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Immunological monitoring of chronic pleural empyema patients

Badania immunologiczne z przewlekłym ropniakiem opłucnej

Chronic pleural empyema (CPE) is not a very common, but it is one of the most difficult thoracic diseases to be treated. Thoracic surgery, pneumonia, and trauma are responsible for most cases of empyemas (13). Alfageme et al. noted that 82% patients had one or more underlying illnesses. They include neoplasms, pulmonary diseases, cardiac disorders, diabetes mellitus, alcoholism, drug abuse, inflammatory bowel disease, neurologic diseases and immunosuppression (4). CPE is the main complication following pulmonary tissue resection and occurs in 2–1.6% cases after pneumonectomy. Its mortality ranges from 1% to 19%, but in immunocompromised patients can be as high as 40% (4).

Postsurgical empyemas constitute approximately 20% (10). Empyema following pneumonectomy is very often associated with bronchopleural fistula (about 80%) and has significant mortality (13). Preoperative irradiation, pulmonary resections for inflammatory conditions, right pneumonectomy, a long bronchial stump, the gross contamination of the pleura, and sputum positive for tuberculosis are the most predisposing factors for postpneumonectomy empyema (13).

The immunological system plays a crucial role in the protection against infection and neoplasm. Decreased immune surveillance is believed to play an important role in tumour development (1).

The interactions between immunological cells and infective agents are responsible for their proper elimination or the appearance of resistance against them (10). This suggests that the immunological system is crucial for the appearance and healing the pleural empyema. The main aim of this study was to clarify the immunological status in chronic pleural empyema.

MAT ERIAL AND METHODS

Fifty consecutive patients (male and female), aged 32–75 years, suffering from chronic pleural empyema (CPE) with (35) or without (15) broncho–pleural fistula (BPF) were treated using well–vascularized thoracic wall muscle flaps in our department in the years 1995–98. We applied our own modification of the procedure described by Pairolero et al. from Mayo Clinic (USA) (9) to treat

them. Our own supplement to this procedure was: a/ no thoracostomy performed before myoplasty; b/ never stick bronchial stump wall to wall, but covering fistula with muscle suture it around the fistula to the mediastinum; c/ a modification of Clagett procedure – postponed filling of pleural cavity with an antibiotic solution (mainly Dab's fluid– gentamycin, neomycin and polymyxin B).

The patients were divided into two groups: post–cancer empyema patients (they had undergone surgical interventions due to cancer – 35 cases) and parapneumonic empyema patients (15 cases).

Peripheral vein blood samples were obtained on heparin (final concentration 50 IU/ml) from all patients a day before operation, a week, a month and 6 months after operation for immunological monitoring. Blood samples from 50 healthy donors matched in age and sex were considered as a control. We measured the leukocyte subpopulations using the flow cytometry assays. The whole blood was incubated with monoclonal antibodies (IgG1/IgG2, CD3, CD4, CD8, CD19, CD15/56, CD25, CD122, HLADR) conjugated with PE or FITC (DAKO, Ortho–diagnostic Systems or Becton–Dickinson) in the dark, at the room temperature. After 20 min. erythrocytes were lysed by Ortho–Mune Lysing Reagent (Ortho–Diagnostic Systems) for 10 min. Cells were washed in PBS twice just after lysing solution removal by centrifugation. Cell analyses were performed immediately after incubation with the help of Cytoron Absolut (Ortho–Diagnostic Systems) flow cytometer and Immunocount II software. Mann–Whitney test for matched variables, Wilcoxon test for no–matched variables and Statistica 5.0 software were applied for statistical analysis.

Immunological conditions were also evaluated by determining the immunoglobulin fraction using standard Manchini method. The levels of IgA, IgG, and IgM were measured. Immunostimulation treatment was applied in 12 patients. It started a week after operation. Patients were given the 10–mg thymic factor X (TFX Polfa) injections daily for a month.

RESULTS

The subpopulations of fresh isolated peripheral blood lymphocytes (PBLs) were examined by two colour flow cytometry assays. The mean proportions of positive cells for each marker are shown in Table 1.

