

Endothelial progenitor cell mobilization by preoperative exercise: a bone marrow response associated with postoperative outcome

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Editor's key points

- Mobilization of endothelial progenitor cells (EPCs) by preoperative exercise was studied as a predictor of postoperative complications.
- In 60 subjects undergoing major thoracic surgery, the exercised-induced increase in EPC number correlated with reduced postoperative complications.
- Whether this response is causative or associative is unknown, but it could provide a useful marker for preoperative risk stratification.

Background. Preoperative anaemia is associated with increased morbidity in patients undergoing major surgery. Whether erythrocytes are the only bone-marrow-derived cell lineage that associates with increased surgical complications is unknown. This prospective observational trial studied the mobilization of endothelial progenitor cells (EPCs) in response to exercise in association with postoperative complications.

Methods. After IRB approval, 60 subjects undergoing major thoracic surgery were exercised to exhaustion (peak \dot{V}_{O_2}). Peripheral blood collected before and after peak exercise was quantified for EPC lineages by fluorescence-activated cell sorter analysis. Complication analysis was based on the Clavien–Dindo classification.

Results. Exhaustive exercise increased EPC [CD45–133+34+ cells=150 (0.00–5230) to 220 (0.00–1270) cells μl^{-1} ; median change (range)=20 (–4,180–860) cells μl^{-1} ; $P=0.03$] but not mature endothelial cell (EC) subpopulations. Pre-exercise levels [odds ratio (OR)=0.86, 95% confidence interval (CI): 0.37–2.00, $P=0.72$], change after exercise as a continuous variable (OR=0.95, 95% CI: 0.41–2.22, $P=0.91$) and a positive response after exercise (change >0 cells μl^{-1} ; OR=0.41, 95% CI: 0.13–1.28, $P=0.12$) were not statistically significantly associated with the incidence of postoperative complications. Post-hoc receiver operating characteristic curve analyses revealed that subjects with a CD45–133+34+ increase ≥ 60 cells μl^{-1} in response to exercise suffered fewer postoperative complications [86% sensitivity, 48% specificity and AUC=0.67 (95% CI: 0.52–0.81)].

Conclusions. Preoperative exercise induces EPC into the peripheral circulation. Subjects with a poor EPC response had a pre-existing propensity for postoperative complications. This warrants further research into the role of bone marrow function as a critical component to endothelial repair mechanisms.

Clinical trial registration. IRB 2003-0434 (University of Texas M.D. Anderson Cancer Center, Houston, TX, USA).

Keywords: training; vascular

Accepted for publication: 1 February 2014

A bone marrow-derived response that mobilizes cells into the peripheral circulation [e.g. reticulocytes with blood loss, leucocytes with infection, and endothelial progenitor cells (EPCs) for vascular repair] is crucial to regenerative responses that follow injury.¹ This cellular response is less appreciated (compared

with the humoral/endocrine/metabolic response) component of the surgical stress response and the ensuing repair phase. Failure of a cellular response can have detrimental consequence(s). Preoperative anaemia is associated with increased morbidity and mortality in patients undergoing major surgery.²

Whether erythrocytes are the only bone marrow-derived lineage that associates with increased postoperative complications is unknown.

The mobilization of bone marrow-derived EPC plays a key role in vascular/endothelial repair. New vessel formation is regulated by paracrine effects via angiogenesis and endothelial colony-forming cells (ECFCs) potentially differentiate into mature endothelial cells (ECs) with vessel-forming ability.³ Poor mobilization of EPCs into the peripheral circulation associates with reduced survival after critical illness, including septic shock;^{4,5} however, it is unknown whether such poor mobilization is associated with postoperative complications.

Given that short-term exercise mobilizes EPCs into the peripheral circulation in healthy volunteers⁶ and that EPC mobilization in response to critical illness has prognostic value,⁵ we hypothesized that preoperative exhaustive exercise to mobilize EPCs, used as a surrogate stressor to mimic surgical stress, has prognostic potential for patients at risk of postoperative complications and potentially provide a novel therapeutic target for reducing postoperative complications. As such, this prospective observational study evaluated the dynamic bone-marrow responsiveness, characterized by EPC mobilization to a planned preoperative stressor, and whether poor bone marrow response is associated with postoperative complications.

