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Assessment of acute phase proteins as prognostic factors in patients surgically treated for non-small cell lung cancer

Abstract

Introduction: The aim of the study was to assess quantitative acute phase protein (APP) level changes in patients with non-small cell lung cancer (NSCLC) subjected to radical resections, as well as their influence on long-term survival. We analysed the correlation between quantitative APP changes and the histological type of the carcinoma, as well as the TNM stage and grade.

Materials and methods: The study group comprised 46 patients subjected to surgical treatment of NSCLC during the period between 2003 and 2004. Average patient age amounted to 61 years (ranging between 45 and 77 years). The most frequent histological types of cancer were: squamous cell lung cancer (24 patients) and adenocarcinoma (17 patients). The majority of patients were diagnosed with stage II B (15 patients) and III A (14 patients). We evaluated the levels of the following APP: C-reactive protein (CRP), α 1-acid glycoprotein (AGP), α 1-antichymotrypsin (ACT), α 1-antitrypsin (AT), α 2-macroglobulin (M), ceruloplasmin (Cp), haptoglobin (Hp), and transferrin (Tf) by means of rocket immunoelectrophoresis (Laurell's method).

Results: The level of AT was significantly higher in patients with adenocarcinoma, as compared to other histological types of cancer. In the case of patients with squamous cell lung cancer, significantly higher M and Cp levels were observed. We found no correlation between the APP level and tumour grading. The levels of five APP: CRP, AGP, ACT, M and Cp were significantly higher in the group of patients with T3 or T4 category, while N1 or N2 patients presented with significantly higher concentrations of AT, CRP and Hp. Multivariate analysis confirmed the influence of the following factors on long-term survival: N stage, histological type of cancer and preoperative serum levels of AGP and Hp.

Conclusions: The serum concentration of some acute phase proteins can correlate with the more aggressive clinical course of non-small cell lung cancer (NSCLC). Patients with adenocarcinoma and local lymph node metastases present with significantly higher levels of AT. Thus, it seems that the elevated preoperative levels of AGP and Hp might unfavourably influence long-term survival. The above-mentioned proteins might prove useful as prognostic factors when assessing the risk of neoplastic recurrence following surgical management.

Key words: acute phase proteins, lung cancer, surgical treatment, prognostic factors

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Introduction

The acute phase reaction is an element of non-specific immunity consisting of early reactions as a response to tissue damage. During the above-

mentioned reaction, numerous endocrine, metabolic and neurological changes can be observed, both local and systemic, occurring immediately after tissue damage, during the early phase of inflammation or inflammatory process. Each form of da-

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mage or tissue structure disturbance can lead towards the initiation of the acute phase reaction. A special form of the acute phase reaction is the coordinated synthesis of several proteins, the so-called acute phase proteins (APP). These proteins are mainly produced in the liver (95% of the total production), as well as by monocytes, leucocytes, lymphocytes, alveoli macrophages and vascular wall cells [1–3]. In order for a protein to be classified as an acute phase protein at least a 25% change in the serous concentration during the acute phase reaction must be observed. Acute phase proteins can be divided into positive and negative proteins, depending whether their concentration increases or decreases during the acute phase reaction. The following are considered as positive proteins: CRP, AGP, ACT, AT, M, Cp and Hp, while transferrin and albumin are classified as negative proteins [4]. Cytokines are responsible for the mobilization of acute phase protein synthesis as a reaction to injury, the following being most important: interleukin 6 (IL-6), interleukin 1 (IL-1) and TNF- α [1, 5, 6].

Quantitative and qualitative cytokine and acute phase protein changes observed during the neoplastic process remain unclear. They can result from the development of immunosuppression connected with the production of immunosuppressive cytokines by the tumour [7, 8].

Lung cancer is one of the most common malignant neoplasms, most often responsible for death regarding neoplastic diseases. For many years the search for factors correlating with the aggressive course of the disease has been under investigation. Publications concerning the role of cytokines and acute phase proteins as prognostic factors following surgical management of malignant tumours are scarce, and their results ambiguous [9–12].

