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The effect of preoperative training on endothelial dysfunction – a way to improve perioperative outcome?

Über den Effekt von präoperativem Training auf die endotheliale Dysfunktion – ein Weg, das perioperative Ergebnis zu verbessern?

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Für meine verstorbene Mutter,
Für unsere verstorbene Ömi,
Für meine Frau Corinna und unsere Tochter Frida,
Für meinen Vater und meine Geschwister

Content

Abbreviations

ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
ASA	American Society of Anesthesiologists
AT	Anerobic threshold
BART	Brachial artery reactivity testing
BOEC	Blood outgrowth endothelial cells
CEC	Circulating endothelial cells
CFC	Colony forming cells
CFU	Colony forming units
cLDL	Low-density lipoprotein cholesterol
CPET	Cardiopulmonary exercise testing
CRP	C-reactive protein
DTM	Digital thermal monitoring
EC	Endothelial cell
ECFC	Endothelial colony forming cells
ECG	Electrocardiogram
E.g.	Exempli gratia
EndoPAT	Endovascular peripheral arterial tonometry
EPC	Endothelial progenitor cells
Et al.	Et alii
FDA	Food and drug administration
FMD	Flow-mediated dilation
GCSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte macrophage colony-stimulating factor
ICU	Intensive care unit
I.e.	Id est
JNK	C-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
NO	Nitric oxide
NP	Nadir to peak
OEC	Late outgrowth endothelial cells
PHC	Proangiogenic hematopoietic cells
PSV	Peak systolic velocity
pVO_2	Peak oxygen uptake
SD	Standard deviation

TF	Temperature fall
Tmax	Maximum temperature
Tmax%	(Tmax/Ts)$*$100
Tmin	Minimum temperature
Tmin%	(Tmin/Ts)$*$100
TR	Temperature rebound (above the baseline)
Ts	Start temperature
TTF	Time to temperature fall
TTR	Time to temperature rebound
VATS	Video-assisted thoracic surgery
VEGF	Vascular endothelial growth factor

1. Introduction

1.1. Background

An increasing number of patients undergoing surgery is predisposed with multiplex preoperative risk factors including hypertension, diabetes, obesity, dyslipidemia and a proinflammatory state[1]. These factors are known to have a negative impact on endothelial function[2-4], especially the inflammatory cascade after major surgery that places the patient at risk for micro-[5] and macrovascular[6,7] related complications (i.e. cardiovascular, wound healing, pulmonary events) postoperatively. Further, this group of patients presents to the preoperative setting with subclinical endothelial dysfunction that may impair perioperative outcome. Without any symptoms or history of cardiac events, these patients oftentimes are classified as American Society of Anesthesiologists (ASA) Class 2 patients, underestimating the underlying risk for complications related to microvascular dysfunction (e.g. wound healing). Therefore, preoperative surrogate markers of endothelial dysfunction, such as BART[8], have gained importance in order to detect patients at risk for endothelial dysfunction-related complications and to adjust perioperative therapeutic strategies to these patients. In addition, preoperative therapeutic strategies as pharmacological treatment (i.e. with statins) or physical exercise training may improve patients' preoperative condition in order to optimize patients postoperative outcome. For example, mobilization of endothelial progenitor cells (EPC), improving their paracrine function, and homing of these cells into the endothelial layer are mechanisms that counteract perioperative endothelial damage or dysfunction. Patients with subclinical endothelial function undergoing major surgery may benefit from interventions like preoperative exercise by improving endothelial function and preventing endothelial-dependent vascular impairment.

1.2. Endothelial dysfunction

Endothelial dysfunction is recognized as one of the earliest events in the pathophysiological process that leads to atherosclerotic disorders[1]. Endothelial dysfunction refers to a condition in which the endothelium loses its physiological properties: the tendency to promote a vasodilatory, anti-inflammatory, and anti-thrombotic (anti-aggregation and anti-fibrinolysis) milieu. Endothelial cells secrete multiple mediators that promote this vasodilatory / anti-aggregation (nitric oxide, prostacyclin, carbon monoxide, endothelium-derived hyperpolarizing factor) or vasoconstriction / aggregation (endothelin-1, thromboxane-A2) milieu. The phenotypic expression of the endothelium can be seen as a dynamic 'set point' that ranges between a quiescent, an activated, or a dysfunctional state (Figure 1). This set point reflects on the balance between the underlying (chronic) health of the endothelium, acute exacerbating triggers such as inflammation and oxidative stress,

and the 'regenerative' ability of the bone marrow through the release of hematopoietic progenitors into the peripheral circulation[9,10]. Transient 'endothelial activation', with decreased bioavailability of homeostatic mediators such as nitric oxide, results in vasoconstriction, proinflammatory and prothrombotic changes to serve as an adaptive physiologic response to acute stressors. This is demonstrated by as much as 50% loss in endothelial vasodilator function within hours of ingesting a meal rich in saturated fat (described as a "Big Mac attack")[11]. The impact of diet on endothelial function is supported by a recent large prospective primary prevention outcome study reporting that, in people at high cardiovascular risk, a Mediterranean diet supplemented with olive oil or nuts associated with a 30% reduction in the incidence of major cardiovascular events when compared to a control diet (advice to reduce dietary fat)[12]. This is further illustrated in patients with type-2 diabetes mellitus, a disease characterized by endothelial dysfunction and impaired EPC mobilization[13], who have a two to four-fold increased risk of cardiovascular disease. As such, endothelial vasodilator dysfunction is a well-established and measurable surrogate that is used as a predictor of cardiovascular morbidity and mortality[14].

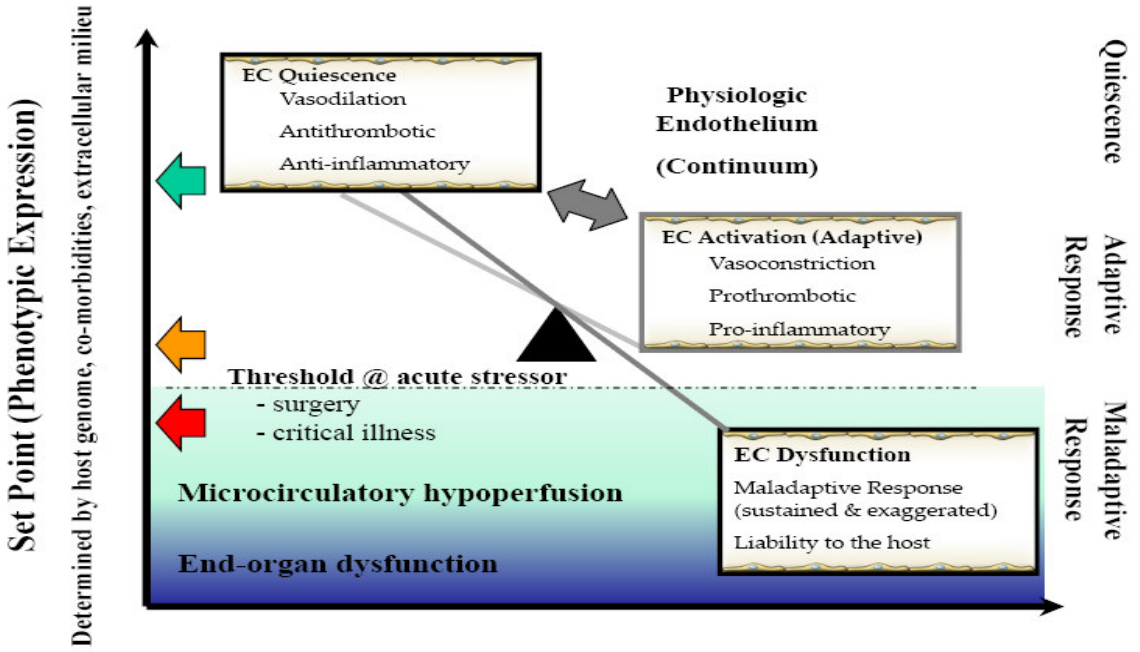


Figure 1. The phenotypic expression of the endothelium can be described as a dynamic 'set point' that ranges between a quiescent, activated or dysfunctional state. This reflects the balance between the underlying health of the endothelium, acute exacerbating triggers such as inflammation and oxidative stress, and the 'regenerative' ability of the bone marrow, which releases hematopoietic progenitors into the peripheral circulation. Inadequate basal endothelial function and/or a maladaptive host response to

pathophysiologic changes that accompany surgical stress may result in deterioration of the endothelial reserve below a critical 'physiologic threshold' that is required to sustain microvascular perfusion[15].

1.3. Pathophysiology of endothelial injury in the perioperative period

Chronic exposure to cardiovascular risk factors and an exacerbated or persistent maladaptive response to acute pathophysiologic stressors, mediated through an inflammatory/oxidant burden and/or the prothrombotic effect of endothelial microparticles, impairs basal nitric oxide bioavailability, a hallmark of endothelial dysfunction[16,17]. Endothelial microparticles are small vesicles that are released from endothelial cells. These vesicles have a membrane containing receptors and other cell surface molecules which enables identification of their endothelial origin[18]. Circulating endothelial microparticles have been identified in individuals with certain diseases, including hypertension and cardiovascular disorders[19]. In addition, endothelial microparticles have been shown to express an array of cell surface molecules that reflect an underlying state of endothelial dysfunction. Therefore, endothelial microparticles may provide a useful index of the underlying functional state of the endothelium in disease, and may potentially play a key role in the pathogenesis of cardiovascular diseases[20]. Furthermore, endothelial dysfunction is likely mediated through an apoptotic process, with inflammatory/oxidant stressors signaling the mitogen-activated protein kinase (MAPK) and the C-Jun N-terminal kinase (JNK) pathways to suppress endothelial-cadherin, and consequent activation of the caspase family leading to intimal denudation through apoptosis[13]. As such, the detachment of entire endothelial cells (circulating endothelial cells, CEC) or apoptotic endothelial microparticles can be characterized and measured.

The endothelium is extremely sensitive to inflammation[21], which occurs almost ubiquitously in the perioperative period. In human volunteers, a challenge with pro-inflammatory cytokines resulted in a transient loss of endothelial vasodilator function, with recovery occurring up to seven days after the inflammatory challenge[22]. Importantly, studies have shown reversibility to inflammation-induced endothelial dysfunction through source removal[23] or through anti-inflammatory strategies (hydrocortisone or high-dose aspirin)[22,24]. This is illustrated by significant improvement in endothelial vasodilator function six months after aggressive treatment of periodontitis (source removal). However, important to the perioperative period, this periodontal treatment resulted in an initial deterioration of endothelial-dependent vasodilator function 24 hours after treatment[25]. These studies demonstrate a temporal link between an acute systemic inflammatory load and acute deterioration of endothelial function. This link is supported by a study that undertook serial

measuring of endothelial function after surgery and found a reduction in endothelial function in the early postoperative period[26]. Of note, decline in endothelial function was greater after laparotomy when compared with laparoscopy, with recovery of endothelial function to baseline by the seventh postoperative day, suggesting that inflammatory load is reduced with less invasive surgery. Similarly, another study displayed a decline in endothelial function 24-48 hours after cardiopulmonary bypass for coronary artery bypass surgery[27].

These observations provide a plausible explanation for the peak incidence of myocardial infarction occurring in the postoperative period rather than intraoperatively, when flow stagnation and increased thrombogenicity prevails[21]; and for lower cardiovascular complication rates observed for laparoscopy compared with laparotomy[22]. This link between inflammation, endothelial dysfunction and vascular events is supported by the observation in population studies of a seasonal variation in myocardial infarction; with increased incidence in the winter months when inflammatory infections occur more frequently[23]. It is this combination of postsurgical inflammation, endothelial dysfunction, and a prothrombotic state that increases the risk of microvascular impairment and postoperative morbidity, and is likely to be more evident in patients with marginal preoperative (baseline) endothelial function[8,24].

1.4. Clinical implications of perioperative endothelial dysfunction

Based on the recommendations of the recent (2014) ESC / ESA Guidelines on non-cardiac surgery, there is a need to refine cardiovascular assessment and management. A lack of data on how non-cardiac risk factors (frailty, extreme low or high body mass index, anemia, immune status) interact with cardiovascular risk factors and how they impact on outcomes of non-cardiac surgery is still a current problem in a perioperative setting[28]. Furthermore, patients undergoing major non-cardiac surgery are at substantial risk for postoperative morbidity, with 30-60% of patients developing complications[29,30].

A recent cohort study conducted by the European Surgical Outcomes Study (EuSOS) group in 498 hospitals across 28 European nations was able to show that 4% of the patients undergoing non-cardiac surgery died before hospital discharge. Interestingly, 73% of the patients who died were not admitted to the Intensive Care Unit (ICU) and only 8% were admitted to the ICU after surgery[31]. Since endothelial dysfunction in the perioperative period contributes to the risk of postoperative complications via impaired vascular homeostasis, increased microvascular permeability and/or thrombogenicity, some of the patients not admitted to the ICU may have suffered subclinical endothelial dysfunction not detected by postoperative surveillance.

Further, in a perioperative setting inadequate basal (preoperative) endothelial function and/or a maladaptive host response to pathophysiologic changes accompanying surgical stress may result in deterioration of the endothelial reserve below a critical 'physiologic threshold' that is likely to be required to sustain microvascular perfusion (Figure 1). As such, the surgical proinflammatory and prooxidant milieu may result in both functional and structural alterations (including cleavage of the glycocalyx) of the endothelium, resulting in hemostatic dysregulation with impaired local tissue perfusion, and consequently micro- and macrovascular related postoperative complications (Figure 2)[32,33].

Methods that characterize the underlying endothelial reserve (e.g. assessment of functionality through endothelial-dependent vasodilation), that quantify the vascular insult (e.g. measuring endothelial, thrombogenic and/or inflammatory biomarkers, and levels of denuded circulating endothelial cells [CEC] or endothelial microparticles), or enumerate the functional regenerative capacity of circulating endothelial progenitor cells (EPC) play a growing role in our clinical armamentarium[34]. These tools are increasingly being explored as methods to improve preoperative risk stratification[15,32,35-37], improving our understanding of the pathophysiology of perioperative endothelial dysfunction[26], and our understanding of potential therapeutic strategies[38]. As such, endothelial vasodilator dysfunction has been shown to be predictive of short- and long-term postoperative cardiovascular events in patients undergoing vascular surgery[32].

