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Neuraxial anesthesia is less harmful to the endothelial glycocalyx during elective joint surgery compared to general anesthesia[1,2]

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Abstract.
BACKGROUND: Damage of the endothelial glycocalyx (EG) has been described during surgery, but the effect of different anesthesia techniques remains unknown. Perfused boundary region (PBR) evaluated by side-stream dark field (SDF) imaging of the sublingual microcirculation enables *in vivo* EG assessment. PBR values are inversely related to the EG thickness.
OBJECTIVE: The aim of the observational study was to evaluate the changes of PBR in patients undergoing elective joint surgery under general (GA) vs. neuraxial anesthesia (NA). Our hypothesis was that PBR will be lower in patients in NA.
METHODS: Sixty consecutive patients (ASA 1–3) undergoing elective total knee or hip replacement under GA or NA were included in this prospective observational cohort study. PBR in the sublingual microcirculation was recorded in each patient using SDF at two time points - before surgery and 2 hours after surgery.
RESULTS: Before surgery, there was no significant difference in baseline PBR between groups (NA: 1.95 μm (±0.24); GA: 2.02 μm (±0.26; $p = 0.098$). Postoperatively (2 hours after surgery) PBR was significantly increased in both groups with respect to baseline values (NA: 2.09 μm (±0.19), GA: 2.20 μm (±0.25); $p < 0.001$). In the GA group, postoperative PBR values were significantly higher than in the NA group ($p = 0.006$).

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CONCLUSION: Joint surgery led to significant increases of PBR. Patients in the GA group had significantly higher PBR values 2 hours after surgery compared to NA group. This might implicate that NA is associated with less EG damage then GA in elective hip/knee surgery.

Keywords: Endothelial glycocalyx, neuraxial anesthesia, joint surgery

List of abbreviations

EG endothelial glycocalyx
GA general anesthesia
ICU intensive care unit
NA neuraxial anesthesia
PBR perfused boundary region
RBC red blood cells.

1. Background

Endothelial glycocalyx (EG) is a gel-like sugar-based structure, lining the apical surface of endothelial cells [1, 2]. It has been considered as a major regulator of endothelial functions [3–5]. The key role of EG is to modulate extravascular fluid shifts and prevent a direct contact between endothelial cells and circulating inflammatory or pro-coagulant triggers [6]. Importantly, EG represents not only a passive barrier between intravascular compartment and the endothelial surface, but it plays an important role in the regulation of inflammatory processes, ischemia and reperfusion injury, and modulation of endothelial production of nitric oxide by mechanotransduction of fluid shear stress [7, 8]. The delicate nature of its carbohydrate-based structure predisposes EG to be fragile and to respond rapidly to noxious stimulation of various origins and to date, the role of EG in clinical medicine is being increasingly appreciated [3]. In addition the composition and dimensions of EG fluctuates even in a physiological condition mainly due to the shear stress from the flowing blood elements and it is continuously regenerated by the synthesis from the endothelial cells [9]. The current evidence suggests that under virtually all critical conditions (e.g. sepsis, trauma, hypoxia) a distortion of the structure and function of EG may occur which in turn can contribute to the distortion of the microcirculation, thrombosis [10] and the subsequent development of organ dysfunction and organ failure [11, 12].

Visualization of the EG in humans is extremely difficult, mainly because of its microscopic dimension and fragility. Until recently, direct *in vivo* visualization of EG in humans has been unsuccessful. The EG is partially accessible to flowing red blood cells at its luminal side, a portion called the Perfused Boundary Region (PBR). The EG forms a mechanical, protective barrier that limits radial motion of flowing red blood cells towards the endothelial surface. Damage to glycocalyx results in increased PBR, which can be measured in human sublingual microvasculature recordings obtained by one of the imaging techniques currently available, e.g. Sidestream Dark Field (SDF) or just recently introduced Incident Dark Field (IDF) imaging technology [13, 14].

Changes in EG related to anesthesia and perioperative medicine are lacking detailed exploration so far [15] and more information about the effect of different treatment strategies [16] may significantly affect the way we look at anesthesia and related interventions in terms of their possible impact on EG.

