ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effects of Anesthesia on Innate Immune Components in Orthopedic Surgery.

Adnan Hamad Aubaid*, and Khalid Lahmood Yaseen.

Department of Medical Microbiology/College of Medicine/University of Al-Qadisiyah, Diwaniyah, Iraq.

ABSTRACT

The present study was carried out to evaluate the possible role of anesthetics and surgery on innate by measuring the levels of TNFα and IFNγ by ELISA. Flow cytometry was used to determine MCP-I and CD16. Serum level of TNFα and IFNγ cytokines were measured by using enzyme linked immunosorbent assay (ELISA) technique. Flow cytometry was used to determine MCP-I and CD16. WBC count and C-reactive protein were determined. There was highly significant rise in CRP blood level post-operatively when compared with pre-operative blood level, (P<0.001). In addition, The results revealed a significant rise in neutrophil count after operation in comparison with its baseline level before operation, (P< 0.001), while the level of lymphocytes showed significant decline following operation, (P<0.001). The level of monocyte and eosinophil also got significantly reduced after operation (P<0.001). CD16 NK cells count reveled significant rise during time of anesthesia. It has been noticed also that the level of MCP-1 got raised significantly in association with anesthesia induction. Analysis of data to correlate the TNFα , IFNγ ,MCP-I and CD16 with types of anesthetic drugs showed no significant association between these components and type of anesthesia (P > 0.05).

Keywords: anesthetics, innate immunity, Interleukins, Monocyte chemoatractant protein, C-reactive protein CPR.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Opioids, inhalational agents, intravenous and local anesthetics have shown different effects on immune system and cytokine expression[1]. General anesthesia accompanied by surgical stress is considered to suppress immunity, presumably by directly affecting the immune system or activating the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system[2]. Surgical stress and general anesthesia may suppress natural killer and cytotoxic T cells and also activating sympathetic nervous system[3]. Clusters of differentiation have numerous physiological function, which acts as receptors or ligands for signal cascade which lead to alter the cell's behavior and its function in cell adhesion[4]. Chemokines play an important role in selectively recruiting monocytes, neutrophils, and lymphocytes, as well as in inducing chemotaxis through the activation of G-protein-coupled receptors[5]. MCP-1 almost made by all cells and tissues upon stimulation by different agents, but it mainly released by monocyte cells, that is why MCP-1 was first designated as monocyte chemotactic and activating factors that could leads to kill tumor targets and considered as the major chemoatractant agent in the human body[6]. The primary aim of this study was to evaluate the effects of different anesthesia techniques on some innate immunity components in orthopedic patients.

MATERIALS AND METHODS

This study was conducted on 30 patients with Orthopedic surgeries and Arthroscopy 16 males (53.3%) and 14 females (64.7%) with age range 10-72 years old recruited from orthopedic and rheumatology department of AL-Diwaniaya Teaching Hospital during period from first of January of 2018 to the end of April of the 2018.Three types of anesthesia were used, 10 patients anesthetized with general anesthetics, 10 patients with local and the 10 patients with local anesthesia with duration of anesthesia range 75 minutes (15-90 minutes). Sample were collected at three timing intervals 24 hr. before, during and 12 hr. after surgery. The study population was assessed by questionnaire regarding age, gender, type of surgery, duration of anesthesia and clinical history of other disease. Kits of ELISA are used in this study depending on sandwich enzyme immunoassay method. Micro ELISA plate provided in this kit has been pre-coated with an antibody specific to (TNFα and IFNγ). OD for each well is calculated at once by using a micro-plate reader spectrophotometer at wave length 450nm.

Flow-Cytometry*:* Flow Cytometry assay kits that have been used in this study are Flowcytometry kit for CD16 and hematological WBC count.

