MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 5320-5328
DOI: 10.12659/MSM.909385

Received: 2018.02.06
Accepted: 2018.04.18
Published: 2018.07.31

Postoperative Regional Analgesia Is Effective in Preserving Perforin-Expressing Lymphocytes in Patients After Total Knee Replacement

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ACDE 1 Sandra Velcic Brumnjak
CDF 2 Ivan Rakovac
BF 1 Dijana Papez Kinkela
AD 1 Kresimir Bukal
AG 2 Branko Sestan
BCF 3 Vera Tulic
D 4 Elisa Velcic Janjetic
ACE 5 Vlatka Sotosek Tokmadzic

1 Department of Anesthesiology and Intensive Care Medicine, Clinic for Orthopedic and Traumatology Lovran, Lovran, Croatia
2 Department of Orthopedics and Traumatology, Clinic for Orthopedic and Traumatology Lovran, Lovran, Croatia
3 Department of Anesthesiology and Intensive Care Medicine, Clinical Hospital Centre Rijeka, Rijeka, Croatia
4 Department for Mathematics, Physics, Foreign Languages, and Kinesiology, Faculty of Engineering, University of Rijeka, Rijeka, Croatia
5 Department of Anesthesiology, Reanimatology, and Intensive Care Medicine, Faculty of Medicine, Rijeka, Croatia

Corresponding Author: Vlatka Sotosek Tokmadzic, e-mail: vlatkast@medri.uniri.hr
Source of support: This research was supported by the Clinic for Orthopedics and Traumatology Lovran, Lovran, Croatia

Background: Pain and surgical stress cause a pro-inflammatory response followed by downregulation of the immune response, which can increase the incidence of postoperative complications, such as infections or prolonged wound healing. T lymphocytes and natural killer (NK) cells have cytotoxic potential and are crucial components of cellular immunity, which is important for maintenance of immune balance. The aim of this study was to analyze the effects of 3 types of postoperative analgesia on the preservation and cytotoxic potential of T lymphocytes, NK cells, and their subpopulations, as well as NKT cells, in patients after total knee replacement (TKR) to find the most effective analgesic technique for mitigating immune suppression.

Material/Methods: Forty-eight patients scheduled for TKR were randomly allocated to Group 1 (patients received epidural analgesia), Group 2 (patients received sciatic and femoral nerve block), or Group 3 (patients received multimodal systemic analgesia). Pain intensity was assessed at rest and on movement before, immediately after, and at 24 and 72 h after surgery. Blood samples were collected at the same time points and peripheral blood mononuclear cells were isolated. The frequencies of T lymphocytes, NK cells, and NKT cells, as well as their perforin expression, were simultaneously detected and analyzed by flow cytometry.

Results: Patients in Group 1 and Group 2 experienced less severe pain than those in Group 3. The frequencies and perforin levels of T lymphocytes, their subsets, and NKT cells were significantly lower in Group 3 than in Group 1 and Group 2.

Conclusions: The present study confirmed that regional analgesia is more effective in maintaining cell-mediated immunity and perforin expression in peripheral blood lymphocytes in patients after TKR.

MeSH Keywords: Anesthesia and Analgesia • Immunity, Cellular • Nerve Block • Pain • Perforin

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/909385

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Total knee replacement (TKR) is a common orthopedic reconstructive surgical procedure that improves the mobility and the quality of life in patients with degenerative osteoarthritis [1]. It is generally associated with strong postoperative pain at the surgical site [1]. Endogenous danger-associated molecular patterns produced and released after the tissue injury are recognized by pattern recognition receptors, such as Toll-like receptors (TLRs), expressed on the surface of local antigen-presenting immune cells and non-immune cells, including primary sensory neurons, synoviocytes, fibroblasts, chondroblasts, and osteoblasts [2]. After engagement of the TLRs, the joint cells support the immune response by secreting cytokines, leading to local and systemic pro-inflammatory states [3], which cause pain [4]. Clinically manifested pain sensation is mediated by an increase in pro-inflammatory cytokines and chemokines, such as interleukin (IL)-1, IL-6, IL-8, IL-17, and tumor necrosis factor-alpha (TNF-α), in body fluids [5]. IL-6 is positively correlated with IL-17 and Western Ontario and McMaster Universities Arthritis Index [5], and IL-8 amplifies the pain mediated by IL-6 and IL-17 [6]. Periodically increased serum TNF-α in osteoarthritis patients is also correlated with pain sensation [7]. Additionally, IL-17 enhances the expression of main pro-inflammatory mediators IL-1, IL-6, IL-8, TNF-α, C-reactive protein, monocyte chemo-attractant protein 1, and matrix metalloproteinases, and supports the recruitment of neutrophils, $CD4^+$ and $CD8^+$ T lymphocytes, and monocytes toward local inflammatory sites [7]. Cytokines have an immunoregulatory function and are main mediators between the central nervous and immune systems [8,9]. Circulating cytokines pass directly through the blood–brain barrier by active transport or indirectly by stimulation of the vagal nerve [10]. In the brain, cytokines change the concentration of neurotransmitters [11] and play a key role in the activation of the hypothalamic-pituitary-adrenal axis as observed in stressed and depressed patients [12]. Pain increases neuroendocrine activation and catecholamine release [12–16], which potentiate the inflammatory response [12]. Catecholamines inhibit T lymphocyte immune function and decrease NK cell activity directly through β-adrenergic receptors or indirectly by inhibiting cytokines, such as IL-12 and interferon-γ, which are essential for their activity [15].

Since surgical trauma and pain suppress cellular and humoral immunity in postoperative patients, effective anesthesia and postoperative analgesia are critical. Major surgical procedures, such as TKR, are associated with neuroendocrine, metabolic, and immunological alterations that result from tissue damage, anesthesia, and pain [17,18]. If not effectively treated postoperatively, pain could additionally suppress the immune response, causing delayed wound healing, infection, and poor outcomes. Therefore, the most effective analgesia technique with minor side effects is required.