2. Objective

The aim of this study was to evaluate PBR value changes as *in vivo* marker of EG thickness in patients undergoing elective hip/knee surgery under general (GA) and neuraxial anesthesia (NA). Our primary hypothesis was that NA affects EG less than GA due to the omission of oxidative stress from general anesthetics.

3. Methods

3.1. Study design and setting

A prospective observational study that has been approved by a local ethical committeeand registered at ClinicalTrials.gov (https://clinicaltrials.gov, ClinicalTrials.gov Identifier: NCT02783443). The study was performed at the University Hospitals Hradec Kralove and Plzen (Czech Republic).

3.2. Participants

Adult patients scheduled for elective total knee/hip replacement under GA and NA (either epidural or spinal) were included in this prospective observational study conducted at two university hospitals. Patients were recruited consecutively during a pre-admission clinic visit from October 2015 to December 2016. Inclusion criteria were: age above 18 years, American Society of Anesthesiologists Physical Status (ASA) 1–3, no history of any oncological disease, and not an active smoker. Patients were investigated after obtaining informed consent. Patients were excluded in the case of surgery cancellation or refusal of the second recording of the microcirculation. After enrolment to the study a designated physician followed patients until hospital discharge. GA group of patients were premedicated with morphine 0.1 mg/kg (Morphine, BB Pharma, Prague, Czech Republic) intramuscular injection. Induction of anesthesia by propofol 2 mg/kg (Propofol, Fresenius Kabi, Bad Homburg, Germany), sufentanil 0.2 μg/kg (Sufentanil Torrex, Chiesi CZ, Prague, Czech Republic) and atracurium 0.4 mg/kg (Tracrium, Aspen Pharma, Dublin, Ireland). The airway was secured by orotracheal intubation. Anesthesia and analgesia was maintained by sevoflurane 0.7–1 MAC (Sevorane, AbbVie, Prague, Czech Republic) in a fresh gas mixture with air and 0.4 F_iO_2 and sufentanil 0.1 μg/kg. Balanced crystalloid solution was used for fluid therapy (Ringerfundin, BBraun, Melsungen, Germany) at basal rate 2 ml/kg/hour with additional boluses as required during surgery according to the blood loss. NA group of patients were premedicated with oral bromazepam 1.5 mg (Lexaurin, Kabu Pharma, Prague, Czech Republic). Subarachnoid anesthesia was done by lumbar pucture (Quinke needle, 25 G, BBraun, Melsungen, Germany) and injection of 2ml of 0.5% levobupivacaine (Chirocaine, AbbVie, Prague, Czech Republic) and 2.5 μg of sufentanil intrathecally. Balanced crystalloid solution was used for fluid therapy (Ringerfundin, BBraun, Melsungen, Germany) at basal rate 2 ml/kg/hour with additional boluses as required during surgery according to the blood loss and blood pressure drop after subarachnoid blockade. Intraoperative sedation was induced and maintained by midazolam 1 mg intravenously (Accord Healthcare, North Harrow, UK) and sufentanil 5 μg intravenously. Supplementary oxygen was provided by face mask with flow of 5 L/minute. In both groups the blood pressure was kept in a range ± 20% from baseline. If vasopressor was required, an intravenous dose of 10mg of ephedrine (Ephedrin biotika, BB Pharma, Prague, Czech Republic) was used. The tourniquet was used only in knee replacement in patients in NA group on the thigh of the operated leg with pressure of 220 mmHg. The bone cement was used according to the type of alloplastic.