The aim of the study was to assess quantitative acute phase protein level changes in patients with non-small cell lung cancer (NSCLC) subjected to radical resections, as well as their influence on long-term survival. We analysed the correlation between quantitative APP changes and the histological type of the carcinoma, as well as the TNM stage and grade.

Material and methods

The study group comprised 46 patients subjected to surgical treatment of primary NSCLC during the period between 2003 and 2004 at the Department of Thoracic Surgery, Medical University, Poznań. The study group consisted of 41 male and 5 female patients, aged between 45 and 77 years (mean age: 61 yrs.). Patients were diagnosed

with the following histological types of carcinoma: 24 — squamous cell lung cancer, 17 — adenocarcinoma, 1 — giant cell carcinoma, 1 — clear cell carcinoma, and 3 with undifferentiated non-small cell carcinoma. None of the patients was subjected to preoperative chemotherapy or radiotherapy. Six patients underwent mediastinoscopy prior to radical surgical treatment. None presented with N2 lymph node metastases. Lobectomy was the most commonly performed procedure (23 patients). Fifteen patients underwent pneumonectomy, 5 — sleeve lobectomy, 1 — bilobectomy, and 2 — sleeve pneumonectomy. Four patients additionally underwent resection of the infiltrated ribs, and one — wedge resection of the other pulmonary lobe. Carcinoma staging was determined on the basis of the TNM classification updated in 1997. The majority of patients were diagnosed with stage IIB (15 patients) and IIIA (14 patients) (tab. 1). Five of the operated patients were classified as stage IIIB (2 with infiltration of the tracheal bifurcation were subjected to sleeve pneumonectomy, 2 with infiltration of the left atrium wall underwent pneumonectomy with partial resection of the atrial wall, and 1 with a satellite neoplastic tumour of the same lobe). Perioperative mortality amounted to 6.5% (3 patients). Eight (17.4%) patients were additionally subjected to adjuvant radiotherapy and 1 (2.2%) to

Table 1. Clinical data

Age	Ranging between 45–77 years (mean 61 yrs.)
Gender M/F	41/5
Histological type	
Adenocarcinoma	17
Squamous cell lung cancer	24
Ca clarcocellulare	1
Giant cell carcinoma	1
Ca solidum nondifferentiatum	3
Clinical stage	
IA	2
IB	10
IIB	15
IIIA	14
IIIB	5
Grading	
G1	6
G2	21
G3	19
Type of surgery	
Pneumonectomy	15
Sleeve pneumonectomy	2
Lobectomy	23
Sleeve lobectomy	5
Bilobectomy	1

postoperative chemo- and radiotherapy. None of the study group patients were diagnosed with autoimmune or chronic inflammatory diseases, or received chronic glucocorticosteroid therapy.

All study group patients signed an informed consent form regarding their participation in the clinical investigation. Serum levels of selected acute phase proteins were subjected to analysis. Prior to the surgical intervention 10 ml of blood was collected from every patient. The serum underwent centrifugation and was stored at a temperature of -40°C . Rocket immunoelectrophoresis by Laurell was the method used to determine the level of the following acute phase proteins: C-reactive protein (CRP), $\alpha 1$ -acid glycoprotein (AGP), $\alpha 1$ -antichymotrypsin (ACT), $\alpha 1$ -antitrypsin (AT), $\alpha 2$ -macroglobulin (M), ceruloplasmin (Cp), haptoglobin (Hp) and transferrin (Tf).

Analysis of survival was based on medical documentation obtained from the oncological and thoracic outpatient clinics, as well as on the basis of questionnaires filled out by the patients. Three patients who died during the perioperative period were excluded from the analysis.

Statistical analysis was based on the "Statistica" computer program. The distribution of variables was verified by means of the Shapiro-Wilk test. The dependency between selected variables was determined on the basis of Person's linear correlation coefficient or Spearman's correlation coefficient. The Mann-Whitney and *t*-student tests were used to determine difference significance, while the former was used to determine the survival curve.