Preoperative assessment of endothelial function and reserve will likely be particularly useful for the refining of risk stratification in the increasing number of patients who present for major non-cardiac surgery and have multiple preoperative risk factors, including hypertension, diabetes, obesity, dyslipidemia (metabolic syndrome), yet are without overt symptoms or history of cardiovascular events. These patients are often classified as American Society of Anesthesiologists (ASA) Physical Class 2, thereby underestimating the propensity toward complications. In a large, retrospective analysis of patients with metabolic syndrome, patients with diabetes, hypertension or obesity who underwent major joint replacement surgery were at greater risk of postoperative complications[39]. This increased risk for postoperative morbidity may be partly attributed to underlying endothelial dysfunction, as supported by the fact that patients with the metabolic syndrome have impaired endothelial function and decreased circulating EPC levels[13]. Similarly, endothelial damage and microcirculatory impairment are early pathogenic events in the end-organ damage (cardiomyopathy, nephropathy, retinopathy, and neuropathy) associated with diabetes, likely mediated through impaired mobilization, proliferation, survival and homing of EPC resulting in reduced capillary density, increased fibrosis, and impaired end-organ function[13].

Apart from a diagnostic and prognostic role, the mature endothelium and increasingly the hematopoietic progenitor cells (e.g. EPC) also provide an attractive therapeutic target to stimulate angiogenesis, vasculogenesis and overall endothelial function[40]. Improved risk stratification and the opportunity to modulate the endothelial-thrombotic-inflammatory cascades will likely lead to improved perioperative outcomes. The importance of improved risk stratification and therapeutic modulation of endothelial function is reflected in the ubiquitous presence of underlying subclinical microvascular endothelial dysfunction in patients following surgery. Endothelial dysfunction is likely to have impact on perioperative morbidity, contributing to complications such as impaired wound healing, microvascular endorgan dysfunction and devastating macrovascular events like myocardial infarction.

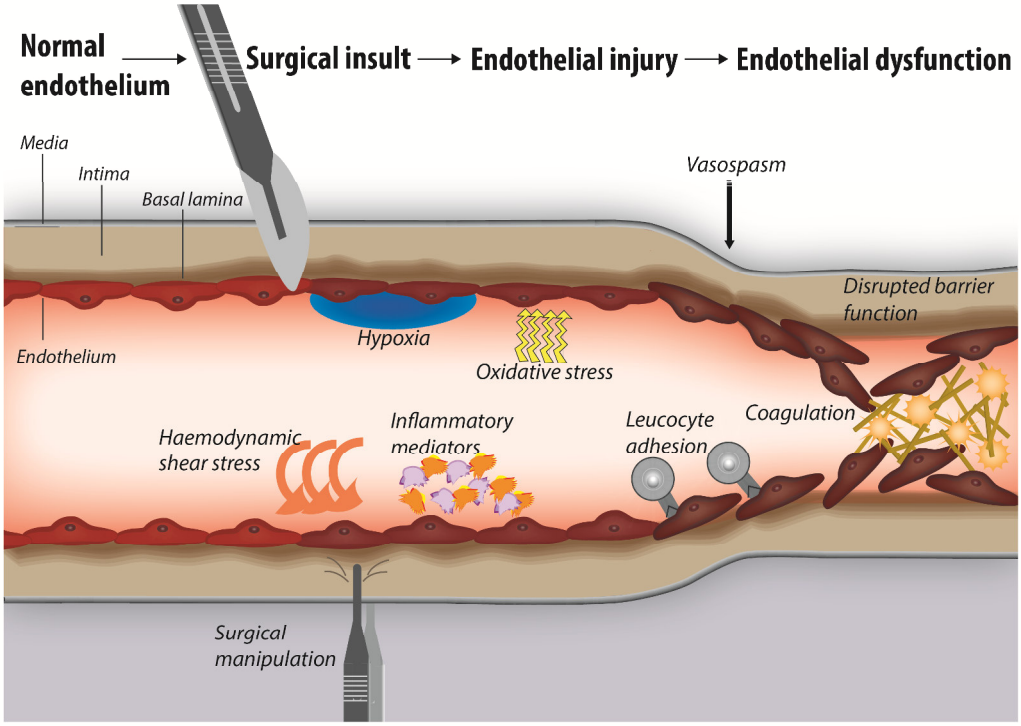


Figure 2. The surgical proinflammatory and prooxidant milieu may result in both functional and structural alterations in the endothelium, resulting in hemostatic dysregulation and impaired local tissue perfusion, with consequent microvascular and macrovascular related postoperative complication (Illustration courtesy of Dr. Marissa Ferguson)[15].

1.5. Non-invasive assessment of endothelial vasodilator function

An indirect assessment of endothelial function can be obtained through examination of endothelial-dependent vasodilator response to the increase in sheer stress that follows induced hyperemia[41]. The tractive force of fluid flow stimulates the endothelium to release vasodilators, most prominently nitric oxide. This phenomenon of induced vascular reactivity can be observed directly by ultrasound imaging of brachial artery diameter[41] or indirectly through monitoring of peripheral temperature change[42], or change in peripheral artery tonometry (EndoPAT / Itamar Medical, Israel).

This methodology has been widely utilized to document the correlation of endothelial vasodilator dysfunction with cardiovascular risk factors and various biomarkers, such as CRP, or asymmetric dimethylarginine (the endogenous antagonist of nitric oxide synthase). Surveillance of therapeutic effects of nutritional and lifestyle (exercise) modifications on endothelial function is technically more challenging and the reliability of the methods may be questionable in terms of their accuracy, sensitivity and specificity.

1.5.1. Brachial artery reactivity testing (BART)

The most prevalent method used to assess endothelial function has been to assess vascular reactivity of the brachial artery in response to a hyperemic challenge. Duplex ultrasonography is used to measure the baseline and post hyperemic diameter of the brachial artery. As such, the BART assesses flow-mediated dilation (FMD) and peak systolic velocity (PSV) in response to hyperemia induced by limb occlusion using a blood pressure cuff inflated to suprasystolic levels. BART emerged as a clinical research tool for studying endothelium-dependent vasomotor function in the early 1990s[41], and FMD was used as a non-invasive method for examining endothelial function, as an early surrogate of atherosclerosis that correlates well with endothelial dysfunction in the coronary circulation and with overall cardiovascular outcome[43,44]. It is now a commonly used research tool to evaluate risk factor status and preclinical disease states, and to monitor improvement in endothelial function with specifically targeted interventions and risk factor modifications[45]. However, despite its deceptively simple appearance, ultrasonographic assessment of brachial artery reactivity is technically challenging, requiring expertise and sensitive ultrasound equipment, and hence has restricted its use to expert vascular laboratories and research settings. In an attempt to standardize the techniques and allow more routine diagnostic use of BART recent guidelines were published for the ultrasound assessment of endothelial-dependent FMD of the brachial artery[46].

FMD has been found to improve non-invasive preoperative risk stratification in patients scheduled for vascular surgery, with impaired brachial artery derived endothelial function

independently predicting postoperative cardiac events[32]. Similarly, in a recent clinical study among major thoracic surgical patients, we demonstrated that BART was a good predictor of postoperative morbidity[36]. Whether FMD can provide further insight into the pathophysiology of endothelial dysfunction or contribute to day to day risk-stratification and prognostication preoperatively to guide clinical decision-making, requires larger studies[37].

1.5.2. Digital thermal monitoring (DTM)

DTM, a novel non-invasive, FDA approved method is currently under evaluation in clinical trials for the assessment of peripheral vascular function, and the improvement of cardiovascular risk assessment[42,43]. This method indirectly measures changes in skin blood flow following reactive hyperemia, utilizing a thermo-coupling method to measure temperature. A recent study showed that the DTM measured temperature rebound closely correlates with Doppler flow velocity[47]. We observed a lower DTM signal in patients with cardiovascular risk factors (abdominal obesity, smoking) and this may contribute to further risk stratification of these patients. However, unlike the findings of Hu et al.[26], we were unable to show significant DTM changes in the perioperative period following major thoracic surgery[15].

1.5.3. Peripheral artery tonometry (EndoPAT)

This novel technique measures peripheral vasodilator response using fingertip pulse amplitude tonometry for non-invasive assessment of vascular function. The technique is based on a system of inflatable latex air cuffs placed on the middle finger and connected by pneumatic tubes to an inflating device. Using a constant counter pressure through the air cushions on the finger and thus preventing venous pooling, venoarteriolar reflex vasoconstriction and occlusion of arterial blood flow, the device senses pulsatile volume changes of the distal digit induced by pressure alterations in the finger cuffs[48]. Impairment of pulse amplitude hyperemic response has been demonstrated among patients with multiple traditional metabolic risk factors[44] and patients with demonstrated coronary artery endothelial dysfunction[49].

Whether a predominantly microvascular hyperemic response, as measured at the fingertip by DTM or EndoPAT, correlates with the gold standard of brachial artery FMD, a macrovascular measure, remains contentious. One study investigated pulse wave form analysis and refuted the claims that large (macrovascular) and small (microvascular) arterial stiffness are substitute measures to sonographic assessments of brachial FMD[50]. Moreover, whether either of these non-invasive techniques are a useful clinical tool, in

order to refine preoperative patient risk assessment and care, has yet to be investigated in large clinical trials.

1.5.4. Plasma biomarkers of endothelial dysfunction

The shift of the normal endothelium to a damaged, procoagulant, pro-inflammatory, vasoconstricted state, with decreased regenerative and reparative capacity, can occur in chronic disease states, cancer, and after following inflammatory insults e.g. following major surgery. Functional assessment (using non-invasive techniques such as FMD) or assessment of inflammatory markers (e.g. CRP levels) are relatively insensitive and non-specific and fail to provide a dynamic assessment of the functional environment or information regarding the pathophysiology of endothelial dysfunction. There is need to explore sensitive and specific biomarkers that can be utilized real-time in routine diagnostic laboratories. Biochemical and cellular "biomarkers" of endothelial (dys)function, such as dimethyl arginine levels, markers of lipid peroxidation, circulating levels of inflammatory mediators, P-selectin, indices of coagulation, and cellular surrogates such as endothelial microparticles, CEC and EPC, may provide greater insight into the mechanistic process and thus provide better risk prediction and guidance of endothelial function and optimization strategies[13,51]. A number of these biomarkers are currently under investigation in various disease states, including surgical populations[52,53], but that is beyond the scope of this habilitation thesis. Many of these biomarkers are still considered investigational and only available in highly specialized laboratories. An area of developing interest lies in endothelial microparticles for their diagnostic and prognostic value[52,53], and in EPC mobilization and functionality for their prognostic[10,54] and therapeutic capacity[55].

1.6. Perioperative therapeutic strategies to improve endothelial function

Current clinical strategies applicable to the perioperative setting that may modulate endothelial dysfunction or preserve microvascular health are centered on reduced inflammatory burden and/or up-regulation of endothelial nitric oxide synthase (eNOS). These strategies are aimed at preserving NO bioavailability and include: preoperative exercise therapy, avoidance of drug withdrawal (e.g. aspirin, statins)[56], anti-inflammatory medications (e.g. aspirin, statins), and other pharmacologic interventions (e.g. novel NO-enhancing ß-blockers). Many of these strategies are still in their infancy and large prospective trials investigating the impact of these therapeutic options on postoperative outcome are eagerly awaited, particularly in patients identified preoperatively with endothelial dysfunction. Data supporting interventional strategies include: studies that have shown reversibility of inflammation-induced endothelial dysfunction through source

removal[23] or anti-inflammatory strategies including hydrocortisone, aspirin and statin therapy[22,27].

1.6.1. Pharmacological intervention: statin therapy

Statins are indicated for primary or secondary prevention of cardiac events[57]. In addition to lowering cholesterol, numerous studies have demonstrated a benefit through the pleiotropic effect, which includes a strong anti-inflammatory effect and improved endothelial regeneration via EPC mobilization. These effects lead to reduced coronary artery plaque formation and stabilization of existing coronary plaques[58,59], contributing to a reduction in short- and long-term cardiovascular complications and deaths.

A randomized placebo-controlled clinical trial showed that preoperative statin use for a median of 37 days before non-cardiac vascular surgery significantly lowered the incidence of myocardial ischemia (statin, 10.8% versus placebo, 19%), myocardial infarction (statin, 4.8% versus placebo, 10.1%), and cardiac death[60]. Interleukin-6 and high-sensitive CRP levels were lower in patients randomized to a statin treatment. Meta-analyses of studies investigating the effect of statin therapy on postoperative outcome suggest that statin administration is associated with decreased postoperative cardiac events[61-63]. A recent meta-analysis of preoperative statin therapy in cardiac surgery reported a 0.9% absolute risk (2.6 vs. 3.5%) and 31% odds reduction for early all-cause mortality with preoperative statin use[64]. In addition, postoperative atrial fibrillation, stroke, intensive care unit and in-hospital stay were also substantially reduced. In a randomized study, cardiac surgery patients assigned to preoperative statin therapy exhibited fewer declines in endothelium-dependent FMD (60.1+15% decline in the placebo group compared with 45.8+16.6% in the atorvastatin group; $p<0.05$) after cardiopulmonary bypass[27]. These data support the endothelial protective benefit attributed to statins. Unfortunately, some of the literature base has questionable scientific validity due to that published by a discredited researcher[65,66].

A withdrawal effect for statins, with increased risk of postoperative cardiac complications, suggests that patients under chronic statin treatment should continue taking statins in the perioperative period to prevent such an adverse outcome[67]. In patients with no history of prior statin use but with multiple cardiac risk factors and elevated levels of inflammatory markers (interleukin-6 and CRP) initiation of statin use at least 30 days prior to the planned surgical procedure could be considered[68], however a shorter duration may be feasible, as a rapid physiological effect has been demonstrated in a prospective trial in which a single oral dose of pravastatin (40mg) significantly attenuated acetylcholine-mediated vasoconstriction after 24 hours[69].

