(Acta Anaesth. Belg., 2021, 72, 7-15)

A preliminary study to assess neutrophil and endothelial response to knee arthroplasty with the use of a tourniquet : effects of spinal or sevoflurane anesthesia

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Abstract : *Background :* During orthopedic surgery, the use of a pneumatic tourniquet results in side effects secondary to ischemia-reperfusion phenomena. We tested the hypothesis that total knee arthroplasty with a tourniquet is associated with increase in plasma concentrations of biomarkers of neutrophil activation and endothelial injury. The second aim was to compare these changes during spinal or general inhalational anesthesia.
Methods : 40 adult ASA I-II patients scheduled for total knee arthroplasty with a tourniquet under spinal or sevoflurane anesthesia were included. Venous blood samples were collected before surgery, 1 h, 3 h, and 24 h after tourniquet deflation. To assess neutrophil activation, plasma concentrations of total and active fractions of myeloperoxidase, as well as elastase concentrations and proteolytic activity were measured. Endothelial injury was assessed by measurement of plasma concentrations of syndecan-1, soluble thrombomodulin, soluble E-selectin, and vascular endothelial growth factor. Results were analyzed with a two-way analysis of variance. $P< 0.05$ was considered statistically significant.
Results : Plasma concentrations of active but not total myeloperoxidase and elastase significantly increased following tourniquet deflation. The level of syndecan-1, soluble thrombomodulin, soluble E-selectin, but not vascular endothelial growth factor, significantly decreased postoperatively. These changes of biomarkers were similar during spinal and sevoflurane anesthesia.
Conclusions : Total knee arthroplasty with pneumatic tourniquet is associated with systemic release of markers of neutrophil activation which was comparable during spinal or sevoflurane anesthesia. Systemic expression of endothelial injury was not detected in our clinical conditions.

Keywords : Knee arthroplasty ; pneumatic tourniquet ; ischemia-reperfusion ; neutrophils ; endothelium.

Introduction

During total knee arthroplasty (TKA), a pneumatic tourniquet is used to achieve a bloodless surgical field and to improve visualization of anatomical structures. However, a pneumatic tourniquet is associated with deleterious clinical consequences among which limb swelling and pain, increased risk of deep venous thrombosis, nerve palsies, muscle weakness, higher incidence of postoperative wound complications, acute pulmonary embolism, and lung injury (1). Most of the symptoms of this so-called tourniquet syndrome result from ischemia-reperfusion that amplifies surgical inflammatory response and contributes to vascular dysfunction (2). In case of ischemia-reperfusion, activated polymorphonuclear neutrophils play a major role and participate to tissue injury by releasing cytotoxic granular enzymes such as myeloperoxidase (MPO) and elastase (EL). Concomitantly, structure and function of endothelial cells are damaged which allows release of fragments of the glycocalyx such as syndecan-1 (SD-1), surface anticoagulant and adhesion molecules including the soluble form of thrombomodulin (s-TM) and soluble E-selectin (s-ES) as well as mitogenic factors like the vascular endothelial growth factor (VEGF) (3, 4). Volatile

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Paper submitted on December 1, 2020 and accepted on March 10, 2021.
Conflict of interest : None

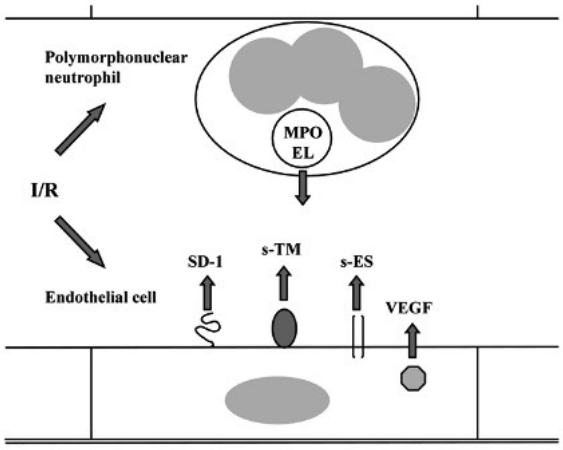

Fig 1. — Schematic illustration of the investigated pathways. The figure shows a simplistic representation of a blood vessel in the operated limb. During tourniquet use, ischemia-reperfusion (I/R) leads to intravascular activation of polymorphonuclear neutrophils and injury to endothelial cells. So, myeloperoxidase (MPO) and elastase (EL) from primary granules of neutrophils are released systemically. Concomitantly, endothelial biomarkers such as syndecan-1 (SD-1), soluble thrombomodulin (s-TM), soluble E-selectin (s-ES), and vascular endothelial growth factor (VEGF) may also be shed in the circulating blood.

anesthetics have cytoprotective immunomodulatory properties in numerous conditions of surgical ischemia-reperfusion (5, 6, 7) and could reduce these biochemical phenomena.

