPC are still unclear. The signal transduction from the local tissue ischemia caused by RIPC seems to be multifactorial and involves both humoral and neuronal mechanisms inducing cyto-protection in remote organs and tissues [3–5].

In a randomized clinical trial, we included patients above 45 years of age with known cardiovascular comorbidity undergoing hip fracture surgery and showed that RIPC immediately prior to surgery reduced the risk of myocardial injury within four days of hip fracture surgery [6].

Accordingly, RIPC prior to primary percutaneous coronary intervention has been shown to reduce infarct size in patients with first acute myocardial infarction [7]. The vascular endothelium plays a key role in haemostasias and thromboembolic disease and there is much evidence of RIPC attenuating vascular endothelial dysfunction in healthy people and patients with cardiovascular disease [4,8–12]. Several experimental and clinical studies have indicated that RIPC affects the haemostatic system [13–18]. A systematic review showed that RIPC attenuated platelet activity and reduced the risk of arterial thromboembolism in patients undergoing surgery or cardiac procedures [19]. RIPC is also capable of increasing fibrinolysis in patients with cerebrovascular and cardiovascular disease when applied daily over a period [16,20]. However, a majority of studies have exclusively investigated the effect of RIPC on specific haemostatic proteins [13–18].

Surgery itself has been associated with a postoperative fibrinolytic shutdown [21,22], which is thought to be

driven by the inhibition of tissue type plasminogen activator (tPA) by a rapid release of plasminogen activator inhibitor type 1 (PAI-1) [21–23]. An alternative or maybe supplementary explanation for the impaired fibrinolysis could be changes in the fibrin structure properties [24]. Thrombin generation as well as fibrinogen/fibrin turnover are central players in the coagulation system [25,26], and alterations in these processes together with fibrin structure have been associated with the pathophysiology of arterial and venous thromboembolic diseases [24,27,28].

In the present study, we investigated the effect of RIPC on thrombin generation, fibrinogen/fibrin turnover, tPA and PAI-1 concentrations and fibrin structure to clarify whether alterations in the central part of haemostasis are related to the cardioprotective effect of RIPC in patients undergoing hip fracture surgery.

Materials and methods
Trial design
The present single-centre, observer-blinded trial was a predefined sub-study of a multicentre, randomized clinical trial (PIXIE trial) designed to test the hypothesis that RIPC, compared with standard treatment, reduces the incidence of myocardial injury after hip fracture surgery [6].

The trial was approved by the Regional Ethics Committee of Region Zealand Denmark (no. SJ-428), and by the Danish Data Protection Agency (no. Reg-115–2014). The trial was reported according to the CONSORT Statement [29] and registered on ClinicalTrials.gov (no. NCT02344797).

Participants
Patients of age more than 45 years undergoing hip fracture surgery (including nails, cemented and uncemented prothesis insertion) were included if they fulfilled at least one of four inclusion criteria being ischemic heart disease defined by angina pectoris, prior myocardial infarction, prior percutaneous coronary intervention or prior coronary artery bypass grafting; peripheral arterial disease defined by either intermittent claudication, reduced peripheral arterial blood flow or previous vascular surgery due to peripheral arterial disease; prior stroke; and any of the following cardiovascular risk factors: previous transient ischemic attack, age at least 70 years, congestive heart failure, diabetes and currently taking antidiabetics, hypertension or preoperative creatinine concentration more than 175 μmol/l.

Patients were not eligible if any of the exclusion criteria were met: history of peripheral arterial disease affecting both arms, renal failure (estimated glomerular filtration rate <30 ml/min/1.73 m^2), cardiogenic shock or cardiac arrest during the current hospital stay (before inclusion), another surgical procedure during the current hospital stay (before inclusion), a condition that prevented the performance of RIPC, not capable of giving informed consent or previously enrolled in the trial. Extended exclusion criteria for this sub-study were active cancer and treatment with therapeutical doses of low molecular weight heparin (LMWH).

The patient recruitment and data collection was conducted at the Regional Hospital West Jutland, a secondary referral centre, between September 2015 and September 2017. The patients were included consecutively. Only per-protocol patients, defined as those undergoing RIPC as described in the protocol and having both a preoperative baseline blood sample and a 2-h postoperative blood sample collected, were included for analyses in this study.

Randomization and blinding
Patients were allocated by randomization to intervention or control. A third party generated the allocation sequence using the electronic generator www.randomization.com with allocation ratio 1 : 1 in blocks of six. A sealed, opaque envelope was opened in the operating theatre by the nurse anaesthetist. Due to the nature of the intervention, the anaesthetist, nurse anaesthetist, surgeon and surgical staff were not blinded. The principal investigator, technicians and data assessors were blinded to the intervention. Patients were blinded to their allocation if operated under general anaesthesia, but not if they were operated under regional anaesthesia without sedation.

Intervention
As described previously [6], patients in both the intervention and control groups received standard care before, during and after surgery permitting the local guidelines to be followed. In addition, patients in the RIPC group received the RIPC procedure consisting of four cycles of forearm ischemia and reperfusion. The procedure was performed by the nurse anaesthetist with an electronic tourniquet device (Tourniquet 4500 ECL; VBM Medizintechnik, Sulz am Neckar, Germany). The tourniquet was inflated to 200 mmHg for 5 min followed by deflation and 5 min of reperfusion. In case of patients having a SBP above 185 mmHg, the tourniquet was inflated to a minimum of 15 mmHg above the patient's SBP. The intervention was performed in the operating theatre after induction of anaesthesia and the first cycle of ischemia and reperfusion was completed prior to skin incision. The exact time of each cycle, technical difficulties and disruptions were noted. Choice of anaesthesia and analgesia were decided by the anaesthesiologist in charge. For type of anaesthesia, see Table 1.

Treatment with tranexamic acid (TXA), vitamin K or blood components was decided by the surgeon in charge and if they were administered, pre, peri and postoperative time and doses were recorded.

