The effects of anesthesia techniques on adaptive immune response in orthopedic surgery

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Abstract

Background: Anesthesia is thought to be main factor affecting patient postoperative outcomes. Anesthetics, surgical stress and trauma have a modulatory effect on immune response which can promote increase or decrease of immune mediators either by direct effect or by stimulation of neuroendocrine system. The mechanism of immune modulation is by disturbing the balance between pro-inflammatory and anti-inflammatory and/or other mediators. **Aim**: The present study was carried out to evaluate the possible role of anesthetics and surgery on cellular immunity by measuring the levels of IL-2 which is pro-inflammatory and IL-10 an anti-inflammatory by ELISA. Flow cytometry was used to determine CD4, CD8. **Methods:** Serum level of IL-2 and IL-10 cytokines were measured by using enzyme linked immunosorbent assay (ELISA) technique. Flow cytometry was used to determine CD4, CD8. Comparisons for statistical significance were performed using Mann-Whitney *U* test. **Result:** Observation of CD4 lymphocytes counts reveled significant rise during time of anesthesia (42.23), however it showed significant reduction post-operatively (37.12), but the reduction did not reach baseline count. In addition, the observation of CD8 lymphocytes counts reveled significant rise during time of anesthesia (28.21), however it showed significant reduction post-operatively (22.91), but the reduction did not reach baseline count. Higher level of cytokine was IL-2 mainly post-operative median level (1257.7), and lower level was seen with IL-10 mainly pre-operative median level (36.08). Although the level of interleukin-2 (IL-2 p=0.393), interleukin-10 (IL-10 p=0.131) all showed no significant change in relation to time of anesthesia whether pre, peri and post-operative ($P > 0.05$) **Conclusion**: Analysis of data to correlate the cytokines level (IL-2, IL-10) and CD4 and CD8 with types of anesthetic drugs (general, local, and regional anesthesia) showed no significant association between these cytokine level and type of anesthesia ($P > 0.05$).

Key words: anesthesia, adaptive, cytokines, cluster of differention CD.

Introduction:

There are two types of immune response mechanisms aimed towards pathogens:, innate reactions, and acquired reactions, Acquired immunity. Cellular response is carried out mainly by various T lymphocyte subpopulations[1]**.** Cell-mediated immunity, mediated mostly by T-lymphocytes and their cytokines, which play an important role in immune cell activation, regulation, and communication[2]. Cytokines are essential

mediators for the regulation of both innate and acquired immunity and hematopoiesis. They modulate immune cell signaling, activation, adhesion and functioning**.** The balance between pro-inflammatory and anti-inflammatory cytokines is critical for the evolution of surgical complications and tumor progression. Several drugs, including anesthetic agents, influence cytokines secretion. Opioids, inhalational agents, intravenous and local anesthetics have shown different effects on immune system and cytokine expression[3]. 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[4]. Surgical stress and general anesthesia may suppress natural killer and cytotoxic T cells and also activating sympathetic nervous system[5]. The T lymphocytes are divided into following subsets: helper (Th1, Th2 and Th17), cytotoxic (CTL), regulatory (T reg) and memory (Tm)[6]. 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[7]. Main function of CD4 is a co-receptor of the T cell receptor (TCR) and assists the latter in communicating with antigen-presenting cells, The CD8 molecule is a marker for cytotoxic T cell population[8]. The primary aim of this study was to evaluate the effects of different anesthesia techniques on cellular immunity in orthopedic patients.

Materials and Methods

Subject **:** 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). The study population was assessed by questionnaire regarding age, gender, type of surgery, duration of anesthesia and clinical history of other disease.

Immunofluorescence: 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 (IL-2, IL-10). OD for each well is calculated at once by using a micro-plate reader spectrophotometer at wave length 450nm.

Flowcytometry: Flow Cytometry assay kits that have been used in this study are Flowcytometry kit for CD4 Thermo Fisher/ Bioscience™ USA and Flowcytometry kit for CD8Thermo Fisher/ Bioscience™ USA.

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.

Result: All results included in this study based on analysis of data belong to 30 patients; mean age was 35.67 ±17.53 years with wide range of age variation from 10 to 72 years. This study included 16 male and 14 female with proportions of 53.3% and 46.7%, respectively; the male to female ratio was 1.14:1. 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).

Higher level of cytokine was IL-2 mainly post-operative median level (1257.7), and lower level was seen with IL-10 mainly pre-operative median level (36.08). Although the level of interleukin-2 (IL-2 p=0.393), interleukin-10 (IL-10 p=0.131). Both showed no significant change in relation to time of anesthesia whether pre, peri and post-operative ($P > 0.05$), as seen in figures (1) and (2). 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 IL-2, IL-10. Analysis of data to correlate the cytokines level (IL-2, IL-10) 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).