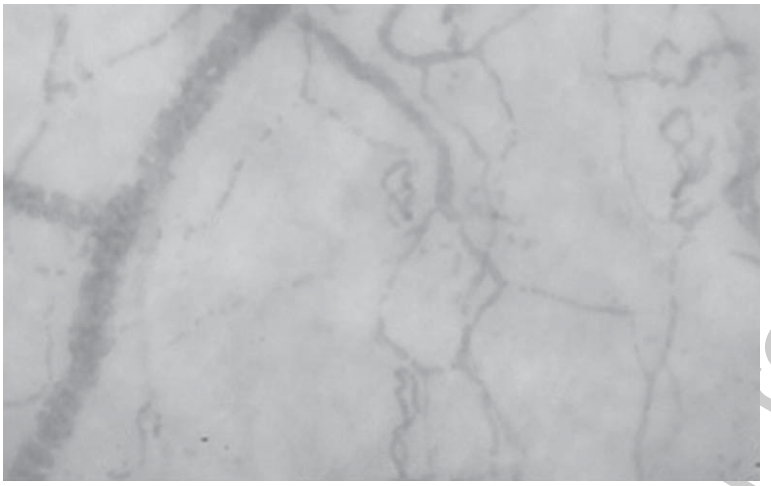

Fig. 1. The original image of the sublingual microcirculation. Original image of the sublingual microcirculation. Surface area: 915x686 Î¼m. Magnification: 325x. Recorded by SDF camera.

The primary outcome of the study was the change in PBR values 2 hours after surgery. Selected clinical and laboratory variables were also recorded preoperatively (age, gender, patient's history - any type of vascular disease, hypertensive on medication, diabetes mellitus (on insulin, oral antidiabetic agents or diet), concomitant medication - beta-blockers, statins, insulin, ACE inhibitors, calcium channel blockers, steroids or immunosuppressing drugs taken longer than one week prior the surgery, routinely ordered laboratory values), during and after anesthesia/surgery (heart rate, blood pressure, pulse oximetry, body temperature, type of anesthesia, type and length of surgery, major and minor intraoperative adverse events (a major adverse event was defined as a complication leading to unplanned admission to intensive care unit (ICU) within 48 hours, a minor adverse event was defined as a complication in the patient documentation that needed therapeutic intervention without admission to ICU), estimated blood loss, amount of fluids/colloids given, amount of transfusion units given during the surgery and up to 2 hours after the surgery.

3.3. Perfused boundary region

For each patient, we scheduled two successive visits at the bedside to obtain recordings of the capillary web of the sublingual microcirculation. The first visit was before surgery (T1) and the second 2 hours after surgery (T2). Patients were given a glass of lukewarm water to rinse the saliva off the sublingual mucosa. On each visit, we performed two recordings from one side of the sublingual area. Microcirculation videos were recorded by specialized hand-held video microscope (KK camera, Research Technology Limited, Alliance Court, Honiton, UK; Fig. 1) connected to a laptop computer with specialized recording and analysis software (GlycoCheck, Maastricht, the Netherlands).

Perfused boundary region (PBR) describes the extent of penetration of the flowing red blood cells (RBC) in μm into the luminal surface of the EG by measuring the radial motion of RBC away from the central flow towards the endothelial cells. The more the EG is injured, the deeper RBC penetrates into the glycocalyx and the higher the PBR is. The software automatically measures PBR in vessels of diameter ranging from 5 to 25 μm (capillaries exclusively) and the resulting number stands for an average of PBR that is corrected for the potential changes in the distribution of vessel diameters. A detailed description of PBR calculation has already been described elsewhere [17]. Briefly, the software identifies all available vessels and places 10 μm long vascular segments along them (Fig. 2).

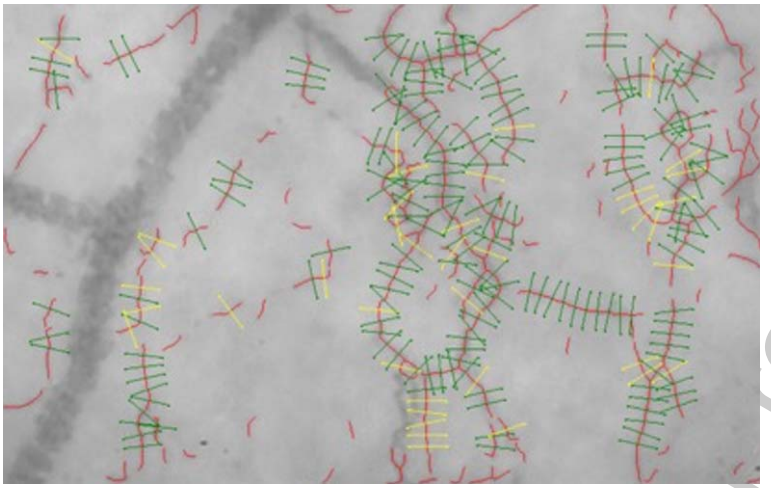

Fig. 2. Image of the software processing of the Fig. 1. Image processing by GlycoCheck analysis software. Red lines trace all available vessels with diameters from 5 to 25 Âμm. Green lines are demarking vascular segments selected for the analysis. Yellow lines are bordering invalid segments.

