Detection of Thoracotomy-Induced Alterations In Cell- and Humoral Mediated Immune Response

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It is well known that thoracotomy leads to several complications. In this study, effects of thoracotomy on cellular and humoral immunity has been investigated. Leukocyte count, lymphocyte count and lymphocyte ratio of 100 patients undergoing thoracotomy have been determined preoperatively and on the postoperative 3rd hour and 1st, 2nd, 3rd 5th days. Lymphocyte surface antigens (CD3, CD4, CD8, CD4/CD8, CD19, CD16/CD56) and immunoglobulin levels (IgG, IgA, IgM, IgE) of 40 patients in the preoperative period and postoperatively twice on 7th day and then in the third week have been measured. After thoracotomy, marked increases in leukocyte counts while marked decreases lymphocyte counts and lymphocyte ratios have been detected (p< 0.001). There was not any significant alteration in the levels of lymphocyte surface antigens and immunoglobulin levels in the postoperative period (p>0.2). According to these results, leukocytosis occurs in the early postoperative period however lymphocyte count decreases within the first week following thoracotomy. Subpopulation of lymphocytes and immunoglobulins are not affected from the operative stress.
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Key words: Thoracotomy, immune response.

Thoracotomy is a common operative procedure used for diagnosis or therapy of any intrathoracic pathology[1]. Thoracic surgical patients are susceptible to pneumonia because of impaired systemic and lung host defenses[2]. The incidence of pneumonia is higher with more extensive lung resections[2]. Also many complications concerning respiratory and circulatory systems have been observed after these operations.[3,4] These complications may be due to impairment of cellular and humoral immune response resulting from the thoracic surgery stress.

In this study, effects of thoracotomy operation on cellular and humoral immune system in different patient groups have been detected. In this respect, lymphocyte surface antigens (CD3, CD4, CD8, CD4/CD8, CD19, CD16/56) for cellular immunity detection and immunoglobulin levels (IgG, IgA, IgM, IgE) for humoral immunity detection have been measured. Also leukocyte counts, lymphocyte counts and lymphocyte ratios were analyzed.

MATERIALS AND METHODS

This study has been performed on 100 patients selected randomly among people operated for thoracotomy at Department of Thoracic Surgery in GATA Haydarpaşa Training Hospital between 1995-1998. Ninety-two patients were men and the remaining 8

patients were women. Average age was 34.4 years and age range was 18-73. Leukocyte and lymphocyte counts, and lymphocyte ratios have been measured in all patients. Lymphocyte surface antigens and immunoglobulin levels were measured in 40 patients. Of these 40 patients male to female ratio was 7:1 and average age was 36.2 (20-68).

A detailed history was taken from the patients by a physician. Physical examination and laboratory analysis were performed routinely. None of the patients had diabetes mellitus, renal insufficiency, seropositive HIV and other chronic diseases affecting immune system. None of the patients have had thoracotomy, except for tube thoracostomy before. Patients were divided into 7 groups (Table 1). Posterolateral thoracotomy was performed to all of the patients except those operated for axillary thoracotomy in the first group.

Blood samples were obtained from all patients in the preoperative period (one day before operation) and postoperative period (3rd hour, 1st day, 2nd day, 3rd day, 5th day after operation) within a 2,5 ml. tube containing EDTA (Vacutainer). Leukocyte counts, lymphocyte counts and lymphocyte ratios were measured with Coulter MD 16 hematology analyzer.

For quantitative immunoglobulin detection blood samples were collected once preoperatively and twice postoperatively, first on the 7th day and second in the third week. IgG, IgM and IgA quantitative measurements were made with immunoprecipitation technique (Turbox Orion). For IgE measurement ELISA method (Abbot Quantum) was used.

For detection of lymphocyte surface antigens, blood with EDTA were analyzed with direct method. 100 m l of blood was added to a combination of a directly conjugated monoclonal antibody with fluorochrom (anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-CD16/56) or isotype control antibodies (DAKO Ltd, UK). Following gentle mixing, the samples were left at room temperature for 10 minutes before addition of 200 m l of FACSlyse solution (Becton Dickinson, UK.) Following gentle resuspension, the samples were incubated for a further 10 minutes before the addition of 250

Table 1. Properties of patient groups performed thoracotomy.