Statistical Analysis: Data were translated into a computerized database structure. An expert statistical advice was sought for. All data were analyzed by using Statistical Package for Social Sciences (SPSS) software version 20 in association with Microsoft Excel 2016. To measure the strength of association between categorical variables, such as the effect of anesthetic techniques on cellular response the odds ratio (OR) was used. Log transformation was carried out in order to make the distribution of variables related to CD16 natural killer cells and the level of MCP-1, normal.

RESULTS

According to type of anesthesia, this study enrolled 10 patients with general anesthesia, 10 patients with regional anesthesia and 10 patients with local anesthesia. The mean duration of anesthesia was 44.33 ±19.85 minutes and it ranged from 15-90 minutes. Hypertension was seen in 3 patients (10%), diabetes was seen also in 3patients (10%), ischemic heart disease was seen in a single patient (3.3%), a single patient (3.3%) suffered from asthma and agranulocytosis was seen in a single patient (3.3%), as explained in table (1).

ISSN: 0975-8585

Table 1: General characteristics of the patients

Characteristic	Value
Number of cases	30
Age	
Mean ±*SD* (years)	35.67 ±17.53
Range (Min.-Max.) years	62 (10-72)
Gender	
Male, *no* (%)	16 (53.3)
Female, *no* (%)	14(46.7)
M:F ratio	1.14:1
Type of anesthesia	
General, *no* (%)	10 (33.3%)
Local, *no* (%)	10 (33.3%)
Regional, *no* (%)	10 (33.3%)
Duration of anesthesia	
Mean ±SD (Minute)	44.33 ±19.85
Range (Min.-Max.) minutes	75 (15-90)
Chronic illness	
Hypertension, *no* (%)	3 (10%)
Diabetes mellitus, *n* (%)	3 (10%)
IHD, *no* (%)	1 (3.3%)
Asthma, *no* (%)	1 (3.3%)
Agranulocytosis, *no* (%)	1 (3.3%)

The levels of Tumor necrosis factor alpha (TNF-α) and Interferon gamma (IFN-γ) cytokines were analyzed, (TNF-α p=0.741) and (IFN-γ), all showed no significant change in relation to time of anesthesia whether pre, peri and post-operative (P > 0.05), as seen in figures (1). The result listed in tables (2) and (3) which associate between cytokines serum level (pre, peri and post-operatively) with gender and age, showed no significant association between male and female as well as no significant correlation with age of patients (p>0.05) for both cytokines including TNFα and IFNγ. Analysis of data to correlate the cytokines level (IFN-γ and TNF-α) with types of anesthetic drugs (general, local, and regional anesthesia) showed no significant association between these cytokine level and type of anesthesia (P > 0.05), as described in table (4). Considering the time of duration of anesthesia, the result revealed there is no significant association between cytokines level and duration of anesthesia as in table (5).The results of this statistical analysis, that correlate the association between CRP blood level and cytokines serum concentration, showed no significant association as described in table (6).

ISSN: 0975-8585

Table 2: Association of cytokine level and gender

Cytokine	Male (n=16)		Female (n=14)		*P*
	Median	IQR	Median	IQR	
TNF-α pre	58.08	46.84	61.02	28.41	0.835
TNF-α peri	53.50	31.62	56.55	13.14	0.560
TNF-α post	55.02	14.23	51.97	26.13	0.519
IFN-γ pre	140.82	106.51	147.52	64.60	0.835
IFN-γ peri	130.40	71.91	137.35	29.88	0.560
IFN-γ post	133.88	32.35	126.93	59.39	0.519

Table 3: Correlation between age and cytokine levels

Cytokine	r	*P*
Log TNF-α pre	-0.109	0.565
Log TNF-α peri	0.091	0.632
Log TNF-α post	-0.052	0.784
Log IFN-γ post	-0.109	0.568
Log IFN-γ peri	0.087	0.649
Log IFN-γ post	-0.047	0.803