Post–cancer empyema patients before their treatments have significant higher percentages of CD3+, CD19+, CD8+, CD3+, CD3+/HLADR+, CD25 (in all $p<0.05$), CD122+ ($p<0.005$) cells and lower percentage of NK cells ($p< 0.0005$) than healthy individuals. One month after operation similar differences were noticed between CD3+ ($p<0.05$), CD8+ ($p<0.005$), CD3+/HLADR+ ($p<0.05$) and NK ($p<0.0005$) cells, but five months later the only decrease of NK cell percentage was observed in post–cancer empyema patients. The percentage of CD3+, CD19+, CD122+ and NK (in all $p<0.05$) cells was significantly higher in the group of parapneumonic empyema before treatment compared with healthy controls. No significant differences were observed in follow–up examinations, as well as between post–cancer and parapneumonic empyema patients in the corresponding time of treatment. The only immunological result of TFX intake was a significant increase of IgM levels 1 and 6 months after operations (in both $p<0.05$). Flow cytometry screen picture of NK cells is shown in Figure 1.

DISCUSSION

There are lots of reports concerning the immunological basements of neoplastic and inflammatory diseases but there is no information about immunological changes in pleural empyema. Neither local immunological status, nor immunological malformations in peripheral blood are described. The

Tab.1. The flow cytometry results of empyema patients and healthy individuals presented as mean percentage of positive cells ± standard deviation

	CD3+	CD19+	CD4+	CD8+	NK	CD3+/ /1656+	HLADR	3+/ /HLADR+	CD25+	CD122+
Healhty control	67.5 ± 10.3	4.0 ± 2.8	42.1 ± 9.2	28.9 ± 8.0	19.2 ± 8.2	2.3 ± 32.1	5.4 ± 2.1	2.7 ± 1.2	5.4 ± 2.8	9.7 ± 32.3
Post–cancer empyema patients before operation	77.6 ± 11.5	6.4 ± 4.6	41.1 ± 10.4	38.0 ± 11.3	10.8 ± 8.5	3.4 ± 3.6	20.1 ± 17.4	12.0 ± 7.6	8.4 ± 4.1	6.2 ± 3.8
Post–cancer empyema patients a week after operation	81.8 ± 9.1	5.6 ± 4.4	36.3 ± 7.3	39.7 ± 10.1	8.8 ± 9.3	3.1 ± 7.2	10.7 ± 5.3	2.6 ± 4.4	5.1 ± 3.2	4.9 ± 2.2
Post–cancer empyema patients a month after operation	78.5 ± 10.7	6.6 ± 5.4	40.6 ± 6.9	46.4 ± 10.8	9.9 ± 9.9	2.2 ± 2.8	17.1 ± 25.1	4.7 ± 3.4	6.8 ± 3.4	6.5 ± 5.1
Post–cancer empyema patients 6 months after operation	76.0 ± 5.4	7.6 ± 4.2	40.7 ± 10.8	42.8 ± 14.8	9.6 ± 6.7	3.1 ± 3.0	12.3 ± 5.0	2.4 ± 7.0	13.1 ± 1.9	8.5 ± 3.7
Parapneumonic empyema patients before operation	77.2 ± 10.8	8.7 ± 3.6	45.8 ± 10.7	29.1 ± 7.3	7.2 ± 4.2	7.0 ± 7.7	15.4 ± 8.8	3.1 ± 8.1	4.0 ± 0.7	6.3 ± 2.9
Parapneumonic empyema patients a week after operation	88.9 ± 12.3	4.8 ± 1.6	45.8 ± 15.0	33.8 ± 14.3	13.7 ± 8.4	1.5 ± 3.2	17.4 ± 6.4	13.7 ± .60	6.6 ± 4.7	4.5 ± 0.4
Parapneumonic empyema patients a month after operation	61.1 ± 7.7	13.5 ± 9.9	32.8 ± 12.1	33.7 ± 3.8	27.4 ± 12.7	3.5 ± 7.1	16.2 ± 3.1	5.2 ± 4.2	5.7 ± 2.9	7.9 ± 0.6
Parapneumonic empyema patients 6 months after operation	72.0 ± 5.1	11.1 ± 3.2	42.3 ± 4.5	35.6 ± 4.9	14.2 ± 4.2	2.8 ± 34.5	19 ± 34.1	10 ± 3.7	5.5 ± 32.7	8.2 ± 33.4

