# Studying macro- and microvascular responses to major surgery to develop preventive strategies for perioperative complications

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# Chapter 4

## Plasma from patients undergoing coronary artery bypass graft surgery does not activate endothelial cells under shear stress *in vitro*

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**Abstract**

Background: Cardiac surgery with cardiopulmonary bypass is commonly associated with acute kidney injury and microvascular endothelial inflammation is a potential underlying mechanism. We hypothesized that pro-inflammatory components of plasma from patients who underwent CABG surgery with CPB induce endothelial adhesion molecule expression when incorporating altered shear stress in the *in vitro* model.

Methods: Clinical characteristics and markers of systemic inflammation and kidney injury were analysed pre- and postoperatively in 29 patients undergoing coronary artery bypass grafting with cardiopulmonary bypass. The effects of tumour necrosis factor (TNF)-a and patient plasma on the expression of endothelial inflammation and adhesion markers were analysed *in vitro*.

Results: Plasma TNF-α was elevated 6 h post-operation (median: 7.3 pg/ml (range: 2.5 – 94.8 pg/ml)). Neutrophil gelatinase-associated lipocalin in plasma peaked 6 h (99.8 ng/ml (52.6 – 359.1 ng/ml)) and in urine 24 h post-operation (1.6 ng/mg (0.2 – 6.4 ng/mg)). Urinary kidney injury molecule-1 concentration peaked 24 h post-operation (0.5 ng/mg (0.2 – 1.2 ng/mg). *In vitro*, expression of E-selectin was induced by 20 pg/ml TNF-α. In addition, expression of interleukin-8, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 was induced by 100 pg/ml TNF-α. Compared to healthy control plasma exposure, postoperative plasma did not increase the expression of markers of endothelial inflammation and adhesion under shear stress *in vitro*.

Conclusion: Patients undergoing cardiopulmonary bypass surgery showed mild systemic inflammation and kidney injury. However, plasma components did not stimulate endothelial inflammation and adhesion molecule expression *in vitro*.

## 1 Introduction

Cardiopulmonary bypass (CPB) allows for coronary artery bypass graft (CABG) surgery on the non-beating heart. The use of CPB is associated with systemic inflammation, a prevailing response to major surgery (1,2). During CPB, systemic inflammation is induced due to contact between blood components and the surface of the extracorporeal circuit, changes in shear stress, haemodilution and ischemia of the heart and other organs (3). Systemic inflammation is associated with postoperative organ failure, which results in reduced benefits for the patient after cardiac surgery (2). Postoperative organ failure can affect all organs and is associated with increased mortality, particularly when multiple organs are affected (4,5). Following CABG surgery, acute kidney injury (AKI) has an incidence of up to 30 % (6). AKI is defined as an abrupt loss of kidney function measured by changes in urine output and serum creatinine concentrations (7). The integration of kidney injury markers, such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule (KIM)-1 into the risk stratification of patients has assisted in the early diagnosis of patients suspected of AKI following cardiac surgery (8). Moreover, work by Lassnigg et al. (2008) and Haase et al. (2011) strongly suggested that even minor kidney injury, though not recognized by AKI classification criteria, is also linked to increased morbidity in cardiac surgery patients (9,10). Therefore, it is important to identify mechanisms initiating and maintaining kidney injury and to develop preventive and therapeutic approaches for patients that develop AKI after undergoing CABG surgery with CPB.

Pro-inflammatory activation of the microvascular endothelium has been observed in AKI in animal models of cardiac surgery using CPB and is likely involved in the initial adverse renal response to CPB in patients (1,11). Endothelial pro-inflammatory activation involves the orchestrated expression of the adhesion molecules E-selectin, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1. Leukocytes express complementary sialyl-lewis$^X$, integrins and other ligands and adhere to the activated endothelium. Consequently, leukocytes infiltrate the underlying tissue whereby inflammation is maintained and propagated. The activation of the endothelium can be triggered by alterations in shear stress as observed during shock and subsequent reperfusion (12). Contrary to laminar flow, altered shear stress renders the endothelium susceptible to pro-inflammatory plasma components such as lipopolysaccharide and tumor necrosis factor (TNF)-$\alpha$, which subsequently evoke an inflammatory response. Endothelial interactions with cells circulating in the blood,

which are activated by the use of CPB, are another potential source for endothelial activation. However, in the perioperative context, it remains unclear which of these factors induce the pro-inflammatory response of endothelial cells in specific organ injury (13,14).

In the present study using existing patient material, we hypothesized that pro-inflammatory components of plasma from patients who underwent CABG surgery with CPB induce endothelial adhesion molecule expression when incorporating altered shear stress in the *in vitro* model.