Methods

Study population

After Institutional Review Board (The University of Texas, M.D. Anderson Cancer Center) approval, 60 consecutive adult subjects undergoing major thoracic surgery, including oesophagectomy, or lung resection (wedge resection, lobectomy or pneumonectomy), were enrolled in this prospective observational study. Major thoracic surgery was defined as procedures requiring thoracotomy. Thoracoscopic surgeries were not included taking into account the differences in complication rates between minimally invasive and open surgical procedures. A detailed list of surgery types is presented in Table 1.

Each subject gave written informed consent after receiving a thorough explanation of the study design and protocol. Predefined exclusion criteria included: inability to exercise above their anaerobic threshold (AT), thereby ensuring a valid cardiopulmonary exercise test (CPET) of sufficient exercise load was achieved, and any medical condition that deemed subjects unsatisfactory for surgery after their pre-anaesthetic evaluation, including a recent (<3 months prior) history of myocardial infarction, venous thromboembolism, and 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), dysrhythmias, congestive heart failure, and postoperative requirement of vasopressors; *pulmonary events*, including prolonged intubation, postoperative re-intubation, pneumonia, acute lung injury (ALI), and acute respiratory distress syndrome; *wound healing events*, including wound infection, empyema, and sepsis; and *surgical events*, including prolonged air leak (>5 days), oesophageal leak, and any other re-operative event. Detailed definitions of these postoperative complications are displayed in the Supplementary Appendix. Complications were analysed according to the Clavien–Dindo classification.⁷

A blinded researcher reviewed the medical records for occurrence of these predefined perioperative comorbidities and postoperative complications according to the Clavien–Dindo classification. These data were collected for the period of subjects' hospital stay.

Study design and intervention

Cardiopulmonary exercise testing

Before exercise, baseline observations (heart rate, arterial pressure, pulse oximetry, ECG) and static pulmonary function tests (forced expiratory volume at 1 s, forced vital capacity, maximal voluntary ventilation) were recorded for all subjects. 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, MN, USA) 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 5 min. After acclimation the subject pedalled in the upright position at 60 rpm with minimal resistance (unloaded work) for 3 min. After 3 min, loaded work (increasing pedal resistance, watts per minute) followed a standardized ramp protocol to maximal symptom limited exertion that typically lasted 9–12 min. Exercise was terminated by the subject or by the study investigator if symptoms of cardiovascular, pulmonary distress, fatigue, or both were observed. Gas exchange analysis recorded oxygen consumption (\dot{V}_{O_2} , ml min kg⁻¹) and carbon dioxide production (\dot{V}_{CO_2} , ml min kg⁻¹) at all phases of exercise. AT (ml min kg⁻¹) was defined as peak \dot{V}_{O_2} at the inflection point as determined by the modified V-slope method of plotting carbon dioxide excretion (\dot{V}_{CO_2}) against oxygen uptake (\dot{V}_{O_2}) during increasing exercise intensity, as described by Wassermann.⁸ The peak \dot{V}_{O_2} was defined as the highest oxygen consumption achieved during the exercise test.

To ensure a comparable amount of perceived exertion, subjects were required to reach the individual AT during the exercise session and encouraged during exercise to achieve true peak \dot{V}_{O_2} . Subjects failing to reach the AT were excluded.

EPC analysis by flow cytometry

Blood was collected before and 10 min after peak exercise, using EDTA as an anticoagulant. Blood samples were frozen according to the freezing/thawing procedure described by Norden-Zfoni and colleagues.⁹ In brief, blood was collected in

Table 1 Summary table of subject baseline characteristics and exercise capacity. *Ethnic group was self-reported. AT, oxygen consumption at anaerobic threshold; \dot{V}_{O_2} max, maximal oxygen consumption during exercise