Daily medication
For patients taking daily medications affecting blood coagulation [acetylsalicylic acid ($n = 34$); platelet inhibitors:

Table 1 Baseline and per-operative characteristics of patients stratified by intervention (remote ischemic preconditioning and controls receiving standard care)

Baseline characteristics	RIPC (n = 65)	Control (n = 72)
Sex, no. (%)		
Males	18 (27.7)	22 (30.6)
Females	47 (72.3)	50 (69.4)
Age, Median (range); years	81.5 (54.5–97.8)	80.0 (58.7–92.3)
BMI, Mean (SD); kg/m^2	23.5 (4.8)	24.1 (4.8)
Daily smoking, No. (%)	17 (26.2)	19 (26.4)
Alcohol abuse, No. (%)	7 (10.8)	10 (13.9)
Comorbidity[a] No. (%)		
Hypertension	45 (69.2)	48 (66.7)
Hypercholesterolemia	45 (69.2)	48 (66.7)
Diabetes mellitus	6 (9.2)	11 (15.3)
Ischemic heart disease	9 (13.8)	9 (12.5)
Peripheral arterial disease	3 (4.6)	6 (8.3)
Congestive heart failure	4 (6.2)	6 (8.3)
Atrial fibrillation	22 (33.8)	13 (18.1)
Stroke	10 (15.4)	12 (16.7)
Transient cerebral ischemia	3 (4.6)	1 (1.4)
Autoimmune diseases	9 (13.8)	6 (8.3)
Daily Medicine intake (dosing range/day), No. (%)		
Beta-blocker	22 (33.8)	25 (34.7)
Calcium antagonist	23 (35.4)	26 (36.1)
ACE-1/ARB	31 (47.7)	33 (45.8)
Acetylsalicylic acid (75 mg)	17 (26.2)	17 (25.0)
Platelet inhibitors (dipyridamol 200–400 mg, clopidogrel 75 mg)	6 (9.2)	8 (11.1)
Vitamin K antagonist (1.25–10 mg)	12 (18.5)	5 (6.9)
DOAC (rivaroxaban 15–20 mg, dabigatranetexilat 110–150 mg, apixaban 2.5–5 mg)	7 (10.8)	10 (13.9)
NSAID (ibuprofen 400–1200 mg, diclofenac 100 mg, naproxen 500mg)	4 (6.2)	4 (5.6)
Glucocorticoids	1 (1.5)	4 (5.6)
Methotrexate (7.5–10 mg once per week)	1 (1.5)	2 (2.8)
Anesthesia, No. (%)		
Epidural	14 (21.5)	16 (22.2)
Spinal	16 (24.6)	23 (31.9)
TIVA	3 (4.6)	2 (2.8)
Volatile	32 (49.2)	31 (43.1)
Norepinephrine infusion	2 (3.1)	6 (8.3)
Bleeding		
No. of patients (%)	55 (84.6)	57 (79.2)
Amount [ml, median (range)]	200 (25–2720)	150 (25–900)
Per-operative transfusions and medications[b], No. (%)		
Erythrocyte suspension	2 (3.1)	1 (1.4)
Plasma	1 (1.5)	0 (0)
Tranexamic acid	25 (38.5)	19 (26.4)
Duration of surgery (min, median [range])	52 (16–197)	55 (11–201)

ACE-1, angiotensin-converting enzyme 1; ARB, angiotensin II receptor blockers; DOAC, direct oral anticoagulants; SD, standard deviation; TIVA, total intravenous anaesthesia. [a] No patient in either group had blood coagulation disorders as a comorbidity. [b] No patients in either group received platelets.

dipyridamol ($n = 4$) and clopidogrel ($n = 10$); vitamin K antagonist ($n = 17$); or direct oral anticoagulants (DOAC): dabigatranetexilat ($n = 5$), rivaroxaban ($n = 5$) and apixaban ($n = 8$)], doses and time of pre and postoperative administration were recorded.

Outcomes

Outcomes assessed in this trial were changes in the levels of thrombin generation, fibrinogen/fibrin turnover, tPA, PAI-1 and fibrin structure determined preoperatively and 2 h postoperatively in RIPC patients and controls, as listed in Table 2.

Blood sampling

Citrate stabilized venous blood samples were collected according to international guidelines [30] using minimal

Table 2 Study outcomes

Thrombin generation	
Prothrombin fragment 1+2	F1+2
Lag time thrombin generation (s)	TGT$_{lag}$
Time to thrombin peak (min)	TTP
Thrombin peak height (nmol/l)	Peak
Endogenous thrombin potential (nmol/l × min)	ETP
Time for thrombin generation to terminate (min)	Trail
Fibrinogen/fibrin turnover	
Tissue-type plasminogen activator concentration (ng/ml)	tPA
Plasminogen activator inhibitor type 1 concentration (ng/ml)	PAI-1
Fibrinogen (μmol/l)	FIBF
Lag time fibrin generation (s)	FIBR$_{lag}$
V$_{max}$ (OD/min)	V$_{max}$
D-dimer	D-dimer
Lysis (% in 30 min)	Lysis
Fibrin fibre structure	
Mass-length ratio (× 10^{12} Dalton/cm)	ML
Diameter (mg/l)	Diam
Density (× 10^{22} Dalton/cm^3)	Dens

stasis prior to RIPC/surgery (baseline) and 2 h postoperatively. Blood samples were withdrawn from a large antecubital vein, the first blood sampled was disposed and a maximum of 30 ml from each participant was collected. Citrate plasma was obtained by centrifugation of samples at 2000 g for 20 min. Subsequently, the plasma was aliquoted and stored at -80°C in cryotubes. The samples were thawed for 5 min at 37°C before analysis, kept at room temperature and analysed within 1 h of thawing.

