Mediastinal lymphadenectomy causes immunosuppression after lung cancer surgery

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Abstract

The aim of this study was to estimate the amount of surgical injury caused by systematic lymphadenectomy of mediastinum in patients with lung cancer. Twenty-three patients undergoing uncomplicated resection due to lung cancer (11 right lung cancer, 12 left lung cancer) were analyzed. In patients with right lung cancer systematic lymphadenectomy, while in patients with left lung cancer systematic sampling was performed. Serum IL-6 and IL-1ra concentration was measured before and after surgery, and on postoperative day 1, 3, and 7, as well as in sputum at the end of surgery and in pleural fluid on postoperative day 1 by ELISA test. Peripheral blood lymphocyte (PBL) count was measured with flow cytometry. Time of surgery was higher in patients after right than after left thoracotomy (154.1±31.29 vs. 119.6±24.81 minutes; P=0.008). The number of resected mediastinal lymph nodes was higher in patients after right than left thoracotomy (27.6±7.6 vs. 11.1±8.1; P=0.00006). Postoperative decrease of PBL was significantly higher in patients after right than left thoracotomy (1.25±0.37 vs. 1.75±0.64 $\times 10^3/\mu L$; p=0.04). No significant differences were found in serum, pleural fluid and sputum concentration of IL-6 and IL-1ra between patients after right and left thoracotomy, but a negative correlation between concentration of these cytokines in pleural fluid and a number of resected mediastinal lymph nodes was found (Spearman test for IL-6: r=-0.723; P<0.001; for IL-1ra: r=-0.768; P<0.001). Number of "positive" N2 lymph nodes did not correlate with cytokines' pleural fluid concentration. Systematic lymphadenectomy of the mediastinum causes immunosuppression, measured by decrease of PBL and negative correlation between the number of resected mediastinal lymph nodes and concentration of IL-6 and IL-1ra in pleural fluid on postoperative day 1.

Key words: *NSCLC, lung cancer, mediastinal lymphadenectomy, IL-6, IL-1ra.*

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Introduction

Systematic mediastinal lymphadenectomy is a recommended completion of radical surgical resection of lung cancer [1]. However, with the introduction of computerized tomography for lung cancer screening, a growing number of small pulmonary neoplasms is observed which could be treated with less invasive methods. Surgeons' efforts to minimize surgical injury concentrate on three fields: surgical approach, extent of pulmonary resection and extent of mediastinal lymphadenectomy. The first goal was achieved by developing technique of VATS lobectomy [2-4]. To reach the second goal, multicenter surgical trials assessing the value of conservative pulmonary resections (segmentectomies and wedge resections) for lung cancer are organized [5, 6]. To achieve the third goal, sentinel node technique has been investigated [7], to establish in which cases mediastinal lymphadenectomy could be avoided. Avoiding lymphadenectomy would not merely decrease the time of operation, but even more importantly – it is believed to diminish the amount of surgical injury. Even with good availability of immunological tests, it is still difficult to assess how much of

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immune response is the result of lymphadenectomy, because lymphadenectomy is not a separate procedure, but is always performed together with thoracic incision and pulmonary resection.

The aim of this study was to estimate the amount of surgical injury caused by systematic lymphadenectomy of mediastinum in patients with cancer of the right lung, comparing with surgical injury in patients with cancer of the left lung (where systematic sampling of mediastinal lymph nodes was performed), and benign pulmonary diseases (no mediastinal lymphadenectomy).