PATIENT GROUPS	SURGICAL OPERATION
Patient group with axillary thoracotomy (n1: 8)	Thoracic sympathectomy (7) Open lung biopsy (1)
Patient group with bronchiectasis (n2: 6)	Segmentectomy (1) Lobectomy (4) Pneumonectomy (1)
Patient group with decortication (n3: 11)	Decortication (11)
Patient group with hydatid cyst (n4: 18)	Enucleation of hydatid cyst (3) Pericystectomy/cystectomy (13) Wedge resection (1) Lobectomy (1)
Patient group with primary lung cancer (Stage IA, stage IB, stage IIA, stage IIB, stage IIIA) (n5: 21)	Lobectomy (4) Bilobectomy (2) Pneumonectomy (15)
Patient group with advanced malignant lung cancer (Stage IIIB primary lung cancer and secondary lung cancer) (n6: 11)	Resection of metastatic lesion (4) Explorative thoracotomy (7)
Patient group with other diseases (n7: 25)	Wedge resection (4) Lobectomy (2) Bilobectomy (1) Pneumonectomy (1) Other surgical operations (17)

ᴍ l of 0.2% formaldehyde in PBS. Samples were analyzed by flow cytometry (Becton-Dickinson FACScan) within 2-4 hours of blood sampling.

Statistical Analysis. Values, given as mean ± standard deviation, were calculated with Excel for Microsoft Office 1997 for each group. The significance of differences between preoperative values and each of the postoperative values was tested using Student's t test; a p value of < 0.05 being defined as statistically significant.

RESULTS

Leukocyte counts increased markedly (+126%) within the initial hours following operation, then declined to +77% on the postoperative 1st, 2nd, 3rd days. At the 5th postoperative day, it fell below the level of preoperative value (Table 2). For all patient groups at the first hours following operation, leukocyte counts increased markedly, but the greatest increase in leukocyte count occurred when there was a coexisting chronic infection as decortication (+180%) and bronchiectasis (+176%). For leukocyte analysis there were statistically significant differences for postoperative 3rd hour and 1st, 2nd, 3rd days ($p<0.001$).

Lymphocyte counts increased (+2%) in the initial hours after operation, on the next day decreased below the preoperative level (-29%), after postoperative 3rd day began to increase (Table 2). When we divided patients into groups, in the resection applied group, lymphocyte count decreased in the first hours after operation (-25%), and in the other groups there was an increase in various degrees (+1% and +27%). For lymphocyte analysis there is a statistically significant difference for postoperative 1st, 2nd, 3rd, and 5th days ($p< 0.001$).

Similarly, when we examined lymphocyte ratios of 100 cases, there was a decrease (-54%) in the first hours after operation, then it began to increase and reached to 80% of preoperative value on the postoperative 5th day (Table 2). When we divided patients into groups, although lymphocyte ratio decreased in all patient groups in the first hours after operation, the greatest decrease was in the decortication group, and the least decrease was in the axillary thoracotomy group. For lymphocyte ratios there is statistically significant difference at all postoperative times ($p< 0.001$).

Table 2. Mean values (c), standard deviation values (SD), postoperative alteration percentages and probability values by student t test are given for leukocyte count, lymphocyte count, lymphocyte ratio in patients underwent thoracotomy. Preoperative values were accepted as %100.