## 2 Methods

### 2.1 Patients

Twenty-nine patients who underwent CABG surgery with CPB were selected for this study. These patients were the CPB subgroup of a prospective randomized controlled trial comparing the outcome of CABG surgery with or without CPB (15,16). CABG with non-pulsatile CPB was used. Blood and urine samples were obtained at the arrival of the patient in the operating room ("pre-op"), at 6 h ("6 h post-operation (6 h post-op)") and 24 h post-operation ("24 h post-op"). Arterial blood samples were collected in ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1,000 rpm for 10 minutes at 4°C (Thermo Fisher Scientific, Waltham, MA, USA). The plasma was aliquoted and stored at -80°C for further analysis. The original study protocol was approved by the local Medical Ethical Committee and written consent was given by each patient. The study is registered on ClinicalTrials.gov (NCT01347827).

### 2.2 TNF-α quantification in plasma

Concentrations of TNF-α were measured in plasma using a commercially available multiplex immunoassay (human CVD panel 3, EMD Millipore, Corporation, Billerica, MA, USA) and were previously published for both cohorts with CABG surgery, with or without CPB (16).

### 2.3 Renal injury marker measurement in plasma and urine

Concentrations of the renal injury markers neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule (KIM)-1 were measured in plasma (NGAL) and urine (NGAL and KIM-1) samples by ELISA (Quantikine ELISA kits DLCN20,

DKM100, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. NGAL and KIM-1 concentrations were corrected for urine creatinine concentration (ng/ml per mg creatinine), assessed with the Creatinine Parameter Assay Kit (KGE005, R&D Systems).

### 2.4 Human umbilical vein endothelial cell (HUVEC) culture

HUVEC (#C2519A, Lonza, Breda, The Netherlands) were cultured in EGM-2 BulletKit Medium (CC-3202, Lonza), in a humidified incubator at 37°C, in 5% CO2. In all experiments, confluent HUVEC were used at passage 5.

### 2.5 *In vitro* HUVEC stimulation with TNF-α

To determine the effect of TNF-α on the pro-inflammatory response of endothelial cells, HUVEC were cultured under static conditions in 12-well plates (Corning, Amsterdam, The Netherlands) and starved in fetal calf serum (FCS)-free EGM-2 BulletKit medium for 1 h. As control plasma, pooled plasma from five healthy humans was purchased from Innovative Research (IPLAK2E, Le-Perray-en-Yvelines, France). HUVEC were exposed to FCS-free medium containing 20% control plasma, 1 IU/ml heparin (Leo Pharma, Amsterdam, The Netherlands) and TNF-α (0.25 to 100 pg/ml, Boehringer Ingelheim, Ingelheim am Rhein, Germany) for 3 h. Thereafter, the medium was replaced by EGM-2 BulletKit medium for 1 h. The TNF-α concentrations used *in vitro* are comparable to concentration ranges reported for patient plasma during and after CABG surgery with CPB (16).



### 2.6 Experimental set-up to study HUVEC exposure to patient plasma and shear stress

An *in vitro* model was used to analyse the effects of combined exposure to plasma and shear stress on HUVEC behaviour (**Figure 1**). µ-Slides I0.4 Luer (80176, ibidi, Gräfelfing, Germany) were used as previously described (17). Confluent monolayers of HUVEC were starved for 1 h in FCS-free medium and, subsequently incubated with FCS-free medium containing 20% control or patient plasma and 1 IU/ml heparin for 3 h. Thereafter, cells were exposed to 20 dyn/cm2 shear stress for 1 h, to mimic the change of shear stress inherent to the CABG surgery with CPB procedure.

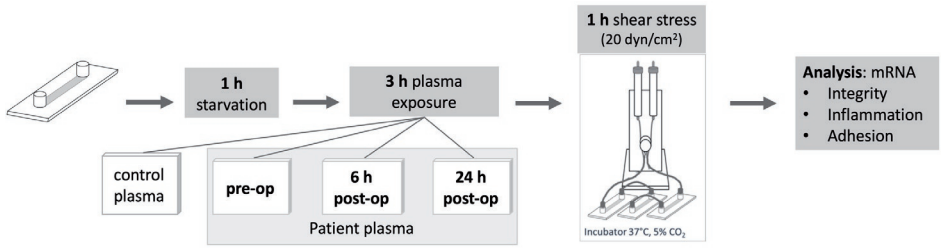


**Figure 1. Experimental set-up to analyse the effects of perioperative patient plasma on endothelial cells.** Cells were exposed to FCS-free medium for 1 h, and subsequently to plasma (20 % plasma in FCS-free medium) from three perioperative time points (pre-op, 6 h and 24 h post-op) for 3 h. Thereafter, cells were exposed to shear stress (20 dyn/cm2) for 1 h and analysed for mRNA expression indicative of changes in endothelial integrity (housekeeping), inflammation, and adhesion behaviour.

