Concentrations of interleukin 6 and interleukin 1 receptor antagonist in pleural fluid as early markers of complications after lung cancer surgery

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Abstract

The objectives of the study was to assess value of Interleukin 6 (IL-6) and Interleukin 1 receptor antagonist (IL-1ra) serum, sputum and drained fluid concentrations as markers of complications in patients operated on due to lung cancer. Thirty-eight patients (15 with, 23 without complications) with resectable lung cancer were analyzed. Serum IL-6 and IL-1ra concentrations were 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. In serum, concentrations of IL-6 and IL-1ra were significantly elevated after surgery in all patients but no differences between two groups were found. Patients with complications had higher concentrations of IL-6 in pleural fluid (90048 (33490-94768) vs. 6554.4 (2003-20636) pg/mL; P=0.00006) and in sputum (3509.4 (2434-14168) vs. 367.6 (245.8-1096) pg/mL, P=0.02). Patients with complications had higher concentrations of IL-1ra in pleural fluid (67908 (52638-106694) vs. 16950 (16050-45470) pg/mL; P=0.00007) and in sputum (80109 (52289-212459) vs. 11816.3 (5854-30511.9) pg/mL, P=0.02). On day 1 after surgery a positive correlation between serum and pleural fluid concentration of IL-6 and IL-1ra were observed (Spearman test for IL-6: r=0.47; p=0.02; for IL-1ra: r=0.48; p=0.02) Logistic regression analysis showed that increase of concentration of IL-6 and IL-1ra in pleural fluid increases the risk of complications (P=0.0008 and P=0.03, respectively). Results of multivariate analysis confirmed that concentrations of IL-6 and IL-1ra in pleural fluid on postoperative day 1 are independent risk factors of development of postoperative complications (for IL-6: OR=1.029; CI=1.002-1.058; P=0.036; for IL-1ra: OR=1.024; CI=1.000-1.049; P=0.046). Elevated concentrations of IL-6 and IL-1ra in pleural fluid on postoperative day 1 are promising early markers of postoperative complications in lung cancer surgery.

Key words: *lung cancer, surgery, complications, IL-6, IL-1ra.*

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Introduction

Preoperatively existing immunosuppression in lung cancer patients is deteriorated by surgical injury and may be responsible for development of postoperative inflammatory complications. Nowadays, not only infections [1], but also postoperative atrial fibrillation [2] are considered inflammatory complications. Postoperative complications are observed in over 30% of patients after extensive pulmonary resections and in most specialized centres still about 2% mortality after

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lobectomies and 6% after pneumonectomies is observed [3, 4]. Elevated production of cytokines and their antagonists is inevitably corollary of surgical injury and bacterial contamination and can lead to the development of Systemic Inflammatory Response Syndrome (SIRS) and the Multiple Organ Dysfunction Syndrome (MODS). Postoperative immune response is mediated by proinflammatory cytokines, for example Interleukin-1 (IL-1), Interleukin 6 (IL-6) and tumour necrosis factor (TNF), and modulated by the naturally occurring antagonists of these cytokines i.e. soluble TNF receptor (sTNFR) and Interleukin 1 receptor antagonist (IL-1ra). Observation that levels of these mediators are higher in patients with postoperative complications after major surgery [5, 6], gave rise to an idea that some of them could serve as early markers of complications and enable prompt administration of proper treatment, leading to decreased morbidity and mortality. This observation was also confirmed in our previous studies [7, 8] and metaanalysis [9] concerning patients undergoing abdominal and thoracic operations, which showed that IL-6 and IL-1ra are the most sensitive markers of inflammatory response after minor and major surgical injury. Our preliminary data showed that IL-6 and IL-1ra in pleural fluid are promising early markers of postoperative complications [10]. The aim of this work was to calculate specificity, sensitivity and cut-of point of this novel method.