The primary aim of this preliminary study was to evaluate whether TKA with the use of a pneumatic tourniquet is associated with measurable plasma release of MPO and EL as well as SD-1, s-TM, s-ES and VEGF as biological indicators of neutrophil activation and endothelial injury respectively (Fig. 1). The second aim was to compare the changes of these biomarkers in two different anesthetic techniques, spinal or sevoflurane anesthesia.

Methods

Patients, surgical procedures and anesthesia protocols

This study was approved by the Institutional Ethics Committee of the Centre Hospitalier Universitaire de Liège (Liège, Belgium, Chair : Prof. V. Seutin, No. 2018-270) and registered on Clinical Trials (ref : NCT03470363) prior to patient enrolment. Forty adult ASA physical status I-II patients scheduled for TKA with the use of a pneumatic tourniquet gave their written informed consent and were included from 2 April 2018 to 28 December 2018. Exclusion criteria were : age < 18 y, chronic conditions or medications susceptible to affect neutrophil or endothelial functions, acquired immune disorders including diabetes, renal failure and liver cirrhosis, lower limb arterial diseases, cardiorespiratory diseases restricting the functional capacity below 4 metabolic equivalents and pre-operative administration of anti-inflammatory or immunosuppressive drugs. The surgical procedures were performed by the same surgeon using the same techniques and prosthesis. Tourniquet pressure was set at 250 mmHg.

All patients received oral premedication with 0.5 mg alprazolam and 50 mg hydroxyzine, one hour before surgery. In all patients, unilateral ultrasound-guided femoral block was performed pre-operatively and a catheter was inserted to provide postoperative analgesia. After an initial bolus injection of 100 mg lidocaine 1% and 100 mg ropivacaine 1% a continuous infusion of ropivacaine 0.2% at a rate of 7 ml/h was started until the first postoperative day. Anesthesia consisted of either spinal anesthesia (SA group, n=17) or sevoflurane inhalation anesthesia (GA group, n=23). Patients were provided with information about anesthesia techniques during the pre-anesthetic consultation and were assigned to the SA or GA group according to their preference. In the SA group, spinal anesthesia was performed using a 27 gauge needle with an intrathecal injection of 12.5 mg hyperbaric bupivacaine, 2.5 µg sufentanil, and 30 µg clonidine. In the GA group, anesthesia was induced with 10 µg intravenous sufentanil and 1-3 mg/kg propofol and maintained with sevoflurane in a mixture air/oxygen (50%/50%) adjusted to keep the State Entropy and the Response Entropy (GE Entropy™, GE Healthcare, Machelen, Belgium) between 40-50 (8). In both groups of patients, intraoperative mean arterial pressure was kept within 70% to 110% of the pre-induction levels with intravenous administration of 500 ml isotonic crystalloid (Plasmalyte™, Baxter, Lessines, Belgium) or 10 mg ephedrine according to the judgement of the attending anesthesiologist and repeated as required. During spinal and sevoflurane anesthesia, oxygen administration was adjusted to achieve a target oxygen saturation of 94-98%. In both anesthesia groups, postoperative analgesia was provided with systematic administration of oral acetaminophen 1 g four times a day, oral celecoxib 200 mg twice daily, and a continuous intravenous infusion of tramadol 300 mg/day. Rescue analgesia consisted of 10 mg oral oxycodone, up to four times a day, as required.

Measurements

The primary endpoints were total and active plasma MPO concentrations, EL plasma concentration and activity. Secondary endpoints were plasma concentrations of four endothelial biomarkers : syndecan-1 (SD-1), soluble thrombomodulin (s-TM), soluble E-selectin (s-ES), and vascular endothelial growth factor (VEGF). These enzymes and biomarkers were measured upon patient arrival in the operating room (baseline, T0), 1 h (T1), 3 h (T3), and 24 h (T24) after tourniquet deflation.