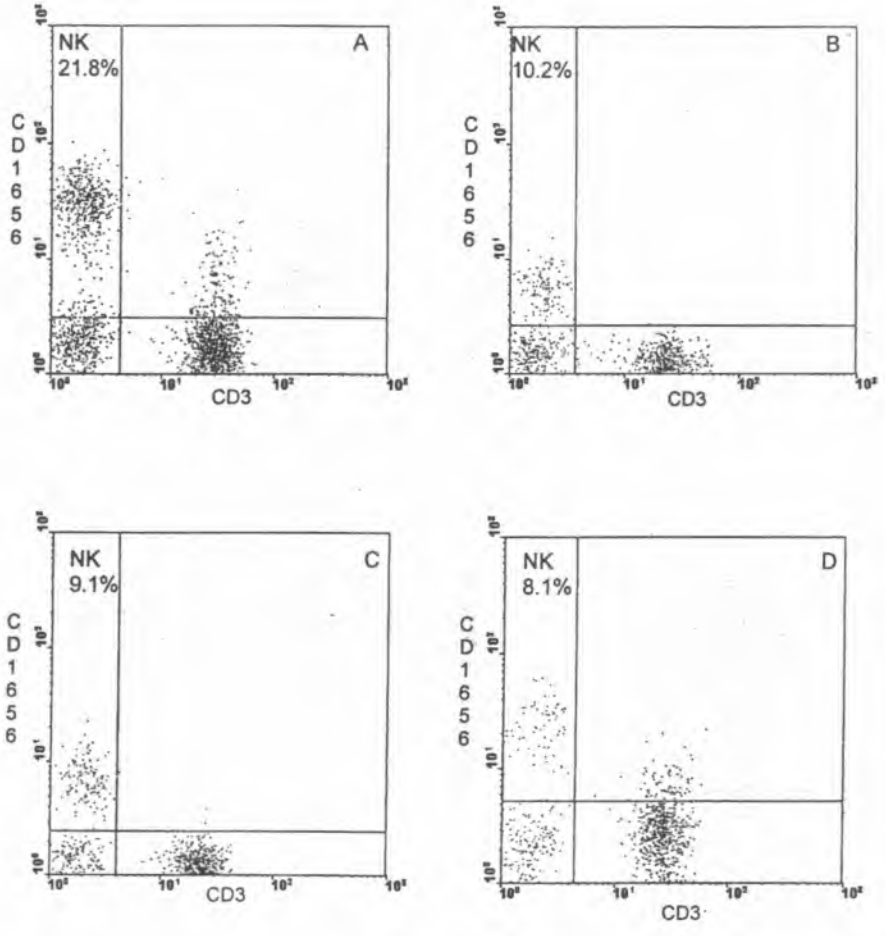

Fig. 1. NK cells in a healthy individual (A) and in a post–cancer pleural empyema patient before operation (B), a week (C) and 6 months after operation (D)

most important is local immunological response, but immunological disturbances also could be observed in peripheral blood (1). They are presented in this study, too.

A significantly higher number of activated peripheral blood cells (CD3+/HLADR+, CD25+, CD 122+, CD3+, CD8+ and CD19+) observed in our study in post–cancer empyema patients before their treatment and healthy people could be explained not only by their neoplastic pass, but also more likely, by the presence of a chronic inflammation. The first hypothesis was not consistent with the report of Wesselius et al., in which he showed no changes in CD3+, CD4+, CD8+, CD19+ cell subpopulations in patients with limited non–small cell lung cancer (NSCLC) and control or even a decreased number of helpers and suppressor/cytolitic cells in advanced ones (15). Another report on CD8+ cell decrease number was presented by Semenzato (8). Moreover, patients examined by us had unde-

tectable cancer (after cancer removal with no evidence of metastases presence). It means that the cancer influence upon the immunological system did not exist or was very little. Our second hypothesis is also consistent with the absence of significant differences between post–cancer and parapneumonic empyema patients. A good confirmation of this is the disappearance of significant changes described above in follow–up examinations after the successful treatment of parapneumonic patients, too.