Methods

Data were collected prospectively from 69 patients (age 61.2±8.1 years), operated on due to primary lung cancer. Out of this group, 21 developed postoperative complications and 3 patients died, due to stroke, pneumonia and acute respiratory distress syndrome (ARDS). Clinical and laboratory data, as well as biological material for immunological investigations were collected simultaneously. For the final analysis only patients who were operated through muscle sparing postero-lateral thoracotomy, without epidural analgesia, who did not have operations in general anaesthesia (i.e. mediastinoscopy, mediastinotomy, videothoracoscopy) within a month before pulmonary resections, were included in the immunological study. Patients receiving immunosuppressive therapy, with lung metastases from other organs, with renal, circulatory or hepatic insufficiency or with insulin-dependent diabetes were excluded. Finally, 38 patients with lung cancer fulfilled inclusion and exclusion criteria, including 15 with and 23 without postoperative complications. Characteristics of patients with immunological studies without (C–) and with (C+) postoperative complications, and final histology are presented in Table 1 and Table 2. Concomitant diseases and number and types of postoperative complications are presented in Table 3 and Table 4.

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 with saline a catheter routinely used for removing sputum from bronchial tree. Diluted sputum was frozen at –80°C. Before freezing, mucus was removed by centrifuging and

Table 1. Clinical characteristics of patients without (C–) and with (C+) postoperative complications after lung cancer surgery

Characteristics of patients	C–	C+	P
number of patients	23	15	NS
women : men	3 : 20	2 : 13	NS
age (years), mean ± SD	58.3±8.8	65.5±4.6	0.01
lobectomies + bilobectomies ÷ pneumonectomies	16 : 7	11 : 4	NS
right lung ÷ left lung	11 : 12	13 : 2	NS
squamous cell cancer ÷ adenocarcinoma	14 : 9	12 : 3 *	NS
preoperative FVC (% normal)	94.0±15.6	77.8±16.5	0.01
preoperative FEV_1 (% normal)	84.4±18.2	69.6±19.7	0.04
atelectasis or fever before surgery	4	7	NS
bronchial asthma	1	3	NS
concomitant circulatory system diseases	29	8	0.01
no concomitant disease	2	4	NS
time of surgery (min)	136.1±32.6	181±54.0	0.003
intraoperative blood loss (mL)	252.2±196.3	427.3±382.0	NS
total postoperative drainage (mL)	1145.7±670	2371±1119	0.0009
days of postoperative drainage	3.13±1.22	9.27±11.4	0.01
postoperative hospitalization (days)	8.57±1.4	16.47±11.84	0.003
treatment with antibiotics (days)	2.13±2.3	3.73±2.19	NS
primary tumour diameter (mm)	42.83±16.6	48.6±34.8	NS
intrapulmonary and hilar (N1) nodes excised	8.8±5.6	7.5±4.4	NS
mediastinal (N2) nodes excised	19.0±11.4	22.2±14.5	NS

** including one patient with small cell lung cancer; SD – standard deviation; FEV_1 – percent of normal value of preoperative FEV_1; FVC – Percent of normal value of preoperative FVC.*

Table 2. Final pathology in 38 patients with immunological studies

Final pathology	Without complications (C–) (n=23)	With complications (C+) (n=15)
squamous cell carcinoma	13	12
adenocarcinoma	9	2
adenosquamous carcinoma	1	
Small cell lung cancer		1

filtering through gauze. Concentrations of IL-6 and IL-1ra were determined using commercially available enzyme immunoassay kits. 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.

Informed consent was obtained from every patient accrued. The study as approved by the local Ethics Committee in Olsztyn. (Decision No 109/2004/II; June 29, 2004).

Results were expressed as median and first and third quartile, or mean values ±SD. To evaluate statistical significance of difference between preoperative and postoperative results of cytokines concentrations Wilcoxon test with Bonfferroni correction was used. Fisher exact test for categorical parameters was used. The differences between groups without and with postoperative complications were analyzed with Mann-Whitney *U*-test. Impact of selected clinical and immunological factors on the risk of development of postoperative complications was analyzed using logistic regression method. Results are presented as odds ratio with 95% confidence intervals.