Different types of anesthesia and postoperative analgesia in patients undergoing surgery have been shown to maintain a better balance of cellular and humoral immunity [19]. Intravenous analgesia with opioids can attenuate postoperative pain, but there is strong evidence that it downregulates the immune response and leads to higher incidence of complication and prolonged recovery [20,21]. Recently, it has been shown that regional analgesia techniques can reduce the pro-inflammatory response caused by surgery and pain [12,22,23], but little is known about their effect on cell immunity and its cytotoxic potential, which is important for wound healing and prevention of infections, particularly in patients undergoing TKR. T lymphocytes, natural killer (NK), and NKT cells have cytotoxic potential; upon stimulation, they induce apoptosis and necrosis by secreting cytotoxic mediators, such as perforin, which form pores in the target cell membrane [24]. Perforin, also called cytolisin, is a 66–70-kDa glycoprotein stored in granules in the cytotoxic lymphocytes and released from the granules at the immunological synapse [25].

This study aimed to find the most effective analgesic technique – epidural analgesia *vs.* peripheral nerve blockade of sciatic and femoral nerves *vs.* multimodal systemic analgesia – for preserving perforin-expressing T lymphocytes, NK, and NKT cells in peripheral blood after TKR.

Material and Methods

Patients

Forty-eight patients scheduled for TKR at the Clinic of Orthopedics and Traumatology Lovran, Lovran, Croatia, were included in this prospective randomized study. The patients were randomly allocated using Datlnf Ranolist software (Datlnf GmbH, Tubingen, Germany) to 1 of 3 groups (n=16 each) according to the type of postoperative analgesia received. The study included primary degenerative osteoarthritis patients who underwent TKR, were of American Society of Anesthesiologists physical statuses I to III, and provided informed consent. Patients aged <50 and >75 years, patients allergic to the anesthetics and analgesics used in this study, patients who received blood transfusion, patients with immunological and malignant disease, and those with secondary form of knee osteoarthritis and rheumatoid arthritis were excluded (Figure 1). The study was approved by the local ethics committee of the Clinic for Orthopedics and Traumatology Lovran, Lovran, Croatia and the Faculty of Medicine, University of Rijeka, Rijeka, Croatia according to the World Medical Association criteria in the declaration of Helsinki. Healthy, sex- and age-matched volunteer blood donors comprised the control group (Group 0, n=16). All subjects provided written informed consent.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

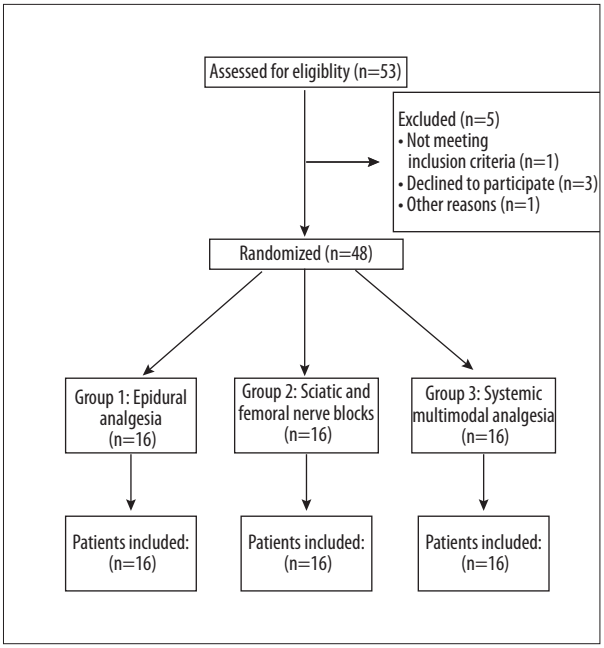

Figure 1. Flow diagram of the study.

Demographic data on sex, age, operative duration, anesthesia duration, and duration of hospitalization were recorded. The subjective perception of pain intensity for each patient was assessed using a 10-cm visual analogue scale (VAS) with endpoints labeled "no pain" and "the worst possible pain" before surgery (T1), immediately after surgery (T2), and 24 h (T3) and 72 h (T4) after surgery at rest and on movement.

Anesthesia procedure

For all patients, the same anesthesia procedure was applied. Twelve hours before surgery, 3800 IU of dalteparin (Fraxiparine; Aspen Pharma Trading Limited, Braine-L'Alleud, France) was administered subcutaneously, and 10 mg diazepam (Roche, Basel, Switzerland) was administered orally. The same surgeon and anesthesiologist performed all operations and anesthetization. All patients underwent regional lumbar spinal anesthesia, before which 3 mg of midazolam (Midazolam 5 mg/mL; B. Braun Melsungen AG, Germany) was administered to all patients intravenously as premedication. The lumbar spinal anesthetization was performed in an aseptic condition with patients in the lateral decubital position, using an atraumatic 25-G needle. When the subarachnoid space was reached, 3 mL of 0.5% bupivacaine (Marcaine 0.5% Spinal; Astra Zeneca, Courbevoie, France) was applied. A midline approach at the L3–L4 level was used for spinal blockade.