Table 4: Correlation between cytokine levels and type of anesthesia

Cytokine	General anesthesia		Local anesthesia		Regional anesthesia		*P*
	Median	IQR	Median	IQR	Median	IQR	
TNF-α pre	62.11	35.12	63.64	41.34	45.86	14.01	0.115
TNF-α peri	51.53	19.63	58.51	42.70	56.22	12.54	0.558
TNF-α post	50.88	22.31	54.58	13.69	55.02	28.85	0.686
IFN-γ pre	150.00	79.85	153.46	93.99	113.04	31.87	0.115
IFN-γ peri	125.94	44.63	141.81	97.07	136.61	28.52	0.558
IFN-γ post	124.46	50.71	132.88	31.12	133.88	65.59	0.686

*Significant at p≤0.05.Values were expressed as median (IQR); n: number of the cases;
† Kruskal Wallis H test.

Table 5: Correlation between cytokine levels and duration of anesthesia

Cytokine	r	*P*
Log TNF-α pre	-0.190	0.315
Log TNF-α peri	-0.155	0.412
Log TNF-α post	-0.105	0.580
Log IFN-γ pre	-0.189	0.317
Log IFN-γ peri	-0.159	0.401
Log IFN-γ post	-0.112	0.557

*Significant only at p≤0.05.

Table 6: Correlation between Cytokine levels and CRP level

Cytokine	r	*P*
Log TNF-α pre	-0.042	0.826
Log TNF-α peri	0.152	0.421
Log TNF-α post	0.045	0.812
Log IFN-γ pre	-0.033	0.862
Log IFN-γ peri	0.143	0.451
Log IFN-γ Post	0.049	0.799

*Significant only at p≤0.05. r: correlation coefficient; IL: Interleukin; TNF: tumor necrosis factor; IFN:

Results of CD16 NK cells count reveled significant rise during time of anesthesia (21.05) however, it showed significant decrease post-operatively (13.350), but the reduction did not reach baseline count, It has been noticed also that the level of MCP-1 got raised significantly in association with anesthesia induction (13.24), however it returned back to its baseline level following surgery, as outlined in table (7). Table (8) showed that the count of CD16 NK cell before, perioperative and post-operatively, had no significant association with gender (p-value > 0.05). It was also obvious, that the level of the chemotactic chemokine MCP-1 remains insignificantly altered before, within and after operation (P > 0.05). Neither CD16 natural killer cells nor MCP-1 showed significant correlation with age of patients (P > 0.05), as demonstrated in table (9). CD16 NK cells did not vary significantly in relation to type of anesthesia, whether local, regional or general, in all situations whether before, at time or after operation (P > 0.05), also the level of the chemotactic chemokine MCP-1 showed no significant difference with respect to type of anesthesia, general versus regional versus local, whatever the time in relation to anesthesia was, pre-operatively, peri-operatively and post-operatively, (P > 0.05), as demonstrated in table (10). Regarding the correlation of immune marker with time duration of anesthesia, the results showed that immune cells, lymphocytes, showed no statistical significance correlation with duration of anesthesia (P > 0.05) table (11). There was highly significant rise in CRP blood level post-operatively when compared with pre-operative blood level, 2.65 (4.99) and 3.99 (5.64) respectively (P <0.001) as in figure (3).

Table 7: Median level of immune markers in relation to operation timeline

Marker	Pre-operatively	Peri- operatively	Post- operatively	*P*†
CD16, median (IQR)	**12.45 (8.14)**	**21.05 (13.36)**	**13.35 (5.79)**	**<0.001**
MCP-1, median (IQR)	**11.14 (12.80)**	**13.24 (7.13)**	**10.79 (11.64)**	**<0.001**

† Friedman test; CD: Cluster of designation; IQR: inter-quartile range.