Table 4. Types of complications in 14 patients operated on due to lung cancer

Type of complication	Number of patients
respiratory	18
pneumonia	2
atelectasis requiring bronchoaspiration	4
prolonged parenchymal air leak (>6 days)	6
residual space	4
ARDS	2
other infectious	2
wound suppuration	2
surgical	6
postoperative bleeding	4
early bronchial fistula	1
bronchial stenosis	1
circulatory	4
atrial fibrillation	4
other (stroke)	1
death	3

Table 3. Concomitant diseases in patients with (C+) and without (C–) complications.

Type of concomitant disease	C–	C+
FEV_1<80% normal value	10	11
atelectasis or fever before surgery	4	7
bronchial asthma	1	3
history of heart infarct	3	0
coronary insufficiency	7	1
lower limb ischemia	5	2
arterial hypertension	7	1
varicous veins of lower limbs	5	3
mitral valve insufficiency	2	1
ulcer disease history	3	3
epilepsy	1	0
history of other cancer	0	1
total	**45**	**32**
no concomitant diseases	2	2

Results

In the whole group of patients, median serum concentration of both IL-6 and IL-1ra at the end and after surgery were significantly higher (P<0.05) compared to preoperative values (Figure 1).

In patients with complications (C+), differences of IL-6 concentrations between groups (C+ vs. C–) were not significant (Figure 2).

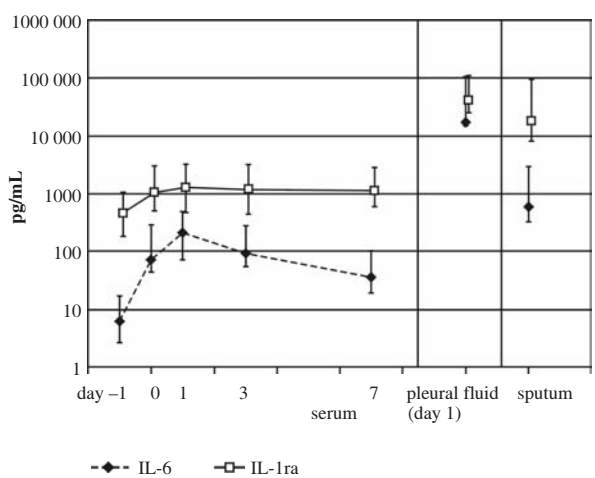

Figure 1. Changes in IL-6 and IL-1ra concentrations (pg/mL, median, 1st and 3rd quartile) in serum, drained fluid and sputum in the whole group of patients after lung cancer surgery

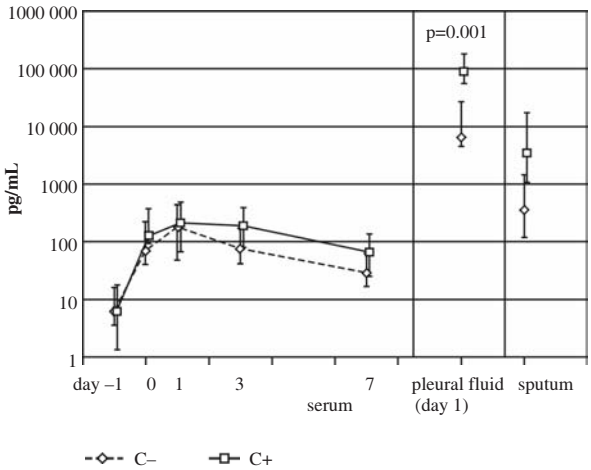

Figure 2. Changes in IL-6 concentrations (pg/mL, median, 1st and 3rd quartile) in serum, drained fluid and sputum in patients without (C–) and with (C+) postoperative complications