An ELISA was used to measure total human MPO (MPO ELIZEN, Zentech, Liege, Belgium). The active MPO fraction released by neutrophils was measured by a SIEFED (Specific Immunological Extraction Followed by Enzymatic Detection) method (9). EL plasma concentration was measured by ELISA (ab 119553 kit, Abcam, Cambridge, UK). EL activity in plasma samples was measured with the ab 204730 kit (Abcam, Cambridge, UK). The four endothelial biomarkers were measured by ELISA (10) : syndecan-1 (reference : KA 3851, Abnova, Taipei, Taiwan), soluble thrombomodulin (reference : DTHBD0, R&D Systems, Minneapolis, USA), soluble E-selectin (reference : DSL00, R&D Systems, Minneapolis, USA), and VEGF (reference : DVE00, R&D Systems, Minneapolis, USA).

The following demographic, anesthetic, and surgical data were recorded : patient age, gender, body mass index, duration of anesthesia, surgery and tourniquet inflation, the volume of fluids and red blood cells infused as well as blood loss and hematocrit changes from baseline to the first postoperative day. The following cardiopulmonary parameters were recorded every 15 min during surgery : heart rate, mean arterial pressure, and arterial oxygen saturation. In addition, end-tidal sevoflurane concentration was measured in the GA group.

Statistical analysis

The distribution of numerical values were tested using the Kolmogorov-Smirnov test of normality. Demographic, anesthetic and surgical characteristics were reported as mean $\pm$ SD and analysed with a Student's t-test for quantitative data, and with a Fisher exact test for categorical data. Intraoperative cardiopulmonary parameters were normally distributed and presented as mean $\pm$ SD. Biochemical data were not normally distributed and were presented as median and interquartile range [p25-p75]. Time effect and anesthetic technique effect on cardiopulmonary parameters and on log-transformed biochemical values were analyzed with two-way analysis of variance (ANOVA) for repeated measures followed by Tukey's multiple comparisons test. In addition, a multiple linear regression model was applied and reported with ß regression coefficients and corresponding 95% confidence intervals [95% CI] to assess the relationships between the maximum increase of active MPO as the outcome variable and the following potential confounding factors : duration of surgery and tourniquet inflation, the volume of fluids infused, blood loss and hematocrit drop from baseline to the first postoperative day. $P< 0.05$ was considered statistically significant. A local pilot study determined that active MPO increased from 1.61 [0.83 - 2.94] ng/ml before TKA surgery to 3.71 [1.94 - 7.8] ng/ml 24 hours after tourniquet deflation. We calculated that a sample size of 40 patients would be sufficient to provide a 80% power for detecting a 50% increase of active MPO postoperatively.

Results

Demographic characteristics

Demographic, anesthetic and surgical characteristics are presented in table 1. These variables were similar in both anesthesia groups, except for the duration of surgery that was longer in group GA than in group SA mainly due to differences in the time required to suture wounds.

Plasma concentrations of total MPO

Total MPO concentration in serum was 14.83 [11.09 - 18.45] ng/ml at T0 and did not significantly increase after tourniquet deflation (Fig. 2).

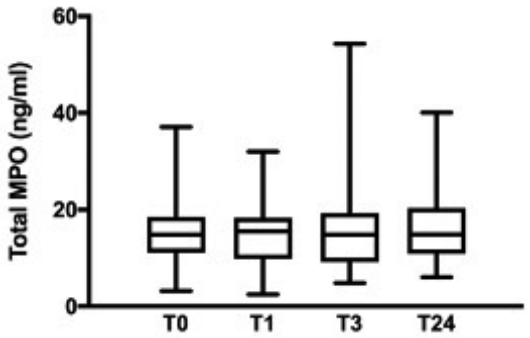

Fig. 2 — The effect of total knee arthroplasty with the use of a pneumatic tourniquet on plasma concentrations of total myeloperoxidase (MPO) at baseline (T0) and 1 h (T1), 3h (T3) and 24 h (T24) after tourniquet deflation. Data are median, boxes 25th and 75th percentiles, whiskers min and max.