### 2.7 Gene expression analysis by quantitative reverse transcriptase-PCR

Cells were harvested in TRIzol™ Reagent (Thermo Fisher Scientific) and total RNA was isolated according to the manufacturer's instructions. RNA concentrations (OD260) were measured by NanoDrop® ND-1000 US-Vis spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). cDNA synthesis was performed using random hexamer primers (Promega, Leiden, The Netherlands) and SuperScript III (Invitrogen, Breda, The Netherlands). The assay-on-demand primers/probe sets E-selectin Hs00174057_m1, GAPDH Hs99999905_m1, ICAM-1 Hs00164932_m1, IL-6 Hs00174131_m1, IL-8 Hs00174103_m1, platelet endothelial cell adhesion molecule (PECAM)-1 Hs00169777_m1, VCAM-1 Hs00365485_m1 and vascular endothelial (VE)-cadherin Hs00174344_m1 (TaqMan® Gene Expression, Thermo Fisher Scientific) were used. Quantitative reverse transcriptase-PCR was carried out on the ViiATM 7 real-time PCR System (Thermo Fisher Scientific) with the following settings: 15 min 95°C, followed by 40 two-step cycles of 15 seconds at 95°C, and 60 seconds at 60°C. Average cycle threshold values (CT) were obtained for sample duplicates and normalized to the housekeeping gene GAPDH. The expression relative to GAPDH was calculated by $2^{-\Delta CT}$. Fold change in expression was calculated as quotient of $2^{-\Delta CT}$ value of the gene of interest and the $2^{-\Delta CT}$ value of the control plasma.

### 2.8 Statistical analyses

For experiments incorporating patient-derived material non-parametric testing was applied, therefore, whisker-box plots were used with whiskers from 10 to 90

percentiles and individual points represent values below and above the cut-offs. Due to lacking data points, the data were analyzed with the Skillings-Mack test by using the R package "Skillings.Mack: The Skillings-Mack Test Statistic for Block Designs with Missing Observations" by Patchanok Srisuradetchai (18). Using the Durbin test, post-hoc comparisons were done with a Bonferroni correction. Data of TNF-α stimulated endothelial cells were analysed by one-way analysis of variance with a Šidák correction. Appropriate post-hoc comparisons were done and reported only when the p-values of the statistical tests were significant. Differences were regarded as significant when $p < 0.05$. Graph plotting and one-way analysis of variance with a Šidák correction analyses were performed using GraphPad Prism software 8.2.1 (GraphPad Prism Software Inc., San Diego, California, USA).



## 3 Results

### 3.1 Patient characteristics

The average age of the 29 patients was 63 years (range 47-77, **Table 1**). Ninety percent were male, which is in line with the fact that more males than females undergo CABG surgery (19). The total procedure time was 188 ± 31 minutes and the mean CPB time was 82 ± 23 minutes, with average aortic cross-clamp times of 53 ± 15 minutes. As indicated by the preoperative European System for Cardiac Operative Risk Evaluation (EuroSCORE) I of 2.3 % and the post-hoc calculated EuroSCORE II of 1.1 %, this cohort had a low preoperative risk of postoperative mortality after cardiac surgery which corresponded to no observed patient mortality within 30 days post-op (11,20).