The recording is stopped when 3000 segments are acquired in focus and without movement. Then the software selects segments with sufficient contrast with the background and counts the median RBC column width and its distribution from the intensity profile. From this intensity profile, the perfused diameter of the vessel is calculated by a linear regression analysis. The PBR stands for the distance between RBC column width and perfused diameter according to the equation: (Perfused diameter – median RBC column width)/2.

3.4. Statistical analysis

Based on the expected difference in PBR between patients under GA and NA, the alpha error I = 0.05 and study power = 0.99, sample size calculation was 52 patients. We aimed to enrol 60 consecutive patients to reach 30 patients with each type of anesthesia, either general anesthesia or neuraxial.

For statistical analysis, we used Graph Pad Prism v6.0c (GraphPad Software, Inc., CA, USA). All data were tested for normality prior testing by D'Agostino-Pearson omnibus normality test. Data are expressed as mean (and standard deviation) or median (and interquartile range), a value of $p \leq 0.05$ was considered as statistically significant for paired and unpaired *t*-tests. Z-test we used for two populations proportion analysis.

4. Results

Sixty-three patients were initially enrolled; one patient was canceled before surgery due to a myocardial infarction and two patients refused to have their microcirculation be recorded again due to the irritating sensation in the mouth and nausea during the first recording. Thirty patients underwent hip/knee replacement surgery under general anesthesia (GA group) and thirty patients under neuraxial anesthesia (NA group). Baseline clinical characteristics of both groups are summarized in Table 1, except for bodyweight (significantly higher weight in GA group) there were no significant differences. There were also no significant differences in selected preoperative and postoperative variables - glycemia, natremia, creatinine and hemoglobin levels, and blood loss or fluid balance response. (Table 2).

Table 1
Basic demographic and surgery data

Patients' data	*GA*	*NA*	*p value*
Age [years]	66.63 (7,08)	67.13 (9.81)	0.82
Gender [%]	F 53, M 47	F 47, M 53	0.19
Weight [kg]	91.46 (17.29)	81.30 (19.87)	0.04
Height [cm]	172.10 (8.42)	168.60 (10.92)	0.17
ASA			
I	2	0	0.07
II	21	19	0.54
III	7	11	0.13
Type of sugery			
Hip replacement	18	21	0.21
Knee replacement	12	9	0.21
Prime - implantation (hip or knee)	27	24	0.14
Revision surgery (hip or knee)	3	6	0.14

Baseline clinical characterictics of both groups. GA - general anesthesia, NA - neuraxial anesthesia. ASA - American Society of Anesthesiologists physical status. M - male, F - female. Data are presented as mean (standard deviation).

Table 2
Selected laboratory and intraoperative course data

Patients' data	*GA*	*NA*	*p value*
Glycemia [mmol/l]	5.4 (5–6)	5.55 (4.88–6.43)	0.53
Natremia [mmol/l]	140 (2.42)	139.50 (2.35)	0.42
Creatinine [mmol/l]	73 (62.70–77.00)	77 (67.50–86.50)	0.17
Hemoglobin [g/l]	140.90 (13.72)	139.30 (15.97)	0.68
Hematocrit [%]	41.23 (3.72)	42.03 (4.46)	0.45
Blood loss [ml]	400 (237.50–600)	300 (300–500)	0.68
Fluid balance [ml]	1175 (909.80–1638)	1550 (975–1800)	0.22

Variables related to the intraoperative course. GA - general anesthesia, NA - neuraxial anesthesia. Data are presented as mean (standard deviation) or median (interquartile range).