1.6.2. Cellular aspects: endothelial progenitor cells (EPC)

Endothelial dysfunction can be improved by the reconstitution of the endothelial layer, which generally involves the biologic paradigms often described as angiogenesis and vasculogenesis. Angiogenesis refers to the neovascularization occurring via migration and proliferation of endothelial cells in preexisting vessels. The capacity of mature endothelial cells to proliferate, however, depends on the presence of endothelial colony forming cells (CFC) that give rise to endothelial progeny[70-72]. In this context, adult vasculogenesis, the *de novo* formation of blood vessels from endothelial progenitor cells, has been demonstrated to play an important role.

The presence of circulating blood cells with the ability to promote vascular repair and regeneration was first described in 1997[9]. These identified cells displayed a variety of seemingly endothelial-specific cell surface antigens, and were therefore referred to as EPC. Since then, an accumulation of experimental studies have been performed to assess the mechanism by which bone marrow-derived EPC may be recruited and incorporated into sites of active neovascularization during tissue ischemia, vascular trauma, tumor growth and inflammation. In parallel, a multitude of clinical studies have identified EPC as a biomarker for clinical disorders such as cardiovascular disease[10], cerebrovascular disease[73,74], sepsis[75], and numerous types of cancer[76,77]. In all of these studies, the concentration of circulating EPC inversely correlates with the risk of adverse outcome. Subsequently, experimental data from marrow transplantation have shown that marrow-derived cells are recruited to sites of active neovascularization and can differentiate into vascular cells in situ, the frequency of this phenomenon and the identification of the cell type involved are yet to be fully determined[78].

1.6.2.1. Different populations of EPC

A major limiting factor in this field has been the lack of a specific marker to identify circulating EPC. Furthermore, different methods have been applied to enumerate EPC (flow cytometry, culture methods, immunostaining) and to determine their functional capacity, rendering comparison difficult. However, the definition of three functional populations of EPC has been generally accepted. The first population expresses the phenotype CD34+ AC133+ KDR+ and has gained wide use as a means to measure circulating EPC in human subjects[79]. These cells may be recruited to denuded vessels in ischemic sites, but they may not directly become persistent vascular endothelial cells or display de novo in-vivo vasculogenic potential. Rather, they display potent paracrine properties regulating new vessel formation via angiogenesis[80,81]. Yoder et al. refer to these cells as proangiogenic hematopoietic cells (PHC)[40,82,83].

Other populations of EPC are identified using colony-forming assays, in which plated human CD34+ peripheral blood cells form cellular clusters on fibronectin-coated dishes in-vitro. These clusters, binding a low-density lipoprotein cholesterol (cLDL), were presented in the initial study[9] as evidence of CD34+ peripheral blood cells differentiating into spindle-shaped endothelial cells. The emerging cell clusters are referred to as EPC colony forming units (CFU). The third population of EPC has been identified as yet another type of cell colony emerging from plated peripheral blood mononuclear cells (PBMC). This cell colony emerges as tightly adherent with a typical cobblestone appearance and is referred to as endothelial colony forming cells (ECFC), late outgrowth endothelial cells (OEC) or blood outgrowth endothelial cells (BOEC). These cells have vessel-forming ability, but also connect to the vessels to become part of the systemic circulation of the host animal[84]. Among all current putative EPC subtypes, ECFC appear to function as a circulating precursor with in-vivo human vessel-forming ability and exhibits the most features of human postnatal vasculogenic cells.

EPC enumeration has been correlated with cardiovascular risk factors, extent of coronary disease, and future cardiovascular events[10]. Given that EPC enumeration and functional characterization represent the only assessment on the reparative side of the balance between damage and regeneration, this technique may offer independent and different assessment of propensity to cardiovascular injury, greatly improving risk stratification of patients. Attempts to stimulate mobilization and homing of bone marrow-derived endothelial progenitor cells or exogenously administered cell-based (progenitor) therapy will likely also emerge in the next decade as peripherally circulating EPC and intrinsic stem cells play an important role in accelerating endothelialization and tissue remodeling at areas of vascular damage in both disease, and following toxic insults and stress[55,85,86].

Comorbid disease states and aging associate with decreased regenerative ability by EPC and may account for increased risk of postoperative complications and delayed recovery. As such, diabetes is characterized by weak bone marrow mobilization, decreased proliferation, and shortened survival of EPC[13]. Inhibition of oxidant stress normalizes post-ischemic neovascularization in diabetics by positive EPC modulation. Bone marrow EPC mobilization was partially rescued in diabetic rats treated with insulin[87]. It is not known whether this favorable effect is mediated by insulin itself or by improved glucose control.

1.6.2.2. Mobilization of EPC with exercise

Many factors have been described as playing an important role in the mobilization of EPC[88,89]. Among them are growth factors, such as the vascular endothelial growth factor (VEGF), placental growth factor, erythropoietin, and angiopoietin-1, proinflammatory

cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (GCSF), chemokines such as stromal cell-derived factor-1, hormones such as estrogens, lipid-lowering and anti-diabetic drugs, as well as physical activity[90]. The stimulatory effect of exercise on EPC has been shown not only in highly trained athletes[91] and healthy subjects[92], but also in patients with known cardiovascular disease[93]. However, little is known about the benefit of exercise to endothelial health in patients with subclinical cardiovascular disease that are predisposed to endothelial dysfunction due to comorbidities including metabolic syndrome or in patients subjected to an acute inflammatory insult around the time of surgery. Further, it is unclear if preoperative exercise ("prehabilitation") sufficiently improves patients' physical status, e.g. exercise capacity (anerobic threshold [AT] and peak oxygen uptake [pVO_2]) and endothelial function in the perioperative period.

Exercise has been shown to have an effect on endothelial regeneration but the duration and intensity of exercise that is needed to adequately stimulate endothelial repair mechanisms (via EPC) still remains unclear. In a study with healthy subjects, Laufs et al. reported increased circulating EPC levels after moderate and intense running for 30 minutes (80-100% velocity of individuals' anerobic threshold), but not after short term running for 10 minutes[92]. In elderly patients with documented coronary artery disease, a 4-week exercise program resulted in increased levels of circulating EPC. More recently, a study showed that even a short (15 days) cardiac rehabilitation program increased EPC in relation to improved exercise capacity. A 3-month cardiac rehabilitation program increased EPC 2-fold, colony-forming units 3-fold, increased blood nitrite concentrations, and reduced EPC apoptosis[94]. Despite the information provided by these studies it remains to be determined what minimum threshold of training duration and intensity is required to elicit improvements in endothelial function[95] and whether such prehabilitation strategies, prior to surgery, will reduce perioperative morbidity.

Surgical injury induces the mobilization of EPC, with significantly higher circulating EPC and bone marrow EPC levels observed 24 hours after surgery in an animal model.[96] The ability to mount an EPC response is also seen in critical illness, and the response is significantly greater in patients that survive sepsis[75], and recover from illness, e.g. without fibrotic changes after pneumonia[85].

Given that 'responders' who mount this 'cellular' stress response to injury, with increased EPC mobilization, have improved organ recovery[85] and improved survival[54], intrigues as to the necessity of a bone marrow-derived cellular component to the 'stress response' and whether strategies to improve bone marrow capacity and responsiveness will influence a patient's ability to withstand surgical injury. Increasing this bone marrow-derived regenerative response through preoperative exercise training may be a potential

therapeutic strategy to optimize patients prior to surgery. In a recent pilot study, we were able to demonstrate that patients scheduled for major thoracic surgery that exhibited an EPC response to the 'stressor' of preoperative exhaustive exercise suffered significantly fewer postoperative complications[97].

However, discovering an inadequate EPC response during acute illness e.g. impaired wound healing, pneumonia, acute lung injury[51] or sepsis[98] is too late. Using a surrogate stressor e.g. exercise and prehabilitating patients prior to surgery to improve bone marrow responsiveness is therefore appealing. Some of the endothelial dysfunction observed in the perioperative period may be transient, reversible, or potentially preventable and may not actually involve structural change in the cells of the vascular endothelium.

Importantly, whether this lack of EPC response is an epiphenomenon, or a surrogate marker, or indeed causative of increased postoperative complications, requires further study. The causative nature is supported by animal studies that suggest exogenous EPC administration to rescue endotoxin-induced acute respiratory distress syndrome (ARDS), with reduced inflammation, improved oxygenation, and improved survival[55,86]. Similarly, Jeong et al. investigated whether diabetic neuropathy could be reversed by local transplantation of EPC[99]. They reported that motor and sensory nerve conduction velocities, blood flow, and capillary density were reduced in sciatic nerves of streptozotocin-induced diabetic mice but recovered to normal levels after hind-limb injection of bone marrow–derived EPC. Injected EPC were preferentially and durably engrafted in the sciatic nerves. Finally, they found that portions of engrafted EPC were uniquely localized in close proximity to vasa nervorum. This study shows, for the first time, that bone marrow-derived EPC could reverse various manifestations of diabetic neuropathy. As such, cell-based translational approaches may provide a novel and valid therapeutic alternative in the future.

Exercise[100] and tissue insult from surgery[96] are known to increase the mobilization of EPC. Unlike Laufs et al., who exercised patients to a moderate intensity, the use of CPET, with exercise to maximum capacity, not only increases the EPC population, but also provides incremental information about the capacity of EPC release from the bone marrow in response to a stressor similar to the surgical stress. Additional gas exchange parameters (AT and pVO_2) obtained during diagnostic CPET can be used to determine patients' individual physiologic capacity and the amount of exercise needed in order to stimulate the population of EPC. Preoperatively, exercise training could be used to condition patients' individual capacity ('prehabilitation') and to improve endothelial function by affecting EPC number and function. As such, Cesari et al. reported a significant increase in circulating EPC in those patients that showed improvement in their exercise capacity of more than 23% (as measured by the six minute walk test) after completion of a rehabilitation program[90].

In the present work we tested the hypothesis, that brachial artery reactivity, as a surrogate marker of endothelial function, is decreased in patients with cardiovascular risk factors (i.e. hypertension, diabetes, hyperlipidemia) undergoing major surgery. Further, we tested the hypothesis that preoperative exercise increases EPC, a cell line that plays a key role in the endothelial repair, and that patients with cardiovascular risk factor show a poor increase in EPC that this associated with postoperative complications.

2. Materials and Methods

2.1. EPC mobilization with preoperative exercise

2.1.1. Study patient group

Following Institutional Review Board (The University of Texas, M.D. Anderson Cancer Center) approval, sixty consecutive adult patients, scheduled for major thoracic surgery, including esophagectomy or lung resection (wedge resection, lobectomy or pneumonectomy), were enrolled in this prospective observational study. Major thoracic surgery was defined as procedures requiring a thoracotomy. Thoracoscopical surgeries (i.e. video-assisted thoracic surgery [VATS]) were not included in the study taking into account the difference in complication rates between minimal invasive and open surgical procedures.

Each subject gave written informed consent after receiving a thorough explanation of the study design and protocol. Predefined exclusion criteria included: inability of patients to exercise above their anaerobic threshold, thereby ensuring a valid CPET of sufficient exercise load was achieved, and any medical condition that deemed a patient unsatisfactory for surgery after their preanesthetic evaluation, including a recent (less than three months prior) history of myocardial infarction, venous thromboembolism, or cerebrovascular accident.

Preoperative comorbidities were defined as: history of smoking, diabetes mellitus, cardiovascular disease (presence of hypertension, coronary artery disease, peripheral artery disease), history of chemoradiation therapy, modified Lee cardiac risk index >2, and ASA Physical Status Classification score >2 and the Charlson weighted index of comorbidity.

Postoperative complications were defined as: *cardiac events,* including myocardial ischaemia (with or without myocardial infarction), dysrhythmia, congestive heart failure and postoperative requirement of vasopressors; *pulmonary events*, including prolonged intubation, postoperative re-intubation, pneumonia, acute lung injury (ALI), and ARDS; *wound healing events*, including wound infection, empyema and sepsis; and *surgical events* including prolonged air leak (>5 days), esophageal leak and any other reoperative

event. Complications were analyzed according to the Clavien-Dindo complication classification[101].

A blinded researcher reviewed the medical charts for occurrence of these predefined perioperative comorbidities and postoperative complications. These data were collected for the period of patients` hospital stay.

2.1.2. Cardiopulmonary exercise testing (CPET)

Prior to exercise, baseline vitals (heart rate, blood pressure, pulse oximetry, and electrocardiogram [ECG]) and static pulmonary function tests (forced expiratory volume at 1 second, forced vital capacity, maximal voluntary ventilation) were recorded for all patients. CPET was performed as a multi-stage incremental ('ramp workload') study using a cycle ergometer and a metabolic cart with standardized exercise software (Medgraphic Cardio-2CP system, Medical Graphics Corporation, St. Paul, Minnesota) for breath-by-breath analysis of gas exchange.

An initial acclimation period consisted of breath-by-breath gas exchange analysis performed in the supine, resting position for five minutes. After acclimation the patient pedaled at 60 rpm with minimal resistance (unloaded work) for three minutes. After three minutes, loaded work (increasing pedal resistance, watts per minute) followed a standardized ramp protocol to maximal symptom limited exertion that typically lasted 9-12 minutes. Exercise was terminated by the study patient or by the study investigator if symptoms of cardiovascular, pulmonary distress, and/or fatigue were observed. Gas exchange analysis recorded oxygen consumption (VO_2, mL/kg/min) and carbon dioxide production (VCO_2, mL/kg/min) at all phases of exercise. Anaerobic threshold (AT, mL/kg/min) was defined as the VO_2 at the inflection point as determined by the modified V-slope method of plotting carbon dioxide excretion (VCO_2) against oxygen uptake (VO_2) during increasing exercise intensity, as described by Wassermann et al.[102]. Peak VO_2 was defined as the highest oxygen consumption achieved during the exercise test.

2.1.3. EPC analysis by flow cytometry

Blood was collected before and 10 minutes after peak exercise, using EDTA as an anticoagulant. Blood samples were frozen according to the freezing/thawing procedure described by Norden-Zfoni et al.[103]. In brief, blood was collected in cell processing tubes (Becton Dickinson, Heidelberg, Germany) containing sodium citrate and Ficoll and centrifuged at room temperature for 25 min at 1,600g within 2 hours of collection. The mononuclear cells were transferred into a cryotube and an equal volume of freezing medium (RPMI 1640 with 20% DMSO for a final 10% concentration) was added to the cell

suspension. Samples then underwent a controlled freeze using an isopropanol bath in a –80°C freezer and then stored in liquid nitrogen until batch analysis.