Results

We determined the correlation between acute phase proteins and the histological type of the carcinoma, as well as its TNM stage and grade. Significantly higher levels of AT (positive protein) and lower levels of Tf (negative protein) were observed in patients with adenocarcinomas, as compared to other histological types (AT $p = 0.014$; Tf $p = 0.035$). Whereas, in the case of patients with squamous cell carcinoma, significantly higher M and Cp protein levels were determined ($p = 0.029$ and $p = 0.022$, respectively).

There was no statistically significant difference in the level of acute phase proteins, depending on the grade of the carcinoma.

When evaluating the relationship between levels of selected acute phase proteins and the clinical grade of the tumour, T and N features were considered. Patients were divided into two groups:

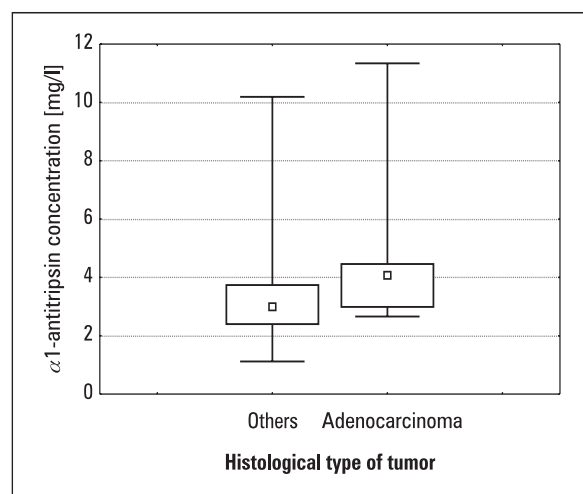


Figure 1. Correlation between AT serum level and histological type of cancer

the first group comprised those with T1 and T2 features (21 patients), while the second group comprised subjects with T3 and T4 features. Regarding patients diagnosed with T3 and T4 tumours, significantly higher levels of the following proteins were noted: CRP ($p = 0.022$), AGP ($p = 0.003$), ACT ($p = 0.008$), M ($p = 0.007$) and Cp ($p = 0.022$).

Depending on the presence of local lymph node metastases, patients were divided into the following groups: stage N0 (22 patients), and those in stage N1 or N2 (24 patients). Patients with local lymph node metastases (N1 or N2) were characterized by significantly higher levels of the following proteins: CRP ($p = 0.034$), Hp ($p = 0.033$) and AT ($p = 0.041$). AT clearly correlated with the higher potential of tumour malignancy, its level being significantly higher in patients with adenocarcinoma (fig. 1), and local lymph node metastases (fig. 2). Similarly, the CRP level correlated with the stage and grade of the carcinoma, being higher in patients with advanced cancer (T3/T4 and N1/N2).

In order to determine the prognostic value of acute phase protein levels we evaluated the three-year survival period. Considering the 43 operated patients, 17 (39.5%) survived three years, 19 (44.2%) died and the fate of 7 (16.3%) remained unknown. The survival median amounted to 31.5 months. The death of 10 (52.6%) of the 19 patients was connected with neoplastic disease recurrence, and all were diagnosed with distant metastases.

Statistical analysis showed a significant relationship with survival of the following four factors: N feature, histological type of carcinoma and preoperative level of two acute phase proteins: AGP (fig. 3) and Hp (fig. 4).

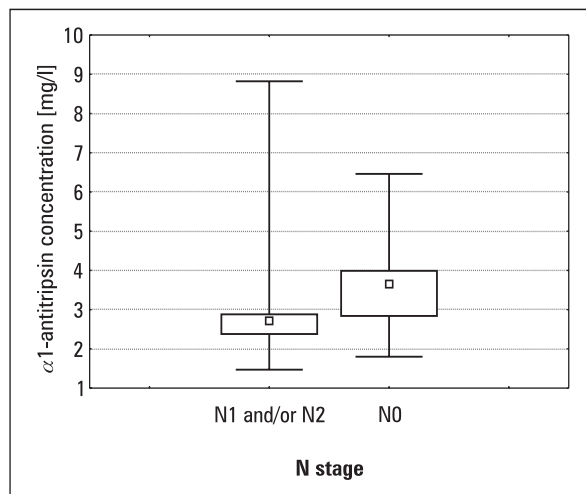


Figure 2. Correlation between AT serum level and N status (0 — absence of lymph node metastases; 1.2 — N1 and/or N2 lymph node metastases)