Observation of CD4 lymphocytes counts reveled significant rise during time of anesthesia (42.23), however it showed significant reduction post-operatively (37.12), but the reduction did not reach baseline count. In addition, the observation of CD8 lymphocytes counts reveled significant rise during time of anesthesia (28.21), however it showed significant reduction post-operatively (22.91), but the reduction did not reach baseline count. Table (6) showed that the count of CD4 lymphocytes and CD8 lymphocytes before, perioperative and post-operatively, had no significant association with gender (p-value > 0.05). CD4 and CD8 lymphocytes showed significant correlation with age of patients ($P > 0.05$), as demonstrated in table (7). Immune marker have been analyzed in relation to type of anaesthesia and the results showed that the count of CD 4 lymphocyte, CD8 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 ($P > 0.05$) as revealed in table (8). 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 (9).

Discussion: The present study showed that the level of cytokines (IL-2 and IL-10) became significantly higher during operation, whatever the type and duration of anesthesia, in comparison with their levels before operation and that their level continued to rise insignificantly after operation, however, it did not return back to the same level before operation. The proposed mechanism for these findings in our study, may be attributed to

surgical stress that enhances a number pro-inflammatory mediators such as IL-2, produced by inflammatory cells which then activate hypothalamic-pituitary-adrenal axis, thereby pro- and anti-inflammatory cytokines and neuro-hormonal system and additively potentiate their abortive effect on the immune system. These results are in agreement with [9-12]. Some authors suggested that opioid use may cause rise in IL-10 through receptor (μ receptors) on the cell surface of macrophages and lymphocytes; these receptors when activated lead ultimately into increase secretion of anti-inflammatory mediator (IL-10) by certain intracellular mechanism that till now is unclear[13]. The current study showed no significant correlation between any of the cytokines and gender of the patients. This finding is in agreement with [14, 15]. This means that gender of patients whether male or females have nothing to do with the level of inflammatory cytokines. 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[16]. The current study showed no significant correlation between any of the cytokines and age of the patients. 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[17]. The current study showed no significant correlation between any of the cytokines and duration of anesthesia. 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[18]. The current study showed no significant correlation between any of the cytokines and type of the anesthesia. Surgical trauma results in a metabolic, hemodynamic, endocrine, and immune reaction that continue for a minimum of several days[2, 12].

The present study showed that the level of immune markers, CD4, CD8 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. The results of study are in agreement with Heimlich *et al*., 1999[19] and Volk *et al*., 2004[20]. The increase in the level of CD4 and CD8 lymphocytes is attributed to increase in the level of cytokines, pro-inflammatory mediators as a response to the stress accompanying surgical operation that is the mirror of humeral and neural stimulation. The rise in cytokines certainly will cause an increment in the number of CD4 and CD8 lymphocyte counts[1]. In the present study, there was no significant correlation between gender and immune markers (CD4, and CD8) and these results are in agreement with Landgraeber *et al*., 2014[21] and Karadeniz *et al*., 2017[22]. 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 count of immune cells, lymphocytes[23]. Moreover, in the present study, there was no significant correlation between age and immune markers (CD4 and CD8) and these results are in agreement with Karadeniz *et al*., 2017[22]and disagree with De Toda *et al*., 2016[17]. The explanation for the lack of significant correlation between these immune markers and the age of the patient is most likely due a relatively small sample size. In the present study, there was no significant correlation between duration of

anesthesia and immune markers (CD4 and CD8) and these results are in agreement with Song *et al*., 2017[24]. In addition there was no significant correlation between type anesthesia and immune markers (CD4 and CD8) and these results are in agreement with Karadeniz *et al*., 2017 [22] and Berger *et al*., 2016[14]. The explanation once tissue injury supervene the level of these cells and markers get rise with disregard to the duration of anesthesia[2].

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.

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%)

SD: Standard deviation; no: number of cases; IHD: ischemic heart disease.

Table (2): Association of cytokine level and gender.

Cytokine	Male (n=16)		Female (n=14)		*P*
	Median	IQR	Median	IQR	
IL-2 pre	1142.10	891.23	1148.90	586.44	0.803
IL-2 peri	1225.10	413.64	973.38	889.19	0.967

IL-2 post	1274.10	300.02	1110.80	764.68	0.677
IL-10 pre	36.08	38.07	36.41	25.11	0.603
IL-10 peri	37.06	22.89	45.19	30.04	0.244
IL-10 post	48.56	32.75	39.55	27.77	0.467

Table (3): Correlation between age and cytokine levels.

Cytokine	r	*P*
LogIL-2 pre	-0.124	0.515
LogIL-2 peri	0.128	0.501
LogIL-2 post	0.149	0.434
LogIL-10pre	-0.082	0.666
LogIL-10 peri	0.140	0.461
LogIL-10 post	0.051	0.788

Table (4): Correlation between cytokine levels and type of anesthesia.