For the analysis, thawing was achieved by washing the cells using the same storage medium without DMSO and samples were enumerated within 60 minutes of thawing. Circulating EPC and mature EC were evaluated by six-color flow cytometry (Figure 3) using a panel of monoclonal antibodies including anti-CD45 (to exclude non-endothelial progenitor cells), anti-CD133 (an EPC marker), anti-CD31, CD34, and CD146 (mature EC markers). Appropriate analysis gates were used to enumerate viable and apoptotic EPC. The combination of Syto16 and 7-AAD was used to gain insight into EC viability according to Van der Pol et al.[104]. Necrotic cells were identified as Syto16^{low}/7-AAD^{+}, apoptotic cells as Syto16^{low}/7-AAD^{-} and viable cells as Syto16^{bright}/7-AAD^{-}.

FACS Canto (Becton Dickinson, Heidelberg, Germany) was used to evaluate cell suspensions after red cell lysis. After acquisition of at least 1×10^{6} cells per blood sample, analysis was considered informative when adequate numbers of cells (>100, typically 300-400) were collected in the enumeration gates. EC were defined as DNA (Syto16) positive, negative for the hematopoietic marker CD45, positive for EC markers CD31, CD34, and CD146 and negative for the EPC marker CD133. EPC were depicted by the expression of the stem cell marker CD133+. Figure 3 displays a schematic flow diagram that summarizes the flow cytometry technique.

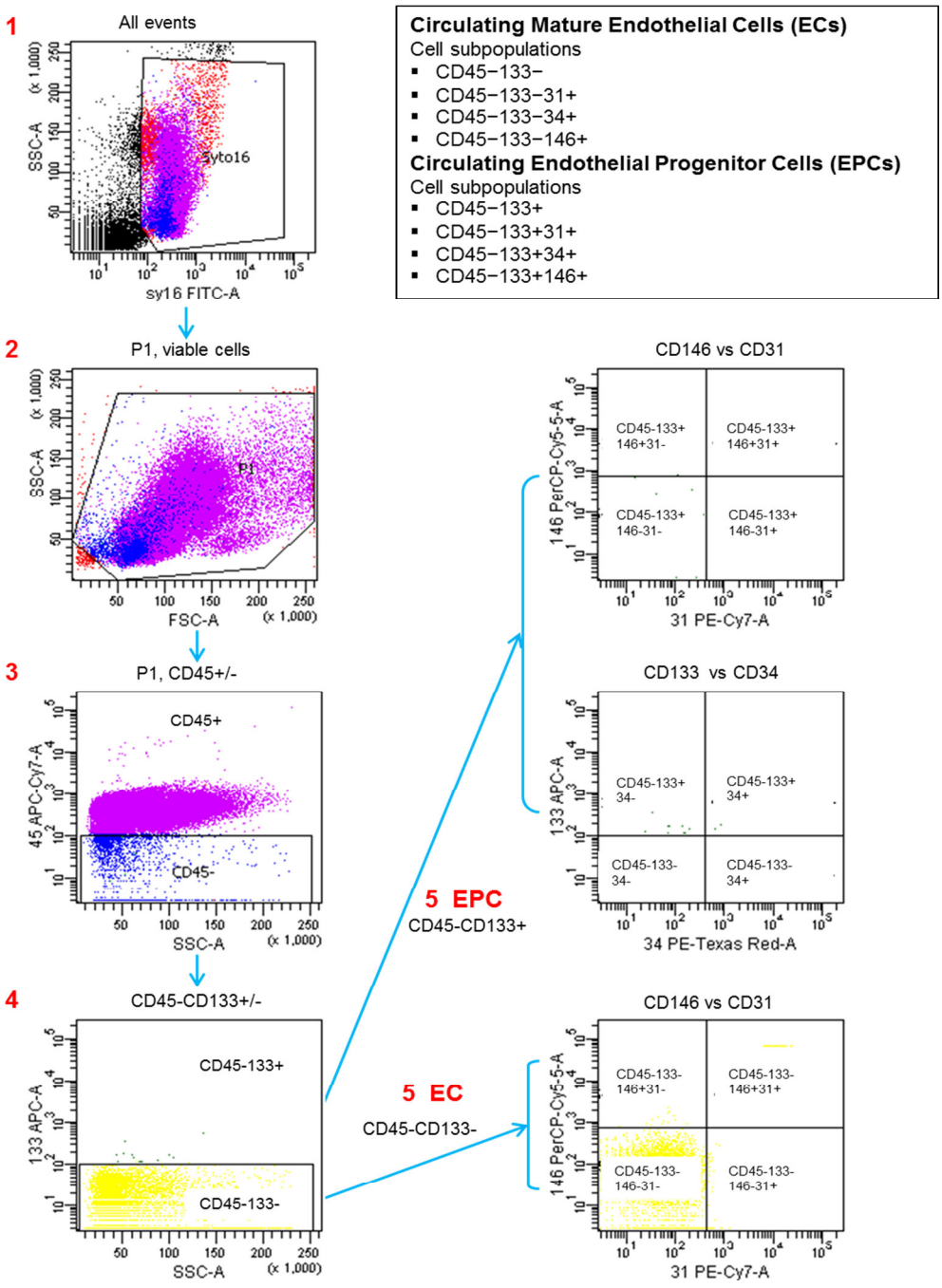


Figure 3. Schematic representation of the strategy used for the quantification of peripheral circulating endothelial progenitor cells (EPC) and mature endothelial cells (EC) by six-color flow cytometry; Step 1., All events: Gates identify CD31bright/Syto16dim platelets (red dots) and CD31+Syto16+ EC/EPC (blue dots) for further phenotypic investigation and enumeration; Step 2., Identification of all viable cells; Step 3., CD45- cells (blue dots) identified to exclude non-endothelial progenitor cells (CD45+, purple dots) cells; Step 4., EPC are identified as CD45-133+ cells and EC as CD45-133- cells; and Step 5., EC/EPC gate show EC (DNA/Syto16+CD31+CD146+) and EPC (DNA/Syto16+CD133+146+31 and DNA/Syto16+CD133+34+)

2.1.4. Statistical analysis EPC

The primary endpoint was defined to be the response of EPC (CD45-133+ lineage) levels to peak exhaustive exercise as compared to pre-exercise (baseline) levels. Sample size for the EPC analysis was calculated using the short-term effect of exercise on EPC release 10 minutes after a symptom-limited dynamic exercise test in volunteers[100].

The changes in EPC and mature EC levels to peak-exhaustive exercise from pre-exercise levels were assessed using the non-parametric Wilcoxon signed-rank test. Associations of patient baseline characteristics with exercise capacity measures (AT and VO_2 max) and EPC levels were investigated using analysis of variance (ANOVA), linear regression or non-parametric methods as appropriate. ROC curve analyses were performed to determine the optimal cut-point for changes in EPC levels to peak-exhaustive exercise in predicting postoperative complications. Binary logistic regression was also used to investigate the association of changes in EPC levels to peak-exhaustive exercise and other preoperative risk indices (AT, modified Lee Cardiac Risk Index and Charlson Comorbidity Index) with the incidence of postoperative complications; odds ratios (OR) were calculated with corresponding 95% confidence intervals (95% CI). A p-value of less than 0.05 was considered to indicate statistical significance. Statistical analyses were carried out using R version 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria).

2.2. Preoperative BART

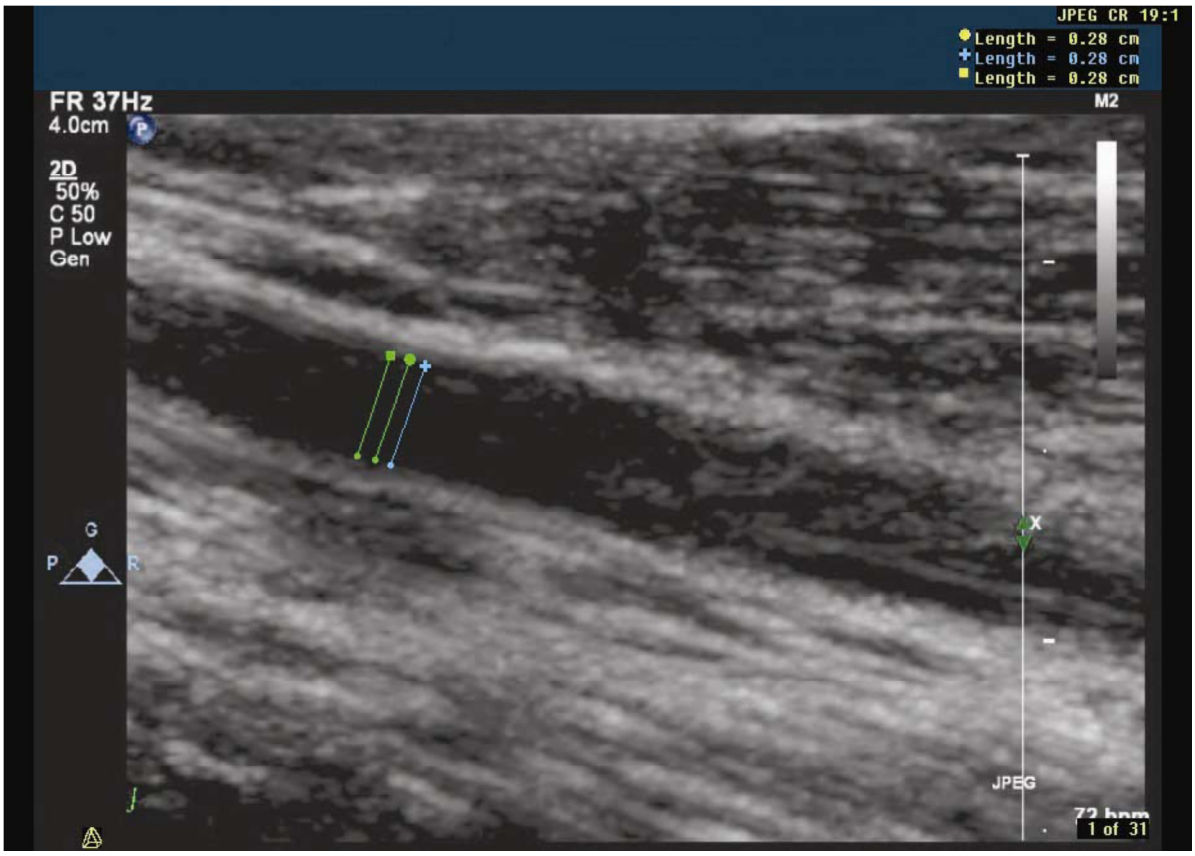
2.2.1. Study patient group

Following IRB approval, 63 consecutive patients scheduled for major thoracic surgery (esophagectomy or major lung surgery e.g. wedge resection, lobectomy or pneumonectomy) at the University of Texas M.D. Anderson Cancer Center were recruited to the study. Exclusion criteria included any condition that deemed a patient unsatisfactory for surgery after the preanesthetic evaluation. Patients were evaluated with standard preoperative risk scores, including the American Society of Anesthesiologists [ASA] Physical Status Classification System, modified Lee Cardiac Risk Index, and American Heart Association/American College of Cardiology [AHA/ACC] Risk Score[105-107].

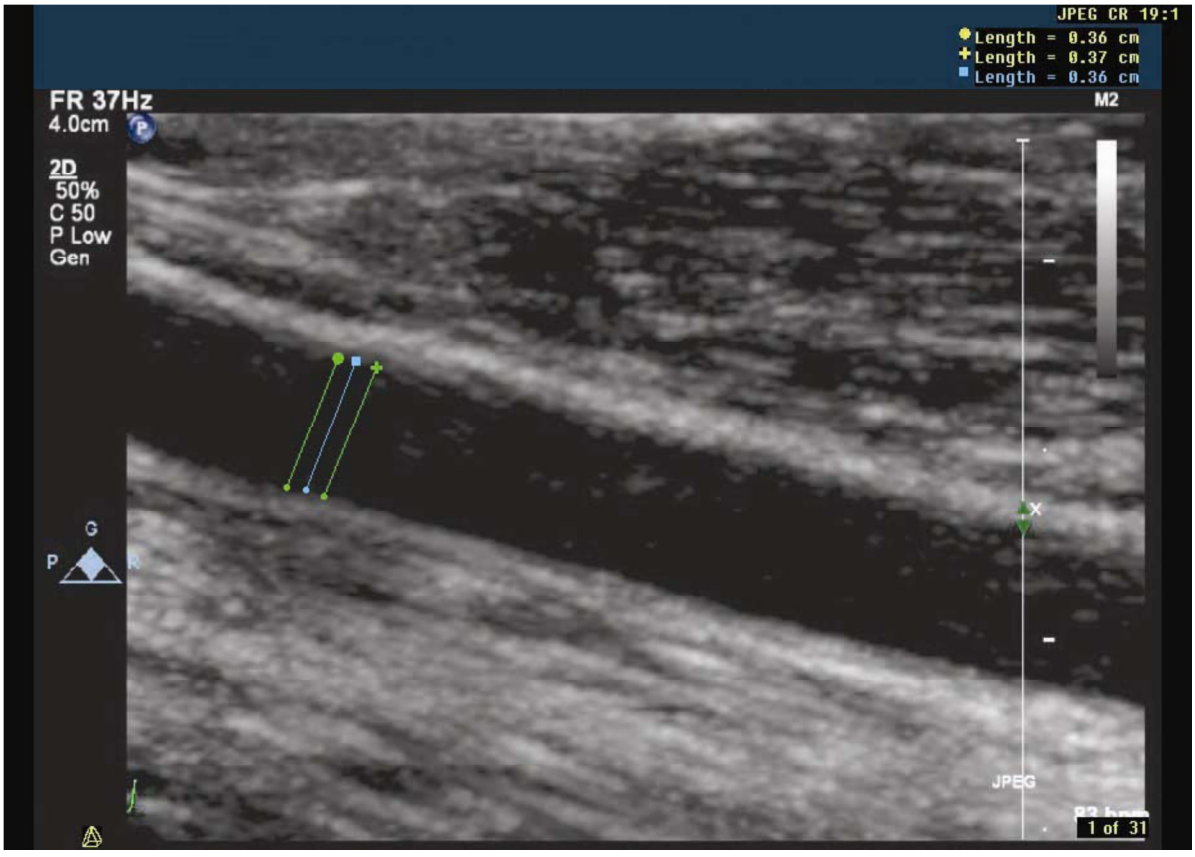
The primary endpoint of this pilot study investigated whether preoperative BART-derived variables (FMD and PSV) would predict postoperative complications, not restricted to cardiovascular events but inclusive of all postoperative complications that commonly occur following major thoracic surgery. The secondary endpoint correlated preoperative BART values to preoperative risk factors and established preoperative risk scores (ASA, ACC/AHA, modified Lee Cardiac Risk Scores)