Material and Methods

Clinical data were collected prospectively from 84 patients (17 women, 67 men, age 29 through 75 years, mean 60.4±9.6 years), treated with anatomical pulmonary resections (at least lobectomy) due to lung cancer or non-malignant diseases. In 69 patients (48 without and 21 with postoperative complications) operation was performed due to lung cancer, while in 15 (13 without and 2 with complications) due to non-malignant diseases. Majority of patients with complications were operated on due to diseases of the right lung (17 cancer patients and one aspergilloma patient). Because immune response to lymphadenectomy is much smaller than immune response to infection or hemorrhage, for further analysis only patients with an uneventful postoperative course were included. To avoid influence of different surgical approach and different type of anesthesia, only patients who were operated through muscle-sparing postero-lateral thoracotomy and without epidural analgesia were selected. Immunological studies were performed only in patients with lung cancer of the right lung who had systematic lymphadenectomy (group 1), in patients with cancer of the left lung who had systematic sampling (group 2) and in patients with non-malignant diseases who did not have any mediastinal nodal dissection (group 3). Patients who had preoperative invasive staging more extensive than bronchoscopy and transthoracic fine needle aspiration (mediastinoscopy, mediastinotomy, thoracoscopy, Daniels procedure) within a month before curative resection, were excluded. Finally, out of the primary group, 33 patients who underwent uncomplicated resections, including 23 with lung cancer (3 women, 20 men; age 58.3±8.8 years) and 10 with benign diseases (3 women, 7 men; age 57.5±12.8 years), fulfilled inclusion and exclusion criteria for immunological studies. In 23 patients with lung cancer (age 58.1±9.96 years) 16 lobectomies (1 left lower, 3 right lower, 7 left upper, 4 right upper, 1 right lower bilobectomy) and 7 pneumonectomies (3 right and 4 left) were performed. In all lung cancer patients mediastinal nodal dissection was performed by the same surgeon (TS). In patients with non-malignant diseases (age 57.5±12.8 years) 10 lobectomies (3 left lower, 2 right lower, 3 right upper and 2 medial) were performed.

Serum IL-6 and IL-1ra concentrations were measured before surgery, at the end of surgery and on postoperative day 1, 3 and 7, as well as in sputum at the end of operation and in pleural fluid on postoperative day 1. After obtaining, venous blood and pleural fluid were cooled to 4°C, centrifuged at a speed 2500/min for 10 minutes, and then preserved in temperature –80°C until further investigations. Sputum was obtained just before extubation, by washing routinely used catheter with saline. Diluted sputum was frozen at –80°C. Before freezing, mucus was removed by centrifuging and filtering through gauze. Concentrations of IL-6 and IL-1ra were determined using enzyme immunoassay kits (Quantikine R&D Systems Europe Ltd, Barton Lane Abingdon, Oxon, UK). Immunological studies were performed with STAT FAX 2100 device.

Degree of dilution of sputum was assessed by comparing concentration of urea in sputum and serum, collected at the end of operation, with urease method (Olympus Diagnostica GmbH, Lismeehan, O'Callaghan Mills, Co. Clare, Ireland). Tests were performed with OLYMPUS AU400 device.

The number of peripheral blood lymphocytes was measured with flow cytometry on postoperative day 1 with ACT 5DIFF Beckman-Coulter device.

Informed consent was obtained from every patient accrued. The study was approved by the local Ethics Committee.

Statistical analysis

Results were expressed as median and first and third quartile, or mean values ±SD or as number and percentage. To evaluate statistical significance of difference between preoperative and postoperative results of cytokines concentration, Wilcoxon test with Bonfferroni correction was used. Fisher exact test for categorical parameters was used. The differences between groups were analyzed with the Mann-Whitney *U* test. Computations were performed using SPSS 12.0 statistical package.

Results

Clinical characteristics of patients from three analyzed groups are presented on the Table 1.

Before surgery, number of peripheral blood lymphocytes (PBL) in the whole group, in patients with cancer, in patients without cancer and in patients with cancer of the right and left lung was 1.9±0.6; 1.9±0.5; 2.0±0.7; 1.9±0.4 and 2.0±0.5 (×109), respectively. Differences between groups were not significant. After operation, the number of PBL was 1.5±0.6; 1.5±0.6; 1.4±0.5; 1.2±0.4 and 1.7±0.6 (×109), respectively. In all groups, significant decrease of PBL count on postoperative day 1 was noted (P=0.0000002; P=0.00002; P=0.005; P=0.004 and P=0.002, respectively). Patients from group 1 had lower postoperative number of PBL than patients from group 2 (1.2±0.37 vs. 1.7±0.64 ×109; p=0.04). Decrease in PBL was significant in the whole group of 33 patients (P=0.001), in 23 patients with cancer (P=0.02) and in patients