Measurement of thrombin generation

The plasma concentration of prothrombin fragment 1+2 was determined using the Enzygnost F1+2 kit (Siemens Healthcare Diagnostics, Marburg, Germany). Thrombin generation was analysed by the calibrated automated thrombin generation assay (Thrombinoscope BV, Maastricht, The Netherlands) employing the Fluoroskan Ascent microplate fluorometer (Thermo Fisher Scientific, Hvidovre, Denmark). The Thrombinoscope software was used for calculating the lag time of the thrombin formation process (TGT_{lag}), time to reach peak thrombin concentration (TTP), peak thrombin concentration (peak), time for thrombin generation to terminate (trail) and endogenous thrombin potential (ETP) recording the total amount of thrombin formed.

Measurement of fibre structure and degradation of fibrin

The plasma concentration of fibrinogen and D-Dimer was quantitatively determined using the STA-Liquid fib and the Liatest D-Di kits (Diagnostica Stago, Asnieres-sur-Seine, Paris, France), respectively, and a STA-R coagulation analyser (Diagnostica Stago). In-house prepared ELISAs employing specific mAbs were used to determine the plasma concentration of tPA and PAI-1 [31].

The rate of fibrin formation (Vmax) and fibrin structure measurements (mass-length ratio [(ML), diameter (Diam) and density (Dens)] and fibrin clot lysis were determined according to Sjøland *et al.* [32] using turbidity measurements

Fibrin clot lysis was measured by mixing 60 μl of plasma with 120 μl of a reaction mixture consisting of 1 IU/ml of thrombin, 15 mmol/l $CaCl_2$, 50 mmol/l Tris-HCL buffer, 150 mmol/l NaCl and 0.6 mg/ml recombinant tPA (Boehringer Ingelheim, Ingelheim am Rhein, Germany). The optical density at 340 nm (OD340) was recorded for 30 min, and the percentage of the clot lysed thereafter was determined. Measures of fibrin clot properties was determined using a similar setup, but replacing rtPA with Tris-HCl buffer. The fibrin fibre properties (i.e. Vmax, mass-length, Diam and Dens) were determined by measurements of OD405, OD560, OD608 and OD690 of the fibrin clot and calculated according to Carr *et al.* [33,34].

Statistical analyses

As the study was a predefined explorative sub-study of a larger randomized clinical trial, sample size calculation

was not performed. All eligible patients from one study centre were included during the 2-year study period.

Categorical baseline data were presented as frequencies (N and %) and analysed using χ^2 test with Yates correction.

The distribution of continuous data was depicted using histogram plots. Normally distributed data were compared by Student's *t*-test and presented as mean (SD), while nonnormally distributed data were compared by the Mann–Whitney *U*-test and presented as median (range). Bonferroni correction was applied to significant results as an adjustment for multiple testing. The Spearman's rank correlation was performed to explore the associations between nonnormally distributed results.

Subgroup analyses were performed post hoc on patients receiving either warfarin therapy, DOAC therapy or no anticoagulant treatment.

All statistical analyses were performed and all graphs were constructed using RStudio (RStudio Team [2019] [35]). A two-sided *P* value less than 0.05 was considered statistically significant.

Results

Baseline and per-operative characteristics

Between September 2015 and September 2017, 206 patients who underwent hip fracture surgery at Regional Hospital West Jutland and fulfilled the inclusion criteria were consecutively included in the study. Due to lack of blood samples, 49 patients were excluded, leaving 157 patients in the intention-to-treat population. Hereafter, 13 patients were excluded as they did not meet the per-protocol criteria (including three patients experiencing discomfort to the intervention; Fig. 1), six patients were excluded due to active cancer and one was excluded due to treatment with therapeutically doses of LMWH. Finally, 137 patients were eligible for per-protocol analyses (RIPC group: $n = 65$, control group: $n = 72$, Fig. 1). A comparison of the baseline characteristics between patients included and those excluded is available in the Appendix Table 1, http://links.lww.com/BCF/A112. Overall, the groups were comparable with one exception that a higher number of patients included in the study had a daily intake of angiotensin receptor blockers or angiotensin-converting enzyme inhibitors than the excluded patients ($P = 0.04$).

The demographics of the included population are presented in Table 1. The majority of the included patients were female, and the median age (range) was 81.5 (54.5–97.8) years in the RIPC group and 80.0 (58.7–92.3) years in the control group. Hypertension and hypercholesterolemia were the most common cardiovascular morbidities in both groups. No patients suffered from blood coagulation disorders. Per-operative characteristics were similar in the two groups (Table 1). One hundred and twenty-six patients had surgery within 24 h from admission, and the remaining 11 patients had surgery within 72 h from admission.