Postoperative analgesia

Postoperative pain was managed with epidural analgesia (Group 1, n=16), blockade of sciatic and femoral nerves (Group 2, n=16), or multimodal systemic analgesia (Group 3, n=16). In Group 1, the epidural catheter was applied immediately after surgery with an 18-gauge Tuohy epidural needle (Espocan; B. Braun Melsungen, Germany) through loss-of-resistance technique with air. It was inserted between the L3-L4 lumbar vertebrae, with the catheter tip located 2–3 cm cranially in the epidural space. Immediately after catheterization, continuous epidural analgesia was initiated. A mixture of 0.25% levobupivacaine (Chirocaine 0.5%; AbbVie S.r.l. Campoverde Di Aprilia, Italy) and 0.5 µg/mL sufentanil (Sufentanil Renaudin, Paris, France) (2–15 mL/hour depending on the patient's VAS score) was applied. After 24 h, intermittent epidural boluses of 10 mL 0.25% levobupivacaine every 4–6 h (depending on the patient's VAS score) were applied. The catheter was removed on postoperative day 3. In Group 2, peripheral nerve blockade of sciatic and femoral nerves in the operated leg was used. The blocks were performed using ultrasound-guided "in plane" technique for displaying the needle on an ultrasound instrument (Sonosite EDGE Ultrasound system; SonoSite Inc., WA, USA). Sciatic blockade was performed in the lateral recumbent position with knee and hip flexure. By setting the ultrasound convex 2–5-MHz transducer (C60x/5-2MHz transducer; SonoSite, Washington, USA) in the subgluteal region and displaying the sciatic nerve in the line formed by the ischial tuberositas and posterior superior iliac spine, a 22-gauge, 100-mm-long neurostimulator needle (Stimuplex^{R} A; B. Braun Melsungen, Germany) connected to the nerve stimulator (Stimuplex^{R} HNS 12; B. Braun Melsungen, Germany) was inserted for easy identification of the nerve (0.5–1 mA, 0.1 millisecond). The needle was positioned near the sciatic nerve and 20 mL of 0.5% levobupivacaine was injected. The femoral blockade was performed in the supine position, using a high-frequency linear ultrasound probe (HFL50x/15-6-MHz Transducer; SonoSite, WA, USA), without nerve stimulation. The transducer was placed in the inguinal region and the femoral nerve was identified. Femoral nerve blockade was assessed with 10 mL of 0.5% levobupivacaine near the nerve. After bolus injection, the femoral catheter (Contiplex Tuohy; B. Braun Melsungen, Germany) was set up under the femoral nerve. The catheter tip was placed 3–4 cm from the needle tip. Intermittent boluses of 10 mL 0.25% levobupivacaine every 4 to 6 h daily were applied on the femoral catheter based on VAS score. The catheter was removed on postoperative day 3. In Group 3, multimodal systemic analgesia was used to manage postoperative pain. Initially, during surgical wound closure, paracetamol (Paracetamol Kabi; Fresenius Kabi, Friedberg, Germany) was intravenously administered. Postoperative pain in these patients was managed with intravenously administered 1 g paracetamol every 6 h, 200 mg tramadol every 10–12 h (Tramal; Grunenthal GmbH, Aachen, Germany), and 2.5 g metamizolsodium (Alkagin; Alkaloid-Int d.o.o., Ljubljana, Slovenia).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Demographic and clinical parameters of patients in all groups.

	Group 1	Group 2	Group 3	P value
Age (years)*	68.5 (64–72)	67 (62–70)	70.5 (64–74.5)	Ns
Gender (M/F)	6/10	6/10	7/9	Ns
Duration of surgery (min)	72.5 (65–88.5)	67.5 (64.5–77.5)	65.5 (60–73)	Ns
Duration of anaesthesia (min)	316 (277.5–407.5)	339 (299–369.5)	318 (294–359.5)	Ns

Group 1: patients received epidural analgesia; Group 2: patients received sciatic and femoral nerve block; Group 3: patients received multimodal systemic analgesia. Data are presented as median (25^{th}–75^{th} percentile). Differences between groups were not significant (NS; $p>0.05$).

Isolation of peripheral blood mononuclear cells (PBMCs)

We drew 20-ml blood samples from each patient. Blood was overlaid onto Lymphoprep (Lymphoprep; Fresenius Kabi, Oslo, Norway). PBMCs were isolated through a centrifugation procedure.

Cell surface and intracellular labeling of PBMCs

The isolated PBMCs (2×10^5/sample) were incubated for 20 min at room temperature (RT) with 10% heat-inactivated human AB serum to block non-specific Fc receptor binding. Then, the cells were washed in fluorescence-activated cell sorting (FACS) buffer containing NaCl (140 mM), KH_2PO_4 (1.9 mM), Na_2HPO_4 (16.5 mM), KCl (3.75 mM) (all from Kemika, Zagreb, Croatia), Na_2-EDTA (0.96 mM) (Fluka, Buchs, Switzerland), and NaN_3 (1.5 mM) (Difco, Detroit, MI, USA) and were fixed with 4% paraformaldehyde (pH 7.4) for 10 min at RT. Thereafter, the cells were washed twice in FACS buffer and permeabilized for 20 min at RT with saponin buffer (0.1% saponin; Sigma, Poole, Dorset, USA), 2% goat serum in phosphate-buffered saline (PBS; NaCl 8 g, KCl 0.2 g, $Na_2HPO_4{\cdot}12H_2O$ 2.87 g, and KH_2PO_4 0.2 g (all from Kemika, Zagreb, Croatia) dissolved in 1 L of distilled water). To appropriate cell samples (3 µg diluted in 200 µl of saponin buffer), mouse dG9 IgG2b anti-perforin monoclonal antibody or isotype-matched control (mouse MA-21, IgG2b; BD Biosciences, Erembodegem, Belgium) conjugated with fluorescein isothiocyanate (FITC) was added and incubated at +4°C for 30 min. Then, the samples were washed twice with saponin buffer and resuspended in 1 mL of FACS buffer. A combination of CyChrome Phycoerythrin-5 (Cy-PE5) conjugated anti-CD3 (mouse UCHT1, IgG_1) and PE-conjugated anti-CD4 mAb (mouse RPA-T4, IgG1), Cy-Pe5 anti-CD3 and PE anti-CD8 (mouse RPA-T8, IgG1), or Cy-Pe5 anti-CD3 and PE anti-CD56 (mouse B159, IgG1) were used for surface labeling. As controls, we used PE-, FITC-, or CY-PE5-conjugated mouse isotype-matched antibodies (all from eBioscience, San Diego, CA, USA). After fixing the labeled samples in 400 µL of 2% paraformaldehyde, they were analyzed using flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA, USA).