Table 1. Clinical parameters in patients with systematic lymphadenectomy, in patients with systematic sampling and in patients without lymphadenectomy (n=33)

Parameter	Group 1 (n=11)	Group 2 (n=12)	Group 3 (n=10)	P value (1 vs. 2)	P value (1 vs. 3)
age	58.9±8.65	57.8±9.2	57.5±12.8	0.78	0,77
sex	10M/1F	8M/2F	7M/3F	0.3	0.6
number of packyears of cigarettes	39±22	32±13	36±74	0.7	0.4
time from quitting smoking (years)	1.9±6.0	3.1±7.4	5.8±11.9	0.7	0.4
preoperative FEV_1 % normal value	85.8±22.9	83.2±13.4	74.0±15.3	0.7	0.2
preoperative FVC % normal value	96.4±16.8	91.8±14.9	78.4±14.9	0.5	0.03
time of surgery (hours)	154.1±31.3	119.6±24.8	128.9±34.3	0.008	0.1
intraoperative blood loss	282±253	225±131	230±162	0.5	0.6
pneumonectomies	3/11	4/10	0	0.2	0.09
postoperative drainage (ml)	1132±630	1158±733	980±395	0.9	0.5
postoperative drainage (days)	3.1±0.9	3.2±1.5	4.0±1.3	0.9	0.07
antibiotics (days)	3.36±2.29	1.0±1.71	2.2±2.62	0.01	0.3
PBL before surgery	1.9±0.4	2.0±0.5	2.0±0.7	0.5	0.6
PBL after surgery	1.2±0.4	1.7±0.6	1.4±0.5	0.04	0.6
squamous cell carcinoma	3	9	0	0.009	<0.001
tumor diameter	45.6±18.2	40.3±15.4	–	0.45	<0.001
number of resected N2 nodes	27.6±7.6	11.1±8.1	0	0.00005	–
number of positive N2 nodes	3.5±8.6	0.3±0.6	0	0.2	–
number of resected N1 nodes	10.0±5.6	7.3±5.9	1.1±2.1	0.3	–
number of positive N1 nodes	0.9±1.3	0.5±1.4	0	0.5	–
number of units of blood transfused	1.67±1.41	1.1±1.8	0.44±0.9	0.5	0.04
time of hospitalization after surgery	8.36±1.5	8.75±1.36	8.1±1.4	0.5	0.7

with cancer of the right lung (P=0.003). Decrease of PBL was not significant in patients with cancer of the left lung (P=0.3) and in patients with benign diseases (P=0.05).

In the whole group, median serum concentration of IL-6 before surgery, at the end of operation and on postoperative day 1, 3 and 7 was 6.5 (range: 2.8-17.8); 73.3 (32.1-123.5); 206.1 (139.8-350.7); 82.9 (41.1-130.4) and 27.4 (12.5-36.6) pg/mL, respectively. Serum concentrations of IL-6 before operation and on all subsequent days, as well as concentrations of IL-6 in sputum, were not statistically different between groups. Pleural fluid concentration of IL-6 was higher in group 3 than 1 (22975 (19980-37613) vs. 2080 (1942-12297) pg/mL; p=0.0003). The difference between group 2 and 1 was not significant (17282 (2128-23881) vs. 2080 (1942-12297) pg/mL; p=0.09) (Figure 1).

Median serum concentration of IL-1ra was 436.5 (262.6-540); 955.6 (441.9-1581.4); 944.9 (782.9-1447.5); 848.6 (562.5-1302.5) and 662.6 (408.8-1205.2) pg/mL, respectively. Serum concentrations of IL-1ra before operation and on all subsequent days, as well as concentrations of IL-1ra in sputum, were not different between groups. In pleural fluid on postoperative day 1, level of IL-1ra was significantly higher in group 3 than 1 (76666 (53618-89618) vs. 16560 (15840-18333) pg/mL; p=0.0004). The difference between group 2 and 1 was not significant (35026 (16740-50515) vs. 16560 (15840-18333) pg/mL; p=0.09) (Figure 2).