Immune Parameter	Number of Patient	Preoperative	Postoperative 3rd hour	Postoperative 1st day	Postoperative 2nd day	Postoperative 3rd day	Postoperative 5th day
Leukocyte count	100						
Mean ± SD		8.463 ± 2.941	19.153 ± 6.728	14.957 ± 4.149	11.957 ± 3.455	9.720 ± 2.860	8.228 ± 2.701
Postoperative alteration		% 100	% 226.9	% 177.2	% 140.2	% 114.6	% 97.6
Probability			p< 0.001	p< 0.001	p< 0.001	p< 0.001	p> 0.2
Lymphocyte count	100						
Mean ± SD		2.285 ± 623	2.348 ± 1.074	1.624 ± 615	1.575 ± 773	1.731 ± 672	1.850 ± 574
Postoperative alteration		% 100	% 102	% 71	% 68.9	% 75.7	% 80.9
Probability			p> 0.2	p< 0.001	p< 0.001	p< 0.001	p< 0.001
Lymphocyte ratio	100						
Mean ± SD		28.9 ± 8.8	13.1 ± 6.3	11.4 ± 5.1	13.8 ± 7.7	18.4 ± 7.4	23.2 ± 6.2
Postoperative alteration		% 100	% 45.4	% 39.5	% 47.6	% 63.5	% 80
Probability			p< 0.001	p< 0.001	p< 0.001	p< 0.001	p< 0.001

Preoperative and postoperative mean values, standard deviation and probability values of lymphocyte surface antigens are given with Table 3 and of immunoglobulins in Table 4. There is not any statistically significant difference between preoperative and postoperative lymphocyte surface antigen and immunoglobulin levels (p> 0.2).

DISCUSSION

Relation between surgical trauma and immune system has been investigated for many years. The first studies reported that the operative stress caused decrease in the immune resistance against cancer cells in laboratory animals[5] and an increase in the growth of tumor[6] after surgical trauma. In later studies, it was established that lymphocytes lost their immunological ability after several surgical operations under general anesthesia. This immunosuppression did not influence the postoperative morbidity[7], however lymphocyte response against mitogens fell down markedly[8]. When T lymphocyte subgroups were studied, it was reported that surgical stress down-regulated the immune response. This down-regulation was more frequent in gastrointestinal malignancy patients than in the benign patients[9]. Özmen et al. searched influence of surgical trauma on immune system and suggested that cellular immunity was depressed markedly in laboratory animals with malignancy[10,11]. In another study of this group,

Table 3. Mean values (c), standard deviation values (SD), and probability values by student t test are given for lymphocyte surface antigens.

	CD3 (60-85%)		CD4 (29-59%)		CD8 (19-48%)		CD4/CD8 (> 1)		CD19 (11-16%)		CD56+CD16 (6-29%)	
	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative
Mean Value	62.7	64.5	38.4	41.2	22.4	22.6	2.05	2.01	16.8	18.2	16.5	15.5
± SD	± 14	± 17	± 15	± 18	± 11	± 10	± 1.5	± 1.1	± 8	± 7	± 9	± 13
Probability	p> 0.2		p> 0.2		p> 0.2		p> 0.2		p> 0.2		p> 0.2	

Table 4. Mean values (c), standard deviation values (SD), and probability values by student t test are given for immunoglobulin levels.

	IgG (gr/L) (7-15)		IgA (gr/L) (0.8-4.0)		IgM (gr/L) (0.4-2.5)		IgE (IU/mL) (10-180)	
	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative
Mean Value	8.1	8.5	1.5	1.7	0.53	0.57	>114	>117
± SD	± 3.5	± 3.3	± 1.2	± 1	± 0.4	± 0.3	± 75	± 78
Probability	p> 0.2		p> 0.2		p> 0.2		p> 0.2	

immunoglobulin levels, delayed type hypersensitivity and T/B lymphocyte ratios were investigated on operated patients and no significant difference could be determined[12].