Subject characteristics	All subjects (n = 53)	
	N	%
Age [mean (sd), yr]	60.3 (10.3)	
<50	9	17
50–59	15	28
60–69	22	42
70+	7	13
Gender		
Male	34	64
Female	19	36
Ethnic group		
Caucasian	43	81
Other	10	19
Height [mean (sd), cm]	170.5 (10.7)	
Weight [mean (sd), kg]	82.3 (17.6)	
ASA physical status		
I–II	3	6
III–IV	50	94
Modified Lee Cardiac Risk Index		
2	43	81
3	8	15
4	2	4
Previous neoadjuvant therapy		
None	18	34
Radiotherapy	3	6
Chemotherapy	8	15
Chemoradiation	24	45
Smoking		
Never	15	28
Current/former	38	72
Coronary artery disease		
Yes	29	55
No	24	45
Diabetes mellitus		
Yes	8	15
No	45	85
Hyperlipidaemia		
Yes	9	17
No	44	83
Exercise capacity		
AT (ml min kg ⁻¹)		
Mean (sd)	9.8 (2.7)	
Median (range)	10.0 (5.0–17.4)	
Peak \dot{V}_{O_2} (ml min kg ⁻¹)		
Mean (sd)	17.8 (4.2)	
Median (range)	18.4 (9.1–28.5)	
Surgery type		
Oesophagectomy	26	49
Pulmonary wedge resection (thoracotomy)	1	2

Continued

Table 1 Continued

Subject characteristics	All subjects (n = 53)	
	N	%
Pulmonary lobectomy (thoracotomy)	22	42
Pneumonectomy	4	8
One-lung ventilation	53	100

cell processing tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium citrate and Ficoll and centrifuged at room temperature for 25 min at 1600g within 2 h 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) 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 analysis, thawing was achieved by washing cells using the same storage medium without DMSO, and samples were enumerated within 60 min of thawing. Circulating EPCs and mature ECs were evaluated by six-colour flow cytometry (Fig. 1) using a panel of monoclonal antibodies including anti-CD45 (to exclude non-EPCs), anti-CD133 (an EPC marker), anti-CD31, CD34, and CD146 (mature EC markers). Appropriate analysis gates were used to enumerate viable and apoptotic EPCs. The combination of Syto16 and 7-AAD was used to gain insight into EC viability¹⁰ Necrotic cells were identified as Syto16^{low}/7-AAD⁺, apoptotic cells as Syto16^{low}/7-AAD⁻, and viable cells as Syto16^{bright}/7-AAD⁻.

FACSCanto (Becton Dickinson) 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. ECs were defined as DNA (Syto16) positive, negative for the haematopoietic marker CD45, positive for EC markers CD31, CD34, and CD146 and negative for the EPC marker CD133. EPCs were depicted by the expression of the stem cell marker CD133+. Figure 1 displays a schematic that summarizes the flow cytometry technique.

Statistical analysis

The primary endpoint was defined as the response of EPC (CD45–133+ lineage) levels to peak exhaustive exercise compared with pre-exercise (baseline) levels. Sample size for the EPC analysis was calculated using the short-term effect of exercise on EPC release 10 min after a symptom-limited dynamic exercise test in volunteers.⁶ Secondary endpoints included: changes with exercise in other EPC subpopulations (CD45–133+31+ and CD34–133+34+) and mature EC subpopulations (CD45–133–146+, CD45–133–31+ and CD45–133–146+31+), the incidence of postoperative complications and the severity of complications classified in three categories (Clavien-Dindo grade 0, grade I–III, grade IV–V) over the