Fig. 1
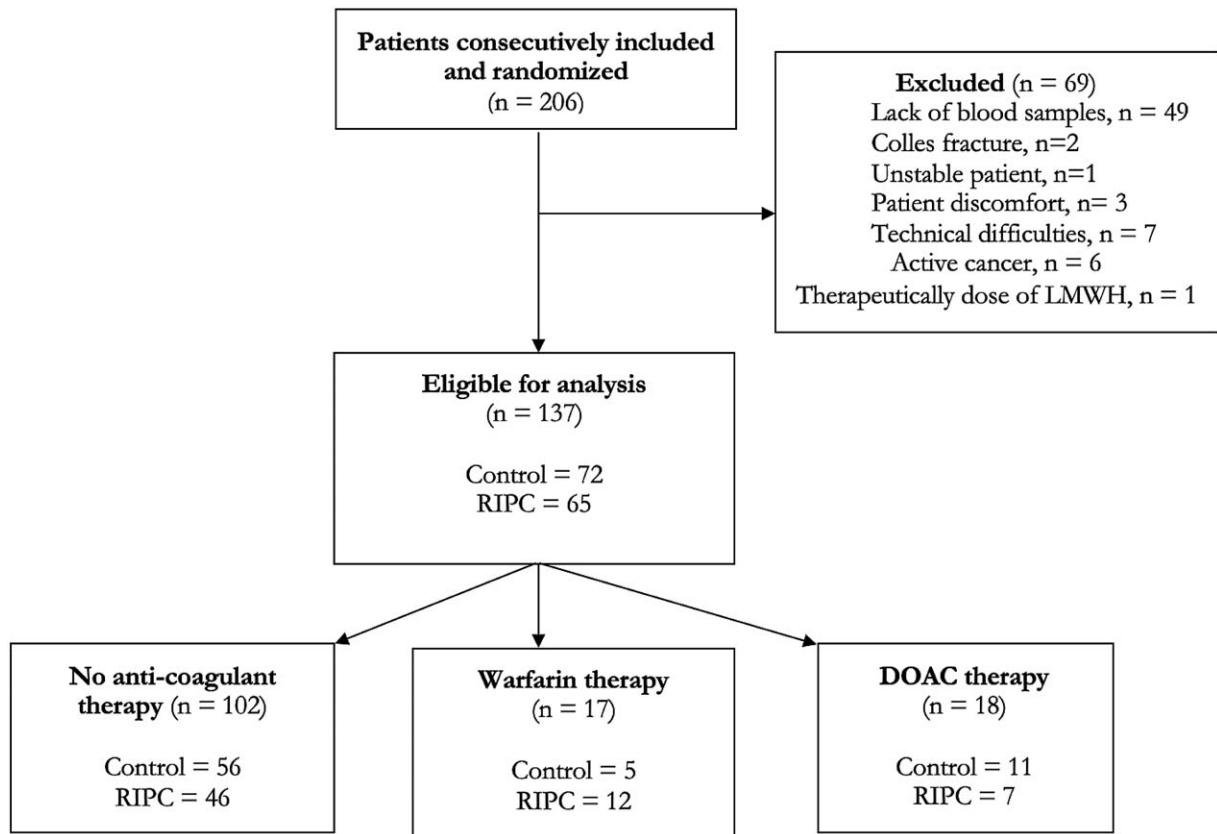
Flow-chart for patient selection.

LMWH was either administered more than $6\,T_{1/2}$ prior to or after RIPC, blood sampling and surgery. Thus, LMWH did not affect the outcome of our analysis.

Forty-four patients received one dose of TXA per-operatively (RIPC = 25 and control = 19). D-dimer level and lysis of fibrin were not analysed on patients receiving TXA due to the impact of TXA on these measures [36].

Fourteen patients received vitamin K preoperatively and prior to baseline blood sample collection (RIPC = 9 and control = 5). The 14 patients who were administered warfarin were not excluded from the subgroup analysis, as they all had an international normalized ratio above normal range (in this case defined as 0.8–1.2) at pre and postoperative blood sample collection in spite of the vitamin K administration. Thus, the patients treated with vitamin K were still responding to warfarin and were included in the warfarin-sub-group analysis.

Thrombin generation

No differences were observed in levels of markers of thrombin generation from baseline until two hours after the hip fracture surgery between patients receiving RIPC and those receiving standard care: F1+2 ($P = 0.30$), TGT_{lag} ($P = 0.75$), TTP ($P = 0.17$), peak ($P = 0.35$), trail ($P = 0.32$) and ETP ($P = 0.46$) (Table 3). Likewise, no changes were observed in the sub-groups; neither in the sub-group of patients not receiving anticoagulant therapy [F1+2 ($P = 0.88$), TGT_{lag} ($P = 0.30$), TTP ($P = 0.15$), peak ($P = 0.13$), trail ($P = 0.92$) and ETP ($P = 0.57$) (Appendix Table 2, http://links.lww.com/BCF/A112)], in patients with a daily intake of warfarin [F1+2 ($P = 0.38$), TGT_{lag} ($P = 0.96$), TTP ($P = 0.83$), peak ($P = 0.51$), trail ($P = 0.15$) and ETP ($P = 0.72$) (Appendix Table 3, http://links.lww.com/BCF/A112)] or patients with an intake of DOACs [F1+2 ($P = 0.13$), TGT_{lag} ($P = 0.33$), TTP ($P = 0.71$), peak ($P = 0.21$), trail ($P = 0.44$) and ETP ($P = 0.33$)] (Appendix Table 4, http://links.lww.com/BCF/A112).

Fibrinogen/fibrin turnover

Fibrinogen/fibrin turnover did not change from baseline until 2 h after the hip fracture surgery between patients receiving RIPC and those receiving standard care [tPA ($P = 0.10$), PAI-1 ($P = 0.11$), fibrinogen ($P = 0.87$), $FIBR_{lag}$ ($P = 0.72$), Vmax ($P = 0.06$), lysis ($P = 0.93$) and D-dimer ($P = 0.83$) (Table 3)].

Table 3   Effect of remote ischemic preconditioning on thrombin generation and fibrinogen/fibrin turnover stratified by intervention [remote ischemic preconditioning ($n = 65$) and control ($n = 72$)]