Cytokine	General anesthesia		Local anesthesia		Regional anesthesia		*P*
	Median	IQR	Median	IQR	Median	IQR	
IL-2pre	1064.50	998.72	1448.20	491.88	1125.80	412.28	0.136
IL-2peri	905.34	597.33	1291.80	797.34	1147.50	830.00	0.227
IL-2post	1226.50	392.55	1406.10	408.87	1113.50	459.21	0.199
IL-10pre	35.00	34.81	36.41	33.40	37.49	21.80	0.885
IL-10pri	32.07	15.68	42.92	14.43	56.91	29.93	0.139
IL-10post	35.98	46.69	38.79	20.44	54.30	29.18	0.110

*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 IL-2 pre	-0.223	0.236
Log IL-2 peri	-0.078	0.681
Log IL-2 post	0.105	0.581
Log IL-10 pre	0.142	0.454
Log IL-10 peri	-0.079	0.678
Log IL-10 post	0.046	0.809

*Significant only at p≤0.05.

Table (6): Lymphocytes immune markers in relation to gender.

Marker	Total *n* = 30	SD	Male *n* = 16	SD	Female *n* = 14	SD	*P*†
	Mean		Mean		Mean		
CD4Pr	32.62	7.80	35.84	6.79	32.45	12.90	0.252
CD4Pe	42.23	17.08	46.10	17.49	35.68	16.26	0.070

CD4Po	37.12	15.11	37.12	16.09	37.37	17.94	0.454
CD8Pr	19.62	7.67	20.87	6.54	18.12	11.57	0.589
CD8Pe	28.21	12.18	29.48	7.75	26.99	15.49	0.236
CD8Po	22.91	12.40	25.93	14.92	19.14	10.53	0.328

*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 (7): Correlation of immune markers with age.

Marker	r	*P*
Log CD4Pr	0.339	0.067
Log CD4Pe	0.324	0.081
Log CD4Po	0.337	0.068
Log CD8Pr	0.186	0.325
Log CD8Pe	0.203	0.282
Log CD8Po	0.231	0.219

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

Table (8): Immune markers in relation to type of anesthesia

Marker	General		Local		Regional		*P*
CD4Pr	32.79	16.53	33.42	5.15	32.33	13.05	0.810
CD4Pe	40.23	17.68	40.26	17.48	42.23	17.72	0.830
CD4Po	36.70	19.63	38.56	12.03	34.46	20.84	0.940
CD8Pr	18.74	9.82	21.26	7.67	21.76	14.79	0.369
CD8Pe	29.34	14.70	27.13	6.47	29.52	19.08	0.844
CD8Po	22.08	17.69	25.98	11.50	18.17	15.44	0.368

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

Table (9): Correlation of immune markers with duration of anesthesia.

Marker	r	*P*
Log CD4Peri	0.186	0.325
Log CD4Post	0.093	0.627
Log CD8Peri	-0.276	0.139
Log CD8Post	-0.358	0.052

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

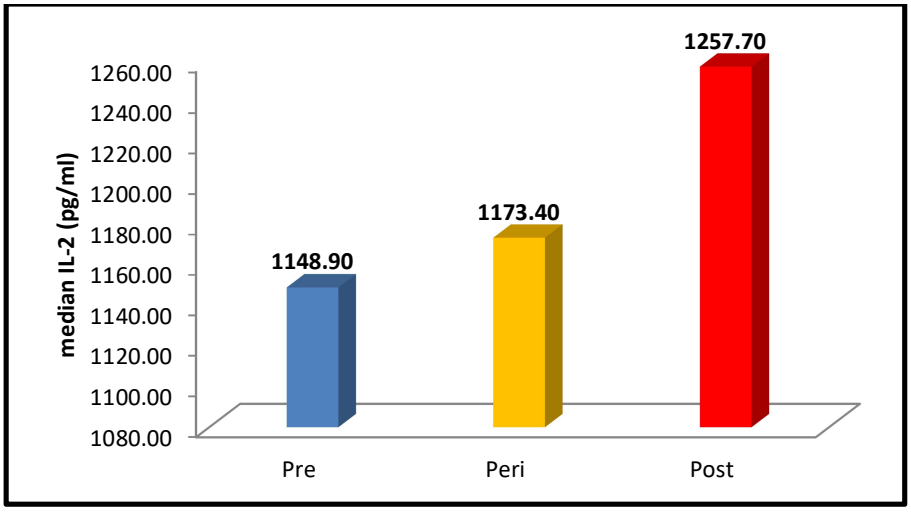
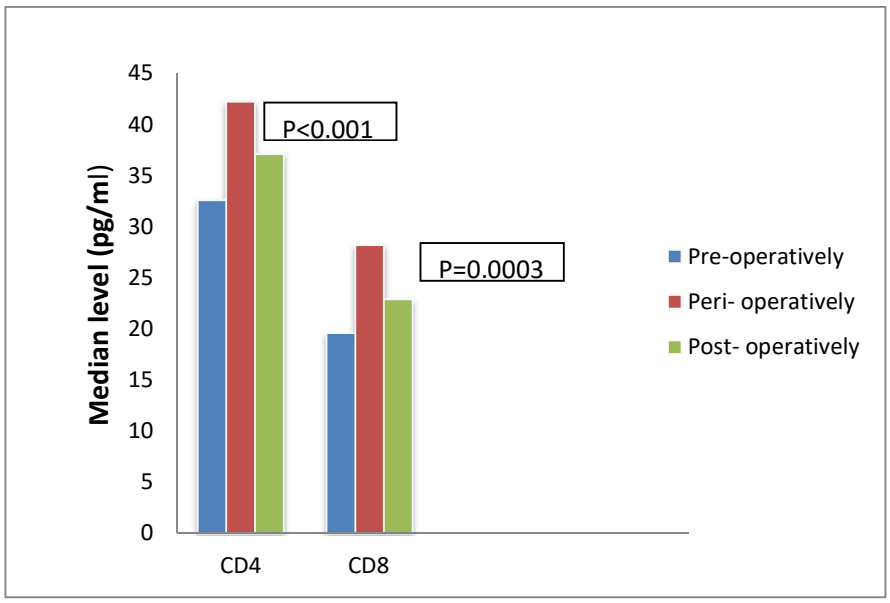