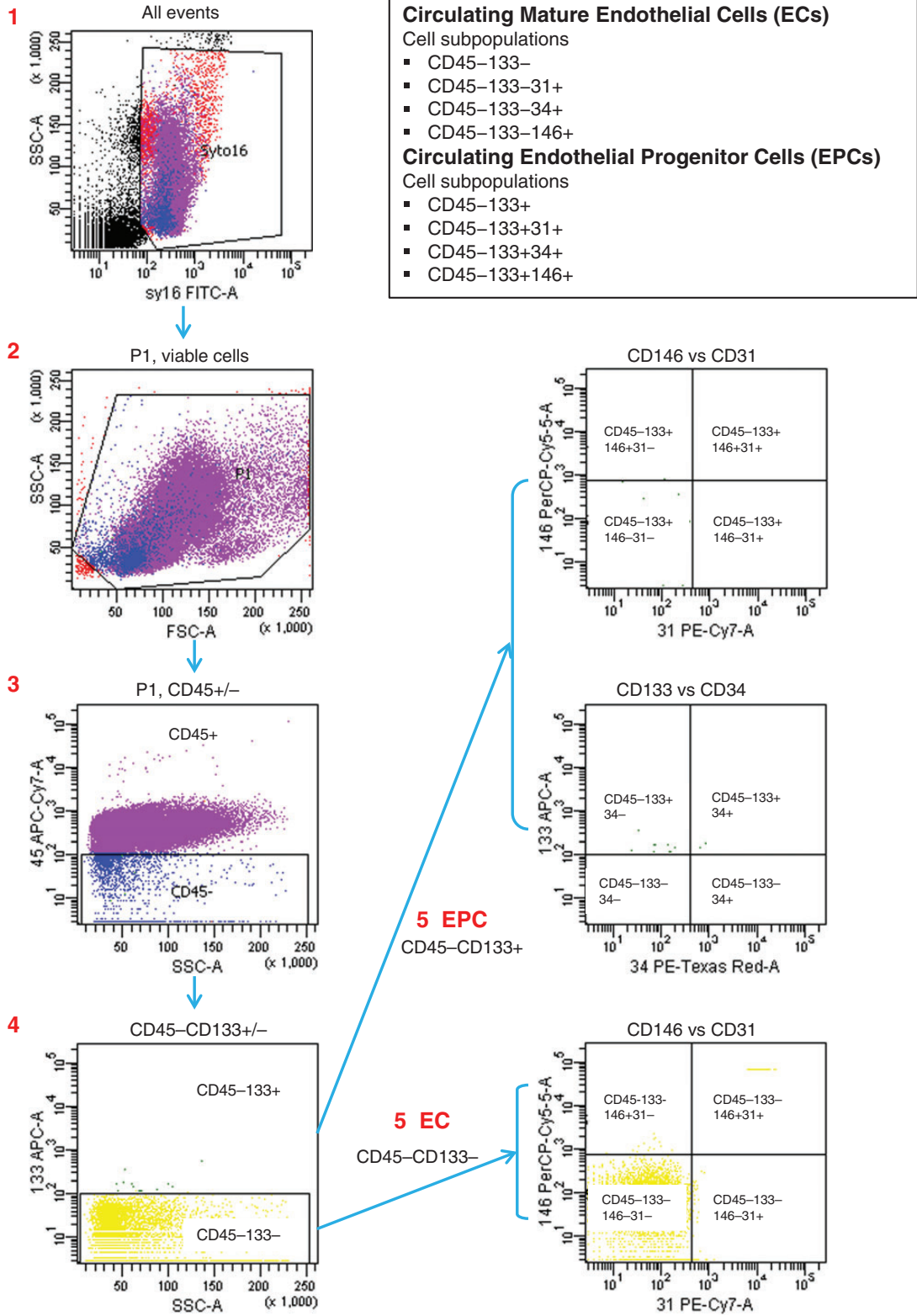


Fig 1 Schematic representation of the strategy used for the quantification of circulating EPCs and ECs by six-colour flow cytometry.

duration of the subjects' hospital stay, and the length of hospital stay.

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 subject baseline characteristics with CD45-133+34+ levels were investigated using analysis of variance (ANOVA), linear regression or non-parametric methods as appropriate. Binary logistic regression was used to investigate univariate associations of changes in CD45-133+34+ levels to peak-exhaustive exercise with the incidence of postoperative complications; odds ratios (ORs) were calculated with corresponding 95% confidence intervals (95% CIs). Post-hoc receiver operating characteristic (ROC) curve analyses were also performed to determine the optimal cut-point for changes in CD45-133+34+ levels to peak-exhaustive exercise in predicting postoperative complications. Ordinal logistic regression was then used to investigate associations of changes in CD45-133+34+ levels to peak-exhaustive exercise with the Clavien-Dindo severity of postoperative complications. A *P*-value of <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).

Results

Sixty consecutive eligible subjects undergoing major thoracic surgery were enrolled in this study (Fig. 2). Three subjects were excluded from data analysis because the procedure was declined for surgical reasons. An additional four subjects were excluded from EPC analysis because they did not have a blood draw because of subject refusal or unavailability of laboratory personnel to process the samples. Subject baseline characteristics are summarized in Table 1.

Circulating EPC and EC levels in response to exhaustive exercise

Compared with baseline levels, exhaustive exercise to peak \dot{V}_{O_2} significantly increased circulating levels of EPC subpopulations [CD45-133+34+ cells: pre-exercise (median [range]) 150 [0.00-5230] cells μl^{-1} to post-exercise 220 [0.00-1270] cells μl^{-1} ; median change [range] 20 [-4180 to 860] cells μl^{-1} ; *P*=0.03] but not that of mature EC subpopulations (Table 2). No statistically significant associations between subject baseline characteristics and change in CD45-133+34+ cell number in response to exhaustive exercise were identified (Table 3).