2.2.2. BART values: flow-mediated dilation (FMD) and peak systolic velocity (PSV)

To ensure consistency, all ultrasound measurements of the brachial artery were performed within one week of scheduled surgery. Measurements were performed in a quiet, dimmed room at a controlled ambient temperature (20 - 25°C). Resting blood pressure was measured by placing a blood pressure cuff on the right forearm. The right brachial artery was then imaged using a 10 MHz linear array vascular transducer connected to an ultrasound machine (Philips IE33, Philips Electronics North America Corporation, Andover, MA USA). After baseline diameter and velocity had been obtained, a Hokanson blood pressure cuff occluder was inflated to 50 mmHg above systolic blood pressure for 5 minutes and then rapidly deflated. Subsequent longitudinal digital scans of the brachial artery diameter and velocity spectral displays were obtained at 30, 60, 90 and 120 seconds after cuff deflation (hyperemic phase measurements). Diameter and flow velocity ($cm.s^{-1}$) were measured in straight segments of the brachial artery, approximately two cm above the antecubital fossa and perpendicular to the ultrasound beam along its longitudinal axis (Figure 1). The same operator, blinded to the patients enrolled in the study and to the data collection, obtained all brachial artery diameter (mm) and peak systolic measurements by acquiring digital clips triggered with ECG synchronization. Diameter measurements were obtained off-line (Philips Excelera workstation, Philips Electronics North America Corporation, Andover, MA, USA) using electronic calipers at the onset of the ECG-derived QRS complex. A representative image of the ultrasound measurements is displayed in Figure 4. Post-occlusion (hyperemic) FMD and PSV were expressed in absolute values and as a percentage increase (in diameter and flow velocity) in relation to each patient's pre-ischemic (baseline) measurement.



a)



b)

Figure 4. Brachial Artery before (a) and after (b) 5 minutes occlusion

2.2.3. Statistical analysis BART

All statistical analyses were performed using the SPSS statistical software version 17.0 (IBM Corporation, Armonk, NY, USA). Sample size calculation and power analysis were based on a 4.8-10.9% chance for postoperative complications[108], ß-error of 0.8, and a significance level of 0.05. A total of 65 patients needed to be enrolled to provide a 90% power of observing 5 or more postoperative complications. The Pearson chi-square test was used to test for differences in distribution between the presence (or absence) of a particular event (cardiac, pulmonary, wound healing and surgical complications). A two-tailed t-test was used to analyze the postoperative course. The Analysis of Variance (ANOVA) with post hoc Bonferroni test was utilized for multiple comparisons between groups with low and high flow-mediated dilation and the general linear model (GLM) to assess the predictive value of low FMD for adverse postoperative events[109].

2.3. Preoperative DTM

2.3.1. Study patient group

Following IRB approval (The University of Texas M.D. Anderson Cancer Center, study protocol No. 2003-0434), thirty consecutive patients scheduled for major non-cardiac surgery (esophagectomy or major lung surgery, e.g. lobectomy or pneumonectomy) were prospectively enrolled into this observational trial. Exclusion criteria were any condition that deemed a patient unsatisfactory for surgery after the preanesthetic evaluation. Patients were evaluated with standard preoperative risk scores, including the ASA Physical Status Classification System and modified Lee Cardiac Risk Index[105,106].

2.3.2. Reactive hyperemia measurements

The primary endpoint of our study investigated whether acute exercise increases reactive hyperemia, a surrogate marker of vascular function, and if this effect was blunted in the presence of preoperative cardiovascular risk factors (i.e. coronary artery disease, hypertension, diabetes, obesity). To ensure consistency, all measurements of reactive hyperemia were performed within one week of scheduled surgery. Measurements were performed before and 10 minutes after exercise (as described in 2.1.2.) in a quiet dimmed room at a controlled ambient temperature (20 – 25 °C) using a VENDYS® 5000BC DTM system (Endothelix, Inc., Houston, TX, USA). This FDA approved device consisted of a computer-based thermometry system (0.006° C thermal resolution), with two special thermocouple fingertip probes designed to minimize the area of skin-probe contact and fingertip pressure. A standard sphygmomanometer cuff and a compressor unit, to control

cuff inflation and deflation, was included to facilitate the occlusion-hyperemia protocol. The test was conducted with the patient at rest for 30 minutes in the supine position, in a quiet, dimmed room with an ambient temperature of 24°C to 26 °C. VENDYS® DTM probes were affixed to the index finger of each hand and after a period of stabilization of basal skin temperature (defined as stabilization within a 0.05 °C threshold) the temperature was measured at the index fingers of both hands (of which the right arm only is subjected to occlusion-hyperemia) and documented in an automated, operator-independent protocol. The right upper arm cuff was rapidly inflated to ≥50 mmHg above systolic pressure for 2 minutes, and then rapidly deflated to invoke reactive hyperemia distally. Thermal tracings were measured continuously and digitized automatically using a computer-based thermometry system with 0.006 °C thermal resolution. Dual channel temperature data was simultaneously acquired at a 1 Hz sample rate. Figure 5 shows a representative example of a temperature–time trace and the primary DTM-derived measures, related to thermal debt and recovery that were recorded and calculated.

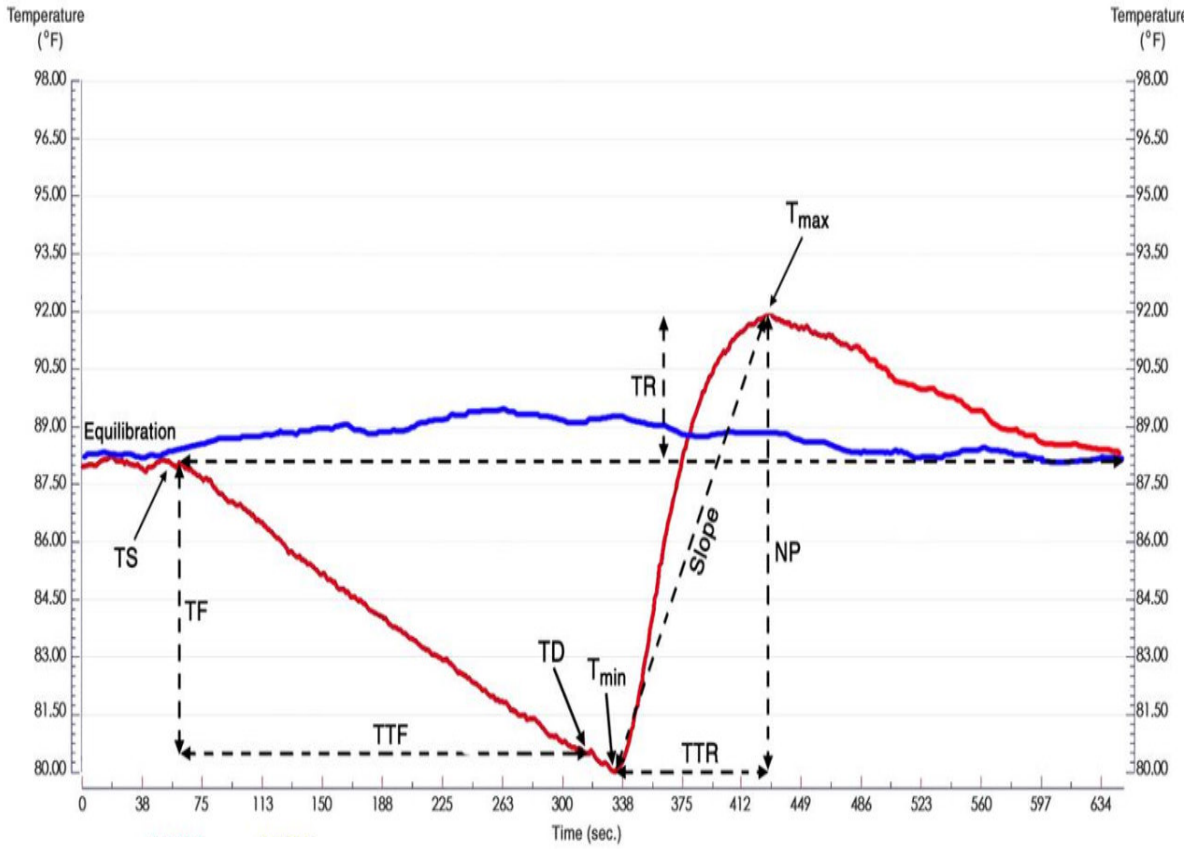


Figure 5. Representative example of a temperature-time trace in response to occlusion-hyperemia. TF= Temperature fall, TR = Temperature rebound (above the baseline), TTF = Time to temperature fall, TTR = Time to temperature rebound, NP = Nadir to peak, Slope= NP / TTR, Tmax = Maximum temperature, Tmin = Minimum temperature, Ts = Start temperature, Tmax% = (Tmax/Ts)∗100, Tmin% = (Tmin/Ts)∗100

2.3.3. Statistical analysis DTM

The study sample size determination was based on data from a previous study by Rakobowchuk et al.[110] who enrolled nine patients to detect an increase of reactive hyperemia, as measured by flow-mediated dilation of the brachial artery, immediately after 45 minutes of exercise on a treadmill at 50 % of their VO_2 peak. We calculated that a sample of thirty patients would need to be enrolled to achieve 80 % power to detect a log-linear trend in the primary endpoint, assuming that the percentage increase of reactive hyperemia after exercise, as measured by TR, was 50 percentage points. Descriptive statistics were used to summarize the patients' demographic, clinical, and TR measures. The relative changes from baseline (before exercise) and post-exercise (10 minutes after

peak exercise) were analyzed using repeated measures (ANOVA) and Wilcoxon-Signed-Rank-Test.

Fisher's exact test was used to analyze for an association of perioperative variables — including patients' comorbidities (i.e. obesity, abdominal obesity, coronary artery disease, and Modified Lee Cardiac Risk Index) with TR measures while tertiles were used as cutoff points. A P-value of less than 0.05 was considered to indicate statistical significance. Statistical analyses were carried out using SAS 9.1 (SAS Institute, Cary, NC, USA) and S-Plus (version 8; Insightful Corp., Seattle, WA, USA).

3. Results

3.1. EPC analysis

Sixty consecutive patients scheduled for major thoracic surgery, that met the eligibility criteria, were enrolled in this study. Three patients were excluded from data analysis because the procedure was declined for surgical reasons. An additional four patients were excluded from EPC analysis because they did not have a blood draw due to patient refusal or unavailability of laboratory personnel to process the samples within the two-hour time frame.

3.1.1. Circulating EPC and EC levels in response to exhaustive exercise

Compared to baseline levels, exhaustive exercise to peak VO_2 statistically significantly increased the circulating levels of EPC subpopulations (CD45-133+34+ cells: pre-exercise (median [range]) 150 [0.00 – 5,230] cells/μL vs. post-exercise 220 [0.00 – 1,270] cells/μL; median change [range] 20 [-4,180 to 860] cells/μL; p=0.03) but not that of the mature EC subpopulations (Table 1).

	N	Pre-exercise Median[Range]	Post-exercise Median [Range]	Change (post-pre) Median [Range]	*P*-value
EPC variables [cells/µl]					
CD45-133+	53	300 [20 to 6,800]	380 [40 to 10,200]	20 [-6,430 to 9,580]	0.07
CD45-133+31+	53	100 [0 to 4,440]	142 [0 to 2,150]	15 [-3,389 to 1,140]	0.06
CD45-133+34+	53	150 [0 to 5,230]	220 [0 to 1,270]	20 [-4,180 to 860]	**0.03**
EC variables [cells/µl]					
CD45-133-146+	53	100 [0 to 3,560]	110 [0 to 3,510]	10 [-2,200 to 3,340]	0.26
CD45-133-31+	53	2,010 [120 to 32,250]	2,530 [200to 48,760]	110 [-20,630 to 48,050]	0.33
CD45-133-146+31+	53	30 [0 to 2,190]	20 [0 to 2,220]	0 [-1,310 to 300]	0.37

Table 1. Effect of preoperative peak exhaustive exercise on circulating levels of endothelial progenitor cell (EPC) and mature endothelial cell (EC) subpopulations; data are presented as median and range. Reported *P*-values for the respective parameters based on Wilcoxon signed-rank test comparing change in postoperative levels compared to preoperative levels.

3.1.2. EPC and the incidence of postoperative complications

Univariate associations of changes in CD45-133+34+ levels in response to exhaustive exercise and the incidence of postoperative complications are summarized in Table 2. Pre-exercise levels (OR=0.86, 95% CI: 0.37–2.00, *P*-value=0.72), change following exercise as a continuous variable (OR=0.95, 95% CI: 0.41–2.22, *P*-value=0.91) and a positive response following exercise (change > 0; OR=0.41, 95% CI: 0.13–1.28, *P*-value=0.12) were all not statistically significant associated with the incidence of postoperative complications.

ROC curve analysis identified an optimal cut-point of 60 cells/μL for EPC CD45-133+34+ mobilization in response to exercise to predict postoperative complications, achieving 86% sensitivity, 48% specificity and AUC = 0.67 (95% CI: 0.52 – 0.81); Figure 6). Patients who exhibited a change in CD45-133+34+of at least 60 cells/μL with exercise suffered statistically significantly fewer postoperative complications (17% vs. 54%, OR=0.17, 95% CI: 0.04–0.69, *P*-value=0.006, Table 2). These patients also had a shorter length of hospital stay (median=6, range [2 – 21] days vs. median=9, range [2 – 77] days), although this did not reach statistical significance (*P*-value=0.08).