**Table 1. Perioperative patient characteristics**

| Preoperative characteristics | units | numbers |
|---|---|---|
| Male/female | n | 26/3 |
| Age (yrs.) | Mean (range) | 63 (47-77) |
| Body mass index (kg/m$^2$) | Mean ± SD | 28.1 ± 4.0 |
| Smoking | n (%) | 19 (66) |
| European System for Cardiac Operative Risk Evaluation I | Mean ± SD | 2.3 ± 1.7 |
| Post-hoc calculated European System for Cardiac Operative Risk Evaluation II | Mean ± SD | 1.1 ± 0.6 |
| *Medical and surgical history* | | |
| Cardiovascular disease | | |
| Coronary disease | n (%) | 29 (100) |
| Hypertension | n (%) | 10 (35) |
| Percutaneous transluminal angioplasty | n (%) | 2 (7) |
| Myocardial infarct | n (%) | 0 |
| Pericarditis/myocarditis | n (%) | 0 |
| Venous thrombosis | n (%) | 1 (3.4) |
| Respiratory disease | | |
| Chronic obstructive pulmonary disease | n (%) | 2 (6.9) |
| Emphysema | n (%) | 1 (3.4) |
| Pneumonia | n (%) | 1 (3.4) |
| Left ventricular ejection fraction | | |
| < 40% | n (%) | 2 (7) |
| 40-55% | n (%) | 10 (35) |
| 55-70% | n (%) | 17 (59) |
| Haemoglobin (mmol/L) | Mean ± SD | 9.1 ± 0.8 |
| **Operative characteristics** | | |
| Cardiopulmonary bypass time (min) | Mean ± SD | 82 ± 23 |
| Aortic cross-clamp time (min) | Mean ± SD | 53 ± 15 |
| Total operation time (min) | Mean ± SD | 188 ± 31 |
| Grafts (n) | Median (range) | 3 (2 - 5) |
| Haemodilution factor | Mean ± SD | 0.7 ± 0.1 |
| **Postoperative characteristics** | | |
| Haemoglobin (mmol/L) | Mean ± SD | 6.2 ± 1.1 |
| Intensive care unit length of stay (days) | Median (range) | 1 (0.5 – 12) |
| Hospital length of stay (days) | Median (range) | 9 (3 – 29) |
| Reoperation | n (%) | 3 (10) |

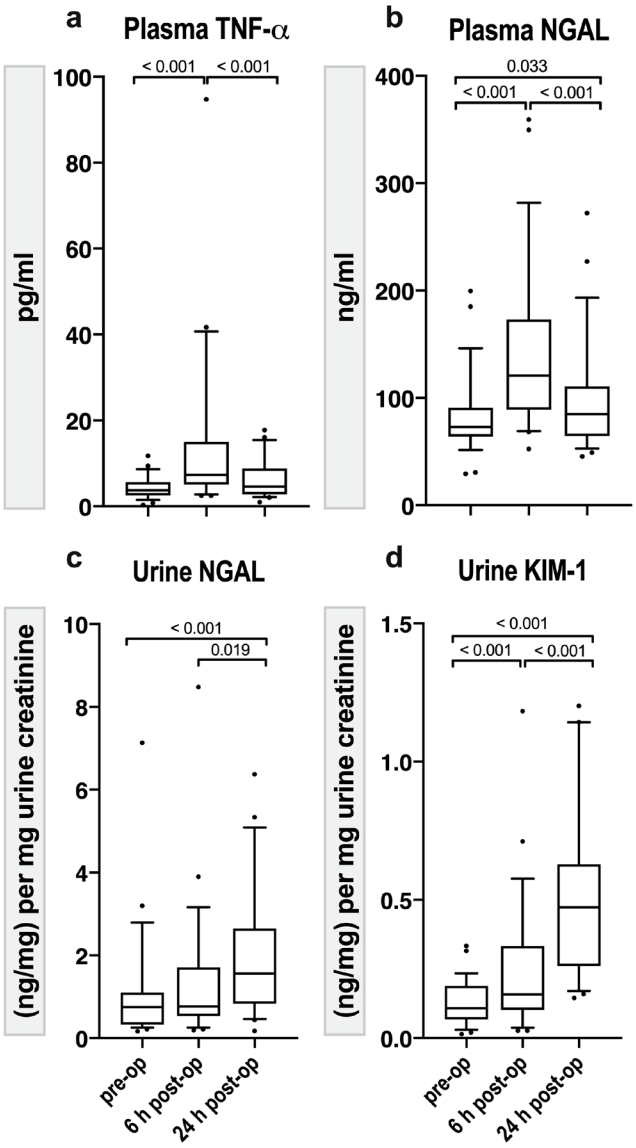


**Figure 2. Markers of systemic inflammation and renal injury in plasma and urine from patients undergoing CABG surgery with the use of CPB.** Plasma and urine were obtained at three perioperative time points. Measurements of TNF-α in plasma (a), NGAL in plasma (b) and in urine (c), and KIM-1 in urine (d) are depicted (n=29). Urine marker measurements were corrected for urine creatinine levels. The whisker-box plots are shown with 10th to 90th percentile and points represent individual values below and above the 10th to 90th percentile cut-offs. Post-hoc comparison p-values are shown in the graph.

### 3.2 Pro-inflammatory and renal injury markers

A temporary increase of plasma TNF-α 6 h post-op was observed (**Figure 2 a**). The median plasma TNF-α concentrations were 3.7 pg/ml (range, 0.3 – 11.7 pg/ml) pre-op, 7.3 pg/ml (2.5 – 94.8 pg/ml) at 6 hours (h), and 4.6 pg/ml (1.0 – 17.7 pg/ml) at 24 h post-op. At 6 h post-op, concentrations were higher than pre-op ($p < 0.001$) and 24 h post-op ($p < 0.001$).