ISSN: 0975-8585

Table 8: immune markers in relation to gender

Marker	Total *n* = 30 Mean	SD	Male *n* = 16 Mean	SD	Female *n* = 14 Mean	SD	*P*†
CD16Pr	12.45	8.14	11.57	8.74	12.84	8.76	0.934
CD16Pe	21.05	13.36	21.16	14.18	20.89	15.78	0.662
CD16Po	13.35	5.79	11.18	7.22	14.66	5.92	0.934
MCP-1Pr	11.14	12.80	11.25	13.27	9.94	12.84	1.000
MCP-1Pe	13.24	7.13	13.24	11.13	13.65	6.56	0.771
MCP-1Po	10.79	11.64	11.02	10.10	10.65	11.23	0.394

*Significant at P< 0.05. SD: standard deviation. Values were expressed as median (Inter-quartile range); n: number of the cases; † Mann Whitney U test.

Table 9: Correlation of immune markers with age

Marker	r	*P*
Log CD16Pr	0.209	0.268
Log CD16Pe	-0.011	0.953
Log CD16Po	0.127	0.503
Log MCP-1Pr	0.020	0.915
Log MCP-1Pe	-0.170	0.370
Log MCP-1Po	-0.007	0.969

*Significant at P< 0.05 *r*: correlation coefficient; CD: cluster of designation.

Table 10: Immune markers in relation to type of anesthesia

Marker	General		Local		Regional		*P*
CD16Pr	13.24	5.51	15.09	6.47	14.33	7.77	0.145
CD16Pe	20.09	17.68	22.98	15.83	21.16	11.99	0.557
CD16Po	12.85	7.06	13.35	7.70	13.28	5.88	0.866
MCP-1Pr	2.32	11.72	11.33	6.23	11.70	13.62	0.084
MCP-1Pe	13.98	15.19	15.00	5.77	12.46	6.50	0.673
MCP-1Po	5.01	10.41	11.44	8.77	10.97	9.82	0.093

*Significant at P< 0.05 Values were expressed as median (Inter-quartile range); *n*:number of the cases; † Kruskal Wallis H test.

Table 11: Correlation of immune markers with duration of anesthesia

Marker	r	*P*
Log CD16Peri	-0.508	0.174
Log CD16Post	0.049	0.799
Log MCP-1Peri	0.069	0.719
Log MCP-1Post	0.095	0.618

*Significant at P< 0.05 *r*: correlation coefficient; CD: cluster of designation

ISSN: 0975-8585

Table 12: WBC count before and after anesthesia

WBC	Before	After	*P*
Neutrophil, Mean ±SD	5.62 ±2.75	6.74 ±2.89	<0.001
lymphocyte, Mean ±SD	3.03 ±1.15	2.85 ±1.13	<0.001
Monocyte, Mean ±SD	0.65 ±0.25	0.63 ±0.24	<0.001
Eosinophil, Mean ±SD	0.31 ±0.40	0.30 ±0.40	<0.001
Basophil, Mean ±SD	0.03 ±0.03	0.03 ±0.04	0.687

WBC: white blood cells; SD: standard deviation

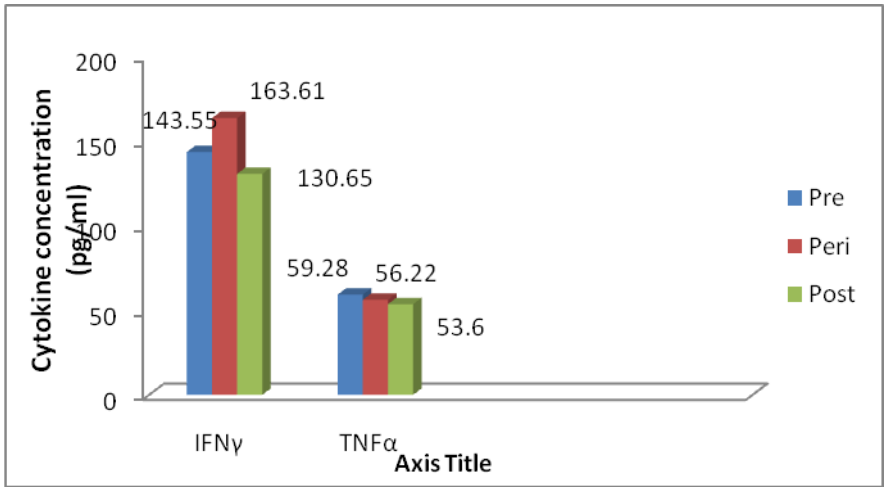

Figure 1: Level of TNFα and IFNγ in relation to time of anesthesia.