Table I

Patients, anesthetic and surgical characteristics in the global population and in the two anesthesia groups. Data are mean ± SD. * P< 0.05 as compared with group SA. BMI= body mass index ; RBC= red blood cells ; Hct= hematocrit.

	All patients *(n= 40)*	Group SA *(n= 17)*	Group GA *(n= 23)*
Male/female	26/14	10/7	16/7
Age (year)	66 ± 8	66.3 ± 6.7	65.9 ± 9.0
BMI (kg/m^2)	29.7 ± 4.6	31.3 ± 5.1	28.5 ± 3.9
Tourniquet time (min)	46.5 ± 10.6	44.2 ± 7.2	48.1 ± 12.5
Operative time (min)	95.6 ± 15.5	89.6 ± 10.7	100.1 ± 17.1*
Fluid infusion (mL)	2155 ± 375	2063 ± 250	2223 ± 438
RBC transfusion (mL)	0	0	0
Blood loss (mL)	296 ± 151	300 ± 124	294 ± 151
Hct changes (%)	- 8.1 ± 2.9	- 8.4 ± 3.3	- 8 ± 2.7

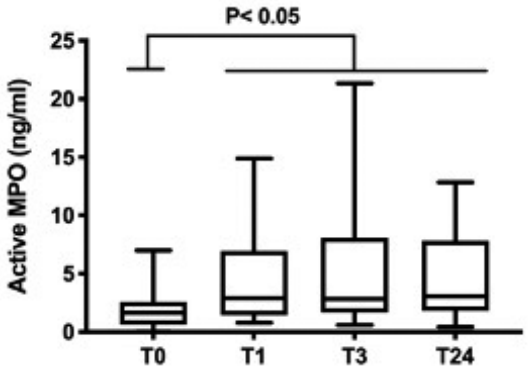

Fig. 3 — The effect of total knee arthroplasty with the use of a pneumatic tourniquet on plasma concentrations of active myeloperoxidase (MPO) at baseline (T0) and 1 h (T1), 3 h (T3) and 24 h (T24) after tourniquet deflation. Data are median, boxes 25th and 75th percentiles, whiskers min and max.

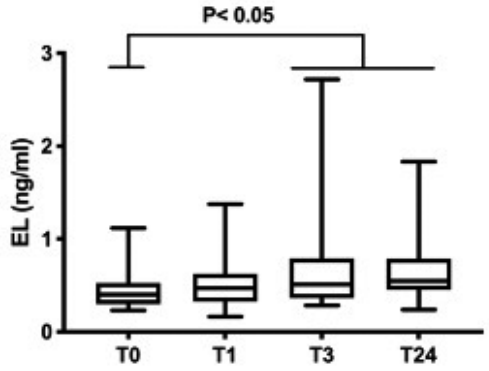

Fig. 4 — The effect of total knee arthroplasty with the use of a pneumatic tourniquet on plasma concentrations of elastase (EL) at baseline (T0) and 1 h (T1), 3h (T3) and 24 h (T24) after tourniquet deflation. Data are median, boxes 25th and 75th percentiles, whiskers min and max.

Plasma concentrations of active MPO

Active MPO in serum was 1.77 [0.69 - 3.44] ng/ml at T0. After tourniquet deflation, we observed a significant increase of active MPO that reached 2.9 [1.46 - 6.95] ng/ml (P= 0.0035) at T1, 2.87 [1.73 - 8.79] ng/ml (P= 0.0014) at T3 and 3.06 [1.83 - 7.82] ng/ml (P = 0.0016) at T24 (Fig. 3). This increase of active MPO did not differ between anesthesia groups. There was no association between the maximum increase of active MPO and the duration of surgery (ß= – 0.04 [– 0.35 - 0.28]), tourniquet time (ß= – 0.28 [– 0.54 - 0.03]), volume of fluids infused (ß= 0.01 [– 0.31 - 0.33]), blood loss (ß= 0.04 [– 0.29 - 0.36]) or hematocrit changes (ß= 0.15 [– 0.17 - 0.44]).