The median plasma NGAL concentrations were 72.8 ng/ml (range 29.3 – 199.6 ng/ml) pre-op, 120.8 ng/ml (52.6 – 359.1 ng/ml) at 6 h, and 99.8 ng/ml (45.4 – 272.1 ng/ml) at 24 h post-op (**Figure 2 b**). The median plasma NGAL concentrations were increased postoperatively. All time points were significantly different from each other, $p = 0.033$ for start vs 24 h post-op and the others $p < 0.001$. The median urine NGAL concentrations were 0.8 ng/mg (range, 0.2 – 7.1 ng/mg) pre-op, 0.8 ng/mg (0.2 – 8.5 ng/mg) at 6 h and 1.6 ng/mg (0.2 – 6.4 ng/mg) at 24 h post-op (**Figure 2 c**). At 24 h post-op, concentrations were higher than pre-op ($p = 0.002$) and 6 h post-op ($p = 0.019$). The median urine KIM-1 concentrations were 0.1 ng/mg (range, 0.1 – 0.3 ng/mg) pre-op, 0.3 ng/mg (0.1 – 1.2 ng/mg) at 6 h, and 0.5 ng/mg (0.2 – 1.2 ng/mg) at 24 h post-op (**Figure 2 d**). The median KIM-1 concentrations at all time points were significantly different ($p < 0.001$) from each other. Collectively, these data show postoperatively elevated marker concentrations of inflammation and kidney damage.

### 3.3 *In vitro* effect of low TNF-α concentrations on endothelial inflammatory response

HUVEC were exposed to varying concentrations of TNF-α *in vitro*. The concentrations of TNF-α used to stimulate the HUVEC were based on the patient cohort's range of plasma TNF-α (0.3 to 94.8 pg/ml) (**Figure 3**). In response to TNF-α exposure, mRNA expression of the (housekeeping) endothelial integrity markers VE-cadherin and PECAM-1 did not change, except for an upregulation seen for PECAM-1 mRNA at 5 pg/ml TNF-α ($p = 0.009$). The pro-inflammatory cytokine IL-6 expression varied with TNF-α, but was not significantly different from the unstimulated condition. A TNF-α concentration-dependent increase in mRNA expression was seen for the chemokine IL-8 and the endothelial adhesion molecules E-selectin, ICAM-1 and VCAM-1. Compared to the unstimulated control, mRNA expression of E-selectin at 20 pg/ml TNF-α ($p = 0.002$) and of IL-8, E-selectin, ICAM-1 and VCAM-1 at 100 pg/ml TNF-α ($p \leq 0.001$) were upregulated compared to unstimulated control.

Concentrations of TNF-α used in this experiment were comparable to TNF-α concentrations in patient plasma and were sufficient to activate HUVEC *in vitro* in the experimental set-up employed.