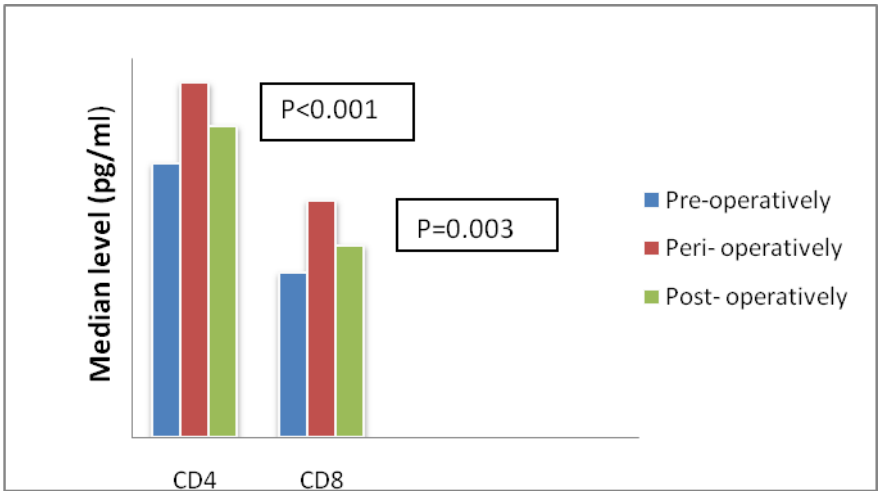

Figure 2: Median level of immune markers in relation to operation timeline

ISSN: 0975-8585

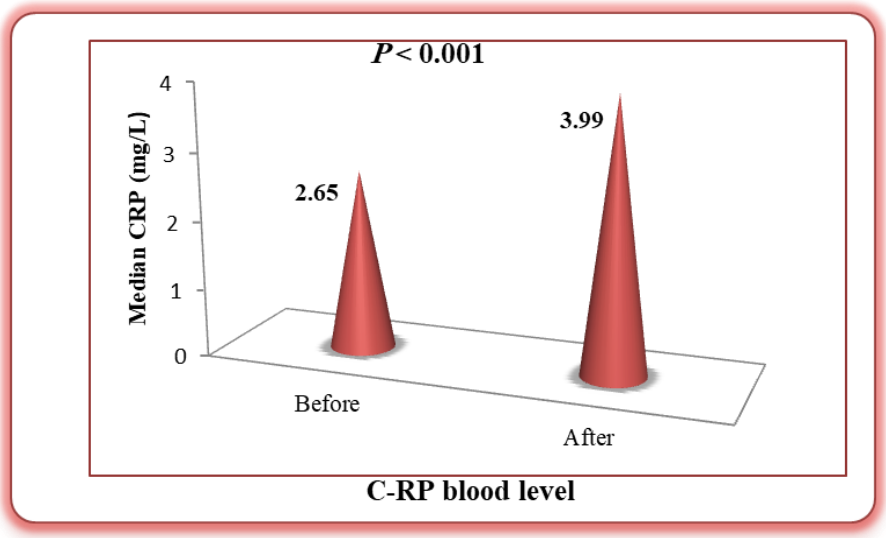

Figure 3: C-RP level before and after surgery.