Figure 3. Correlation between survival and preoperative serum level of AGP

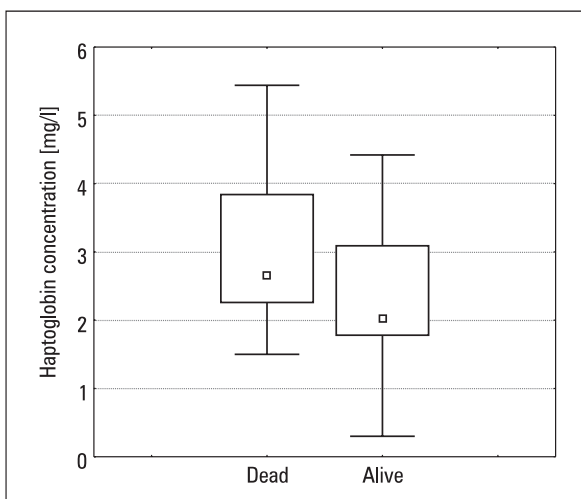


Figure 4. Correlation between survival and preoperative serum level of Hp

Significantly shorter survival was observed in patients with local lymph node metastases, patients diagnosed with adenocarcinoma and those with elevated preoperative AGP and Hp levels. The following factors had no significant influence on survival: T feature, cellular differentiation, patient age and gender, type of operation and adjuvant therapy (radiotherapy and/or postoperative chemotherapy).

Discussion

The significance of cytokine and acute phase protein secretion during the process of cancerogenesis remains unclear. We have already observed for a long time the coexistence of chronic inflammatory processes with neoplastic disease [3, 6]. We do not know whether chronic inflammation can induce the neoplastic process, whether inflammation results from the progression of the tumour, or whether both factors superimpose on one another. Many authors have confirmed the higher level of cytokines and acute phase proteins in patients with diagnosed lung cancer, as compared to values obtained from healthy subjects [10, 13–17]. Siemens and co-authors observed higher levels of CRP in patients with lung cancer, which might suggest that CRP can be a biomarker of chronic inflammation, preceding the development of lung cancer [15]. Neoplastic tumours, in order to avoid the immunological response of the host, can produce factors masking their own antigens and hindering the presentation of these antigens towards immunocompetent host cells [10]. Some cytokines, such as IL-4, IL-5, IL-6, IL-10 and IL-13 can be secreted not only by host cells but also by cancer cells with evident immunosuppressive activity. Their higher serum level and that of selected acute phase proteins can inhibit the cellular response, lymphocyte T proliferation, antigen presentation, cytotoxic cell activation and production of cytokines stimulating the cellular response (IL-2, interferon), which leads to anti-neoplastic host immunity reduction [10]. Similarly, their excessive release as a consequence of significant operative injury can lead to immunity reduction, which increases the risk of distant metastases [18–20]. Thus, high cytokine and acute phase protein levels are not favourable in patients subjected to surgical intervention, due to malignant neoplasms. Some authors suggest that preoperative levels of pro- and anti-inflammatory cytokines, as well as acute phase proteins can be malignant prognostic factors of lung cancer [7, 21–23]. Tissue injuries connected with operative trauma can disturb the balance be-