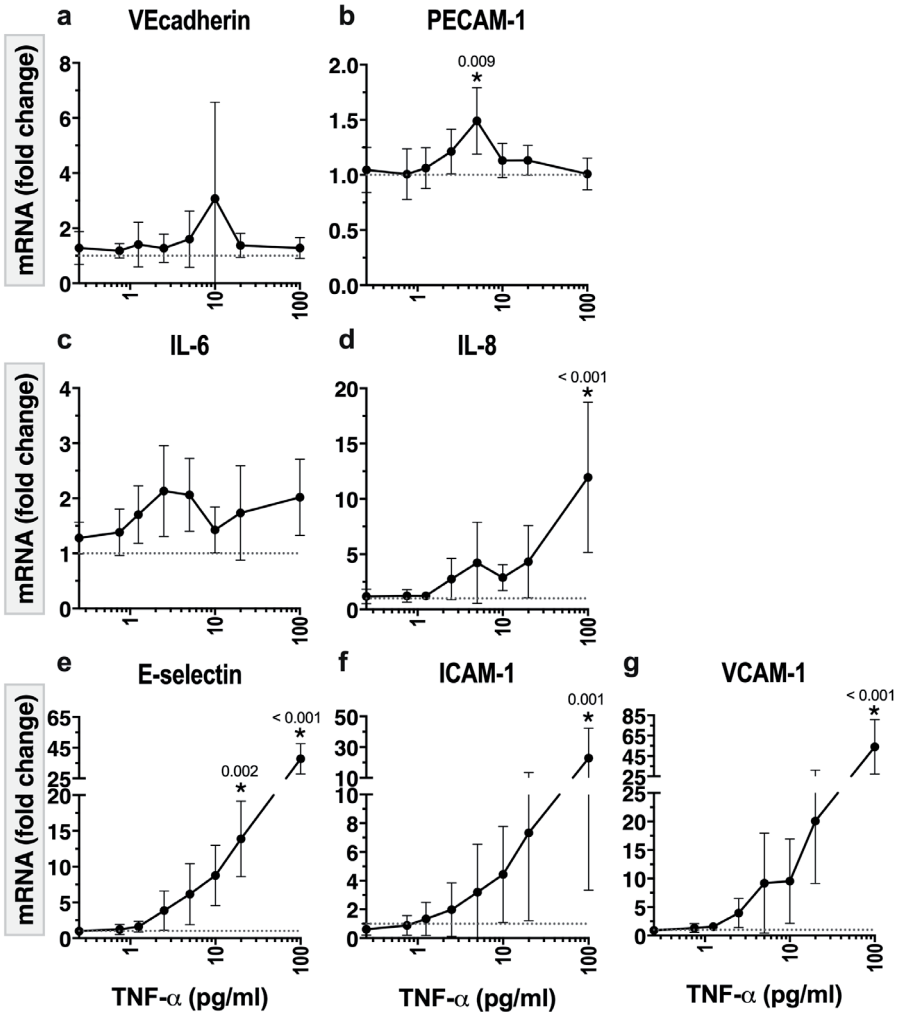


**Figure 3. Changes in inflammation and adhesion of HUVEC in response to TNF-α.** Under static conditions, HUVEC were stimulated with TNF-α (0.25 to 100 pg/ml) for 3 h. mRNA fold-changes compared to the untreated control (dotted line at 1) of VE-cadherin (a) and PECAM-1 (b), IL-6 (c) and IL-8 (d), E-selectin (e), ICAM-1 (f), and VCAM-1 (g) are shown. Data are depicted as the mean of four biological replicates ± SD. * control vs TNF-α stimulated. Post-hoc comparison p-values are shown in the graph.