Plasma measure (median, IQR)	Baseline	2 h postoperative	Delta-value	P_{DELTA}
Prothrombin fragment 1+2 (pmol/l)				
RIPC	368 (361)	682 (429)	180 (319)	0.30
Control	438 (378)	843 (431)	316 (487)	
TGT_{lag} (s)				
RIPC	3.50 (1.25)	3.50 (1.00)	0.00 (0.25)	0.75
Control	3.50 (1.00)	3.50 (1.00)	0.00 (0.50)	
TTP (min)				
RIPC	7.00 (1.50)	6.50 (1.50)	0.00 (0.50)	0.17
Control	6.50 (1.75)	6.50 (1.58)	−0.25 (0.50)	
Peak (nmol/l)				
RIPC	228 (85.9)	229 (79.3)	3.04 (25.9)	0.35
Control	248 (82.5)	250 (77.9)	6.81 (23.5)	
ETP (nmol/l × min)				
RIPC	1488 (335)	1509 (262)	21.0 (111)	0.46
Control	1568 (298)	1594 (315)	26.5 (112)	
Trail (min)				
RIPC	25.0 (4.00)	25.5 (3.00)	0.50 (1.50)	0.32
Control	25.5 (4.75)	26.0 (4.50)	0.00 (1.75)	
tPA (ng/ml)				
RIPC	12.2 (5.40)	14.3 (7.70)	2.00 (4.70)	0.10
Control	12.8 (5.73)	15.8 (7.78)	2.90 (5.20)	
PAI-1 (ng/ml)				
RIPC	21.8 (17.3)	35.0 (28.9)	9.50 (22.5)	0.11
Control	23.6 (18.4)	39.4 (38.9)	19.4 (30.6)	
FIBF (μmol/l)				
RIPC	11.9 (3.40)	11.5 (3.50)	−0.30 (1.10)	0.87
Control	11.2 (2.40)	10.9 (3.12)	−0.25 (1.12)	
$FIBR_{lag}$ (s)				
RIPC	15.0 (0.00)	15.0 (1.00)	0.00 (1.00)	0.72
Control	15.0 (1.00)	15.0 (1.00)	0.00 (1.00)	
Vmax (OD/min)				
RIPC	1.11 (0.31)	1.15 (0.29)	0.004 (0.12)	0.06
Control	1.09 (0.30)	1.05 (0.37)	−0.012 (0.15)	
D-dimer (mg/l) [a]				
RIPC	4.62 (9.84)	7.40 (9.20)	0.58 (3.32)	0.83
Control	4.07 (10.1)	7.66 (9.40)	0.82 (5.56)	
Lysis/30 min (%) [a]				
RIPC	50.2 (29.6)	49.0 (20.5)	1.05 (13.4)	0.93
Control	52.3 (32.9)	53.0 (33.4)	0.60 (17.3)	
Diam (μm)				
RIPC	0.14 (0.02)	0.14 (0.02)	−0.01 (0.01)	0.29
Control	0.13 (0.02)	0.13 (0.01)	0.00 (0.01)	
Dens (×10^{22} Dalton/cm^3)				
RIPC	3.90 (0.70)	4.00 (0.70	0.10 (0.30	0.45
Control	3.95 (0.75)	4.00 (0.80)	0.20 (0.43)	
ML (×10^{22} Dalton/cm)				
RIPC	5.90 (1.10)	5.80 (1.00)	−0.20 (0.60)	0.19
Control	5.65 (0.93)	5.75 (1.10)	0.00 (0.63)	

P values are given for the difference between the delta-values of the plasma markers in the two groups. Delta-value = value$_{2\text{-h postoperative}}$ − value$_{baseline}$. Dens, density; Diam, diameter; ETP, endogenous thrombin potential; FIBR, fibrinogen; $FIBR_{lag}$, lag time fibrin generation; ML, mass-length ratio; PAI-1, plasminogen activator inhibitor type 1; RIPC, remote ischemic preconditioning; TGT_{lag}, lag time thrombin generation; tPA, tissue-type plasminogen activator; TTP, time to thrombin peak. [a] Forty-four patients excluded from analysis due to tranexamic acid administration (RIPC = 25, control = 19).

Although there were no differences between the groups, both tPA and PAI-1 increased after surgery. For the RIPC group, tPA increased from preoperative median of 12.2 (IQR 5.40) ng/ml to 14.3 (IQR 7.70) ng/ml postoperative ($P < 0.001$), and for the control group, tPA likewise increased from a preoperative median of 12.8 (IQR 5.73) ng/ml to 15.8 (7.78) ng/ml postoperative ($P < 0.001$).

PAI-1 increased from preoperative values of 21.8 (IQR 17.3) ng/ml to 35.0 (IQR 28.9) ng/ml postoperative ($P < 0.001$) in the RIPC group, and for the control group, a similar increase was seen from a preoperative level of 23.6 (IQR 18.4) ng/ml to a postoperative level of 39.4 (IQR 38.9) ng/ml ($P < 0.001$).

In addition, PAI-1 was negatively correlated to lysis ($\rho = -0.26$; 95% confidence intervals = −0.46, −0.09; $P = 0.01$).

Sub-group analyses of patients not receiving anticoagulant therapy revealed no significant changes in any of the fibrinogen/fibrin turnover measurements from pre to postoperative values: patients not receiving anticoagulant therapy [tPA ($P = 0.18$), PAI-1 ($P = 0.16$), fibrinogen ($P = 0.43$), $FIBR_{lag}$ ($P = 0.50$), Vmax ($P = 0.50$), lysis ($P = 0.68$) and D-dimer ($P = 0.91$) (Appendix Table 2,

http://links.lww.com/BCF/A112)]; patients with a daily intake of warfarin [tPA ($P = 0.96$), PAI-1 ($P = 0.72$), fibrinogen ($P = 0.10$), FIBR$_{lag}$ ($P = 0.94$), Vmax ($P = 0.94$), lysis ($P = 0.89$) and D-dimer ($P = 0.33$) (Appendix Table 3, http://links.lww.com/BCF/A112)] and patients with a daily intake of DOAC [tPA ($P = 0.43$), PAI-1 ($P = 0.33$), fibrinogen ($P = 0.39$), FIBR-$_{lag}$ ($P = 0.50$), Vmax ($P = 0.50$), lysis ($P = 1.0$) and D-dimer ($P = 0.81$) (Appendix Table 4, http://links.lww.com/BCF/A112)].

Fibrin structure

There were no significant differences in changes of fibrin structure measurements or lysis [Diam ($P = 0.29$), Dens ($P = 0.45$), mass-length ($P = 0.19$), lysis ($P = 0.93$) (Table 3)] between patients receiving preoperative RIPC and controls receiving standard care. These findings did not change in subgroup analyses of patients not receiving anticoagulant therapy [Diam ($P = 0.32$), Dens ($P = 0.42$), mass-length ($P = 0.07$) and lysis ($P = 0.68$) (Appendix Table 2, http://links.lww.com/BCF/A112)], in patients with a daily intake of warfarin [Diam ($P = 0.74$), Dens ($P = 0.83$), mass-length ($P = 0.75$), lysis ($P = 0.89$) (Appendix Table 3, http://links.lww.com/BCF/A112)] or in patients with a daily intake of DOACs [Diam ($P = 0.78$), Dens ($P = 0.96$), mass-length ($P = 0.86$), lysis ($P = 1.0$) (Appendix Table 4, http://links.lww.com/BCF/A112)].

