Interleukin 6 and interleukin 1 receptor antagonist as early markers of complications after lung cancer surgery

Tomasz Jaroslaw Szczyesny, Robert Slotwinski, Aleksander Stankiewicz, Bruno Sczygieł, Marzanna Zaleska, Maria Kopacz

Abstract

Objective: To assess changes of interleukin 6 (IL-6) and interleukin 1 receptor antagonist (IL-1ra) in serum, sputum, and drained pleural fluid of patients operated on due to lung cancer. Methods: Twenty-seven patients treated with lobectomy or pneumonectomy, including 14 with complications and 13 without complications, were analyzed. Serum IL-6 and IL-1ra concentration was measured before, at the end of surgery, and on postoperative day 1, 3, and 7. Additionaly, concentration of IL-6 and IL-1ra was measured in sputum at the end of surgery and in pleural fluid on postoperative day 1. Results: In the entire group serum concentrations of IL-6 and IL-1ra were significantly elevated after surgery, in comparison with preoperative values. Serum IL-6 concentration was higher in patients with complications only on day 7 (median 59.0 (range: 41.25–76.65) pg/ml vs 20.6 (range: 9.87–35.0) pg/ml; p=0.012). Patients with complications had higher concentration of IL-6 in pleural fluid (91312 (51812–94872) pg/ml vs 66470 (15930–108564) pg/ml; p=0.00008). Serum IL-1ra concentration was higher in patients with complications on day 1 (1832.4 (1144.7–2362.2) pg/ml vs 1088.4 (817.5–1312.5) pg/ml; p=0.01). Concentration of IL-1ra in drained fluid was higher in patients with complications (68128.8 (48104–108564) pg/ml vs 16470 (15930–16875) pg/ml; p=0.0003). On day 1 after surgery a significant correlation between serum and pleural fluid concentration for IL-6 as well as for IL-1ra were observed (Spearman test for IL-6: r=0.47; p=0.02; for IL-1ra: r=0.48; p=0.02). Conclusions: Elevated concentrations of IL-6 and IL-1ra in pleural fluid on postoperative day 1 are promising early markers of postoperative complications. Elevated concentrations of IL-6 and IL-1ra in serum are good early markers of severity of surgical injury and may reflect development of postoperative complications.

Keywords: Lung cancer; Surgery; Complications; IL-6; IL-1ra

1. Introduction

Preoperatively existing immunsuppression in cancer patients is deteriorated by surgical injury and postoperative infectious complications. Over 30% of patients after anatomical lung resections with mediastinal lymphadenectomy due to lung cancer develop life-threatening complications, for example pulmonary infections, cardiac arrhythmias, and atelectasis requiring bronchoaspiration [1]. In most specialized centers, about 2% mortality after lobectomies and 6% after pneumonectomies is observed [2,3]. Surgical injury and postoperative complications may stimulate cytokines and cytokine antagonists production in the early postoperative period, leading to the development of systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). These changes in immune system can be monitored by measurement of immunocompetent cells’ activity or concentration of mediators of inflammation in serum. Special attention was paid to mediators, especially cytokines and their inhibitors, which are good markers of severity of surgical injury. The primary postoperative immune response is mediated by the proinflammatory cytokines, among others, interleukin 1 (IL-1), IL-6, and tumor necrosis factor (TNF), and modulated by the naturally occurring antagonists of these cytokines for example soluble TNF receptor (sTNFR) and IL-1 receptor antagonist (IL-1ra) [1,2]. Observation that levels of these mediators are higher in patients with postoperative complications after major surgery [4,5] gave rise to an idea that some of them could serve as early markers of complications and subsequently...
lead to more prompt administration of proper treatment which might decrease morbidity and mortality.

Elevation of cytokines is primarily a local process, as they have mainly autocrine and paracrine activity, and only secondarily their concentration becomes elevated in serum. Based on this concept, unlike majority of other authors, we measured concentration of cytokines not only in serum but also locally (in drained pleural fluid and sputum). In our previous studies concerning influence of surgical trauma on cytokine levels in patients after colorectal cancer surgery and open cholecystectomy we found out that IL-6 and IL-1ra are most sensitive markers of inflammatory response after minor and major surgical injury [6,7]. Therefore, the aim of this study was to assess usefulness of IL-6 and IL-1ra measurement as markers of postoperative complications in lung cancer patients.