A significant negative correlation between the number of resected mediastinal (N2) lymph nodes and level of cytokines in pleural fluid was observed in 23 lung cancer patients (for IL-6: r=-0.44; p=0.04; for IL-1ra: r=-0.57; p=0.01) (Figure 3). No significant correlation between level of cytokines and number of resected intrapulmonary lymph nodes (N1), number of "positive" N2 nodes and number of "positive" N1 lymph nodes was observed.

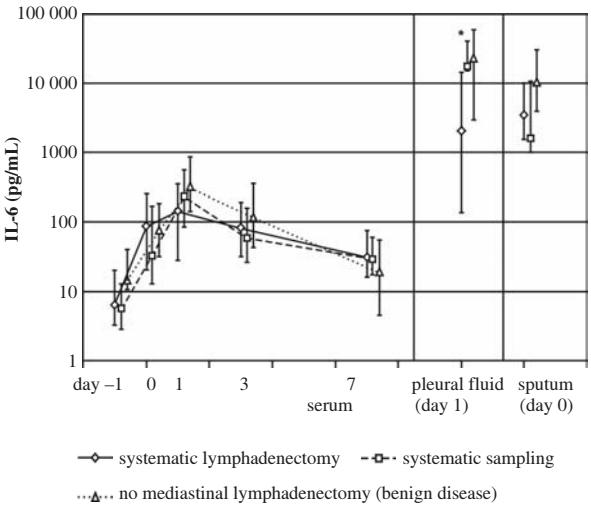

Figure 1. Concentration of IL-6 in serum, pleural fluid and sputum in patients after systematic lymphadenectomy, systematic sampling, and no lymphadenectomy of superior mediastinum. *P<0.05 between group 1 and 3

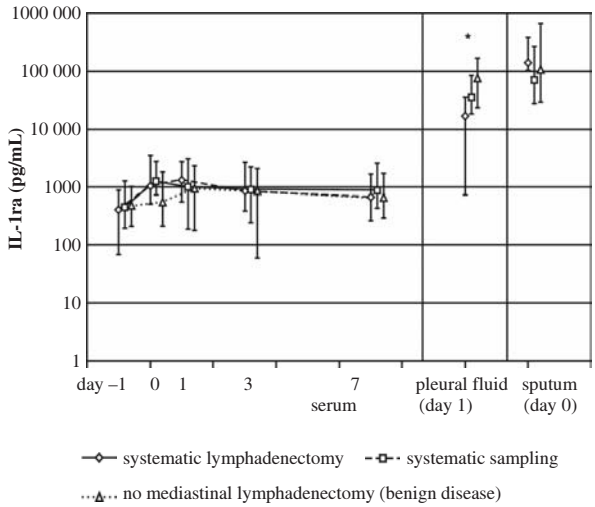

Figure 2. Concentration of IL-1ra in serum, pleural fluid and sputum in patients after systematic lymphadenectomy, systematic sampling, and no lymphadenectomy of superior mediastinum. *P<0.05 between group 1 and 3.

Discussion

Assessing serum concentration of immune response mediators is a widely recognized method of measurement of the amount of surgical injury and SIRS-type reaction (Systemic Inflammatory Response Syndrome) [8-10].

Mediastinal lymph node dissection, first described over 50 years ago [11, 12] is a mandatory completion of radical resection of lung cancer, according to current recommendations. However, it is a different procedure in patients with cancer of right and left lung, due to two main reasons: (1) predominant lymphatic drainage of both lungs through right paratracheal lymph nodes and (2) impaired access to paratracheal lymph nodes through left thoracotomy, due to aortic arch and its large branches. In patients with cancer of the left lung, systematic lymphadenectomy consists of transsection of superior pulmonary ligament and removal of all fatty tissue including lymph nodes, which is considered by some surgeons challenging and time consuming procedure. Additionally, in patients with cancer of the left lung, lymphadenectomy does not influence the late outcome as much as lymphadenectomy in patients with cancer of the right lung [13]. Due to all these reasons, in patients with cancer of the left lung, systematic sampling (consisting of removal of 2-3 lymph nodes from groups 5, 6, and 7, without transsection of superior pulmonary ligament and without removal of left inferior paratracheal lymph nodes – group 4L) is widely accepted. On the background of this study was the assumption that systematic sampling, as a less extensive procedure, causes smaller immune response than systematic lymphadenectomy, and therefore can serve as control group