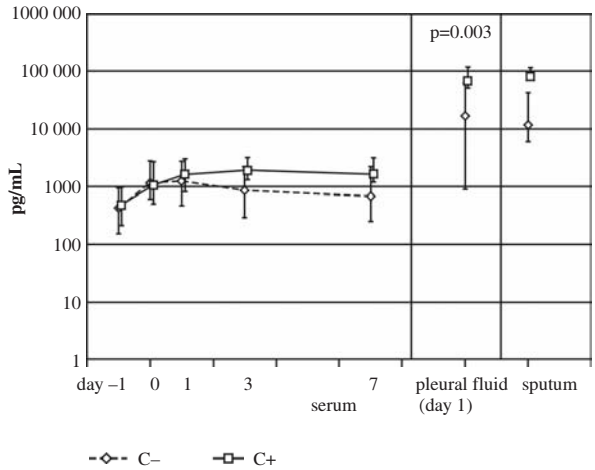

Figure 3. Changes in IL-1ra concentrations (pg/mL, median, 1st and 3rd quartile) in serum, drained fluid and sputum in patients without (C–) and with (C+) postoperative complications

In patients with complications (C+), differences between serum concentrations of IL-1ra in groups C+ and C– were not significant (Figure 3).

Differences in sputum concentration between C+ and C– groups for both IL-6 and for IL-1ra were not statistically significant (Figures 1-3). However, in 6 patients from group C– and in 8 patients from group C+ sputum was so scarce that it as not obtained with the catheter.

Median concentration of IL-6 in pleural fluid in the whole group was 17282 pg/mL (2088-90048), in the group with complications (C+) 90048 (33490-94768), in the group without complications (C–) 6554.4 (2003-20636), difference between C+ and C– group was significant (P=0.001). Median concentration of IL-1ra in pleural fluid in the whole group was 42010 pg/mL (16620-67908); in the group with complications (C+) it was 67908 (52638-106694), in the group without complications (C–) 16950 (16050-45470) pg/mL, difference between C+ and C– group was significant (P=0.003) (Figures 1-3).

The results of logistic regression analysis of influence of early immunological parameters (IL-6 and IL-1ra in serum, sputum and pleural fluid) on the risk of development of postoperative complications were presented in figure 4. It shows that concentration of IL-6 and IL-1ra in pleural fluid on the first postoperative day significantly increased the risk of development of complications.

Sensitivity, specificity and cut-off point for pleural fluid IL-6 concentration was 83%, 87% and 24626.6 pg/mL, and for IL-1ra: 67%, 94% and 59653.2 pg/mL, respectively.

Discussion

Postoperative complications remain a major problem in lung cancer patients. The major factor influencing the risk of complications in these patients is immune response to surgical trauma. Changes in the immune system after major surgery can be monitored by measuring concentration of immune system mediators [11], and number and activity of immunocompetent cells [12]. Major surgical trauma results in migration of immunocompetent cells to the operated area and subsequently in extensive local production of cytokines and their inhibitors, also called tissue hormones. Elevated concentration of these mediators in serum occurs only when their local concentration is extremely high. Extensive cytokinemia leads to systemic inflammatory response syndrome (SIRS) or compensatory anti-inflammatory response syndrome (CARS), both likely to result in multiple organ failure (MOF) and death.

Interleukin-6 regulates immune response, with predominantly proinflammatory, but also antiinflammatory activity. It is produced mainly by monocytes and macrophages, but also by a number of other cells, including pneumocytes [13] and some cancer lines [14]. IL-1ra (also known as IL-1F3: cytokine number 3 from the IL-1 family) is produced by monocytes, macrophages, and neutrophils, upon regulation of IL-1β. It binds to IL-1 receptors but it does not trigger intracellular reactions. To inhibit biological responses to IL-1, at least 100-fold excess of IL-1ra over IL-1 is necessary, both *in vitro* and *in vivo*. The administration of IL-1ra blocks the effects of IL-1 in some animal models of septic shock, inflammatory arthritis, graft-versus-host disease and inflammatory bowel disease. However, results of clinical trials in the treatment of septic shock in humans were negative [15]. Lately, both IL-1ra and IL-6 were tested in diagnostics of inflammatory processes. Both IL-6 and IL-1ra are usually measured by ELISA test, which does not identify the cellular source of secreted cytokines into serum and drained fluid but is most

commonly used because of its relative ease [16]. Flow cytometry identifies intracellular cytokines but it is not obvious whether these cytokines are subsequently secreted into blood stream [17].