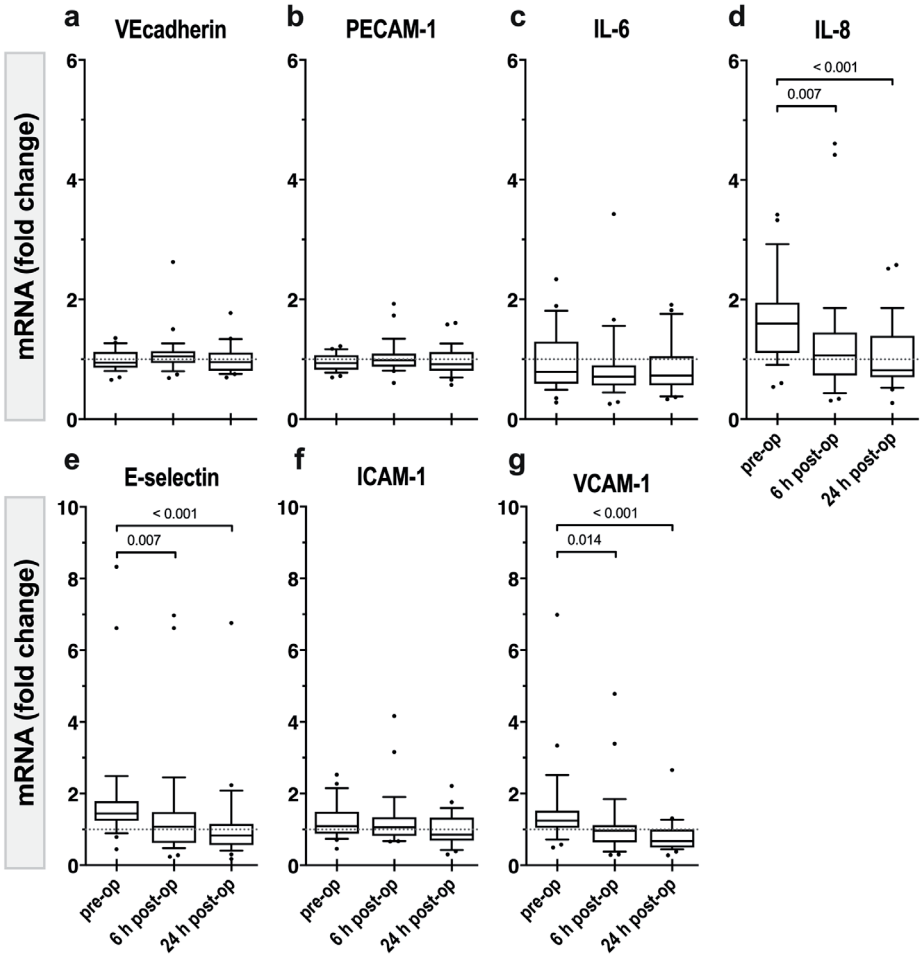


**Figure 4. Effect of patient plasma and shear stress on inflammation and adhesion markers of HUVEC.** mRNA expression of VE-cadherin (a) and PECAM-1 (b), IL-6 (c), IL-8 (d), E-selectin (e), ICAM-1 (f), and VCAM-1 (g) are shown as fold changes normalized to the healthy plasma control (dotted line at 1). The whisker-box plots are shown with 10th to 90th percentile and points represent individual values below and above the 10th to 90th percentile cut-offs. Post-hoc comparison p-values are shown in the graph.

### 3.4 Effect of patient plasma followed by altered shear stress on endothelial behaviour *in vitro*

We next studied the effect CABG plasma has on HUVEC expression of endothelial integrity (housekeeping), endothelial pro-inflammatory cytokines, and endothelial adhesion molecules in combination with shear stress (**Figure 4**). Expressions of the

integrity markers VE-cadherin and PECAM-1, or the cytokine IL-6 and the adhesion molecule ICAM-1 were not observed. Pre-op plasma induced a 1.5- to 2-fold increase in the mRNA expressions of IL-8 (median 1.6, range 0.5 – 3.4), E-selectin (1.4, 0.4 – 8.3), and VCAM-1 (1.3, 0.5 – 7.0) compared to control plasma. Patients' plasma obtained 6 h post-op resulted in lower mRNA expression of IL-8 (median 1.1, range 0.3 – 4.6; $p = 0.007$), E-selectin (1.1, 0.2 – 7.0; $p = 0.007$), and VCAM-1 (1.0, 0.3 – 4.8; $p = 0.014$) than pre-op plasma but similar to control plasma. Patients' plasma obtained 24 h post-op resulted in lower mRNA expression of IL-8 (median 0.8, range 0.3 – 2.6; $p < 0.001$), E-selectin (0.8, 0.2 – 6.8; $p < 0.001$), and VCAM-1 (0.7, 0.3 – 2.7; $p < 0.001$) than pre-op plasma. Collectively, these data indicate that plasma from CABG-CPB patients obtained at 6 h and 24 h post-op in combination with shear stress did not induce an increased endothelial adhesion molecule expression in HUVEC.



## 4 Discussion

Cardiac surgery with CPB is commonly associated with AKI and microvascular endothelial inflammation is a potential underlying mechanism (21–23). We hypothesized that pro-inflammatory components of plasma from patients who underwent CABG surgery with CPB induce endothelial adhesion molecule expression when incorporating altered shear stress in the *in vitro* model. Although the postoperative patient plasma contained elevated concentrations of markers of inflammation and kidney injury, pro-inflammatory endothelial activation, represented by induction of IL-6, IL-8, E-selectin, ICAM-1, and VCAM-1, was not observed following the exposure of endothelial cells to postoperative plasma and altered shear stress.

In a previous analysis of this patient cohort, the pro-inflammatory cytokines TNF-α and IL-6, as well as the anti-inflammatory cytokine interleukin-10 and myeloperoxidase temporarily increased in plasma postoperatively, while endothelial-derived soluble adhesion molecules E-selectin, VCAM-1, and ICAM-1 did not (16). This observation is partially not in line with previous reports that described a postoperative rise in both cytokines and soluble adhesion molecule markers (24,25). This discrepancy indicated that the microvasculature in patients with a low preoperative risk of postoperative mortality undergoing CABG surgery with CPB might not present with a pronounced pro-inflammatory phenotype. This was supported by the fact that only a small number of patients (5/29) had plasma TNF-α concentrations exceeding 20 pg/ml at a single time point, namely, at 6 h post-op. In patients undergoing CABG surgery with CPB,