Plasma concentration of EL

Plasma concentration of EL in serum was 0.40 [0.30 - 0.52] ng/ml at T0 and showed a non-significant increase to 0.47 [0.32 - 0.62] ng/ml one hour after tourniquet deflation. EL concentrations were significantly increased 3 hours (0.51 [0.36 - 0.79] ng/ml, P= 0.0129) and 24 hours (0.55 [0.45 - 0.79] ng/ml, P= 0.0005) after tourniquet deflation (Fig. 4). This increase in EL concentrations was not different between the two anesthesia groups.

Proteolytic activity of plasma EL

No proteolytic activity of EL was detectable in plasma sampled before surgical incision and after tourniquet deflation.

Table II

Plasma concentrations of syndecan-1 (SD-1), soluble thrombomodulin (s-TM), soluble E-selectin (s-ES) and vascular endothelial growth factor (VEGF) at baseline (T0) and 1 h (T1), 3h (T3) and 24 h (T24) after tourniquet deflation. Data are median and interquartile range (75th and 25th percentiles). * P< 0.05 compared with basal pre-anesthetic levels.

	T0	T1	T3	T24
SD-1 (ng/ml)	35.6 (22.2-49.2)	25.4 (17.7-32.1)*	27.9 (21.1-40.8)*	36.2 (25.9-63.1)
s-TM (ng/ml)	3.3 (3-4.4)	3.1 (2.6-3.7)*	3.1 (2.5-3.9)*	3.5 (3-4.1)
s-ES (ng/ml)	30.9 (19.1-44.3)	25.7 (18.9-36)*	26.8 (18.4-35.7)*	24.2 (18.2-32)*
VEGF (pg/ml)	118.5 (55.4-210.1)	83.1 (46.6-145.1)	101.3 (49.5-190.2)	107.5 (66.5-139.7)

Table III

Intraoperative cardiopulmonary parameters

Time (min)	0	15	30	45	60	75	90	105	120
HR (beats/min)	66±11	61±11*	57±9*	57±9*	58±10*	58±12*	60±11*	61±10*	60±11*
MAP (mmHg)	109±14	83±17*	78±15*	83±14*	81±15*	81±13*	76±15*	77±14*	75±12*
SpO_2 (%)	97±2	98±2	98±2	98±2	97±2	98±2	97±2	97±2	97±2
ET SEVO (%)	0	1.2±0.4	1.5±0.6	1.8±0.4	2.1±0.4	2.1±0.2	2.0±0.3	1.8±0,4	1.4±0.7

Data are presented as mean ± SD. HR= heart rate ; MAP= mean arterial pressure ; SpO_2= arterial oxygen saturation ; ET SEVO= end-tidal sevoflurane concentrations in group GA. * P < 0.05 compared with basal pre-anesthetic levels.

Plasma concentrations of endothelial biomarkers

Serum concentrations of endothelial biomarkers are shown in table 2. As compared with pre-anesthetic levels, SD-1 and s-TM decreased significantly at 1 hour and at 3 hours following tourniquet deflation, then returned to baseline levels. Plasma concentrations of s-ES decreased significantly upon the first hour after tourniquet deflation and remained below the baseline levels for at least 24 hours postoperatively. The changes in concentrations of these endothelial biomarkers were not influenced by the anesthesia technique. Plasma concentrations of VEGF remained stable throughout the observation period.

Intraoperative cardiopulmonary parameters

Intraoperative cardiopulmonary parameters are presented in table 3. Compared with baseline preoperative levels, heart rate and mean arterial pressure significantly dropped 15 min following induction of general or locoregional anesthesia, then remained stable until the end of surgery. Mean arterial pressure decreased significantly more in the GA group at 15 min (74 ± 15 mmHg *versus* 95 ± 11 mmHg, P < 0.0001) and at 30 min (69 ± 11 mmHg *versus* 90 ± 9 mmHg, P < 0.0001) than in the SA group. Arterial oxygen saturation remained stable throughout the observation period in all patients. End-tidal sevoflurane concentrations in GA group peaked at 60 min and 75 min after induction of general anesthesia.

Discussion

Main findings

This study demonstrated that total knee arthroplasty with the use of a pneumatic tourniquet results in neutrophil activation evidenced by a rise in blood levels of active MPO and EL after surgery. Spinal anesthesia or sevoflurane general anesthesia were associated with similar neutrophil activation. Finally, biomarkers of endothelial injury, including SD-1, s-TM, s-ES and VEGF, did not increase in the early postoperative period in our clinical conditions.