tween the levels of pro- and anti-inflammatory cytokines, which can lead to the impaired anti-neoplastic defensive response of the organism. Postoperative and post chemotherapy monitoring of the above-mentioned cytokines might serve as early identification factors of neoplastic process recurrence. The high level of IL-6 in patients with NSCLC correlates with elevated levels of selected acute phase proteins (mostly CRP), worse patient condition (according to the WHO) and malnutrition, which plays a significant role in the pathophysiology of cancerous cachexia and can be an independent factor influencing survival [14, 16]. De Vita and co-authors demonstrated the evident correlation between the level of IL-10 and patient response towards chemotherapy. Patients without remission following chemotherapy presented with higher levels of IL-10, both before and after treatment, in comparison to patients with at least partial remission. Additionally, the level of IL-10 proved to be an independent factor influencing survival [10]. On the other hand, Tas and co-authors showed a significant reduction of IL-6 after chemotherapy, and its influence on survival in the case of patients with NSCLC [17]. Other authors also observed the significant influence of selected acute phase protein and cytokine levels on long-term survival. Yildirim and co-authors analysed the level of selected acute phase proteins (AGP, Cp, Tf, ferritin, albumin) in patients with primary lung cancer. They observed higher levels of Cp and ferritin, and lower levels of Tf and albumin in patients with lung cancer. The higher level of AGP and ferritin correlated with the presence of local lymph node metastases, and worse patient condition and prognosis of survival [12]. Similarly, serum AGP levels proved to be an independent factor influencing survival, regarding a group of 180 patients subjected to treatment by means of docetaxel, due to NSCLC [9]. Liu and Khan showed that the level of amyloid A can be a prognostic marker enabling the monitoring of the progression of lung cancer. Its concentration is high in patients with advanced-stage non-small cell lung cancer, decreasing after lung resection or chemotherapy [13, 24].

Regarding the analysed group of patients, we observed a significant correlation between the elevated levels of some acute phase proteins and the high malignancy potential of lung cancer. In cases of patients diagnosed with adenocarcinoma, characterized by a high level of malignancy, and in those with local lymph node metastases, significantly higher serum AT concentrations were observed. On the other hand, elevated levels of CRP were measured in cases of patients with T3/4 and

N1/2 carcinomas. Craig and co-authors came to the same conclusions, demonstrating high CRP levels in patients with advanced lung cancer, independent of whether surgery was performed by means of videothoracoscopy or classical thoracotomy [19].

When analysing distant survival, both local lymph node metastases and the histological type of cancer, as well as preoperative AGP and Hp protein levels, proved to be important factors connected with survival. Yildirim and Bharti came to the same conclusions [12, 25]. The measurement of the above-mentioned proteins in patients with NSCLC qualified towards surgical intervention might be of prognostic value, facilitating the selection of patients with increased risk of neoplastic process recurrence.

Conclusions

The serum concentration of some acute phase proteins can correlate with the more aggressive clinical course of non-small cell lung cancer (NSCLC). Patients with adenocarcinoma and local lymph node metastases present with significantly higher levels of AT. Thus, it seems that elevated preoperative levels of AGP and Hp might unfavourably influence long-term survival. The above-mentioned proteins might prove useful as prognostic factors when assessing the risk of neoplastic recurrence following surgical management.

References

1. Baumann H., Gauldie J. The acute phase response. *Immunol. Today* 1994; 15: 74–80.
2. D'Armiento J., Dalal S.S., Chada K. Tissue, temporal and inducible expression pattern of haptoglobin in mice. *Gene* 1997; 195: 19–27.
3. Kushner I., Mackiewicz A. Acute phase proteins as disease markers. *Dis. Markers* 1987; 5:1–11.
4. Kushner I. The phenomenon of the acute phase response. *Ann. NY Acad. Sci.* 1982; 389: 39–48.
5. Grenier A., Dehoux M., Boutten A. et al. Oncostatin M production and regulation by human polymorphonuclear neutrophils. *Blood* 1999; 93: 1413–1421.
6. Streetz K.L., Wustefeld T., Klein C. et al. Mediators of inflammation and acute phase response in the liver. *Cell. Mol. Biol.* 2001; 47: 661–73.
7. Atwell D.M., Grichnik K.P., Newman M.F. et al. Balance of proinflammatory and anti-inflammatory cytokines at thoracic cancer operation. *Ann. Thorac. Surg.* 1998; 66: 1145–1150.
8. Smith D.R., Kunkel S.L., Burdick M.D. Production of IL-10 by human bronchogenic carcinoma. *Am. J. Path.* 1994; 145: 18–25.
9. Bruno R., Olivares R., Berille J. et al. Alpha-1-acid glycoprotein as an independent predictor for treatment effects and a prognostic factor of survival in patients with non-small cell lung cancer treated with docetaxel. *Clin. Cancer Res.* 2003; 9: 1077–1082.
10. De Vita F., Orditura M., Galizia G. et al. Serum Interleukin-10 levels as a prognostic factor in advanced non-small cell lung cancer patients. *Chest* 2000; 117: 365–373.
11. Kamińska J., Kowalska M., Kotowicz B. et al. Pretreatment serum levels of cytokines and cytokine receptors in patients with non-small cell lung cancer and correlations with clinicopathological features and prognosis. *Oncology* 2006; 70: 115–125.
12. Yildirim A., Meral M., Kaynar H. et al. Relationship between serum levels of some acute-phase proteins and stage of disease