Incidence of postoperative complications

Univariate associations of changes in CD45-133+34+ cell number in response to exhaustive exercise and the incidence of postoperative complications according to the Clavien-Dindo classification are summarized in Tables 4 and 5. Pre-exercise levels (OR=0.86, 95% CI: 0.37-2.00, *P*=0.72), change after exercise as a continuous variable (OR=0.95, 95% CI: 0.41-2.22, *P*=0.91) and a positive response after exercise (change >0; OR=0.41, 95% CI: 0.13-1.28, *P*=0.12) were

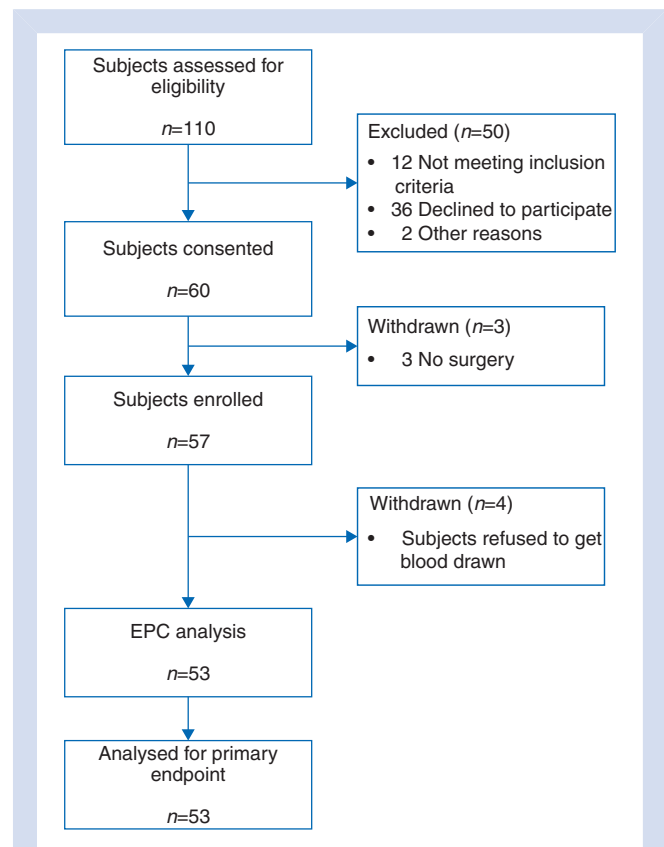


Fig 2 Study flow chart showing subject eligibility and data analysis.

all not statistically significantly associated with the incidence of postoperative complications.

Post-hoc ROC curve analysis identified an optimal cut-off point of 60 cells μl^{-1} for 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). Subjects who exhibited a change in CD45-133+34+ of at least 60 cells μl^{-1} with exercise suffered fewer postoperative complications (17% vs 54%, OR=0.17, 95% CI: 0.04-0.69). These subjects also had a shorter length of hospital stay [median=6, range (2-21) days compared with median=9, range (2-77) days]; however, this did not reach statistical significance (*P*=0.08).

Discussion

The primary analysis of this study found that acute preoperative exhaustive exercise, as a 'physiological stressor', induces an increase in EPC but not mature EC lineages. This bone marrow-derived mobilization likely reflects the regenerative capacity of the subject. As such, secondary analyses found a 'dose-response' effect, with fewer postoperative complications according to the Clavien-Dindo classification in subjects exhibiting an increase in circulating levels of EPC after exercise (responders).

Table 2 Effect of preoperative peak exhaustive exercise on circulating levels of EPC and mature 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 with preoperative levels

	<i>n</i>	Pre-exercise Median (range)	Post-exercise Median (range)	Change (post-pre) Median (range)	<i>P</i> -value
EPC variables (cells μl^{-1})					
CD45 – 133+	53	300 (20 to 6800)	380 (40 to 10 200)	20 (–6430 to 9580)	0.07
CD45 – 133+31+	53	100 (0 to 4440)	140 (0 to 2150)	20 (–3390 to 1140)	0.06
CD45 – 133+34+	53	150 (0 to 5230)	220 (0 to 1270)	20 (–4180 to 860)	0.03
EC variables (cells μl^{-1})					
CD45 – 133 – 146+	53	100 (0 to 3560)	110 (0 to 3510)	10 (–2200 to 3340)	0.26
CD45 – 133 – 31+	53	2010 (120 to 32 300)	2530 (200 to 48 800)	110 (–20 600 to 48 100)	0.33
CD45 – 133 – 146+31+	53	30 (0 to 2190)	20 (0 to 2220)	0 (–1310 to 300)	0.37