Discussion

The present study addresses for the first time the potential association between the cardio protecting effect of RIPC in patients undergoing hip fracture surgery [6] and the effect of RIPC on the haemostatic balance in terms of thrombin generation, fibrinogen/fibrin turnover, tPA, PAI-1 and fibrin structure measures. Our study demonstrates no significant differences in preoperative and postoperative values of the aforementioned outcomes between the patients who received preoperative RIPC and those who received only standard care.

Few other studies have focused on the effect of RIPC on coagulation and fibrinolysis demonstrating either no effect on the fibrinolytic system [17] augmented fibrinolysis [13,16] and prolongation of the prothrombin time suggesting impaired activation of the coagulation system induced by RIPC [15]. These studies however were conducted in animals [13] or included a low number of patients [15,16].

In our study, focusing on the activation of coagulation, we demonstrated that F1+2 concentrations rose in response to surgery as previously described [37–39], but RIPC had no effect on F1+2 levels confirming recent findings in RIPC patients undergoing elective head and neck cancer surgery [17].

Thrombin generation is another step in the central coagulation process wherein RIPC potentially could play a role. Thrombin plays a crucial role in clot formation with a dynamic concentration during the coagulation process [40]. Surgery in cancer patients has been found to impact markers of thrombin generation; although the findings in one study were inconsistent [41], another study confirmed surgery to induce a hypercoagulable state with decreased TGT_{lag} and TTP together with increased ETP and peak during surgery [42]. RIPC did not reduce thrombin generation in our patient population.

Reduced fibrinolytic shutdown instigated by RIPC is an alternative hypothesis on the anticoagulative effect of RIPC. There was no difference in fibrinolytic shutdown between patients receiving RIPC and controls in our study. Our baseline values of D-dimer were very significantly increased compared to the normal range and D-dimer rose further after surgery. The hip fracture trauma occurring hours before surgery may explain the elevated baseline values of D-dimer in our data.

Inhibition of tPA by a rapid release of PAI-1 has been associated with the fibrinolytic shutdown caused by surgery [21–23]. In our results, tPA and PAI-1 increased after surgery and the increase in PAI-1 was negatively correlated to lysis after surgery. This is consistent with findings of the postoperative shutdown in fibrinolysis being more prominent on postoperative day one, correlating to the maximum increase in PAI-1, than that observed later in the postoperative period [43]. The results of other studies investigating the short-term effect of RIPC on fibrinolysis in both healthy men [44] and patients undergoing cancer surgery [17] are similar to ours as they show that RIPC has no evident impact on fibrinolysis. However, a trial demonstrating the positive effect of long-term remote ischemic conditioning found tPA to be increased and PAI-1 to be reduced after 15 days of bilateral remote ischemic conditioning twice daily [20]. Hence, it has been suggested that long-term administration of remote ischemic conditioning may be required to increase fibrinolysis [17]. This is supported by studies on both healthy individuals undergoing RIPC daily for 7 days improving microcirculation [45] and surgical patients exposed to RIPC twice daily for 2 weeks before carotid artery stenting with positive result on the incidence and volume of new ischemic lesions in the brain after surgery [46].

Changes in the properties of the fibrin structure might be another way for RIPC to impair fibrinolysis [24]. Our study is the first to investigate the fibrin fibre properties in relation to RIPC and surgery. We did not find any difference between the patient groups. Notably, a recent study on elective orthognathic surgery opposes these findings [36]. This study revealed clear and unidirectional results of decreased fibrin clot lysis, decreased fibrin fibre mass-length ratio and diameter as well as increased density, Vmax and D-dimer after surgery [36]. These properties are consistent with impaired breakdown of fibrin, and thus, a

haemostatic system tipped in the prothrombotic direction after surgery. However, there are several differences in the patient populations of the study on orthognathic surgery and our study. These differences may contribute to the dissimilar findings: our patients were subjected to sub-acute repair of hip fractures and not elective procedures and suffered a trauma prior to surgery, which might have activated part of the haemostatic and fibrinolytic system long before the surgical trauma. Furthermore, our patients were considerably older, and all of them had pronounced cardiovascular risk factors.

As RIPC seems to protect the heart through induced cytoprotective effects [3–5], we found it interesting, from a clinical perspective, to investigate subgroups of patients receiving either no anticoagulant therapy or daily medication with warfarin or DOACs. However, none of the subgroup analyses showed any significant differences between patients receiving RIPC or controls receiving standard care.

A limitation to our study is the lack of sample size calculation as this was a sub-study and thus there is a risk of type II errors, but the study is the largest so far focusing on the effect of RIPC on coagulation and fibrinolysis. No other studies have investigated both thrombin and fibrin turnover as well as fibrin fibre structure measurements in the same surgical setting or investigated the effect of RIPC prior to subacute surgery on fibrin fibre structure after surgery. The patients were admitted at all hours, and we assume that their daily anticoagulant medication induces a significant effect at the time of intervention and blood sampling. Whether Multiplate or ROTEM/TEG platelet aggregometry was performed was up to the anaesthetist or surgeon in charge. However, neither Multiplate nor ROTEM/TEG platelet aggregometry were performed in any of the patients. It has been concluded in other studies that platelets may play a role in the mechanism behind RIPC [17]. Our focus was specifically on fibrin formation and metabolism, and the influence of platelets was not part of our analyses. A general limitation to clinical studies on this subject is that we cannot mimic the on-site haemostasis including all the components involved. Shear stress and glycocalyx are important components in coagulation, and recently, RIPC has been observed to offer protection against glycocalyx degradation after surgery [47].