2. Materials and methods

Prospective studies were carried out in 57 patients (age 62.2 ± 8.18 years) with lung cancer undergoing elective thoracic surgery. Twenty-three patients were operated on due to cancer of the left lung (13 left superior lobectomies, 3 left inferior lobectomies, and 7 left pneumonectomies were performed) and 34 due to cancer of the right lung (16 right superior lobectomies, 7 right inferior lobectomies, 2 inferior bilobectomies, and 9 pneumonectomies). All resections were performed through standard posterolateral thoracotomy. Out of 57 patients, 16 developed postoperative complications (6 due to respiratory distress syndrome (ARDS). Clinical and laboratory data, as well as biological material for immunological studies (14 with complications and 13 without complications). Patients after immunosuppressive therapy, patients with lung metastases from other organs, with renal, circulatory or hepatic insufficiency, and with insulin-dependent diabetes were excluded from the study. Patients who were operated on through anterolateral or videothoracoscopic approach were also excluded from the study. Both groups were matched according to sex, extent of resection, duration of operation, intraoperative blood loss, and TNM staging. Characteristics of patients without (C−) and with (C+) postoperative complications and types of complications are presented in Tables 1 and 2.

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 min, 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 commercially available enzyme immunonassay kits (Quantikine R&D Systems Europe Ltd., Barton Lane Abingdon, Oxon, UK). Samples were tested in duplicate. Differences within the test and differences between measurements for IL-6 were 4.3% and 6.3% in concentrations 153.0 and 164.0 pg/ml, and for IL-1ra were 6.2% and 6.7% in concentrations 153.0 and 164.0 pg/ml, respectively. The lower limit of sensitivity of the assay was 0.7 pg/ml for IL-6 and 22 pg/ml for IL-1ra. 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).

<table>
<thead>
<tr>
<th>Patients' characteristics</th>
<th>C−</th>
<th>C+</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Number of patients</td>
<td>13</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Women/men</td>
<td>2/11</td>
<td>2/12</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>56.15 ± 9.15</td>
<td>65.14 ± 4.47</td>
<td>0.003</td>
</tr>
<tr>
<td>Lobectomies + bilobectomies/pneumonectomies</td>
<td>8/5</td>
<td>10/4</td>
<td>NS</td>
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<tr>
<td>Right lung / left lung</td>
<td>7/6</td>
<td>12/2</td>
<td>NS</td>
</tr>
<tr>
<td>Squamous cell cancer adenocarcinoma</td>
<td>6/7</td>
<td>11/2*</td>
<td>NS</td>
</tr>
<tr>
<td>Preoperative FEV1 (% normal), mean ± SD</td>
<td>97.5 ± 16.8</td>
<td>78.6 ± 16.8</td>
<td>0.007</td>
</tr>
<tr>
<td>Preoperative FVC (% normal), mean ± SD</td>
<td>90.5 ± 18.2</td>
<td>69.4 ± 20.7</td>
<td>0.01</td>
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<tr>
<td>Atelectasis or fever before surgery</td>
<td>4</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>1</td>
<td>3</td>
<td>NS</td>
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<tr>
<td>Concomitant circulatory system diseases</td>
<td>9</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>No concomitant diseases</td>
<td>2</td>
<td>4</td>
<td>NS</td>
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<tr>
<td>Time of surgery (min), mean ± SD</td>
<td>143 ± 36</td>
<td>183 ± 55.7</td>
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<td>Intraoperative blood loss (ml)</td>
<td>285 ± 230</td>
<td>436 ± 395</td>
<td>NS</td>
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<td>Total postoperative drainage (ml), mean ± SD</td>
<td>1046 ± 620</td>
<td>2371 ± 1119</td>
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<td>Days of postoperative drainage, mean ± SD</td>
<td>3.5 ± 1.4</td>
<td>9.6 ± 12</td>
<td>NS</td>
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<td>Postoperative hospitalization (days), mean ± SD</td>
<td>8.15 ± 0.98</td>
<td>17 ± 12.1</td>
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<td>Treatment with antibiotics (days), mean ± SD</td>
<td>3.3 ± 2.1</td>
<td>3.9 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Primary tumor diameter (mm), mean ± SD</td>
<td>43.5 ± 15.3</td>
<td>47.8 ± 36</td>
<td>NS</td>
</tr>
<tr>
<td>Intrapulmonary (N1) nodes excised, mean ± SD</td>
<td>8.85 ± 6.43</td>
<td>8.36 ± 4.57</td>
<td>NS</td>
</tr>
<tr>
<td>Mediastinal (N2) nodes excised, mean ± SD</td>
<td>20.77 ± 12.47</td>
<td>22.86 ± 14.81</td>
<td>NS</td>
</tr>
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</table>

SD: standard deviation.