- and performance status in patients with lung cancer. *Med. Sci. Monit.* 2007; 13: 195–200.
13. Khan N., Cromer C.J., Campa M. et al. Clinical utility of serum amyloid A and macrophage migration inhibitory factor as serum biomarkers for the detection of non-small cell lung carcinoma. *Cancer* 2004; 101: 379–384.
 14. McKeown D.J., Brown D.J., Kelly A. et al. The relationship between circulating concentrations of C-reactive protein, inflammatory cytokines and cytokine receptors in patients with non-small cell lung cancer. *Br. J. Cancer* 2004; 91: 1993–1995.
 15. Siemes C., Visser L.E., Coebergh J.W. et al. C-reactive protein levels, variation in the C-reactive protein gene and cancer risk: the Rotterdam Study. *J. Clin. Oncol.* 2006; 24: 5216–5222.
 16. Songur N., Kuru B., Kalkan F. et al. Serum interleukin-6 levels correlate with malnutrition and survival in patients with advanced non-small cell lung cancer. *Tumori* 2004; 90: 196–200.
 17. Tas F., Duranyildiz D., Argon A. et al. Serum levels of leptin and proinflammatory cytokines in advanced-stage non-small cell lung cancer. *Med. Oncol.* 2005; 22: 353–358.
 18. Christou N.V., Meakins J.L. Phagocytic and bactericidal functions of polymorphonuclear neutrophils from anergic surgical patients. *Can. J. Surg.* 1982; 25: 444–448.
 19. Craig S.R., Leaver H.A., Yap P.L. et al. Acute phase responses following minimal access and conventional thoracic surgery. *Eur. J. Cardio. Thorac. Surg.* 2001; 20: 455–463.
 20. Pollock R.E., Lotzova E., Stanford S.D. Surgical stress impairs natural killer cell programming of tumour for lysis in patients with sarcomas and other solid tumours. *Cancer* 1992; 70: 2192–2202.
 21. Arias-Diaz J., Vara E., Torres-Melero J. Nitrite/nitrate and cytokine levels in bronchoalveolar lavage fluid of lung cancer patients. *Cancer* 1994; 74: 1546–1551.
 22. Martinet N., Charles T., Vaillant P. Characterisation of a tumor necrosis factor-alpha inhibitor activity in cancer patients. *Am. J. Respir. Cell. Mol. Biol.* 1992; 6: 510–515.
 23. Partanen R., Koskinen H., Hemminki K. Tumor necrosis factor-alpha (TNF-alpha) in patients who have asbestosis and develop cancer. *Occup. Environ. Med.* 1995; 52: 316–319.
 24. Liu D.H., Wang X.M., Zhang L.J. et al. Serum amyloid A protein: a potential biomarker correlated with clinical stage of lung cancer. *Biomed. Environ. Sci.* 2007; 20: 33–40.
 25. Bharti A., Ma P.C., Maulik G. et al. Haptoglobin alpha-subunit and hepatocyte growth factor can potentially serve as serum tumor biomarkers in small cell lung cancer. *Anticancer Res.* 2004; 24: 1031–1038.