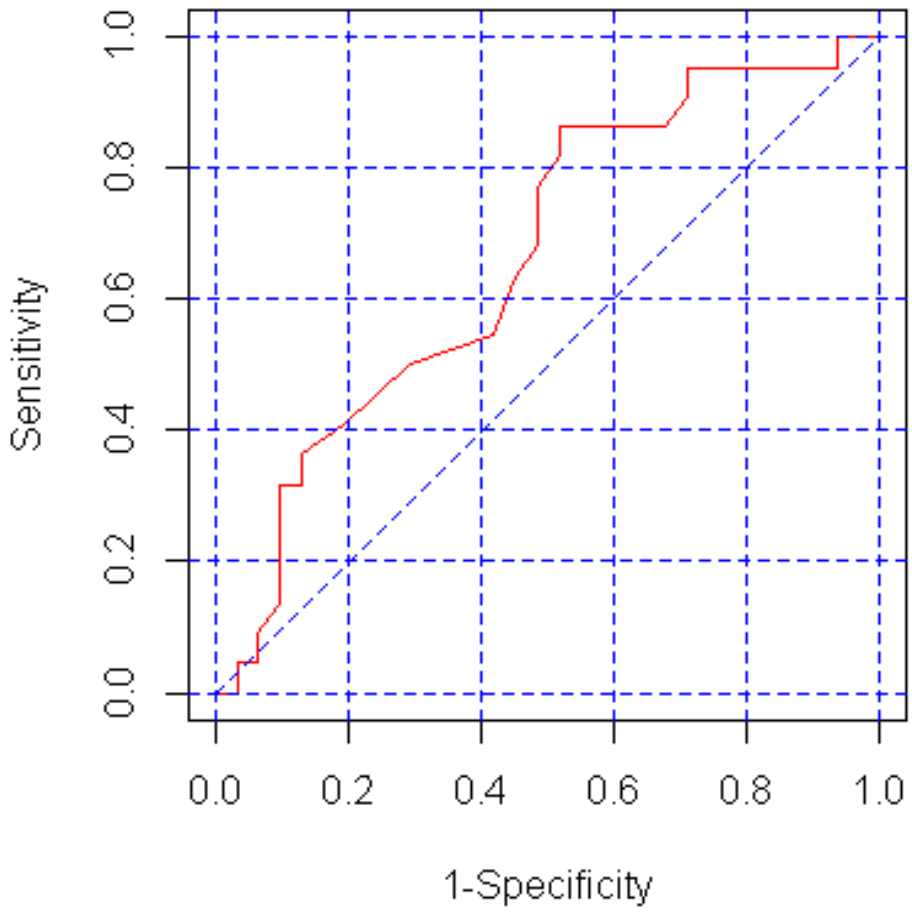


Figure 6. ROC-curve depicting a sensitivity of 86% and a specificity of 48% for CD45-133+34+ change (pre- / post-exercise) in predicting postoperative complications.

	Postoperative complication						
	Clavien grade 0		Clavien grade I–V				
	N	**%**	***N***	**%**	**OR**	**95% CI**	***P*-value**
Pre-exercise CD45-133+34+ levels							
As a continuous variable					0.86	(0.37, 2.00)	0.72
Change in CD45-133+34+ following exercise							
As a continuous variable					0.95	(0.41, 2.22)	0.91
Change ≤ 0 (– response)	9	45%	11	55%			
Change > 0 (+ response)	22	67%	11	33%	0.41	(0.13, 1.28)	0.12
Change <60 cells/µl	16	46%	19	54%			
Change ≥60 cells/µl	15	83%	3	17%	0.17	(0.04, 0.69)	**0.006**

Table 2. Univariate associations of change in CD45-133+34+ in response to exhaustive exercise and the incidence of postoperative complications; odds ratio associated with an increase in 1000 cells/µl.

3.1.3. Clavien severity classification of postoperative complications

Univariate associations of changes in CD45-133+34+ levels in response to exhaustive exercise and the Clavien severity classification of postoperative complications are summarized in Table 3. The optimal cut-point of 60cells/µL for EPC CD45-133+34+ mobilization in response to exercise was statistically significantly associated with reduced postoperative complication severity (OR=0.21, 95% CI: 0.05–0.85, *P*-value=0.02, table 3). The biggest effect appeared to be between none versus any postoperative complication (Clavien grade 0 vs. Clavien grade I–V).

	Postoperative complication								
	Clavien grade 0		Clavien grade I–III		Clavien grade IV–V				
	N	%	*N*	%	*N*	%	OR[a]	95% CI	*P*-value
Pre-exercise CD45-133+34+ levels									
As a continuous variable							0.88	(0.39, 2.02)	0.76
Change in CD45-133+34+ following exercise									
As a continuous variable							0.96	(0.41, 2.26)	0.92
Change in CD45-133+34+ following exercise									
Change ≤ 0 (– response)	9	45%	6	30%	5	25%			
Change > 0 (+ response)	22	67%	4	12%	7	21%	0.50	(0.17, 1.48)	0.21
Change <60 cells/µl	16	46%	10	29%	9	26%			
Change ≥ 60 cells/µl	15	83%	0	0%	3	17%	0.21	(0.05, 0.85)	**0.02**

Table 3. Univariate associations of changes in CD45-133+34+ in response to exhaustive exercise and the Clavien severity classification for postoperative complications; [a]Odds ratio associated with an increase in 1000 cells/µl.

3.2. BART

The study population comprised 63 patients (38 males and 25 females) with mean age of 61±11 years (range, 26 - 80 years) scheduled for major thoracic surgery. Approximately three quarters of the study cohort were at cardiovascular risk: thirty-nine (62%) patients had atherosclerotic risk factors such as hypertension, hyperlipidemia, or diabetes, while six (10%) patients had known coronary artery disease, defined as documented history of angina pectoris and/or myocardial infarction and/or pathologic coronary angiography.

Overall, 44% (n=28) of the study patients had one or more complications (cardiac, pulmonary, surgical and/or wound healing events). Patients who suffered a predefined postoperative complication (event group) were older (64±2 vs. 59 ±2 years; P=0.038), more likely to have received neoadjuvant chemotherapy (n=20 vs. 14; P=0.013), and required a

longer duration of ICU (5.9±2.6 days vs. 0.1±0.1; P = 0.001) and hospital (17.4±4.2 days vs. 6.0±0.6 days, P=0.001) stay compared to the no-event group.

3.2.1. FMD

Percentage increase in FMD values were grouped according to the median FMD value (median FMD = 11.5%). Low FMD group consisted of patients with FMD <11.5% and High FMD group consisted of patients with FMD ≥11.5%. Overall, 59% of the study population had a low FMD (<11.5%, n=37) and 41% had a high FMD (FMD ≥11.5%, n=26). The length of the ICU (3.95± 2.04vs. 0.88± 0.32 days; P=0.015) and hospital (14.00±3.32vs. 6.85± 0.59 days; P = 0.007) stay was significantly greater in the low FMD group.

Table 4 and table 5 summarize the significant clinical characteristics of the study population in terms of the low and high FDM groups and in relation to adverse postoperative events and preoperative comorbidities. In the low FMD group, 54% of the patients had one or more adverse postoperative events (vs. 30% for the high FMD group) and 11% had 3 or more adverse postoperative events (vs. 0% for the high FMD group; P<0.001). Increasing age, hyperlipidemia, and neoadjuvant chemoradiation were observed more frequently in the presence of low FMD patients that suffered events.

		FMD [%]		
	Complications	**Low, <11.5% (n=37)**	**High, ≥11.5% (n=26)**	**P-value**
Overall, N (%)	**0**	17 (46)	18 (70)	**<0.001**
	1-2	16 (43)	8 (30)	
	≥ 3	4 (11)	0(0)	
Preoperative Co-morbidities				
Age, mean ± SE				
	0	59.24± 2.85	58.11± 2.47	0.767
	1-2	69.00 ± 2.22	57.38 ± 2.56	**0.004**
	≥ 3	60.00 ± 6.18	0 (0)	
Hyperlipidemia, N (%)				
	0	7 (19)	2 (8)	**0.042**
	1-2	2 (5)	0 (0)	0.296
	≥ 3	1 (3)	0 (0)	

Table 4. Age and hyperlipidemia as risk factors for impaired FMD (ANOVA analysis of variance with post hoc Bonferroni test)

		Low, <11.5% (n=37)	High, ≥11.5% (n=26)	P-value
Preoperative Comorbidities				
Age, mean ± SE,				
	No event	59.24 ± 2.85	58.11± 2.47	**<0.001**
	Event	67.20 ± 2.23	57.38 ± 2.56	
Hyperlipidemia, N (%)				
	No event	7 (19)	2 (8)	**0.040**
	Event	3 (8)	0 (0)	
Preoperative Risk Scores				
ASA Risk Score ≥3, N (%)				
	No event	16(43)	17 (65)	0.089
	Event	20 (54)	7 (30)	
Lee Cardiac Index ≥3, N (%)				
	No event	1 (3)	1 (4)	0.141
	Event	4(11)	1 (4)	
ACC/AHA Risk Score ≥2, N (%)				
	No event	4(11)	2 (8)	0.133
	Event	6 (16)	0	
Preoperative Therapy				
Chemotherapy, N (%)				
	No event	6 (16)	8 (31)	**0.029**
	Event	14 (38)	6 (23)	
Statin therapy, N (%)				
	No event	5 (14)	5 (19)	0.094
	Event	5 (14)	1 (4)	

Table 5. Preoperative risk factors and FMD values in relation to postoperative complications (General linear model at P<0.15)

Based on the %FMD, approximately 71% of the patients were accurately predicted to develop a postoperative event, with 71.4% (95%CI: 54.7%, 88.2%] sensitivity and 48.6% (95%CI: 32.0%, 65.1%) specificity. Subgroup analysis for specific postoperative complications revealed: 69.2% (95%CI: 44.1%, 94.3%) sensitivity and 42.0% (95%CI: 28.3%, 55.7%) specificity for cardiac events; 66.7% (95%CI: 42.8%, 90.5%) sensitivity and 41.7% (95%CI: 27.7%, 55.6%) specificity for pulmonary events; 100% sensitivity and 43.9% (95%CI: 31.0%, 56.7%) specificity for wound healing events; and 76.9% (95%CI: 54.0%, 99.8%) sensitivity and 44.0% (95%CI: 30.2%, 57.8%) specificity for surgical events.

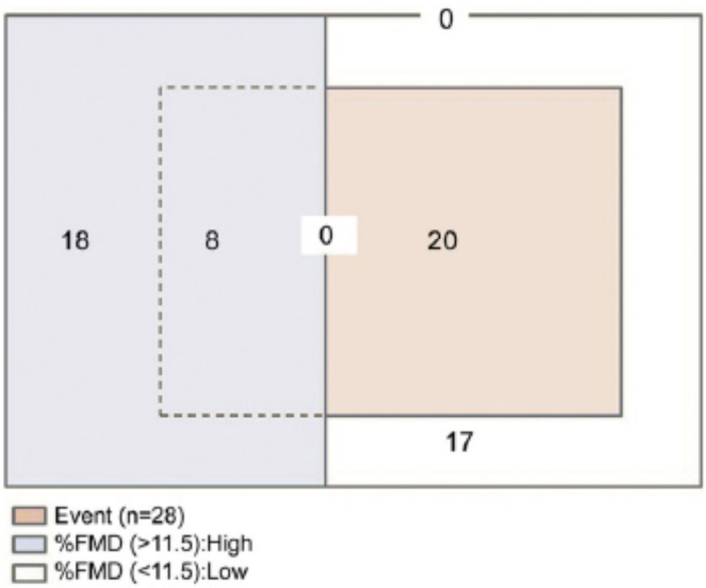


Figure 7: A scaled rectangle diagram (Figure 7) was constructed to illustrate the value of the FMD as a predictive tool for the occurrence of postoperative events, with the large outer rectangle representing the whole study population (n=63), the blue rectangle representing those patients with high FMD (n=26), the white rectangle representing those patients with low FMD (n=37), and the red rectangle, in the center, representing the percentage of patients who were accurately predicted to have an adverse event based on their FMD (n=20 out of the 28).

3.2.2. PSV

BART-derived percentage increase in PSV following hyperemia was grouped according to the median value for the no-event group into low PSV (<77.9%) and high PSV (>77.9%) groups. Overall, female patients were found more frequently in the high PSV group (22 vs. 16; P=0.020). Older age (P=0.027) and neoadjuvant chemotherapy (P=0.046) were

observed more frequently in the low PSV group with postoperative events. Duration of ICU- and hospital length-of-stay were significantly longer in the low PSV group (P=0.047 and P=0.020).

Based on the %PSV had 64.3% (95%CI: 46.5%, 82.0%) sensitivity and 51.4% (95%CI: 34.9.3%, 68.0%) specificity for all-events. Subgroup analysis revealed: 61.5% (95%CI: 35.1%, 88.0%) sensitivity and 46.0% (95%CI: 32.2%, 59.8%) specificity for cardiac events; 60.0% (95%CI: 35.2%, 84.8%) and 45.8% (95%CI: 31.7%, 59.9%) specificity for pulmonary events; 66.7% (95%CI: 29.0%, 100%) and 45.6% (95%CI: 32.7%, 58.5%) specificity for wound healing events; and 69.2% (95%CI: 44.1%, 94.3%) sensitivity and 48.0% (95%CI: 34.2%, 61.9%) specificity for surgical events.

3.3. Preoperative exercise and DTM

Thirty patients (18 males and 12 females) with mean age of 58 ± 10 years scheduled for major non-cardiac surgery were enrolled in the study. Twenty-eight (93%) patients had an increased perioperative risk with an ASA score >2; thirteen (46%) patients had cardiovascular risk factors, for example, hypertension and dyslipidemia; and twenty-one (70%) patients were current smokers.

3.3.1. Reactive hyperemia before and after exercise

Table 6 summarizes the vital signs (heart rate and blood pressure) and the reactive hyperemia measures before and after exercise. The heart rate was significantly increased 10 miuntes after exercise when compared to baseline (mean ± SD: 75±10.58 vs. 76±19.88 min^{-1}; P=0.021). There were no differences in blood pressure before and after exercise. The starting temperature at the beginning of the reactive hyperemia measurement did not differ before and after exercise (mean ± SD: 32.84 ± 1.78 versus 32.23 ± 2.01 °C; P=0.147).