* Plus one patient with small cell lung cancer.
when ammonia produced in the first reaction with water and urease combines with 2-oxoglutarate and NADH in the presence of glutamate-dehydrogenase (GLDH) to yield glutamate and NAD⁺. The decrease in NADH absorbance per unit time is proportional to the urea concentration. Tests were performed with OLYMPUS AU400 device.

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

3. Statistical analysis

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 cytokine concentrations, Wilcoxon test with Bonferroni correction were used. Fisher’s exact test for categorical parameters was used. The differences between groups without and with postoperative complications were analyzed with the Mann—Whitney U-test. Impact of selected clinical and immunological factors on the risk of development of postoperative complications was analyzed using logistic regression. Results of analysis are presented as odds ratio with 95% confidence intervals. All computations were performed using SPSS 12.0 statistical package.

4. Results

In the whole group of patients, median serum concentration of IL-6 before surgery, at the end of operation, and on postoperative day 1, 3, and 7 was 6.25 pg/ml (range: 2.5—8.0), 70.5 (30.05—116.3), 143.5 (107.5—181.5), 61.5 (25.5—95.0), and 21.5 (9.9—35.0), respectively. Differences of IL-6 concentrations between groups (C+ vs C−) were significant on postoperative day 7 (p = 0.012) (Fig. 2).

In patients with complications (C+), median serum concentration of IL-6 before surgery, at the end of surgery, and on postoperative day 1, 3, and 7 was 6.2 pg/ml (4.6—9.2), 94.45 (29.45—245.2), 212.4 (147.1—265.3), 182.9 (102.9—254.4), and 59 (41.3—76.65) pg/ml, respectively. In patients without complications (C−), median serum concentration of IL-6 before surgery, at the end of surgery, and on postoperative day 1, 3, and 7 was 6.25 pg/ml (2.5—8.0), 70.5 (30.05—116.3), 143.5 (107.5—181.5), 61.5 (25.5—95.0), and 21.5 (9.9—35.0), respectively. Differences of IL-6 concentrations between groups (C+ vs C−) were significant on postoperative day 7 (p = 0.012) (Fig. 1).

Serum concentrations of both IL-6 and IL-1ra at the end and after surgery (on day 1—7) were significantly higher (p < 0.05) compared to preoperative values (Fig. 1).

In patients with complications (C+), median serum concentration of IL-6 before surgery, at the end of surgery, and on postoperative day 1, 3, and 7 was 6.25 pg/ml (4.6—9.2), 94.45 (29.45—245.2), 212.4 (147.1—265.3), 182.9 (102.9—254.4), and 59 (41.3—76.65) pg/ml, respectively. In patients without complications (C−), median serum concentration of IL-6 before surgery, at the end of surgery, and on postoperative day 1, 3, and 7 was 6.25 pg/ml (2.5—8.0), 70.5 (30.05—116.3), 143.5 (107.5—181.5), 61.5 (25.5—95.0), and 21.5 (9.9—35.0), respectively. Differences of IL-6 concentrations between groups (C+ vs C−) were significant on postoperative day 7 (p = 0.012) (Fig. 2).

In patients with complications (C+), median serum concentration of IL-1ra before surgery, at the end of surgery, and on postoperative day 1, 3, and 7 was 475.5 pg/ml (364.83—716.2), 976.45 (615.4—2193.7), 1832.4 (1144.7—2362.2), 1934.9 (1433.7—2759.2), and 1605.9 (1013—2897.2) pg/ml, respectively. In patients without complications (C−), median serum concentration of IL-1ra before surgery, at the end of surgery, and on postoperative day 1, 3, and 7 was 405 pg/ml (240—480), 1325.0 (521.3—4760.6), 1088 (817.5—1312.5), 832.5 (562.5—1170), and 525 (420—847.5) pg/ml, respectively.
832.5 (562.5—1170), and 525.0 (420.0—847.5) pg/ml, respectively. Differences of IL-1ra concentrations between groups (C+ vs C−) were significant on postoperative day 1 (p = 0.01) (Fig. 3).

Median concentration of IL-6 in sputum in the whole group was 2524 pg/ml (922.4—6947), in the group with complications (C+) 5676 pg/ml (3221—38657), and in the group without complications (C−/C0) 595.2 pg/ml (415.3—2009). Median concentration of IL-1ra in sputum in the whole group was 72776 pg/ml (32812—190802), in the group with complications (C+) it was 80109 pg/ml (64856—169145), and in the group without complications (C−) 34048 pg/ml (31141—188425). Differences between groups both for IL-6 and for IL-1ra were not statistically significant (Figs. 1—3).