Statistical analysis

Statistical analysis was performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The Kolmogorov-Smirnov test was used to test for normal distribution of the data. Non-parametric tests were used because the data did not demonstrate normal distribution. Friedman's test and the post hoc Wilcoxon rank sum test were used to compare time points within groups. Kruskal-Wallis test was used to analyze differences among groups, followed by Mann-Whitney *U*-test for post hoc analysis. Bonferroni adjustments were applied when multiple comparisons were made.

We calculated the minimum required sample size based on preliminary results obtained for the 3 groups. As we compared the 3 patient groups, we used sample size calculation in Statistica, option several means. We set type I error rate (alpha) at 0.05, power goal at 0.80, RMSSE at 0.6, and the non-centrality parameter (delta) at 7.2. The analysis indicated that 15 patients in each group were required. Correlation between VAS values and the frequency of different leukocyte subpopulations and percentage of perforin expression was tested using Spearman's rank correlation coefficient. A difference was judged to be statistically significant at $p<0.05$. All data are presented as the 25^{th}–75^{th} percentile values.

Results

Clinical parameters

Demographic and surgical data are shown in Table 1. There were no significant differences among Groups 1, 2, and 3 regarding age, sex, operation duration, or anesthesia duration.

Comparison of VAS scores at rest and on movement

Table 2 shows the VAS scores for Groups 1, 2, and 3 at rest and on movement at different time points (T1, T2, T3, and T4). VAS

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Comparison of visual analogue scores (VAS) at rest and on movement in patients of all groups.

	Group 1				Group 2				Group 3				P value
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	
VAS at rest	0 (0–0)	0 (0–1)°	0.5 (0–2.5)#	0 (0–1)	0 (0–0)	0 (0–0)*	0.5 (0–1)$	0 (0–2)	0 (0–0)g,h,i	1 (0–3.5)	3 (2.5–4)	1.5 (0.5–3.5)	g,h,i, °,*,#,$ p<0.05
VAS on movement	0 (0–1)	0 (0–1)a,b,°	1.5 (0.5–3)#	1 (0–3)&	0 (0–1)	0 (0–0)c,*	1 (0–2)$	2 (0–3)%	0 (0–1)d,e,f	2 (0.5–6)	4 (3–5)	4 (2.5–4)	a,b,c,d,e,f °,#,*,&,$,% p<0.05

Data are presented as median (25th–75th percentile). Statistical significance is shown by *p*-values between VAS on movement at T2 and T3 (a), T2 and T4 (b) in Group 1, at T2 and T4 in Group 2 (c), T1 and T2 (d), T1 and T3 (e), and T1 and T4 (f) in Group 3. Statistical significance between VAS scores at rest in Group 3 between T1 and T2 (g), T1 and T3 (h), and T1 and T4 (i) in Group 3 is shown. When comparing VAS values between groups, statistical significance is presented using *p*-values as follows: * between Group 2 and Group 3 at T2, between Group 1 and Group 3 at time T2, # between Group 1 and Group 3 at T3, $ between Group 2 and Group 3 at time T3, & between Group 1 and Group 3 at T4,% and between Group 2 and Group 3 at T4. * $p<0.05$. VAS – visual analogue scale; T1 – before surgery; T2 – after surgery; T3 – 24 h after surgery; T4 – 72 h after surgery.

scores at rest and on movement recorded at T1 did not differ significantly. In Group 1, the VAS score on movement was significantly higher at T3 and T4 than at T2. In Group 2, the VAS score on movement was significantly higher at T4 than at T2. VAS scores in Group 3 were significantly higher at T2, T3, and T4 than at T1 at rest and on movement. At T2, the VAS score was significantly higher in Group 3 than in Groups 1 and 2 at rest and on movement. At T3, the VAS score was significantly higher in Group 3 than in Groups 1 and 2 at rest and on movement. At T4, the VAS score was significantly higher in Group 3 than in Groups 1 and 2 on movement.

Effects of different types of postoperative analgesia on the frequency of T lymphocytes and their subsets

The frequencies of T lymphocytes (determined as $CD3^+CD56^-$ cells), NK cells ($CD3^-CD56^+$ cells), and NKT cells ($CD3^+CD56^+$ cells) did not differ significantly before surgery (T1) among groups (Figure 2A). At T2, the frequencies of T lymphocytes and their $CD3^+CD4^+$ and $CD3^+CD8^+$ subsets, as well as NK cells, were significantly lower in Group 3 than in Groups 1 and 2 (Figure 2B). At T3, the frequencies of $CD3^+$ and the $CD3^+CD8^+$ subset were lower in Group 3 than in Group 2 (Figure 2C). The frequency of $CD3^+CD8^+$ cells was lower in Group 3 than in Group 2 at T4 (Figure 2D), while at T3 the frequency of NK cells was significantly lower in Group 3 than in Groups 1 and 2 at T3 (Figure 2C) and T4 (Figure 2D). The frequencies of NK cells and their subsets $CD3^-CD56^{+bright}$ and $CD3^-CD56^{+dim}$ (Figure 3C) did not differ significantly among groups (data not shown).

Effects of different types of postoperative analgesia on perforin expression in peripheral blood lymphocytes

Figure 3 shows the changes in $perforin^+$ cell frequency at different time points. $Perforin^+$ cell frequency was significantly lower in Group 3 than in Groups 1 and 2 at T3 and T4.