The study is part of a data-assessor blinded randomized controlled trial design, data were prospectively collected and our population had numerous cardiovascular risk factors and cardiovascular comorbidity, which may affect our outcomes, compared with the studies involving healthier or younger population.

In conclusion, RIPC induced no significant changes in thrombin generation, fibrinogen/fibrin turnover, tPA, PAI-1 or fibrin structure when determined prior to surgery and 2 h after hip fracture surgery compared with controls receiving standard care only. Subgroup analysis of patients receiving either no anticoagulant therapy or those receiving daily warfarin or DOAC therapy showed no differences between patients exposed to RIPC or controls. Having the limitations of our study in mind, our results do not support that the RIPC-mediated cardiac protection in relation to hip fracture surgery is a result of changes in thrombin generation, fibrinogen/fibrin turnover, tPA, PAI-1 or fibrin structure properties within the first two postoperative hours.

Acknowledgements

Conflicts of interest
The authors declare no conflict of interest.

References

1 Kharbanda RK, Nielsen TT, Redington AN. Translation of remote ischaemic preconditioning into clinical practice. *Lancet* 2009; **374**:1557–1565.
2 Sukkar L, Hong D, Wong MG, Badve SV, Rogers K, Perkovic V, *et al.* Effects of ischaemic conditioning on major clinical outcomes in people undergoing invasive procedures: systematic review and meta-analysis. *BMJ* 2016; **355**:i5599.
3 Heusch G, Bøtker HE, Przyklenk K, Redington A, Yellon D. Remote ischemic conditioning. *J Am Coll Cardiol* 2015; **65**:177–195.
4 Kharbanda RK, Peters M, Walton B, Kattenhorn M, Mullen M, Klein N, *et al.* Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans in vivo. *Circulation* 2001; **103**:1624–1630.
5 Pedersen CM, Cruden NL, Schmidt MR, Lau C, Bøtker HE, Kharbanda RK, *et al.* Remote ischemic preconditioning prevents systemic platelet activation associated with ischemia-reperfusion injury in humans. *J Thromb Haemost* 2011; **9**:404–407.
6 Ekeloef S, Homilius M, Stilling M, Ekeloef P, Koyuncu S, Münster AMB, *et al.* The effect of remote ischaemic preconditioning on myocardial injury in emergency hip fracture surgery (PIXIE trial): phase II randomised clinical trial. *BMJ* 2019; **367**:l6395.
7 Botker HE, Kharbanda R, Schmidt MR, Bottcher M, Kaltoft AK, Terkelsen CJ, *et al.* Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet* 2010; **375**:727–734.
8 Loukogeorgakis SP, Panagiotidou AT, Broadhead MW, Donald A, Deanfield JE, MacAllister RJ. Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: role of the autonomic nervous system. *J Am Coll Cardiol* 2005; **46**:450–456.
9 Moro L, Pedone C, Mondi A, Nunziata E. Effect of local and remote ischemic preconditioning on endothelial function in young people and healthy or hypertensive elderly people. *Atherosclerosis* 2011; **219**:750–752.

10 Manchurov V, Ryazankina N, Khmara T, Skrypnik D, Reztsov R, Vasilieva E, *et al.* Remote ischemic preconditioning and endothelial function in patients with acute myocardial infarction and primary PCI. *Am J Med* 2014; **127**:670–673.

11 Tong X, Cui W, Li Y, Su C, Shao Y, Liang J, *et al.* Chronic remote ischemic preconditioning-induced increase of circulating hSDF-1(level and its relation with reduction of blood pressure and protection endothelial function in hypertension. *J Hum Hypertens* 2019; **33**:856–862.

12 Rytter N, Carter H, Piil P, Sørensen H, Ehlers T, Holmegaard F, *et al.* Ischemic preconditioning improves microvascular endothelial function in remote vasculature by enhanced prostacyclin production. *J Am Heart Assoc* 2020; **9**:e016017.

13 Warzecha Z, Dembiński A, Ceranowicz P, Dembiński M, Cieszkowski J, Kuśnierz-Cabala B, *et al.* Influence of ischemic preconditioning on blood coagulation, fibrinolytic activity and pancreatic repair in the course of caerulein-induced acute pancreatitis in rats. *J Physiol Pharmacol* 2007; **58**:303–319.

14 Linden MD, Whittaker P, Frelinger AL, Barnard MR, Michelson AD, Przyklenk K. Preconditioning ischemia attenuates molecular indices of platelet activation-aggregation. *J Thromb Haemost* 2006; **4**:2670–2677.

15 Mayor F, Bilgin-Freiert A, Connolly M, Katsnelson M, Dusick JR, Vespa P, *et al.* Effects of remote ischemic preconditioning on the coagulation profile of patients with aneurysmal subarachnoid hemorrhage: a case-control study. *Neurosurgery* 2013; **73**:808–815.

16 Pryds K, Kristiansen J, Neergaard-Petersen S, Nielsen RR, Schmidt MR, Refsgaard J, *et al.* Effect of long-term remote ischaemic conditioning on platelet function and fibrinolysis in patients with chronic ischaemic heart failure. *Thromb Res* 2017; **153**:40–46.

17 Krag AE, Kiil BJ, Hvas CL, Hvas A-M. Effect of remote ischemic preconditioning on hemostasis and fibrinolysis in head and neck cancer surgery: a randomized controlled trial. *PLoS One* 2019; **14**:e0219496.

18 Lanza GA, Stazi A, Villano A, Torrini F, Milo M, Laurito M, *et al.* Effect of remote ischemic preconditioning on platelet activation induced by coronary procedures. *Am J Cardiol* 2016; **117**:359–365.

19 Krag AE, Hvas A-M. Ischemic conditioning as a hemostatic intervention in surgery and cardiac procedures: a systematic review. *Semin Thromb Hemost* 2017; **43**:716–731.

20 Meng R, Ding Y, Asmaro K, Brogan D, Meng L, Sui M, *et al.* Ischemic conditioning is safe and effective for octo- and nonagenarians in stroke prevention and treatment. *Neurotherapeutics* 2015; **12**:667–677.