TNF-α concentrations above 20 pg/ml were associated with worse outcome compared to lower concentrations (26). We demonstrated that TNF-α in concentrations similar to those observed in our cohort have caused a concentration-dependent increase in endothelial adhesion molecules under static conditions *in vitro*. Contrary to our expectations, the presence of cytokines such as TNF-α in patient plasma did not suffice to activate endothelial cells under shear stress *in vitro*, which is in line with previous findings under static conditions (27). Interestingly, out of all time points pre-op plasma induced the highest expression of IL-8, E-selectin and VCAM-1. This was unexpected as the pro-inflammatory cytokines measured in plasma where lower than those at 6 h post-op. We can only speculate whether pre-op plasma contained unidentified pro-inflammatory mediators or whether post-op plasma contained unidentified anti-inflammatory mediators that balanced out the measured pro-inflammatory marker concentrations. Furthermore, reasons for the absence of postoperatively increased adhesion molecules in response to plasma and the only temporary and moderate rise in cytokines may be related to advances in the anaesthetic and surgical field. Examples of these advances are improved biocompatibility of extracorporeal circuits and hemodynamic management, as well as blood ultrafiltration for cytokine removal and shorter durations of CPB (28,29). Other reasons may be that the presence of anti-inflammatory mediators such as interleukin-10 in plasma, which also peaked at 6 h post-op, may have counterbalanced a pro-inflammatory endothelial response (16). This hypothesis can be studied in future studies using blocking antibodies against IL-10 and other plasma cytokines to see whether an anti-pro inflammatory rebalancing takes place during cardiac surgery. Moreover, cellular whole blood components such as leukocytes may be accountable for postoperative pro-inflammatory endothelial responses (30).

The effect of CABG surgery with CPB on organs was investigated in terms of kidney injury markers, as AKI is a common CPB-associated complication (31). The patients did not fulfil classical “Kidney Disease: Improving Global Outcome” AKI criteria, but a rise of NGAL and KIM-1 suggested mild kidney injury at 6 h post-op, and markers remained elevated at 24 h post-op in urinary measurements. Both NGAL and KIM-1 are detectable in urine within 2 to 6 h after renal injury and originate from renal tubules (31). Additionally, NGAL has extra-renal sources such as various inflammatory cells. Furthermore, the systemically measured concentrations only give limited information about organ-specific expression and hence, it can only be speculated whether the

local renal microvasculature expresses more pronounced signs of pro-inflammatory endothelial response in CABG patients, as observed in rat models of cardiac surgery using CPB (11). An animal model, which mimics human cardiac surgery with CPB and provides perioperative plasma and organ biopsies, might give insight into these mechanisms in future research (32,33).

Based on our current findings, we cannot conclude that microvascular pro-inflammatory activation by plasma components is the mechanism underlying the observed postoperative increase in kidney injury markers NGAL and KIM-1 in plasma and urine. Alternatively, endothelial hyperpermeability facilitating microvascular leakage and systemic hypotension leading to ischemia/reperfusion injury are noxious stimuli that have been suggested to cause postoperative AKI (34–37).

This study has a number of limitations. First, the *in vitro* endothelial cells were exposed to 20% of the plasma that the patient's vasculature was in contact with, due to the experimental requirement to dilute plasma in cell culture medium. This limitation is inherent to an *in vitro* model, which is assessing only a fraction of factors and also excluded leukocyte-endothelial interactions and soluble factors of the complement and coagulation systems. Secondly, other pro-inflammatory plasma factors known to be released during CABG surgery such as the hypochlorous acid or thioredoxin-interacting protein were not studied. Third, the plasma samples collected at specific time points merely represent circulating plasmatic components at a particular time point, rather than the kinetics of plasma components throughout the perioperative time period. Only the inclusion of more time points or a large animal *in vivo* model will provide more information on events in the organ microvasculature during CABG with CPB. Moreover, there is a trend towards the use of pulsatile extracorporeal circulation in patients as this may preserve microcirculatory function better (38). Since our study was conducted with non-pulsatile CPB, the *in vitro* model also incorporated non-pulsatile shear stress. Further, more information on baseline health status and comorbidities would have given a better insight into the preoperative patient characteristics. However, this cohort had a low preoperative risk of postoperative mortality. Further studies may look at patients with a higher risk for perioperative complications, for which additional information on health status and comorbidities would be necessary.

## 5 Conclusion

CABG surgery with CPB patients with low preoperative risk of postoperative mortality postoperatively showed signs of mild systemic inflammation and kidney damage. Plasmatic components do not seem to be accountable for the underlying postoperative microvascular endothelial adhesion molecule expression.

### Research Quality and Ethics Statement

The authors of this manuscript declare that this scientific work complies with reporting quality, formatting and reproducibility guidelines set forth by the EQUATOR Network, notably the CONSORT 2010 Statement. The authors also attest that this clinical investigation was determined to require Institutional Ethics Committee review, which was provided by the Medical Ethical Committee of the University Medical Center Groningen (UMCG, The Netherlands; METc 2011/045). Finally, the authors have registered this clinical study with the following Clinical Trial Registry: ClinicalTrials.gov NCT01347827.

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### Author contributions

SFE, TWLS, GT and MvM contributed to conception, design of the study and interpretation of the data. SFE, RMJ, KN and CLS obtained, analysed, and curated data. SE and MvM performed the statistical analysis. SFE wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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