Perforin-expressing $CD3^+$ cells and their $CD3^+CD8^+$ subset were lower in Group 3 than in Groups 1 and 2 at T3 (Figure 4C) and T4 (Figure 4D). Perforin-expressing $CD3^+CD4^+$ and NKT cells (Figure 4), as well as NK cells and their subsets (data not shown), did not differ significantly at any time point.

Correlation between VAS values and frequencies and perforin expression of different leukocyte subpopulations

VAS values were negatively correlated with the frequencies of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD3^+CD56^+$ cells at both T2 and T3 (Table 3). At T3 and T4, a negative correlation was found between VAS values and all perforin-positive, $CD3^+perforin^+$, and $CD3^+CD8^+$ $perforin^+$ cells (Table 3).

Discussion

Pain management after TKR is mandatory but usually difficult [1], and there are currently no unique postoperative pain management approaches. Despite the reported impacts of different methods of postoperative analgesia in patients undergoing major thoracic and abdominal surgery on cellular- and molecular-level alterations in the immune response, few studies exist on patients undergoing TKR [13,19,22,23]. Here, we report reductions in the frequency of circulating T lymphocytes, their subsets $CD3^+CD4^+$ and $CD3^+CD8^+$ cells, and NKT

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

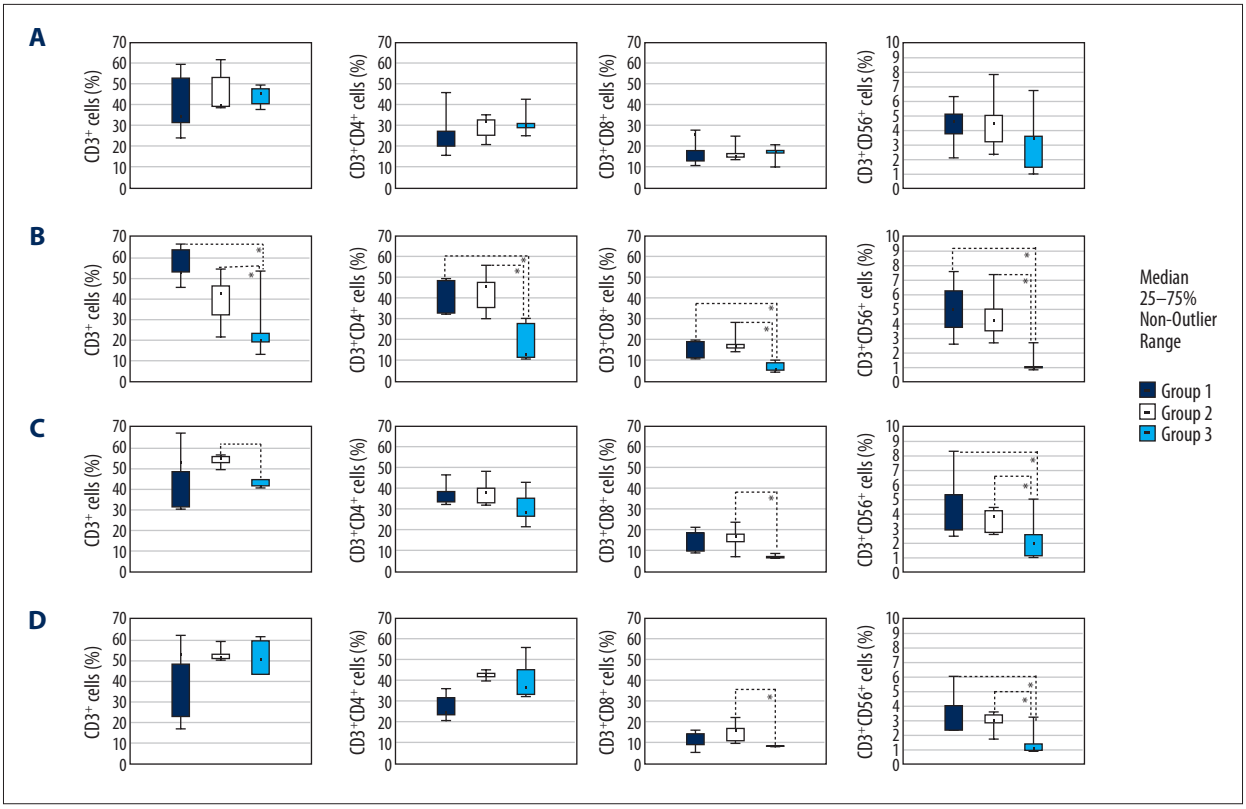

Figure 2. Frequencies of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD3^+CD56^+$ cells in peripheral blood lymphocytes in Group 1 (■), Group 2 (□), and Group 3 (=) at time points T1 (before surgery) (**A**), T2 (immediately after surgery) (**B**), T3 (24 h after surgery) (**C**), and T4 (72 h after surgery) (**D**). Data are presented as median (□), 25^{th}–75^{th} percentile (□), non-outlier range (I), and outliers (○). * $p<0.05$.