21 D'Angelo A, Kluft C, Verheijen JH, Rijken DC, Mozzie E, Mannucci PM. Fibrinolytic shut-down after surgery: impairment of the balance between tissue-type plasminogen activator and its specific inhibitor. *Eur J Clin Invest* 1985; **15**:308–312.

22 Eriksson BI, Eriksson E, Gyzander E, Teger-nilsson AC, Risberg B. Thrombosis after hip replacement: relationship to the fibrinolytic system. *Acta Orthop* 1989; **60**:159–163.

23 Aillaud M, Juhan-Vague I, Alessi M, Marecal M, Vinson M, Arnaud C, *et al.* Increased PA-inhibitor levels in the postoperative period–no cause-effect relation with increased cortisol. *Thromb Haemost* 1985; **2**:466–468.

24 Undas A, Ariëns RAS. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. Arterioscler Thromb Vasc Biol 2011. http://ahajournals.org [Accessed 5 January 2021].

25 Al Dieri R, de Laat B, Hemker HC. Thrombin generation: what have we learned? *Blood Rev* 2012; **26**:197–203.

26 Sidelmann JJ, Gram J, Jespersen J, Kluft C. Fibrin clot formation and lysis: basic mechanisms. *Semin Thromb Hemost* 2000; **26**:605–618.

27 Lim HY, O'Malley C, Donnan G, Nandurkar H, Ho P. A review of global coagulation assays: is there a role in thrombosis risk prediction? *Thromb Res* 2019; **179**:45–55.

28 Lisman T. Decreased plasma fibrinolytic potential as a risk for venous and arterial thrombosis. *Semin Thromb Hemost* 2017; **43**:178–184.

29 Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *Int J Surg* 2011; **9**:672–677.

30 CLSI Standard GP41. Wayne PA. Collection of diagnostic venous blood specimens. PA, USA: Clinical and Laboratory Standards Institute; 2017.

31 Gram AS, Bladbjerg EM, Skov J, Ploug T, Sjödin A, Rosenkilde M, *et al.* Three months of strictly controlled daily endurance exercise reduces thrombin generation and fibrinolytic risk markers in younger moderately overweight men. *Eur J Appl Physiol* 2015; **115**:1331–1338.

32 Sjøland JA, Sidelmann JJ, Brabrand M, Pedersen RS, Pedersen JH, Esbensen K, *et al.* Fibrin clot structure in patients with end-stage renal disease. *Thromb Haemost* 2007; **98**:339–345.

33 Carr ME, Gabriel DA. The effect of dextran 70 on the structure of plasma-derived fibrin gels. *J Lab Clin Med* 1980; **96**:985–993.

34 Carr ME, Hermans J. Size and density of fibrin fibers from turbidity. *Macromolecules* 1978; **11**:46–50.

35 RStudioTeam. *RStudio: integrated development environment for R.* RStudio, Inc.; 2019.

36 Sidelmann JJ, Gram F B, Godtfredsen A C M, Thorn F J. Orthognathic surgery-induced fibrinolytic shutdown is amplified by tranexamic acid. *J Oral Maxillofac Surg* 2020; **78**:1183–1189.

37 Su EP, Mount LE, Nocon AA, Sculco TP, Go G, Sharrock NE. Changes in markers of thrombin generation and interleukin-6 during unicondylar knee and total knee arthroplasty. *J Arthroplasty* 2018; **33**:684–687.

38 Dexter SPL, Griffith JP, Grant PJ, McMahon MJ. Activation of coagulation and fibrinolysis in open and laparoscopic cholecystectomy. *Surg Endosc* 1996; **10**:1069–1074.

39 Bai F, Feng S, Xu C, Xu Z, Chen J, Zheng Y. Transurethral resection versus holmium laser enucleation of the prostate: a prospective randomized trial comparing perioperative thrombin generation and fibrinolysis. *Medicine* 2019; **98**:e15223.

40 Wolberg AS. Thrombin generation and fibrin clot structure. *Blood Rev* 2007; **21**:131–142.

41 Horne MK, Merryman PK, Cullinane AM, Nghiem K, Alexander HR. The impact of major surgery on blood coagulation factors and thrombin generation. *Am J Hematol* 2007; **82**:815–820.

42 Van Poucke S, Huskens D, Van der Speeten K, Roest M, Lauwereins B, Zheng MH, *et al.* Thrombin generation and platelet activation in cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy: a prospective cohort study. *PLoS One* 2018; **13**:e0193657.

43 Kluft C, Verheijen JH, Jie AFH, Rijken DC, Preston FE, Sue-Ling HM, *et al.* The postoperative fibrinolytic shutdown: a rapidly reverting acute phase pattern for the fast-acting inhibitor of tissue-type plasminogen activator after trauma. *Scand J Clin Lab Invest* 1985; **45**:605–610.

44 Kristiansen J, Grove EL, Rise N, Neergaard-Petersen S, Würtz M, Kristensen SD, *et al.* Effect of remote ischaemic conditioning on coagulation and fibrinolysis. *Thromb Res* 2016; **141**:129–135.

45 Jones H, Hopkins N, Bailey T, Green D, Cable N, Thijssen D. Seven-day remote ischemic preconditioning improves local and systemic endothelial function and microcirculation in healthy humans. *Am J Hypertens* 2014; **27**:918–925.

46 Zhao W, Meng R, Ma C, Hou B, Jiao L, Zhu F, *et al.* Safety and efficacy of remote ischemic preconditioning in patients with severe carotid artery stenosis before carotid artery stenting. *Circulation* 2017; **135**:1325–1335.

47 Tosun M, Can MG, Aksu U, Emiroglu R, Toraman F. Remote organ ischaemic preconditioning in human recipients suppresses systemic inflammation and prevents glycocalyx degradation in living-donor liver transplantation A. *Eur J Anaesthesiol* 2021; **38**:667–669.