Median concentration of IL-6 in pleural fluid in the whole group was 15812 pg/ml (2003—94016), in the group with complications (C+) 91312 pg/ml (51812—94872), in the group without complications (C−/C0) 2006 pg/ml (1926—2108) (significant difference between C+/C− group, p = 0.00008). Median concentration of IL-1ra in pleural fluid in the whole group was 16470 pg/ml (15930—16875) (significant difference between C+/C− group, p = 0.0003) (Figs. 1—3).

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

5. Discussion

In patients operated on due to lung cancer postoperative complications remain a major problem. The main factors influencing the rate of postoperative complications in oncological patients are changes in the immune response to surgical trauma. Immunological disorders after major surgery can be monitored by measuring concentration of immune system mediators [8] and immunocompetent cells’ activity [9]. Major surgical trauma results in a migration of immunocompetent cells to the wound and extensive local production of cytokines and their inhibitors, also called tissue hormones. In serum, high levels of cytokines are observed in cases of extremely high stimulation of immune system (major surgery and infections), for example in systemic inflammatory response syndrome and compensatory anti-inflammatory response syndrome (CARS) which can lead to an early multiple organ failure (MOF) and death.

In our study, performed in lung cancer patients, both IL-6 and IL-1ra are significantly elevated in the whole group after surgery, in comparison with preoperative values, which reflects dominating impact of operative injury on releasing of these mediators. Serum levels of IL-6 (on day 7) and IL-1ra (on day 1) were significantly higher in patients with postoperative complications, as compared with patients without complications. These findings are consistent with 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 [6,10].

Interleukin 6 is a multipotent factor, regulating mostly immune response (with predominantly proinflammatory but also anti-inflammatory effect). It is produced mainly by monocytes and macrophages but also by a number of other cells, including pneumocytes [11] and some cancer lines.
TNF and IL-6 are factors responsible for cancer cachexia and fever. IL-1ra is produced by monocytes, macrophages, and neutrophils, upon regulation of IL-1β. Like IL-1α and IL-1β, IL-1ra binds to IL-1 receptors, but it does not trigger secondary interactions necessary to exert cell reactions. To inhibit biological responses to IL-1, at least 100-fold excess of IL-1ra over IL-1 may be 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 [13]. Nowadays, both IL-1ra and IL-6 are tested in diagnostics of inflammatory process, but results of these studies are equivocal. 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 [14]. Flow cytometry identifies intracellular cytokines, but it is not obvious whether these cytokines are subsequently secreted into blood stream [15].

Current 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 [16—18]. Studies of these authors suggested that the highest serum concentrations of IL-6 and IL-1ra were observed at the end of surgery, just after surgery, or on the first postoperative day, which is confirmed in our current data. Assessment of usefulness of these studies as early markers of postoperative complications is negatively influenced by the fact that other authors did not analyze concentrations of cytokines in groups with and without complications.

One of the advantages of this 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 from that obtained for IL-8 and granulocyte elastase in esophageal cancer patients [19], where concentration of these markers in bronchial washings was significantly higher during first postoperative days in patients who later developed pneumonia. This may be an influence of smoking status in lung cancer patients. Also in several patients sputum was so scarce that it was not obtained with the suction catheter. Another 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 — 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.

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 concentrations in pleural fluid are significantly more elevated after surgery (on day 1) in patients with postoperative complications as compared with uneventful postoperative course. It means that these mediators are early sensitive markers of postoperative complications. This was confirmed by logistic regression method which also showed that pleural fluid concentration of IL-6 and IL-1ra significantly correlates with incidence of postoperative complications, similarly as other well-known non-immunological factors (like age, results of pulmonary function test, duration of surgery, or amount of postoperatively drained pleural fluid).

Results of our study are promising and should be confirmed in a larger population of patients undergoing lung cancer surgery, before introducing this test into routine clinical practice.

6. Conclusions

Elevated concentrations of IL-6 and IL-1ra in pleural fluid on postoperative day 1 are promising early markers of postoperative complications. Elevated concentration of IL-6 or IL-1ra in serum is a good early marker of severity of surgical injury and reflects development of postoperative complications.

References

Appendix A. Conference discussion

Dr E. Stoelben (Cologne, Germany): We know that complications after thoracic surgery in smokers and nonsmokers are different. My question is if there are any differences in IL-6 development in smokers and nonsmokers.

Dr Szczesny: This was not a topic of this analysis. To answer your question I can say that patients who were active smokers were not more at risk of developing postoperative complications. We found that patients who were active smokers had lower concentration of IL-6 and IL-1ra in sputum which suggests local immunosuppression, but this is a completely different topic which we plan to publish in the future.