Table 3 Univariate associations of subject baseline characteristics and change in circulating EPC (CD45 – 133+34+) levels in response to exhaustive exercise. BART, brachial artery reactivity testing; FMD, flow-mediated dilation; PSV, peak systolic velocity; x+1 identifies the calculation of change in CD45 – 133+34+ per unit change in BART variable (FMD%, PSV%)

	Reference	Comparator	Mean change in CD45 – 133+34+ levels after exercise (cells μl^{-1})	95% CI	<i>P</i> -value
Subject baseline characteristics					
Age	≤60 yr	>60 yr	226	(–127, 579)	0.21
Gender	Female	Male	173	(–197, 543)	0.35
ASA	≤2	>2	23	(–751, 798)	0.95
Lee risk index	≤2	>2	–208	(–662, 246)	0.36
Treatment	None	Chemoradiation	156	(–255, 566)	0.76
	None	Chemotherapy	173	(–386, 732)	
	None	Radiotherapy	372	(–448, 1193)	
Smoking	Never	Former/current	302	(–86, 691)	0.12
Cardiac disease	No	Yes	–124	(–482, 234)	0.49
Diabetes	No	Yes	–418	(–904, 69)	0.09
Exercise capacity and BART variables					
AT	x	x+1	63	(3, 128)	0.06
Peak \dot{V}_{O_2}	x	x+1	38	(–104, 180)	0.60
FMD%	x	x+1	–15	(–116, 85)	0.76
PSV%	x	x+1	2	(–11, 14)	0.79

Our 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—epinephrine, cortisol, mobilization of erythrocytes, and leucocytes 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 anaemia and tissue injury (including EPC cell lineages).

Endothelial dysfunction is recognized as a risk predictor for adverse cardiovascular events,^{11–14} adverse postoperative events,¹⁵ and increasingly implicated in the pathogenesis of

sepsis^{16 17} and ALI.^{18 19} Subjects 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 ALI bone marrow-derived progenitor cells sequester within the inflammatory site and differentiate into alveolar epithelial and capillary ECs.²⁰ 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.²⁰ In humans, critically ill patients with pneumonia,²¹ ALI²² and sepsis⁵ respond

Table 4 Summary of postoperative outcomes

Postoperative outcomes	All subjects (N=53)	
	n	%
Major complications		
Yes	22	42
No	31	58
Cardiac complications		
Yes	11	21
No	42	79
Pulmonary complications		
Yes	11	21
No	42	79
Surgical complications		
Yes	10	19
No	43	81
Clavien complication worst grade		
Grade 0	31	58
Grade I	1	2
Grade II	4	8
Grade IIIa	1	2
Grade IIIb	4	8
Grade Iva	5	9
Grade IVb	7	13
Grade V	0	0
Length of hospital stay (day)		
Mean (sd)	10.5 (12.9)	
Median (range)	8 (2–77)	
0–5 days	18	34
6–10 days	19	36
11–20 days	13	25
>20 days	3	6
Length of ICU stay (days)		
Mean (sd)	2.6 (9.9)	
Median (range)	0 (0–60)	
0 days	40	75
1–5 days	8	15
6–10 days	3	6
>10 days	2	4

with increased levels of circulating EPCs. Those subjects exhibiting lowest levels of EPC had persistent pulmonary fibrotic changes despite recovering from pneumonia and also poorer survival after ALI and sepsis.^{21 22} These studies support a prognostic value to the magnitude of EPC release in response to a stressor (e.g. surgical trauma, pneumonia or sepsis).

A prospective analysis of subjects undergoing vascular surgery demonstrated that preoperative endothelial dysfunction, as assessed by flow-mediated dilation, also provides independent prognostic information.¹⁵ Risk for cardiovascular events within 30 days of surgery was five-fold higher in those subjects with flow-mediated dilation in the lower 2 tertiles (<8%) than in those in the upper tertile (OR 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-effector organ interfacing between the blood vessel itself and blood-borne elements in all organs, lies central to vascular–haemostatic–inflammatory homeostasis.