Biomarkers of neutrophil activation

During orthopedic surgery, bones and soft tissues trauma leads to activation of inflammatory

pathways. Furthermore, the use of a pneumatic tourniquet and the subsequent ischemia reperfusion injury represent an additional inflammatory stimulus dependent on the extent and duration of tissue ischemia (1). Hence, during minor arm surgery, regional (i.e. circumscribed to the operated limb) neutrophil degranulation has been demonstrated in correlation with the duration of tourniquet ischemia although neutrophil activation could not be detected systemically (11). However, during TKA performed with a pneumatic tourniquet, various manifestations of neutrophil activity have been described in the systemic circulation. In a study by Kageyama *et al.* (2) neutrophil-platelet aggregates, a witness of inflammation-mediated cell interactions, were evidenced in arterial blood sampled within 2-3 h following TKA with a tourniquet but not without a tourniquet. Similarly, in the study of Katsumata *et al.* (12) systemic neutrophil degranulation measured on the first postoperative day was eightfold increased in TKA with a tourniquet as compared to no tourniquet. Together, these data suggest that limb ischemia-reperfusion represents the main trigger of the early neutrophil response to TKA with a tourniquet. Accordingly, we report an increase in active MPO and EL in case of TKA with a tourniquet. Although we had no control group without tourniquet, one may speculate that the changes observed are attributable to ischemia-reperfusion secondary to tourniquet, since no signs of neutrophil activation were reported by others in the absence of tourniquet (2, 12). MPO and EL are not only markers of neutrophilic inflammation, but also play a significant role in the pathophysiology of ischemia-reperfusion injury (3, 13). Indeed, MPO, a pro-oxidant enzyme from primary granules is responsible for the oxidation, nitration and chlorination of a wide variety of molecules when released in the extracellular space during inflammation (14). The use of two different but complementary immunological techniques (i.e. ELISA and SIEFED respectively) allows to determine the effects of TKA with tourniquet on both total and active MPO released by neutrophils. Differentiation of active MPO from total MPO is clinically relevant for two reasons. First, active MPO is a more sensitive marker of neutrophil degranulation than total MPO (15). The better sensitivity of the SIEFED assay over the ELISA is further confirmed in the present work where active but not total MPO increased after tourniquet deflation. Second, active MPO may be a more accurate biomarker than total MPO to predict the clinical consequences of neutrophil activation (16, 17). Elastase (EL) is a broad-spectrum serine protease co-localized with MPO in primary granules of neutrophils. Following ischemia-reperfusion, EL can damage various cell types and the extracellular matrix (18). Furthermore, the activating effect of EL on the coagulation cascade may contribute to the increased incidence of thromboembolic events reported when TKA is performed with the use of a pneumatic tourniquet (12). We observed an increased plasma concentration of EL that persisted for at least one day after surgery, but no increased proteolytic activity. Accordingly, elevated plasma levels of EL do not always correlate with an increased protease activity in the blood. Indeed, the potential damaging effects of EL released in the blood is considerably limited by endogenous EL inhibitors, mainly α-1 protease inhibitor, and secondarily α-2 macroglobulin (19). However, these results do not rule out increased EL proteolysis in the extravascular compartment, particularly in the reperfused limb where reactive oxygen species produced locally can inactivate α-1 protease inhibitor and allow the cytotoxic activity of EL (20).

Biomarkers of endothelial injury

Surgical dissection together with ischemia-reperfusion can damage the vascular endothelium (2). In particular, MPO and EL from intravascular activated neutrophils could participate to endothelial injury (21, 22). Several studies have used plasma biomarkers to assess the consequences of tourniquet ischemia on endothelium during orthopedic surgery. After upper limb surgery, Kamat *et al.* (23) showed that a mean tourniquet inflation time of 2h was not associated with changes in plasma levels of SD-1 and heparan sulfate, markers of endothelial glycocalyx injury, during the first 10 min after reperfusion. During knee surgery with 2-hour of tourniquet-induced ischemia, Huda *et al.* (24) reported a decrease in plasma soluble fraction of endothelial ICAM-1 (*Intercellular Adhesion Molecule-1*) during the 4 first hours of reperfusion. In similar surgical conditions, Hallström *et al.* (25) showed that the soluble plasma fractions of the adhesion molecules E-selectin et VCAM-1 (*Vascular Cell Adhesion Molecule-1*) commonly released by activated endothelium did not raise after 90 min of ischemia and two hours of reperfusion. Beyond the duration of ischemia, the volume of ischemic tissues seems critically determinant to detect endothelial damages using systemic biomarkers. So, abdominal aortic aneurysm surgery with infra-renal ischemia of only 40 min resulted in a two-fold and 15-fold increase in plasma concentrations of heparan-