In our study, performed in lung cancer patients, both IL-6 and IL-1ra concentrations were significantly elevated in serum in the whole group after surgery, in comparison with preoperative values. This proves the dominating role of operative injury on the release of these mediators. Serum levels of IL-6 and IL-1ra were not different between patients with and without postoperative complications. This result is different than results of our own investigations in patients after major abdominal cancer surgery which showed higher serum concentration of IL-6, IL-1ra and sTNF RI in patients with postoperative complications [7, 18]. A possible explanation of this fact is a hypothesis that in patients undergoing thoracotomies, secretion of cytokines is elevated even in uncomplicated cases, due to production of these cytokines by pneumocytes and macrophages as a result of direct injury to the operated lung. A high proportion of complications, both after abdominal and thoracic operations, are pulmonary complications. Therefore at least some serum cytokines in patients with complications after abdominal surgery may be derived from lungs.

Data from the literature show that increased serum concentrations of IL-6 and IL-1ra are good markers of severity of surgical stress after thoracic cancer surgery [19-21]. Results of these studies suggest that the highest serum concentrations of IL-6 and IL-1ra are observed at the end of surgery, just after surgery or on the first postoperative day, which is confirmed in current data. Other authors did not analyze concentrations of cytokines in groups with and without complications therefore it is not possible to assess usefulness of these tests as early markers of postoperative complications on the basis of the literature.

One of advantages of our study is assessment of local immune response – in sputum and drained pleural fluid. Despite very high levels, concentration of IL-1ra and IL-6 in sputum was not significantly different in patients with and without complications. This result is also different than obtained for IL-8 and granulocyte elastase in oesophageal cancer patients [22], where concentration of these markers in bronchial washings was significantly higher during first postoperative days in patients who later developed pneumonia. We believe that the main reason is that we collected sputum "blindly" from different parts of bronchial tree. We believe that IL-6 and IL-1ra in sputum should be tested in the future with a more precise protocol (for example with intraoperative bronchoalveolar lavage performed through bronchoscope and washing always similar bronchus: e.g. medium lobe on the right side or lingular bronchus on the left side with the same saline volume). In the current study we intentionally used a less precise method of obtaining sputum, but easier to implement into routine clinical practice. However, we can not recommend this method because in 14 patients sputum was so scarce that it was not obtained with a suction catheter.

The assumption that local immune response (inside the chest) may precede elevated concentrations of cytokines in serum gave rise to the idea of measurement of these cytokines also in pleural fluid. Our study showed that both IL-6 and IL-1ra concentration in pleural fluid are significantly more elevated after surgery (on day 1) in patients with postoperative complications as compared with patients with an uneventful postoperative course. These mediators are early sensitive markers of postoperative complications. This was confirmed by logistic regression method which also showed that increased pleural fluid concentration of IL-6 and IL-1ra significantly increases incidence of postoperative complications, together with non-immunological factors (like age, results of pulmonary function test, duration of surgery or amount of postoperatively drained pleural fluid). In the multivariate analysis only time of surgery, amount of postoperative drainage and concentration of IL-6 in pleural fluid were statistically independent prognostic factors of development of postoperative complications.

This study confirms on a larger population of patients results of our previous paper [10]. It shows that measurement of IL-6 and IL-1ra in pleural fluid on postoperative day 1 is a valuable test for implementation into a routine clinical practice. This test would enable to concentrate time and resources on patients with complications.

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