DISCUSSION

Variation in type of surgical operation was proved by several authors to be associated with different types of responses to same anesthesia techniques [7]. In the current study, there is significant rise in neutrophil count after anesthesia in comparison with baseline level. This is in agreement with Deirmengian, *et al.* 2011[8]. The proposed mechanism of rising neutrophil count is most probably due surgical trauma and associated stress with neurohumoral effect in addition to the possibility of postoperative infection[9]. In the current study, there is significant decline in lymphocyte count after anesthesia in comparison with baseline level. This is in agreement Dąbrowska and Słotwiński 2014, The proposed mechanism for the reduction in lymphocyte count is the disturbance in apoptosis of lymphocyte through bcl2 dependent mechanism, by dysregulation of anti-apoptosis and pro-apoptosis signals equilibrium[10].

In the current study, there is significant decline in monocyte count after anesthesia in comparison with baseline level. The reason for that is most probably due to changes in immune mediators as a response to tissue injury and stress accompanying surgery, also there is significant decline in eosinophil count after anesthesia in comparison with baseline level. The reason for that is most probably due to changes in immune mediators as a response to tissue injury and stress accompanying surgery, Moreover, in the current study, there is no significant change in basophil count after anesthesia in comparison with baseline level. The reason for that is most probably due to changes in immune mediators as a response to tissue injury and stress accompanying surgery This is in agreement with Sayit and Terzi 2017[8]. The present study showed that post-operative WBC count and differential counts were not significantly correlated to age of the patient. This finding disagrees with Chen, Qian *et al.* 2016[11]. The concept of aging of immune system is recent and controversial. Several suggestions have been proposed to explain the reduced number of some cell types that are involved in adaptive and innate immune response and the most widely accepted explanation is the aging of bone marrow, the source of all cells involved in immune system[12]. The present study showed that post-operative WBC count and differential were not significantly correlated to gender of the patient. This is in agreement with Valiathan *et al.*, 2016[13]. The present study showed that post-operative WBC count and differential were not significantly correlated to duration of anesthesia. This finding is in agreement with Costa *et al*. 2013[14]. The present study showed that post-operative WBC count and differential were not significantly correlated to type of anesthesia. This is in agreement Cho *et al.* 2017[15]. It appears that, the changes in WBC counts happened as a response to the stress accompanying surgical operation that is the mirror of humoral and neural stimulation and that trauma and tissue damage associated with surgical incision is the main stimulant factor behind these stress responses[16]. Accordingly, there will be no significant correlation with the count of WBC and the type and duration of anesthesia. In addition, the present study showed that post-operative WBC count and differential were not significantly correlated to CRP. This finding is

in accordance with Boersema *et al.* 2018[17]. The CRP has been shown to rise significantly in the current study, a finding that is similar to Godoy *et al*. 2010[18]. The explanation for the rise of CRP is most likely to inflammation that accompanies tissue injury at time of surgery with increase in hepatic production of this acute phase reactant[17].

The present study showed that the level of cytokines (IFNγ and TNFα) became significantly lower during operation, whatever the type and duration of anesthesia, in comparison with their levels before operation and that their level continued to fall insignificantly after operation, however, it did not return back to the same level before operation. These results are similar to the findings of Cheng *et al*. 2013[19]. The explanation for the fall in the level of these cytokines is most probably due to the anti-inflammatory effect subjected by IL-10. IL-10 is an anti-inflammatory cytokine that acts by autocrine and paracrine mechanisms that causes suppression of secretion of pro-inflammatory cytokines such as (IFN-gamma and TNF-alpha) by the same cell secreting IL-10 and other nearby cells, an effect that is named as shifting from t-helper 1 into t-helper 2 predominance[2]. The current study showed no significant correlation between any of the cytokines and gender of the patients. This finding is in agreement with Berger *et al*., 2016[20]. The explanation for that is that the main difference between male and female patients is represented by certain hormonal levels, namely estrogen, progesterone and testosterone and these hormones have no effect on the level of inflammatory mediators[5]. The current study showed no significant correlation between any of the cytokines and age of the patients which are in agreement with Gołąbek-Dropiewska *et al*., [21]. The explanation for the lack of significant correlation between these cytokines and the age of the patient is most likely due a relatively small sample size; however, substantial amount of published literature document the negative correlation between age and immune markers due the concept of aging of the immune system[22]. The current study showed no significant correlation between any of the cytokines and duration of anesthesia, the duration of anesthesia has nothing to do with the level of inflammatory cytokines; therefore the most likely explanation is that the trigger for the rise in cellular counts and immune marker is the tissue injury produced by the surgical operation and so once tissue injury supervene the level of these markers get changed with disregard to the duration of anesthesia[23]. The current study showed no significant correlation between any of the cytokines and type of the anesthesia. The same previous explanation is proposed to explain the later finding; once tissue injury supervene the level of these markers get changed with disregard to the duration of anesthesia[24].