Allendorf et al. conclude in their study that postoperative cell-mediated immune function varies inversely with the degree of surgical trauma[13]. Results from the minilaparotomy and laparoscopy groups suggest that procedures done through small incisions may result in preservation of postoperative immune function[14]. Da Costa et al. compared the effects of laparoscopy with laparotomy on extraperitoneal tumor growth and immune function in a murine model. There was a significant increase in flank tumor growth in the first 48 hours after laparotomy and laparoscopy compared with controls[14]. De Wilde et al. showed that although surgical trauma might depress various aspects of the immune response in rats, it did not decrease their resistance to intraperitoneal microbial infections[15]. In order to determine the effect of surgical trauma on neutrophil functions, Shijo et al. set up an experimental abdominal surgical model using rats and analyzed neutrophil functions. After the surgical trauma (24-48 hours), blood neutrophil counts significantly increased, and neutrophil chemotaxis, phagocytosis and active oxygen production were markedly enhanced[16]. In another study conducted by Mendoza-Sagaon et al. the effect of different operative procedures on the cell-mediated immune response in a pediatric animal model using the delayed type hypersensitivity (DTH) skin test.[17] A statistically significant difference in DTH skin reaction at 24 and 48 hours was observed between postoperative days 1 to 5 in the extraperitoneal and laparotomy groups with respect to baseline and the control group. According to the results of this pediatric animal model, abdominal surgical procedures accompanied by extensive tissue dissection produce a cellular immunosuppression, lasting up to 7 days, which is not observed in less invasive procedures. Observations concerning lesser immunosuppressive effects of laparoscopy when compared with laparotomy

in adult models, as previously described by the laboratory, were also found in this pediatric model[17]. Daphan et al. reported that the number of peritoneal polymorphonuclear leukocytes was significantly higher in the laparotomy alone group than in the control or any of the insufflation groups. Laparotomy and air insufflation depressed cell-mediated immunity[18]. Immune functions during laparotomy and laparoscopy have been studied extensively. Gitzelmann et al. suggested that cellular immunity was preserved after carbon dioxide pneumoperitoneum compared with extraperitoneal incisions and laparotomy as measured by DTH and the ability to reject an immunogenic tumor[19].

There are several studies about the influence of median sternotomy upon the immune system. A study about this subject revealed that in early period after open heart surgery CD4, CD4/CD8 ratio and immunoglobulin levels fell down markedly[20]. In another study CD3 and CD8 increased, CD4 did not change and CD4/CD8 ratio decreased[21]. Bilal et al. reported that immune system was disregulated after open heart surgery[22]. Naldini et al. declared that there was a significant decrease in IFN-g, TNF-a and IL-2 levels in these operations[23].

Kim et al. reported that total leukocyte count increased and lymphocyte count decreased after operation in operated patients for stage I and II lung cancer[24]. Also they reported that CD8 increased and CD4/CD8 ratio decreased. Ancheva et al. have found a marked decrease in NK activity of peripheral blood mononuclear cells after operation of lung cancer patients[25]. Watanabe et al. reported increase in leukocyte count and decrease in lymphocyte count in 18 patients who had thoracotomy and laparotomy because of esophagus malignancy[26]. But Watanabe et al. conversely suggested that thoracotomy leads to decrease in CD4+ and CD8+ cells and increase in CD4/CD8 ratio[26]. Watanabe et al. reported also a marked decrease in immunoglobulin levels in the first days after thoracotomy[26]. In our study, it was

found that thoracotomy did not cause a significant change on lymphocyte surface antigens and immunoglobulins. This result was not correlated with the findings of Kim et al[24]. However leukocyte count increased and lymphocyte count decreased after operation in our cases similar to results of Kim et al[24]. Also our results were not parallel to Gebhard et al. because they reported decrease in immunoglobulin levels in 34 patients operated for benign and malignant lung disease[27].

Our study suggests that thoracotomy leads to leukocytosis in the early postoperative period, however lymphocyte count decreases within the first week. Among the reasons of leukocytosis; trauma (such as operation, fractures, and burn injury) is one of the leading causes following infectious diseases. There is a neutrophil reserve in the bone marrow and neutrophils are released from bone marrow to the blood in cases of operative stress, infection, and corticosteroid intake. This may be an explanation for leukocytosis in the early postoperative period and lymphocyte count decreases proportionally.

In conclusion, this study gives some clues about reactive immune response against surgical stress by increasing inflammatory cells immediately. Subpopulation of lymphocytes are not affected from the operative stress showing similar percentages before and after thoracotomy. Also levels of immunoglobulins do not change implying the preservation of humoral immune response during thoracotomy.

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