Figure (1): Level of IL-2 in relation to time of anesthesia.



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

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

1. Lisowska, B., et al., *Anesthesiology and the cytokine network.* Postepy Hig. Med. Dosw.(Online), 2013. **67**: p. 761-769.
2. Cruz, F.F., P.R.M. Rocco, and P. Pelosi, *Anti-inflammatory properties of anesthetic agents.* Critical Care, 2017. **21**(1): p. 67.
3. Kitamora, Y., et al., *Anesthesia, cytokines and cancer recurrance.* Glob Anesth Perioper Med, 2015. **1**: p. 84-92.
4. Kurosawa, S. and M. Kato, *Anesthetics, immune cells, and immune responses.* Journal of anesthesia, 2008. **22**(3): p. 263-277.
5. 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**(7): p. 13291-13297.
6. 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.

7. 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**(1): p. 18-28.
8. Osińska, I., K. Popko, and U. Demkow, *Perforin: an important player in immune response.* Central-European journal of immunology, 2014. **39**(1): p. 109.
9. Volk, T., et al., *Postoperative epidural anesthesia preserves lymphocyte, but not monocyte, immune function after major spine surgery.* Anesthesia & Analgesia, 2004. **98**(4): p. 1086-1092.
10. Tureci, E., et al., *The effects of analgesia-sedation on the immune system before and after cerebral digital subtraction angiography.* Turkish neurosurgery, 2011. **21**(3): p. 340-346.
11. Cheng, Y.-C., 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**(8): p. 716.
12. basnawi, E.a.a.A., *Effects of Anesthesia & Anesthetic techniques on Cellular Immunity.* Journal of Anesthesia & Critical Care, 2017. **7**(6).
13. Liang, X., et al., *Opioid system modulates the immune function: a review.* Translational perioperative and pain medicine, 2016. **1**(1): p. 5.
14. 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**(4): p. 1299-1310.
15. 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**(1): p. 42.
16. Liu, J., et al., *Effects of etomidate and propofol on immune function in patients with lung adenocarcinoma.* American journal of translational research, 2016. **8**(12): p. 5748.
17. De Toda, I.M., et al., *Immune function parameters as markers of biological age and predictors of longevity.* Aging (Albany NY), 2016. **8**(11): p. 3110.
18. Frieri, M., K. Kumar, and A. Boutin, *Stress, Trauma, Sepsis, Inflammation, Management in Asthma.* Open J Asthma, 2017. **1**(1): p. 001-006.
19. Heimlich, F., et al., *Immunosuppression caused by head and neck surgery.* HNO, 1999. **47**(10): p. 885-892.
20. Volk, T., et al., *Postoperative epidural anesthesia preserves lymphocyte, but not monocyte, immune function after major spine surgery.: Anesthesia (7).* Pain Practice, 2004. **4**(4): p. 315.
21. Landgraeber, S., et al., *The pathology of orthopedic implant failure is mediated by innate immune system cytokines.* Mediators of inflammation, 2014.
22. Karadeniz, M.S., 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**(4): p. 203.
23. Everett, B.G., et al., *Sexual orientation and gender differences in markers of inflammation and immune functioning.* Annals of Behavioral Medicine, 2013. **47**(1): p. 57-70.
24. 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**(3): p. 2206-2210.