The present study showed that the level of immune markers CD16 and MCP-1 became significantly higher during operation, whatever the type and duration of anesthesia, in comparison with their levels before operation and that their level decreased significantly after operation, however, it did not return back to the same level before operation (with the exception of MCP-1 which returned back almost to the same level before operation). Natural killer cell is an important player of the innate immunity and its count is expected to rise during physiologic stress[18]. In the present study, there was no significant correlation between gender and age with MCP-1, CD16 immune markers and these results are in agreement with Karadeniz *et al*., 2017 and De Toda *et al*., 2016 and [22, 25]. In the present study, there was no significant correlation between duration of anesthesia and immune markers (MCP-1,CD16) and these results are in agreement with Song *et al.,* 2017[26]. therefore the most likely explanation is that the trigger for the rise in cellular counts and immune marker is the tissue injury produced by the surgical operation and so once tissue injury supervene the level of these cells and markers get rise with disregard to the duration of anesthesia[27]. In addition, in the present study, there was no significant correlation between type anesthesia and immune markers (MCP-1,CD16) and these results are in agreement with Berger *et al*.,2018[20].

CONCLUSIONS

Primarily there is no significant effect for anesthesia on immune response in patients undergoing orthopedic operations. And changes in cells, immune markers and cytokines were mainly attributable to tissue trauma during operation that is mediated by neuro-humoral response.

Conflicts Of Interest: There are no conflicts of interest.

REFERENCES

[1] Kitamora Y, et al., Anesthesia, cytokines and cancer recurrance. Glob Anesth Perioper Med, 2015; 1: 84-92.