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. 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. Once causation is proved identification of non-responders to an elective ‘physiologic stressor’ such as exercise before surgery might 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,²³ or cell regenerative therapies] to potentially improve surgical outcomes and reduce healthcare expenditure.

Many factors have been described as having important roles in the mobilization of EPCs.^{24 25} Among them are growth factors, such as VEGF, placental growth factor, erythropoietin, and angiopoietin-1, pro-inflammatory cytokines such as GM-CSF and GCSF, chemokines such as stromal cell-derived

Table 5 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^{-1} .

	Postoperative complication				OR	95% CI	P-value
	Clavien grade 0		Clavien grade I–V				
	n	%	n	%			
Pre-exercise CD45–133+34+ levels							
As a continuous variable					0.86*	(0.37, 2.00)	0.72
Change in CD45–133+34+ after 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

factor-1, hormones such as oestrogens, and lipid-lowering and anti-diabetic drugs, and also physical activity.²⁶

Exercise⁶ and tissue insult from surgery²⁷ are known to increase the mobilization of EPCs. Exercise might enhance endothelial function through: shear stress-associated improvement of endothelial function, with increased endothelial nitric oxide synthase (NOS-3) expression and phosphorylation;^{28, 29} attenuation of vascular oxidative stress by higher local extracellular superoxide dismutase activity;³⁰ 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 ECs.³¹ 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 min after symptom-limited exercise testing.⁶ Further, studies have reported a significant increase in circulating EPC levels after exercise training, ranging in duration from 7 days to 1 yr.^{32–35} Importantly, this stimulatory effect of exercise on EPC mobilization has been reported not only in trained athletes³⁶ and healthy subjects,³⁷ but also in subjects with known cardiovascular disease.³³ However, the duration and intensity of exercise needed to adequately stimulate EPC is still unclear.³⁸

Laufs and colleagues reported increased circulating EPC levels after training with 30 min of moderate exercise (80–100% velocity of the individuals' AT) but not after short term (10 min) of running in healthy subjects.³⁷ In elderly subjects, with documented coronary artery disease, a 4 week exercise programme increased circulating EPC levels, while a more recent study showed that a shorter (15 days) cardiac rehabilitation programme increased EPC in relation to improved exercise capacity.³⁹ A 3 month cardiac rehabilitation programme reported a two-fold increase in circulating EPC levels and a three-fold increase in colony-forming units, with an increase in blood nitrite concentrations and a reduced EPC apoptosis.⁴⁰

Our study has several limitations. (i) The study design was chosen to be observational and exploratory in nature; therefore, cautious interpretation of the results is required because of the lack of characteristics of a randomized, controlled trial. (ii) Our data do not allow us to speculate about the possible effect of exercise on clinical improvement of endothelial function. Although we were able to show a quantitative increase in EPCs shortly after exercise, it remains uncertain whether mobilized EPCs 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. (iii) The investigation period of the EPC analysis was relatively short and limited to the first 10 min after peak exercise. The long-term effects of exercise on EPC release were not tested in our study. (iv) Although all subjects exercised to their peak ability and above their AT, 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. Subjects with impaired mobilization suffered a greater incidence of

postoperative complications according to the Clavien–Dindo classification. To our knowledge, this is the first trial that investigates the preoperative regenerative capacity of surgical subjects. Identification of 'non-responders' to exercise or other physiological stressors before the anticipated insult of major surgery could allow for improved preoperative risk stratification and potentially facilitate timely implementation of preoperative optimization strategies to reduce postoperative complications and thus healthcare expenditure.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

Authors' contributions

R.S.: study design/subject recruitment/data collection/data analysis/scientific input/manuscript writing/reviewing. R.E.Z.: cell analysis/data collection/data analysis/scientific input/manuscript writing/reviewing. A.C.: cell analysis/data collection/data analysis/manuscript writing/reviewing. M.C.: data analysis/scientific input/manuscript writing/reviewing. N.R., P.T., J.V.H., R.M.: scientific input/manuscript writing/reviewing. B.R.: study design/data collection/data analysis/scientific input/manuscript writing/reviewing. H-K.W. performed flow cytometry experiments. M.L.: data analysis/scientific input.

Declaration of interest

None declared.

Funding

This study was funded through an International Anesthesia Research Society (IARS) Clinical Scholar Grant awarded to B.R.

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Handling editor: H. C. Hemmings