Patients' medical history is summarised in Table 3. The tourniquet was used in knee replacement surgery in patients in NA (9 patients) as well as bone cement. Before surgery, there was no significant difference in PBR between groups (1.95 μm (±0.24) in NA vs. 2.02 μm (±0.26) in GA, $p = 0.098$). Two hours after surgery PBR significantly increased in both groups with respect to baseline values (NA: 2.09 μm (±0.19), $p < 0.001$; GA: 2.20 μm (±0.25), $p < 0.001$). Moreover, PBR significantly differed between NA and GA group 2 hours after surgery ($p = 0.006$) (Table 4, Fig. 3). In NA group, 3 patients (10%) were reported to have a postoperative adverse event. In GA group 14 patients (47%), the difference reached statistical significance ($p < 0.001$). Major adverse events were a pulmonary embolism (one patient in NA group), myocardial infarction (one patient in NA group), deep venous thrombosis (one patient in GA group) and supraventricular arrhythmia (one patient in GA group). Table 5 summarizes minor postoperative adverse events in both groups.

Table 3
Summarisation of patients' medical history

Patients' history	*GA*	*NA*	*p value*
Hypertension	21	18	0.42
Antihypertensive drugs	21	18	0.42
Diabetes mellitus	5	9	0.22
PAD/insulin	3	7	0.16
Dyslipidaemia	9	10	0.78
Statins	9	10	0.78

GA – general anesthesia, NA – neuraxial anesthesia, PAD – per oral antidiabetic drugs.

Table 4
The endothelial glycocalyx analysis data

PBR [μm]	*GA*	*NA*	*p value*
PBR	2.02 (0.26)	1.95 (0.24)	0.098
PBR_2	2.20 (0.25)	2.09 (0.19)	0.006
		PBR – PBR_2	<0.001
	PBR – PBR_2		<0.001

Results of the endothelial glycocalyx measurement. PBR - perfused boundary region, PBR_2 – perfoused boundary region in 2 hours after the surgery, GA - general anesthesia, NA - neuraxial anesthesia. Data are presented as mean (standard deviation).

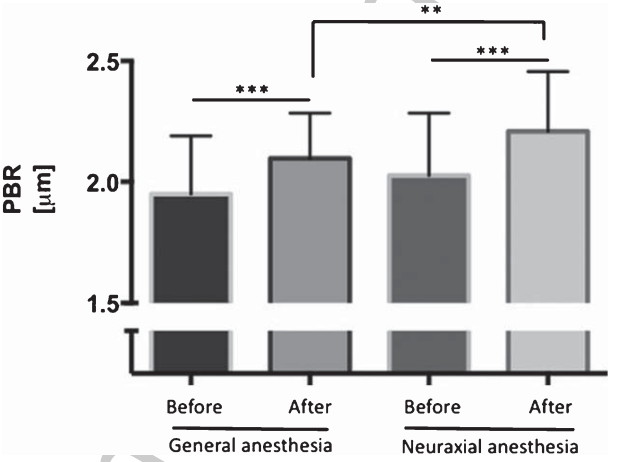

Fig. 3. Graph of the endothelial glycocalyx analysis data. PBR results in both groups. PBR - perfused boundary region. Data are presented as mean (columns) and standard deviation (error bars).

5. Discussion

Our study showed that elective joint surgery led to increased PBR dimensions and that patients in GA group had higher PBR 2 hours after surgery compared to NA group. We have chosen this interval of 2 hours after surgery because patients had entirely recovered from both types of anesthesia, and

Table 5
Postoperative adverse events

Minor postoperative adverse events	*GA*	*NA*	*p value*
Anemia	2	0	0.07
Obstipation	0	1	0.15
PONV	8	0	0.001
Delirium	2	0	0.07
Total	12	1	<0.001

Summary of minor postoperative adverse events.
PONV - postoperative nausea and vomiting.

in addition EG regenerates in days back to full thickness. Therefore, 2 hours after the surgery, the presumed damage would be best detectable [2]. Since PBR is an indicator of endothelial glycocalyx thickness, obtained data supports the hypothesis that neuraxial anesthesia techniques are associated with a lower extent of EG damage compared to general anesthesia for the given surgical procedure. Different types of surgery under general anesthesia have been associated with glycocalyx injury [18, 19] and a term "endotheliopathy" has been just recently introduced in order to describe changes of vascular endothelium with regard to trauma, which has many common features with elective surgery [20]. There is ongoing discussion whether or not the choice of the anesthetic may affect the outcome after total hip replacement [21–24] and there is limited evidence favouring neuraxial anesthesia techniques in term of better postoperative outcome as shown in a recent systematic review by Johnson et al. [24]. The vascular endothelium is one of the earliest sites of injury during inflammation [25, 26], ischemia-reperfusion injury [25, 27] and trauma [12, 15, 28] - all those events are present during surgery to some extent. Attenuating endothelial injury could be the result of better blocking afferent inputs from the surgical field, reduced systemic inflammatory response to surgery and more efficient sympathetic blockade attributed to neuraxial blockade [29]. However, further mechanisms may play a role - less amount of intravenous anaesthetics given, modulating vascular tone or modulating cell to cell interaction by local anaesthetic agents [29, 30].