	Pre-Exercise			***Post-Exercise (10 Min. after)***			
	N	Mean	SD	N	Mean	SD	P-values*
Starting Temperature [C°]	30	32.84	1.78	30	32.23	2.01	0.147
Temperature Rebound [TR C°]	30	0.04	0.42	30	0.53	0.95	**0.035***
Temperature Rebound [TR %]	30	0.14	1.27	30	1.78	3.29	**0.033***
Area under curve after 15 sec.	30	14.89	4.70	30	11.92	5.26	**0.019***
Area under curve after 30 sec.	30	29.01	9.04	30	23.29	10.23	**0.017***
Area under curve after 45 sec.	30	41.50	12.86	30	33.34	14.53	**0.017***
Area under curve after 60 sec.	30	52.11	16.15	30	41.85	18.12	**0.020***
Heart Rate [bpm]	27	75	10.58	28	76	19.88	**0.021**
Systolic Blood Pressure [mmHg]	27	128	16.94	28	132	16.35	0.216
Diastolic Blood Pressure [mmHg]	27	76	6.09	28	79	9.06	0.081
Mean Blood Pressure [mmHg]	27	94	11.59	28	98	2.01	0.094

Table 6. Reactive hyperemia (TR) before and after exercise (Wilcoxon-Signed-Rank-Test)

Reactive hyperemia was significantly increased 10min after exercise with an absolute TR increase of 0.04 ± 0.42 versus 0.53 ± 0.95 °C, P=0.035 and a relative TR increase of 0.14 ± 1.27 versus 1.78 ± 3.29%, P=0.033 (Figure 8). Area under the curve (AUC) of the TR slope was significantly lower after exercise with AUC 15sec: 14.89 ± 4.70 versus 11.92 ± 5.26, P=0.019; AUC 30sec: 29.01 ± 9.04 versus 23.29 ± 10.23, P=0.017; AUC 45sec: 41.50 ±

12.86 versus 33.34 ± 14.53, P=0.017; and AUC 60sec: 52.11 ± 16.15 versus 41.85 ± 18.12, P=0.020. There was no association between clinical characteristics and low TR values (2 lower tertiles) when compared to high TR values (upper tertile), Table 7.

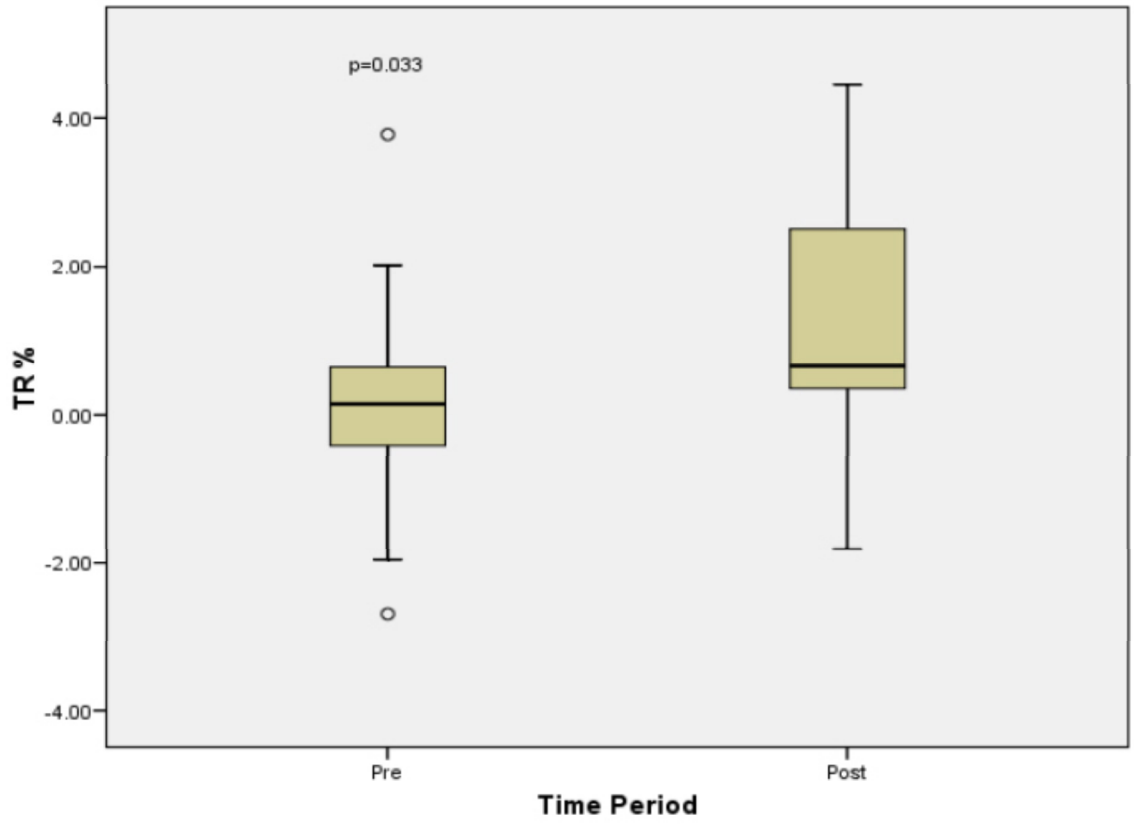


Figure 8. Increase of reactive hyperemia (Temperature Rebound, TR %) 10 minutes after peak exercise. Graph shows median (line in box), 25 %- and 75 %-percentiles (lower and upper border of box), 10 %- and 90 %-percentiles (lower and upper border of leg) and single values (circles) outside of percentile range.

	N	**Lower 2 Tertiles (<-0.0952 and <1.1162)**	**Upper Tertiles (≥1.1162)**	P-Value*
Age, y	30	57.5 ± 11.3	59.1 ± 6.7	0.685
Sex, n (%) female	11	6 (55)	5 (45)	0.425
Height, m	30	1.7 ± 0.1	1.7 ± 0.1	0.589
Weight, kg	30	84.5 ± 19.3	79.5 ± 17.8	0.501
Waist, cm	28	107.5 ± 46.2	95.9 ± 10.1	0.465
BMI, $(kg/m)^2$	30	28.8 ± 5.4	27.7 ± 3.3	0.567
Obesity, n (%)	10	8 (80)	2 (20)	0.419
Abdominal Obesity, n (%)	13	8 (62)	5 (38)	0.505
Smoker, n (%)	21	12 (57)	9 (43)	0.204
Coronary Artery Disease**, n (%)	1	1 (100)	0 (0)	1.000
Hypertension, n (%)	13	11 (85)	2 (15)	0.119
Diabetes, n (%)	4	4 (100)	0 (0)	0.272
Dyslipidemia, n (%)	13	8 (62)	5 (38)	0.705
Statin Therapy, n (%)	5	3 (60)	2 (40)	1.000
ß-Blocker Therapy, n (%)	6	6 (100)	0 (0)	0.074
Aspirin Therapy, n (%)	5	5 (100)	0 (0)	0.140
ACE-Inhibitor Therapy, n (%)	4	3 (75)	1 (25)	1.000
ASA Risk Score >2, n (%)	28	19 (68)	9 (32)	0.615
Lee Cardiac Risk Index >2, n (%)	3	3 (100)	0 (0)	0.107
Chemotherapy, n %	13	7 (54)	6 (46)	0.255
Radiationtherapy, n %	10	7 (70)	3 (30)	0.101
PreOp Echo/EF, %	17	61.4 ± 3.8	62.4 ± 6.9	0.709
PreOp Hemoglobin, mg/dl	30	13.3 ± 1.0	13.5 ± 1.4	0.705
PreOp Fasting Glucose, mg/dl	30	106.7 ± 28.6	95.8 ± 12.0	0.260
PreOp Creatinine, mg/dl	28	1.0 ± 0.3	1.0 ± 0.2	0.509
Length of Hospital Stay, d	30	11.2 ± 11.7	12.6 ± 18.32	0.800
Length of ICU Stay, d	30	2.4 ± 9.0	6.3 ± 19.9	0.462

Table 7. Clinical characteristics and tertiles of TR % change after exercise (pre/post exercise difference); * Fisher's Exact Test, **Patient status post myocardial infarction (with or without intervention)

4. Discussion

4.1. The effect of preoperative exercise on EPC

We found that acute preoperative exhaustive exercise, as a 'physiologic stressor', induces an increase in EPC but not mature EC lineages.

This bone marrow-derived mobilization likely reflects the regenerative capacity of the patient. As such, secondary analyses found a 'dose-response' effect, with fewer postoperative complications as patients exhibited increasing circulating levels of EPC following exercise (responders).

Our underpinning hypothesis is that the surgical stress response is phasic, with both humoral and cellular components, and that all phases are integral to an optimal surgical outcome. Phasic components include: early fight/flight phase (adrenaline, cortisol, mobilization of erythrocytes and leukocytes and their precursors for oxygen transport and immune functions), intermediate procoagulant phase, and a late profibrinolytic 'reperfusion' and repair phase with mobilization of various cell lineages to restore anemia and tissue injury (including EPC cell lineages).

Endothelial dysfunction is recognized as a risk predictor for adverse cardiovascular events[111-114], adverse postoperative events[7], and increasingly implicated in the pathogenesis of sepsis[115,116] and acute lung injury[117,118]. Patients with impaired EPC mobilization are more likely to have underlying endothelial dysfunction. Evidence that endothelial dysfunction and bone marrow responsiveness contribute to adverse outcome (and the converse, that endothelial health promotes recovery) is found in both animal models and in humans. In a murine model of LPS-induced acute lung injury, bone marrow-derived progenitor cells sequester within the inflammatory site and differentiate into alveolar epithelial and capillary endothelial cells[119]. Suppression of progenitor cells by sub-lethal irradiation of the bone marrow impaired recovery, resulting in emphysema-like changes. Reconstitution of the bone marrow prevented these changes[119]. In humans, critically ill patients with pneumonia[85], acute lung injury[120] and sepsis[75] responded with increased levels of circulating EPC. Those patients exhibiting lowest levels of EPC had persistent pulmonary fibrotic changes despite recovering from pneumonia and also poorer survival rates after acute lung injury and sepsis[85,120]. These studies support a prognostic value to the magnitude of EPC release in response to a stressor (e.g. surgical trauma or sepsis).

A prospective analysis of patients undergoing vascular surgery demonstrated that preoperative endothelial dysfunction, as assessed by flow-mediated dilation, also provides independent prognostic information[7]. Risk for cardiovascular events within 30 days of surgery was 5-fold higher in those patients with flow-mediated dilation in the lower 2 tertiles (<8%) than among those in the upper tertile (odds ratio 4.9; 95% CI 1.5–16; p=0.009).

Preserved endothelial function had 95% sensitivity and 98% negative predictive value for cardiovascular events.

These studies emphasize that the vascular endothelium, a critical sensor-effect or organ interfacing between the blood vessel itself and blood-borne elements in all organs, lies central to vascular-hemostatic-inflammatory homeostasis. The 'dose-response' relation observed in our data, with decreasing incidence of postoperative complications observed with increasing bone-marrow responsiveness and EPC mobilization, supports causation. Whether endothelial dysfunction and impaired EPC mobilization and that of other cell lineages is an epiphenomena or causative needs further investigation in terms of mechanism, prognostic value, and therapeutic interventions. Identification of non-responders to an elective 'physiologic stressor' such as exercise prior to surgery may allow for improved preoperative risk stratification and potentially facilitate timely preoperative optimization (e.g. preoperative exercise therapy ('prehabilitation'), statin therapy to promote EPC mobilization through its pleiotropic effects[67], or cell regenerative therapies) to potentially improve surgical outcomes and reduce healthcare expenditure.

Exercise[100] and tissue insult from surgery[121] is known to increase the mobilization of EPC. Exercise may enhance endothelial function through: shear stress-associated improvement of endothelial function, with increased endothelial nitric oxide synthase (eNOS) expression and phosphorylation[122,123]; attenuation of vascular oxidative stress by higher local extracellular superoxide dismutase activity[124] and recruitment of bone marrow-derived circulating progenitor cells to the injured endothelial layer to either promote endothelial repair via paracrine mechanisms or differentiate into mature endothelial cells[125]. The short-term effect of exercise on the release of EPC has been reported in volunteers performing exhaustive dynamic exercise with blood samples obtained 5-10 minutes after symptom-limited exercise testing[100]. Further, studies have reported a significant increase in circulating EPC levels after exercise training, ranging in duration from seven days to one year[93,126-128]. Importantly, this stimulatory effect of exercise on EPC mobilization has been reported not only in trained athletes[129] and healthy subjects[92], but also in patients with known cardiovascular disease[93]. The duration and intensity of exercise needed to adequately stimulate EPC, however, still remains unclear[95].

Laufs et al. reported increased circulating EPC levels after training with 30 minutes of moderate exercise (80-100% velocity of the individuals' anaerobic threshold) but not after short term (10 minutes) of running in healthy subjects[92]. In elderly patients, with documented coronary artery disease, a 4-week exercise program increased circulating EPC levels, while a more recent study showed that a shorter (15 days) cardiac rehabilitation program increased EPC in relation to improved exercise capacity[130]. A 3-month cardiac rehabilitation program reported a 2-fold increase in circulating EPC levels

and a 3-fold increase in colony forming units, with an increase in blood nitrite concentrations and a reduced EPC apoptosis[94].

Our study has several limitations. (1) The study design was chosen to be observational and exploratory in nature therefore cautious interpretation of the results is required due to the lack of characteristics of a randomized, controlled trial. (2) Our data does not allow us to speculate about the possible effect of exercise on clinical improvement of endothelial function. Although we were able to show that the number of EPC increases shortly after exercise, it remains uncertain whether mobilized EPC are fully functional and able to restore endothelial function. Uncertainty remains, whether these cells are able to travel from the bone marrow to the blood vessels and are immured into the endothelial layer, as part of a homing process. (3) The investigation period of the EPC analysis was relatively short and limited to the first 10 minutes after peak exercise. The long-term effects of exercise on EPC release were not tested in our study. (4) Although all patients exercised to their peak ability and above their anaerobic threshold, variability in the intensity of exercise being performed might have influenced the results.