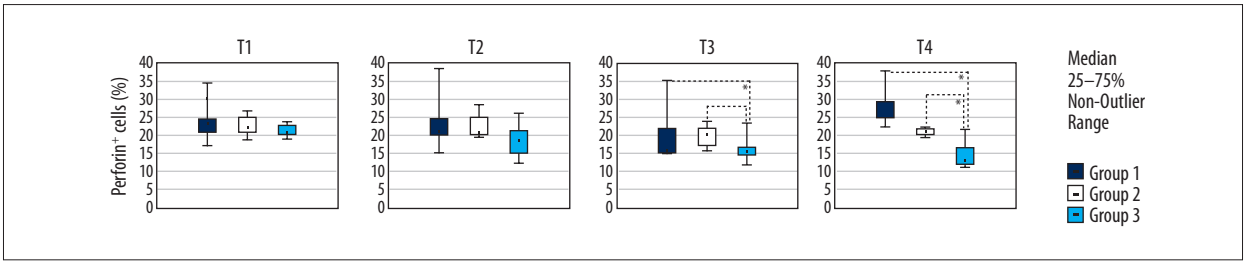

Figure 3. Frequencies of perforin-positive cells within peripheral blood lymphocytes in Group 1 (■), Group 2 (□), and Group 3 (=) at times points T1 (before surgery), T2 (immediately after surgery), T3 (24 h after surgery), and T4 (72 h after surgery). Data are presented as median (□), 25^{th}–75^{th} percentile (□), non-outlier range (I), and outliers (○). * $p<0.05$.

cells in patients receiving multimodal systemic analgesia compared to regional analgesia. These findings indicate that both regional epidural analgesia and blockade of sciatic and femoral nerves efficiently maintain cell-mediated immunity after TKR. Strong negative correlations between pain and decreases in circulating T lymphocytes of both the $CD3^+CD4^+$ and the $CD3^+CD8^+$ phenotype and NKT cells were observed immediately and 24 h after surgery in patients with multimodal systemic, but not regional analgesia. The strong negative correlation found between pain intensity and perforin-expressing cytotoxic $CD3^+CD8^+$ T lymphocytes confirmed the suppression of cell-mediated immunity against intracellular antigens, such as viruses and fungi.

Pro-inflammatory cytokines modulate pain intensity in a dose-dependent manner. Enhanced stimulation of nociceptors and pain recognition in the brain lead to activation of the hypothalamic-pituitary axis [26] and the sympathetic and parasympathetic nerve systems [27], which are involved in the decrease in cell-mediated immune response and lymphocyte count. In turn, pain induces the synthesis of CRP, cytokines, and chemokines at the site of surgical injury, which might support local

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

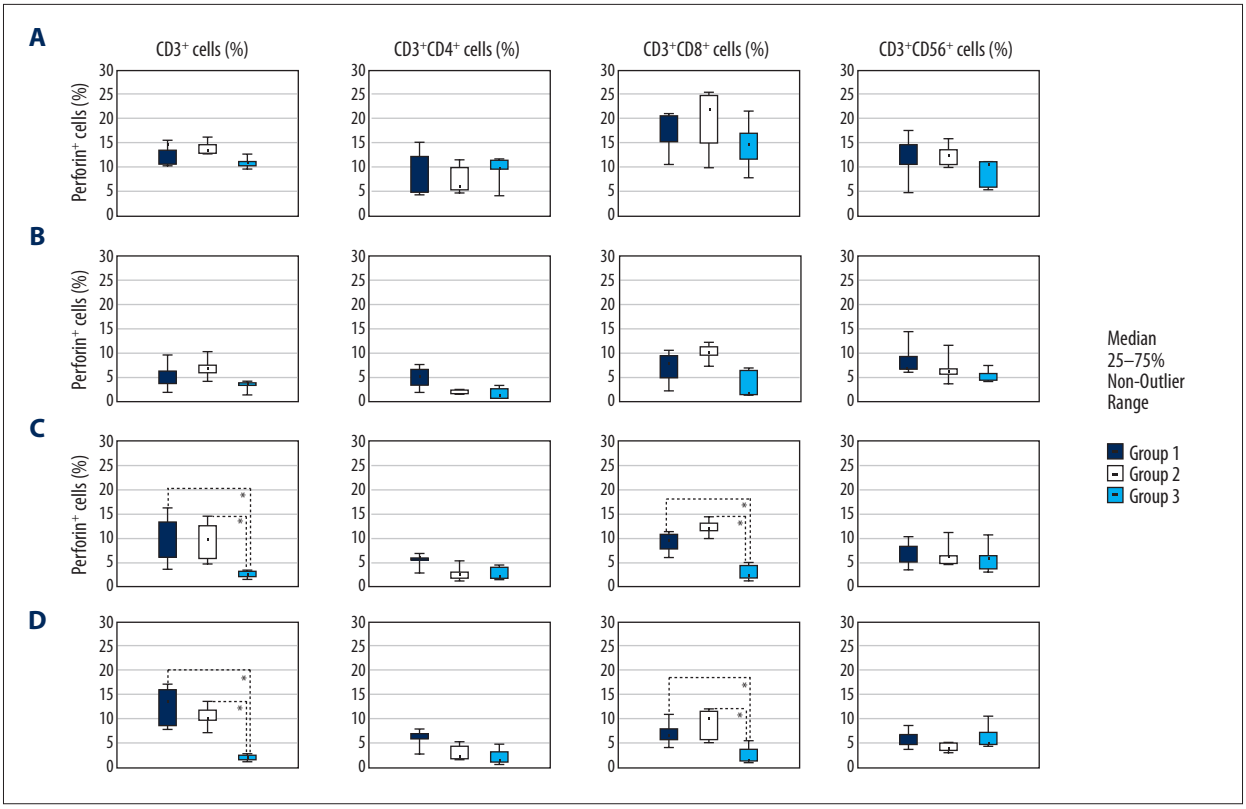

Figure 4. Frequencies of CD3⁺perforin⁺, CD3⁺CD4⁺perforin⁺, CD3⁺CD8⁺perforin⁺ and CD3⁺CD56⁺perforin⁺ cells within peripheral blood lymphocytes in Group 1 (■), Group 2 (□), and Group 3 (=) at times points T1 (before surgery) (**A**), T2 (immediately after surgery) (**B**), T3 (24 h after surgery) (**C**), and T4 (72 h after surgery) (**D**). Data are presented as median (□), 25th–75th percentile (□), non-outlier range (I), and outliers (○). * $p<0.05$.

recruitment of immune cells at the site of pain [26]. Locally recruited cells, predominantly cytotoxic CD3⁺CD8⁺ lymphocytes and NK cells, could be responsible for elimination of injured or strongly activated cells. The cytotoxic activity might be mediated by perforin [25]. Perforin is one of the main cytotoxic mediators of cell-mediated immunity and its downregulation in peripheral blood is associated with increased incidence of systemic infection and poor patient outcome [24]. Here, a greater decrease in perforin was observed in patients receiving systemic multimodal analgesia than in those receiving regional analgesia. This was prominent in T lymphocytes, predominantly those with the cytotoxic CD3⁺CD8⁺ phenotype.