Our study has several limitations. Firstly, we used PBR as the only method for the evaluation of EG damage. PBR describes the extent of penetration of the flowing RBC in μm into the EG by measuring the deviation of RBC from the central flow towards the endothelial cells. EG damage results in deeper penetration of RBC and is reflected by increased PBR value. Despite the fact that PBR was used as an indicator reflecting glycocalyx thickness in various clinical scenarios [9, 35–38], the current report of peri-operative glycocalyx changes would benefit from combining PBR with measuring other humoral markers (e.g. syndecan–1, hyaluronan, heparan sulfate) to estimate glycocalyx shedding *in vivo*. However, none of these circulating glycobiological markers are specific to endothelia. Moreover, they are strongly affected by extravascular tissue damage and changes in hepatic function which is a major obstacle to their use in clinical medicine [26, 35]. Secondly, alterations of EG by factors other than the choice of the anesthetics cannot be ruled out - e.g. preoperative fluid status, the fluid amount given during surgery, types of fluids given, blood loss (in our study none of the patients required transfusion in the first two hours after the surgery), chronic diseases, usage of tourniquet and bone cement (in our study 9 patients out of 60) [30, 36, 37]. We presumed hip and knee surgery were comparable in the severity of surgerical trauma. Thirdly, our study was not randomized, and patients entered the study in consecutive fashion at two different departments that may cause bias in patient selection and therefore affect study results.

Due to the key role of EG in humans in protecting the vascular endothelium against capillary leakage, inflammation, and coagulation, the injury of glycocalyx is expected to lead to an increased capillary permeability resulting in tissue edema, a proinflammatory environment, hypercoagulability and loss of vascular reactivity. Any of these mechanisms may contribute to the development of various postoperative non-surgical complications [3, 6, 38]. Limiting damage to the EG by using neuraxial blockade instead of general anesthesia may, therefore, serve as another piece of the puzzle in searching for an explanation why some authors report better outcome after the use of neuraxial anesthesia [26, 39, 40]. In our opinion, our findings support the idea that EG and its damage/dysfunction may play a non negligible role in the clinical outcome of surgical patients. Searching for the "glycocalyx-friendly" anesthesia techniques in order to mitigate EG damage caused by surgery might, therefore, represent an important research area. Preconditioning the EG (e.g. pharmacologically) before major elective surgery may serve as a future target in perioperative medicine.

6. Conclusion

Elective joint replacement surgery led to significant changes of PBR dimensions indicating EG damage during the procedure. Undergoing the procedure under neuraxial blockade was associated with less EG damage compared to general anesthesia as measured by PBR. Preservation of the EG may be associated with a decreased rate of postoperative complications.

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Authors' contribution: David Astapenko acquired half of the data, collected all data, performed statistics, evaluated the results, was responsible for manuscript writing, editing and submission, images, graph, and tables preparation. Jiri Pouska acquired half of the data. Jan Benes was responsible for manuscript editing and evaluation of the results. Roman Skulec was responsible for manuscript editing. Christian Lehmann evaluated the results, was responsible for manuscript editing and language check. Hans Vink was responsible for manuscript editing. Vladimir Cerny set up the study protocol, calculated the size of the patients' cohorts, evaluated the results and was responsible for manuscript writing, editing, and submission.

All authors read and approved the final manuscript.

David Astapenko, MD, provided images of his sublingual microcirculation for illustration. Petra Hirsova, PhD, contributed to the final shape of the manuscript.

Authors'information: Hans Vink is the Chief Scientific Officer of GlycoCheck BV, Maastricht, The Netherlands.

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