In conclusion, a preoperative 'physiologic stressor' of short, exhaustive exercise resulted in a cellular 'stress response' with increased peripheral circulating EPC levels. Patients with impaired mobilization suffered a greater incidence of postoperative complications. To our knowledge, this is the first trial that investigates the preoperative regenerative capacity of surgical patients. Identification of 'non-responders' to exercise or other physiologic stressors prior to the anticipated insult of major surgery may allow for improved preoperative risk stratification and potentially facilitate timely implementation of preoperative optimization strategies to potentially reduce postoperative complications and thus healthcare expenditure.

4.2. The effect of exercise on reactive hyperemia, a surrogate marker of endothelial function

The principal finding of our reactive hyperemia study, as measured by DTM, is that a single episode of acute exercise above the anerobic threshold enhanced the reactive hyperemia, a surrogate marker of endothelial function. These results imply that a short period of exercise enhances cutaneous perfusion and is associated with an increase in the release and / or bioactivity of endogenous vasodilatative mediators (e.g. NO) in the endothelial cells of skin vasculature. We were able to measure this short-term effect with the use of DTM of temperature rebound (TR), which provides a non-invasive assessment of vascular function.

However, the diagnostic value of our findings in terms of preoperative assessment of endothelial dysfunction warrants further research. Although we found that patients with

preoperative cardiac risk factors and postoperative complications were within the lower 2 tertiles of the study population (ΔTR <1.1%), this observation needs to be validated in a larger patient population.

In agreement with our findings, previous work has demonstrated that acute exercise increases skin blood flow and cutaneous vascular conductance accompanied by enhanced plasma NO metabolite levels and acetylcholine-induced cutaneous perfusion[131]. These authors suggested that endothelium-dependent dilation in skin vasculature is enhanced by moderate exercise training and reversed to the pretraining state with detraining.

Furthermore, our observations suggest that this effect can be reproduced by a single episode of exercise above the anerobic threshold increasing the aerobic capacity and vascular responsiveness to acute exercise.

In contrast, a previous study investigating on the effect of 6 month of aerobic exercise in patients with type 2 diabetes mellitus was not able to show an improvement of microvascular dysfunction[132]. The authors interpreted their negative results with the hypothesis that micro- and macrocirculation respond differently to the exercise stimulus. We were able to observe a significant increase of reactive hyperemia after a short exercise stimulus. However, it remains unclear how long this effect would have lasted on and we suggest that our observed physiological response to exercise has rather diagnostic than therapeutic value.

Our study has implications for preoperative assessment of endothelial function, as the observed increased reactive hyperemic signal shortly after exercise may serve as a diagnostic tool. Impairment of endothelial function is a precursor for cardiovascular disease and precedes the morphological changes associated with atherosclerosis in the blood vessels[133] and the clinical manifestations of its associated complications (e.g. myocardial infarction, stroke)[134,135]. Furthermore, any transient inflammatory burden or a systemic inflammatory state also adversely affects endothelium-dependent vascular function with consequent increase in risk for cardiovascular complications[25,136]. In the perioperative context, inflammatory mediator release associated with surgical trauma, has been shown to impair vascular function and correlate with both the duration and extent of major surgery[25,75,136-138]. This effect may be additive to the underlying endothelial dysfunction that is inherent in certain surgical patients as a result of their preoperative co-morbidity burden and thus plays a significant role in certain perioperative complications (e.g. perioperative myocardial infarction, poor wound healing, ALI, sepsis)[75,137].

Based on our results, we suggest that the preoperative assessment of endothelial function using reactive hyperemia in response to exercise, gains clinical importance as a potential risk assessment tool in the prevention of perioperative complications and should be further studied in a larger patient population.

4.3. The prognostic value of BART in a perioperative setting

Our BART pilot study adds to the current body of knowledge by introducing preoperative microvascular dysfunction as a potential method for improved preoperative risk assessment in the non-cardiovascular surgical population. In patients undergoing major thoracic surgery, those with poor microvascular function, characterized by low FMD or low PSV, were at greater risk for postoperative complications; these included wound healing and surgical complications (including prolonged air leak or esophageal leak, which may reflect 'internal' wound healing) resulting in a significantly greater length of ICU and hospital stay.

Microvascular function was evaluated by BART. This non-invasive, ultrasound-based method measures flow-mediated changes in the brachial artery diameter and is a validated endothelial-dependent measure[139]. Flow-mediated changes in artery diameter are secondary to hyperemia that result in shear stress induced generation of endothelial derived vasoactive mediators, predominantly nitric oxide. Correlation between FMD and endothelial dysfunction in microvessels has been published in several trials[140-142]. In the surgical setting, Gocke et al. reported that BART-derived parameters improved risk prediction for acute and long-term adverse cardiovascular events following vascular surgery[7,35].

Given the ubiquitous nature of endothelium-dependent microvascular function, it is not too surprising that low values of BART-derived variables associate with increased postoperative complications and prolonged ICU- and hospital length-of-stay. Preoperative comorbidities, including age, hyperlipidemia, and neoadjuvant chemoradiotherapy may impair microvascular function, which could impact the incidence of postoperative complications (e.g. wound healing). Several studies report effects of chemotherapeutic agents (i.e. cisplatin, paclitaxel, vinblastine) at the endothelial level preventing tumor neovascularization, endothelization, or causing thrombosis[143-145].

Timely preoperative identification of patients with underlying microvascular dysfunction allows for implementation of strategies to improve microvascular function before surgery, thereby aiming to reduce postoperative complications. Such strategies may include timely smoking cessation, exercise[126,146,147], and possible therapeutic interventions e.g. statins. Dogra et al. reported that FMD improved significantly after statin therapy in patients with diabetes and nephritic syndrome[148,149]. Statins improve microvascular function through numerous pleiotropic effects including up-regulation of endothelial dependent nitric oxide pathway[150-152]. anti-inflammatory effects[153,154], and mobilization of endothelial progenitor cells into the peripheral circulation[155,156]. The effects of such strategies on surgical wound healing and anastomotic dehiscence, however, are unknown and theoretical. Intraoperative strategies, including judicious intraoperative fluid therapy, oxygen delivery and avoidance of vasoconstrictors should be investigated in patients identified with microvascular

dysfunction. Tonetti et al.[25] demonstrated impaired flow-mediated dilation immediately following surgical intervention, with other studies reporting an increased incidence of adverse cardiac events after surgical procedures[157]. As such, anti-inflammatory strategies may preserve microvascular function in the immediate postoperative period.

There are several limitations to our study: 1) The sample size of our prospective, observational study is quite low. While our pilot study is underpowered to show predictability for adverse cardiac outcomes in a major thoracic surgical population, it does identify the optimal size of a dataset (N=165) to adequately (power 0.8) establish the predictive value of BART for postoperative complications in a future study. 2) Our BART data was obtained manually by an operator acquiring digital clips triggered with ECG synchronization. There is controversy in the literature as to what is the best method for obtaining FMD and PSV[45,158]. Some research groups use an automated machine to provide exact and reproducible ultrasound measurements[159]. 3) Our study design was observational and did not allow for any randomization and blinding of patients undergoing major thoracic surgery.

In conclusion, the prevalence of cardiovascular risk factors (i.e. metabolic syndrome with hypertension, diabetes, hyperlipidemia, etc.), predisposing to increased adverse perioperative outcome, is increasing in the non-cardiac surgical population. BART quantifies the underlying impairment in microvascular function associated with these risk factors. Our study supports the utility of BART as a preoperative risk stratification tool in patients undergoing major thoracic surgery, and more specifically for the identification of patients at increased risk for complications of wound healing and surgical complications resulting in a prolonged hospital stay. This pilot study acts as a catalyst for additional larger studies and for discussion concerning the usefulness of BART in patients with subclinical microvascular disease who through timely preoperative identification may benefit from optimization prior to surgery.

5. Summary

An increasing number of patients undergoing surgery is predisposed with multiplex preoperative risk factors including hypertension, diabetes, obesity, dyslipidemia and a proinflammatory state[1]. These factors are known to have a negative impact on endothelial function[2-4], especially the inflammatory cascade after major surgery that places the patient at risk for micro-[5] and macrovascular[6,7] related complications (i.e. cardiovascular, wound healing, pulmonary events) postoperatively.

Therefore, preoperative surrogate markers of endothelial dysfunction, such as BART[8], have gained importance in order to detect patients at risk for endothelial dysfunction-related complications and to adjust perioperative therapeutic strategies to these patients.

These therapeutic strategies may include a preoperative exercise regimen in order to improve patients' physical status and endothelium-related vascular function ("prehabilitation"). Exercise is known to have an effect on the mobilization of EPC, improving their paracrine function, and homing of these cells into the endothelial layer are mechanisms that counteract endothelial damage or dysfunction. The stimulatory effect of exercise on EPC has been shown not only in highly trained athletes[91] and healthy subjects[92] but also in patients with known cardiovascular disease[93]. However, little is known about the benefit of exercise to endothelial health in patients presenting to the operating room with subclinical cardiovascular disease that are predisposed to endothelial dysfunction but not detected by the preoperative anesthesia risk assessment (ASA classification, Lee Cardiac Risk Index and ACC/AHA risk scores). This group of patients may benefit from preoperative exercise by improving endothelial function and preventing endothelial-dependent vascular impairment.

The present work presents preliminary studies that tested the use of diagnostic tools (BART) to detect patients at risk within the preoperative anesthesia setting and further investigated on the effect of preoperative exercise training on endothelial regeneration via EPC.

We found that using BART, preoperative microvascular dysfunction can be identified in patients at increased risk for postoperative complications. Further, we found that preoperative exercise induces EPC into the peripheral circulation. Subjects with a poor EPC response had a pre-existing propensity for postoperative complications, highlighting the important role of bone marrow-released EPC as a critical component to endothelial repair mechanisms. Based on our data, further prospective, randomized, controlled trial studies testing the therapeutic effect of exercise on endothelial function are warranted in order to implement optimization strategies aimed at improving vascular in the perioperative setting.

6. Zusammenfassung

Eine zunehmende Anzahl an Patienten vor chirurgischen Operationen leidet an einer Vielzahl von präoperativen Risikofaktoren wie arterieller Hypertonie, Diabetes Mellitus, Adipositas, Hyperlipidämie und entzündlichen Prozessen[1]. Diese Faktoren beeinträchtigen die Endothelfunktion[2-4], und eine durch das chirurgische Trauma bedingte perioperative, inflammatorische Reaktion verstärkt diesen Effekt. Im Rahmen einer Endothelschädigung steigt das Patientenrisiko, sowohl mikro-[5] als auch makrovaskuläre[6,7] postoperative Komplikationen zu erleiden.

Ziel der wissenschaftlichen Studien im Rahmen der vorliegenden Arbeit war es, die diagnostischen Möglichkeiten zur Analyse der perioperativen Endothelfunktion zu untersuchen, um so eine Verbesserung der Risikostratifizierung von Patienten mit subklinischem Endothelschaden zu bewirken. Untersucht wurden nicht-invasive diagnostische Methoden wie z.B. die Analyse der Reaktivität der Brachialarterie (BART). Basierend auf unseren Daten konnte gezeigt werden, dass durch eine präoperative Untersuchung der Endothelfunktion mit BART, als Surrogatparameter der Endothelfunktion, Patienten mit hohem Risiko für postoperative Komplikationen detektiert werden können. Zudem wurde in der vorliegenden Arbeit der therapeutische Ansatz untersucht, ob eine endotheliale Regeneration auf zellulärer Ebene durch ein präoperatives physiologisches Training positiv beeinflusst werden kann. Basierend auf unseren Daten konnten wir zeigen, dass die Mobilisation von Endothelvorläuferzellen (EPC), welche als pluripotente Stammzellen Potential zur Differenzierung zu maturen Endothelzellen besitzen und somit entscheidend zum Reparaturvorgang des Endothels beitragen, durch eine einmalige physiologische Belastung präoperativ stimuliert werden kann. Ein Anstieg der EPC korrelierte in unseren Untersuchungen mit einem verbesserten postoperativem Ergebnis[97]. Dieser stimulierende Effekt konnte in anderen Studien sowohl an Leistungssportlern[91] und gesunden Probanden[92] als auch an Patienten mit kardiovaskulären Vorerkrankungen[93] nachgewiesen werden.

Wir konnten zeigen, dass nicht-invasive Messmethoden wie BART und DTM zur Quantifizierung der perioperativen Endotheldysfunktion bei Patienten vor großen Operationen als valider Prognosefaktor eingesetzt werden können. Zudem gelang es uns darzulegen, dass bereits eine einmalige präoperative körperliche Belastung die Freisetzung von EPC, welche entscheidend zur Regeneration des geschädigten Endothels beitragen, bewirkt. Dieses vielversprechende Signal wird in einer künftigen translationalen Untersuchung zum Effekt eines präoperativen Trainings auf sowohl zelluläre Mechanismen der endothelialen Regeneration (z.B. via EPC) als auch auf klinische Surrogatparameter der Endothelfunktion (z.B. BART) untersucht werden müssen.

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8. Auszeichnungen

Im Mai 2011 erhielt Herr Dr. Schier auf dem Deutschen Anästhesie Congress (DAC) den **„Klinisch-wissenschaftlichen Forschungspreis 2011“** der Deutschen Gesellschaft für Anästhesiologie und Intensivmedizin e.V. (DGAI), dotiert mit einem Preisgeld von 20.000 Euro. Für seine Arbeit mit dem Thema „Effect of preoperative exercise training on endothelial progenitor cells in patients with metabolic syndrome – a randomised controlled trial” wurde er von einer international besetzten Jury unter dem Vorsitz von Sten G.E. Lindahl, Vorsitzender des Nobelpreis-Komitees für Medizin, ausgezeichnet.
Zudem erhielt er 2011 auf dem amerikanischen Anästhesiekongress (American Society of Anesthesiologists / ASA) in Chicago (USA) die Auszeichnung **“Best Abstract – Clinical Science”** für seine Arbeit “Translational research: Increase of circulating endothelial progenitor cells and reactive hyperemia in response to exhaustive exercise - a predictor of perioperative outcome?”. Die Arbeit wurde 2014 in der hochrangigen Zeitschrift British Journal of Anaesthesiology publiziert[97].

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