sulfate and SD-1 respectively (26). We measured SD-1, s-TM, s-ES and VEGF to assess potential glycocalyx degradation, endothelium damage, activation and permeability during TKA with a tourniquet (10). Like in the studies of Huda *et al.* (24) and Hallström *et al.* (25) with however duration of tourniquet ischemia twice or more longer than in our patients, we did not observe systemic evidence of compromised endothelium within the first postoperative day.

Effects of anesthesia

We also investigated whether sevoflurane anesthesia may affect the release of active MPO and EL as compared with spinal anesthesia. The impact of the anesthetic technique on the pathophysiological components of the tourniquet syndrome remains uncertain. Luchinetti *et al.* suggested potential immunomodulatory and endothelial conditioning properties of volatile anesthetics in the setting of limb ischemia-reperfusion. In healthy volunteers exposed to 15 min forearm ischemia, inhalation of sedative concentrations of sevoflurane (0.5-1%) resulted in a more limited intravascular activation of leucocytes and a better post-occlusive hyperemic response (27). During knee surgery with the use of a pneumatic tourniquet, Carles *et al*. (28) used muscle microdialysis to demonstrate that sevoflurane anesthesia was more effective than propofol or spinal anesthesia to preserve glycolysis metabolites during ischemia and after reperfusion. However, these interesting results are tempered by studies that focused on the impact of volatile anesthetics on the oxidative stress induced by the pneumatic tourniquet. In adults patients scheduled for TKA under tourniquet, sevoflurane anesthesia has been associated with increased plasma biomarkers of oxidative stress as compared with propofol (29) or spinal anesthesia (30). Similarly in a pediatric population undergoing upper and lower extremity surgery, propofol or regional anesthesia provided better antioxidant defenses against tourniquet induced ischemia-reperfusion than sevoflurane anesthesia (31). In our study, sevoflurane administered before tourniquet inflation and after deflation did not change the systemic increase of active MPO and EL as compared with spinal anesthesia. Of note, this study is likely not adequately powered for comparisons between anesthesia techniques.

Limitations

Our study has several limitations. First, since all patients had TKA with a tourniquet, no firm conclusion can be drawn on the causative effects of surgery or the tourniquet on the changes in biomarkers reported. We chose such study design with the objective to limit variability between procedures that were performed by one surgeon and with the same standardized technical approach. However, future studies including groups without and with tourniquet should be conducted to better clarify its ischemia-reperfusion effects on neutrophil activation and endothelial injury. Second, our anesthetic protocol included the perioperative administration of drugs able to blunt the response of neutrophils and endothelium to injury. Local anesthetics such as lidocaine and ropivacaine administered for femoral block as well as non-steroidal anti-inflammatory drugs inhibit neutrophil activation (32, 33). In addition, local anesthetics have been reported to protect the endothelium (34) whereas non-steroidal anti-inflammatory drugs probably have limited effects (35). Moreover, performing a femoral block before TKA reduces the requirements in volatile anesthetics (36) which may have precluded the immunomodulatory effects of sevoflurane. Finally, the lack of randomization may have introduced some selection bias. Nonetheless, we could reasonably rule out differences between the two anesthesia groups in major confounding factors such as inhomogeneous distribution of patient demography, surgical characteristics, or intraoperative cardiopulmonary variables.

Conclusion

Total knee arthroplasty with the use of a pneumatic tourniquet is associated with neutrophil activation and increased plasma concentrations of active MPO and EL. Sevoflurane anesthesia did not change the course of MPO or EL release as compared with spinal anesthesia. Endothelial damage could not be demonstrated in the clinical setting of our study with measurement of plasma concentrations of biomarkers. A prospective randomized study, including groups without tourniquet, should be performed in order to validate our results.

Acknowledgements

This work has been supported by a research grant from the University Hospital of Liège.

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