In patients receiving peripheral nerve blockade, the maintenance of T lymphocytes, NKT cells, NK cells, and their subsets, as well as perforin expression, in the early postoperative period could be explained by prolonged blockade of C-fibers of afferent and efferent nerves with local anesthetics, thereby reducing central nervous system stimulation and subsequent neuro-immunomodulation induced by locally produced pro-inflammatory and pain-inducing cytokines [28–32]. We cannot exclude the possibility that local anesthetics themselves modulate the inflammatory response locally and systemically. However, we did not find significant fluctuation in NK cells (data not shown).

The present results agree with previous findings that patients receiving regional analgesia, including peripheral nerve blockade or epidural analgesia, experience lesser hyperalgesia than those receiving systemic multimodal analgesia [13,33]. In line with these results, techniques of regional analgesia, predominantly epidural postoperative analgesia, can significantly reduce altered inflammatory responses in the early postoperative period after major thoracic, abdominal, and orthopedics surgery [13,19,34,35]. By measuring blood glucose, insulin, and cortisol levels, as well as leukocyte count, as inflammatory markers, Bagry et al. found that continuous lumbar plexus and sciatic peripheral nerve block effectively reduces pain after TKR and can attenuate distinct inflammatory responses [28]. Song et al. reported that epidural analgesia produces milder deleterious effects on cell-mediated immunity in critically ill patients after tumor resection surgery [36]. Our results suggest that maintaining an appropriate balance of the immune system through careful management of postoperative analgesia after TKR can improve patient prognosis, although in our patients, complications such as delayed wound healing and infection did not occur.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. Correlation between VAS values and different lymphocytes subpopulation or their perforin expression after surgery (T2), 24 (T3), and 72 (T4) h.

Comparison	VAS					
	T2		T3		T4	
	r	*p*	r	*p*	r	*p*
$CD3^+$ cells	–0.538	0.038	–0.0617	0.014	0.178	0.525
$CD3^+CD4^+$ cells	–0.083	0.001	–0.0648	0.009	0.078	0.78
$CD3^+CD8^+$ cells	–0.078	0.001	–0.063	0.012	–0.448	0.093
$CD3^+CD56^+$ cells	–0.078	0.001	–0.494	0.041	–0.616	0.054
$CD56^+$ cells	–0.028	0.92	0.124	0.659	–0.454	0.089
$CD56^{dim+}$ cells	–0.999	0.72	0.377	0.16	–0.17	0.54
$CD56^{bright}$ cells	–0.998	0.726	–0.193	0.48	–0.08	0.77
$Perforin^+$ cells	–0.403	0.136	–0.445	0.05	–0.442	0.048
$CD3^+perforin^+$ cells	–0.624	0.13	–0.463	0.05	–0.736	0.002
$CD3^+CD4^+perforin^+$ cells	–0.14	0.616	–0.09	0.72	–0.266	0.33
$CD3^+CD8^+perforin^+$ cells	–0.678	0.051	–0.774	0.001	–0.672	0.006
$CD3^+CD56^+perforin^+$ cells	–0.512	0.052	–0.157	0.575	0.135	0.63
$CD56^+perforin^+$ cells	–0.385	0.156	–0.235	0.398	–0.291	0.358
$CD56^{dim+}perforin^+$ cells	–0.298	0.296	–0.375	0.17	–0.408	0.13
$CD56^{bright+}perforin^+$ cells	0.36	0.183	–0.484	0.067	–0.549	0.054

Statistical significance is shown as *p* and r values.

Conclusions

Our results suggest that peripheral nerve blockade or epidural analgesia are better and more effective in maintaining perforin-expressing peripheral blood T lymphocytes with cytotoxic $CD3^+CD8^+perforin^+$ phenotype and NKT cells than multimodal systemic analgesia after TKR in the early postoperative period.

Acknowledgements

The authors would like to thank Mrs. Ksenija Tulic for technical laboratory work and Prof. Gordana Laskarin for critical review of the manuscript.

Conflicts of interest

None.

References:

1. Ilfeld BM, Shuster JJ, Theriaque DW et al: Long-term pain, stiffness and functional disability following total knee arthroplasty with and without an extended ambulatory continuous femoral nerve block: A prospective one-year follow-up of a multicenter, randomized, triple-masked, placebo-controlled trial. Reg Anesth Pain Med, 2011; 36: 116–20
2. Gómez R, Villalvilla A, Largo R et al: TLR4 signalling in osteoarthritis-finding targets for candidate DMOADs. Nat Rev Rheumatol, 2015; 11: 159–70
3. Klechevsky E: Functional diversity of human dendritic cells. Adv Exp Med Biol, 2015; 850: 43–54
4. Liu T, Gao YJ, Ji RR: Emerging role of Toll-like receptors in the control of pain and itch. Neurosci Bull, 2012; 28: 131–44
5. Liu Y, Peng H, Meng Z, Wei M: Correlation of IL-17 level in synovia and severity of knee osteoarthritis. Med Sci Monit, 2015; 21: 1732–36
6. Fernandes JC, Martel-Pelletier J, Pelletier JP: The role of cytokines in osteoarthritis pathophysiology. Biorheology, 2002; 39: 237–46
7. Zhu S, Qian Y: IL-17/IL-17 receptor system in autoimmune disease: Mechanisms and therapeutic potential. Clin Sci (Lond), 2012; 122: 487–511
8. Rothwell NJ, Hopkins SJ: Cytokines and the nervous system: II. Actions and mechanisms of action. Trends Neurosci, 1995; 18: 130–36
9. Banks WA: Blood-brain barrier transport of cytokines: A mechanism for neuropathology. Curr Pharm Des, 2005; 11: 973–84
10. Maier SF, Goehler LE, Fleshner M, Watkins LR: The role of the vagus nerve in cytokine-to-brain communication. Ann NY Acad Sci, 1998; 840: 289–300
11. Miller AH, Haroon E, Raison CL, Felger JC: Cytokine targets in the brain: Impact on neurotransmitters and neurocircuits. Depress Anxiety, 2013; 30: 297–306