On the other hand, a smaller number of parapneumonic than post–cancer empyema patients could also explain smaller differences between parapneumonic empyema patients and healthy people than between post–cancer empyema patients and healthy ones.

Our research has also revealed a very interesting significant decrease of NK cell percentage only in the group of post–cancer empyema patients (Fig. 1). Moreover, this was present in every examination. NK cells show anti–tumour activities and their number and anti–tumour activity is significantly higher in the blood than in the regional lymph nodes and in tumour infiltration lymphocytes (TILs) in lung cancer patients (5). The reduction of their number, which is presented in this paper, could be explained by their escaping from blood to hypothetical micrometastases, or by general neoplastic sociability in this group of post–cancer empyema patients. Both hypotheses have no confirmation in literature. We most remember that lung cancer frequently occurs in elderly individuals and has been found to be associated with alterations in host immunocompetence, too (11). These significant differences were smaller in the third examination than in the first one, so it is also possible that they would disappear in further examinations.

Thymic factor X (TFX) is a non–specific immunostimulatory factor (9). Its addition to the treatment of empyema patient seems to reduce the number of inflammatory complications in our study (data not shown). TFX can stimulate IgM production, which are crucial in early host defence. Significant IgM level increase observed in these patients can be responsible for their protection against different infections reflecting better clinical results.

We could only speculate about the local immunological status in pleural empyema. Moreover, the differences between PBLs and lymphocytes from pleural fluid (3), regional lymph nodes (12), broncho–alveolar lavage (BAL) (7) and tumour infiltration lymphocytes (TILs) (16) also need further investigations.

CONCLUSIONS

1. The empyema patients reveal a significantly higher percentage of CD3+, CD19+ and activated cells (post–cancer empyema patients additionally CD8+ cells) than normal individuals.

2. No changes in peripheral blood lymphocyte subsets were observed between post–cancer empyema patients and healthy individuals.

3. The differences observed between post–cancer empyema patients and healthy people are probably due to inflammation and not because of their neoplastic pass.

4. The significant NK cells percentage decrease in post–cancer empyema could have an important influence upon the course of their neoplastic diseases.

5. The significant IgM level increase observed in empyema patients previously treated with TFX injections could be responsible for better clinical results observed in this group of patients.

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STRESZCZENIE

Ropniak opłucnej jest niezbyt częstym, lecz bardzo ciężkim schorzeniem klatki piersiowej, na którego powstanie niewątpliwy wpływ mają zaburzenia immunologiczne. W Klinice Chirurgii Klatki Piersiowej AM w Lublinie leczono pięćdziesięciu pacjentów z przewlekłym ropniakiem opłucnej, stosując metodę mioplastyki. W 35 przypadkach byli to chorzy z powikłaniami raka płuca, a u 15 chorych przyczyny miały charakter zapalny. Określano ekspresję CD3, CD4, CD8, CD19, CD16/56, CD25, CD122, HLADR na limfocytach oraz poziom immunoglobulin we krwi obwodowej. Badania wykonywano w dniu poprzedzającym operację oraz w 7, 30 dni i 6 miesięcy po operacji mioplastycznej. Nieswoistą stymulację preparatem TFX zastosowano u 12 pacjentów. U tych chorych stwierdzono istotny wzrost komórek CD3+, CD8+, CD19+ i markerów aktywacji limfocytów. Liczba komórek NK była znacząco mniejsza u pacjentów z ropniakiem będącym powikłaniem raka płuc. Istotny wzrost poziomu IgNt, stwierdzony tylko u leczonych uprzednio TFX, mógł mieć wpływ na korzystniejszy przebieg kliniczny w tej grupie chorych. Przedstawione wyniki wskazują na zasadniczą rolę układu immunologicznego w patogenezie ropniaka opłucnej, choć wymaga to dalszych badań.