[2] Kurosawa S, and M Kato, Anesthetics, immune cells, and immune responses. Journal of anesthesia2008; 22:263-277.
[3] Gu Q, et al., The influence of anesthetic techniques on long-terms survival of colorectal cancer patients undergoing surgery: a meta-analysis. International Journal Of Clinical And Experimental Medicine;2016: 9:13291-13297.
[4] Babatope I, et al., Normal CD4, CD8 T-lymphocytes and leucocyte baseline in healthy HIV-seronegative pregnant women in Ekpoma, Edo State, Nigeria. International Journal of Basic, Applied and Innovative Research 2018; 7:18-28.
[5] Liu J, et al., Effects of etomidate and propofol on immune function in patients with lung adenocarcinoma. American journal of translational research 2016; 8:5748.
[6] Oh YT., et al., Monocyte chemotactic protein-induced protein-1 enhances DR5 degradation and negatively regulates DR5 activation-induced apoptosis through its deubiquitinase function. Oncogene;2018:37:3415-3425.
[7] Eyelade O, et al., Outcome of anesthesia in elective surgical patients with comorbidities. Annals of African medicine 2016: 15:78.
[8] Sayit E, and Y Terzi, The effects of partial hip replacement surgery after hip fractures on complete blood count parameters in elderly. Int J Clin Exp Med 2017; 10:7157-7162.
[9] Deirmengian GK, et al., Leukocytosis is common after total hip and knee arthroplasty. Clinical Orthopaedics and Related Research 2011;469:3031-3036.
[10] Dąbrowska AM and R Słotwiński, The immune response to surgery and infection. Central-European journal of immunology 2014;39:532.
[11] Chen XF, et al., Prognostic value of perioperative leukocyte count in resectable gastric cancer. World journal of gastroenterology 2016; 22:2818.
[12] Ventura MT, et al., Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. Clinical and Molecular Allergy 2017; 15:21.
[13] Valiathan R, M Ashman, and D Asthana, Effects of ageing on the immune system: infants to elderly. Scandinavian journal of immunology 2016; 83: 255-266.
[14] Costa P, et al., Hematologic changes in propofol-anesthetized dogs with or without tramadol administration. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2013; 65:1306-1312.
[15] Cho HY, et al., The Delta Neutrophil Index as a predictive marker of histological chorioamnionitis in patients with preterm premature rupture of membranes: A retrospective study. PloS one 2017; 12: 0173382.
[16] Yuki K and RG Eckenhoff, Mechanisms of the immunological effects of volatile anesthetics: a review. Anesthesia & Analgesia, 2016; 123:326-335.
[17] Boersema G, et al., Systemic Inflammatory Cytokines Predict the Infectious Complications but Not Prolonged Postoperative Ileus after Colorectal Surgery. Mediators of inflammation, 2018.
[18] Godoy D, et al., Relationship between baseline white blood cell and c-reactive protein with mortality in patients with spontaneous intracerebral hemorrhage. J Neurol Neurophysiol, 2010;1:104.
[19] Cheng YC, et al., Combined general and regional anesthesia and effects on immune function in patients with benign ovarian tumors treated by laparoscopic therapy. International journal of clinical and experimental medicine 2013; 6:716.
[20] Berger M., et al., The effect of propofol versus isoflurane anesthesia on human cerebrospinal fluid markers of Alzheimer's disease: results of a randomized trial. Journal of Alzheimer's Disease 2016; 52:1299-1310.
[21] Gołąbek-Dropiewska K, et al., Analysis of selected pro-and anti-inflammatory cytokines in patients with multiple injuries in the early period after trauma. Central-European journal of immunology 2018; 43:42.
[22] De Toda IM, et al., Immune function parameters as markers of biological age and predictors of longevity. Aging (Albany NY) 2016;8:3110.
[23] Frieri M, K Kumar, and A Boutin, Trauma, Sepsis, Innate, Adaptive Immunity and Inflammation. MJ Immu 2017;1:004.
[24] Lisowska B, The Stress Response and Its Functional Implications in the Immune Response After Surgery in Patients with Chronic Inflammation Undergoing Arthroplasty, in Recent Advances in Arthroplasty 2012, InTech.
[25] Karadeniz MS, et al., Comparing the Effects of Combined General/Epidural Anaesthesia and General Anaesthesia on Serum Cytokine Levels in Radical Cystectomy. Turkish journal of anaesthesiology and reanimation 2017; 45:203.

[26] Song P, et al., Effects of different methods of anesthesia and analgesia on immune function and serum tumor marker levels in critically ill patients. Experimental and therapeutic medicine 2017; 14:2206-2210.
[27] Frieri M, K Kumar, and A Boutin, Stress, Trauma, Sepsis, Inflammation, Management in Asthma. Open J Asthma 2017; 1: 001-006.
[28] Tri D W, Erryana M, Diah M L, Immunomodulatory Effects Of Black Cincau (Mesona Palustris Bl.) Supplement On Escherichia Coli Strain O157-Infected Mice. Asian J Pharm Clin Res 2017;10: 326-330
[29] Laxman K S, Priyadarsini S, Effect Of Intravenous Versus Intrathecal Dexmedetomidine On Characteristics Of Hyperbaric Bupivacaine Spinal Anesthesia In Lower Limb Surgeryasian , J Pharm Clin Res 2018;11:427-430.