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

12. Anisman H, Merali Z: Anhedonic and anxiogenic effects of cytokine exposure. In: Dantzer R, Wollman EE, Yirmiya R (eds.). Cytokines, stress and depression. New York, Plenum, 1999; 199–233
13. Ahlers O, Nachtigall I, Lenze J et al: Intraoperative thoracic epidural anesthesia attenuates stress-induced immunosuppression in patients undergoing major abdominal surgery. Br J Anaesth, 2008; 101: 781–87
14. Richard Chapman C, Tuckett RP, Song CW: Pain and stress in a system prospective: Reciprocal neural, endocrine and immune interactions. J Pain, 2008; 9: 122–45
15. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES: The sympathetic nerve – an integrative interface between two supersystems: The brain and the immune system. Pharmacol Rev, 2000; 52: 595–638
16. Elenkov IJ, Iezzoni DG, Daly A et al: Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation, 2005; 12: 255–69
17. Weissman C: The metabolic response to stress: An overview and update. Anesthesiology, 1990; 73: 308–27
18. Hall GM, Peerbhoy D, Shenkin A et al: Hip and knee arthroplasty: A comparison and the endocrine, metabolic and inflammatory responses. Clin Sci, 2000; 98: 71–79
19. Gu CY, Zhang J, Qian YN, Tang QF: Effects of epidural anesthesia and postoperative epidural analgesia on immune function in esophageal carcinoma patients undergoing thoracic surgery. Mol Clin Oncol, 2015; 3: 190–96
20. Weinbroum AA: Superiority of postoperative epidural over intravenous patient-controlled analgesia in orthopedic oncologic patients. Surgery, 2005; 138: 869–76
21. Sacerdote P: Opioids and the immune system. Palliat Med, 2006; 20(8): 9–15
22. Hughes SF, Hendricks BD, Edwards DR et al: Total hip and knee replacement surgery results in changes in leukocyte end endothelial markers. J Inflamm, 2010; 7: 2
23. Choi PT, Bhandar M, Scott J, Douketis J: Epidural analgesia for pain relief following hip or knee replacement. Cochrane Database Syst Rev, 2003; 3: CD003071
24. Sotosek Tokmadzic V, Laskarin G et al: Expression of cytolytic protein – perforin – in peripheral blood lymphocytes in severe traumatic brain injured patients. Injury, 2012; 43: 624–31
25. Voskoboinik I, Whisstock JC, Trapani JA: Perforin and granzymes: Function, dysfunction and human pathology. Nat Rev Immunol, 2015; 15(6): 388–400
26. Schlereth T, Birklein F: The sympathetic nervous system and pain. Neuromol Med, 2008; 10(3): 141–47
27. Tsigos C, Chrousos GP: Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res, 2002; 53(4): 865–71
28. Bagry H, de la Cuadra Fontaine JC, Asenjo JF et al: Effect of a continuous peripheral nerve block on the inflammatory response in knee arthroplasty. Reg Anesth Pain Med, 2008; 33: 17–23
29. Beloeil H, Asehnoune K, Moine P et al: Bupivacaine's action on the carrageenan-induced inflammatory response in mice: Cytokine production by leukocytes after *ex-vivo* stimulation. Anesth Analg, 2005; 100: 1081–108
30. Martin F, Martinez V, Mazoit JX et al: Antiinflammatory effect of peripheral nerve blocks after knee surgery: Clinical and biologic evaluation. Anesthesiology, 2008; 109: 484–90
31. Gentili ME, Mazoit JX, Samii KK, Fletcher D: The effect of a sciatic nerve block on the development of inflammation in carrageenan injected rats. Anesth Analg, 1999; 89: 979–84
32. Chloropoulou P, Iatrou C, Vogiatzaki T et al: Epidural anesthesia followed by epidural analgesia produces less inflammatory response than spinal anesthesia followed by intravenous morphine analgesia in patients with total knee arthroplasty. Med Sci Monit, 2013; 19: 73–80
33. Leduc C, Gentili ME, Estebe JP et al: The effect of local anesthetics and amitriptyline on peroxidation *in vivo* in an inflammatory rat model: Preliminary reports. Anesth Analg, 2002; 95: 992–96
34. Beilin B, Shavit Y, Trabekin E et al: The effects of postoperative pain management on immune response to surgery. Anesth Analg, 2003; 97: 822–27
35. Fant F, Tina E, Sandblom D et al: Thoracic epidural anesthesia inhibits the neuro-hormonal but not acute inflammatory stress response after radical retropubic prostatectomy. Br J Anaesth, 2013; 110: 747–57
36. Song P, Dong T, Zhang J et al: Effects of different methods of anesthesia and analgesia on immune function and serum tumor marker levels in critically ill patients. Exp Ther Med, 2017; 14: 2206–10

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]