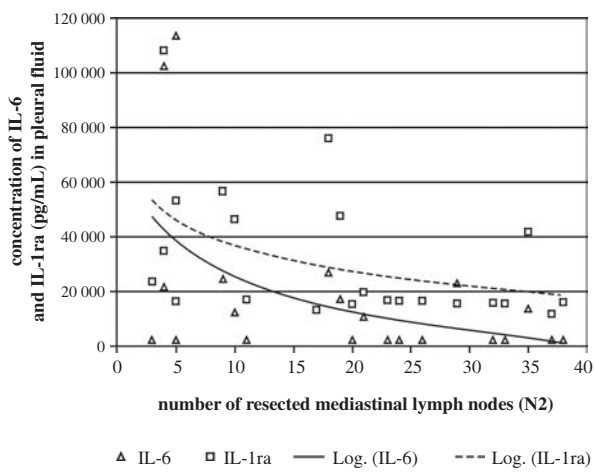

Figure 3. Correlation between concentration of IL-6 and IL-1ra in pleural fluid and the number of resected mediastinal lymph nodes (N2) in 23 patients with lung cancer without complications

in the study evaluating amount of surgical injury caused by systematic lymphadenectomy. Another control group consisted of patients operated on due to benign pulmonary diseases, with no mediastinal nodal dissection performed.

From the analysis of patients with complications who were excluded from the current study, we learned that elevated concentration of IL-6 and IL-1ra in pleural fluid on postoperative day 1 is a sensitive early marker of

postoperative complications [14]. Therefore, negative correlation between number of resected N2 lymph nodes and concentration of these cytokines in pleural fluid of lung cancer patients (Figure 3) might paradoxically suggest that patients who had more extensive lymphadenectomy were of lower risk of developing complications.

Several explanations of this fact are possible: (1) majority of patients with complications were operated on due to cancer of the right lung and due to squamous cell carcinoma (differences were not significant) and excluding these patients from the current study might influence its results; (2) excised lymph nodes or removed lymphatic vessels can be the source of cytokines in pleural fluid; (3) higher number of mediastinal lymph nodes are found in the resected specimen of patients with more advanced cancer which causes more pronounced immunosuppression; (4) at least some cytokines are produced by pneumocytes in the operated lung [15], therefore concentration of cytokines is lower when the route of lymphatic drainage is transsected or removed; (5) lymphadenectomy decreases number of mediastinal dendritic cells which play major role in presenting inhaled bacterial antigens to T cells [16].

The fact that no significant correlations were found between concentration of IL-6 and IL-1ra in pleural fluid and number of "positive" N1 and N2 lymph nodes suggests that the third explanation is not valid. We did not find any other studies in the literature assessing immune response to mediastinal lymphadenectomy.

Another symptom of immunosuppression in patients after systematic lymphadenectomy of the mediastinum (cancer of the right lung) may be significant decrease of PBL after surgery. Decrease of PBL was highly significant in patients after surgery of right lung and not significant in patients after resections of cancer of left lung. Therefore, we believe that negative correlation between concentration of IL-6 and IL-1ra in pleural fluid and number of resected N2 lymph nodes should be interpreted as immunosuppression (which can predispose to postoperative complications), not as decreased risk of development of postoperative complications (in analogy to increased concentration of IL-6 and IL-1ra in pleural fluid observed by us in the study concerning patients with complications).

Conclusions

Systematic lymphadenectomy performed in patients with cancer of the right lung, in comparison with patients with cancer of the left lung, does not result in elevation of IL-6 and IL-1ra concentration in serum, sputum nor pleural fluid which indicates that this procedure is not a major factor influencing changes in the immune response. Systematic lymphadenectomy is accompanied by immunosuppression, as measured by negative correlation between number of resected N2 nodes and concentration of IL-6 and IL-1ra in pleural fluid on postoperative day 1, and by decreased number of peripheral blood lymphocytes in comparison with patients after systematic sampling.

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