Research Article

Prognostic Assessment of the Inflammatory Process Activity in Sarcoidosis of Respiratory Organs: Potential Use of C-reactive Protein and TNF-α

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Abstract
This research work is devoted to the development of new additional criteria for the activity of inflammatory process in sarcoidosis of respiratory organs. The objective is to assess the effectiveness of performed treatment of sarcoidosis of respiratory organs by using low-cost highly-sensitive inflammatory markers.

Materials and methods. The study involved 68 patients with lung sarcoidosis before and after the three-month treatment. In addition to general-clinical methods of examination, patients with sarcoidosis were also determined the levels of TNF-α and CRP.

Results and their discussion. Patients with active lung sarcoidosis had 17.6 times (p < 0.05) increased level of CRP in bronchoalveolar lavage fluid and 9.0 times (p < 0.05) increased levels in peripheral blood serum; the levels of TNF-α increased by 4.98 times (p < 0.05) in bronchoalveolar lavage fluid and by 3.2 times (p < 0.05) in peripheral blood serum as compared to the findings in the control group of patients. The study showed that in the group of patients, where the efficacy of the prescribed therapy was noted, the level of CRP decreased by 2.76 times (p < 0.05) in bronchoalveolar lavage fluid and by 2.58 times (p < 0.05) in peripheral blood serum, and the concentration of TNF-α decreased by 3.87 times (p < 0.05) in bronchoalveolar lavage fluid and by 2.06 times in peripheral blood serum as compared to the initial indices.

Conclusions. The decrease of TNF-α level in bronchoalveolar lavage fluid on the background of three-months treatment correlated (r=0.89; p < 0.05) to the changes in peripheral blood serum; at the same time the decrease of TNF-α level in peripheral blood serum correlated (r=0.82; p < 0.05) to the decrease of CRP in peripheral blood serum of patients with sarcoidosis of respiratory organs.

Keywords
sarcoidosis; activity criteria; prognosis

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Problem statement and analysis of the recent research

For more than a century of study and despite the rapid development of medicine and the introduction of molecular-and-biological methods of investigation into clinical practice, sarcoidosis still remains a disease with unidentified etiology [2, 6].

Many scientists assume that the specific immune response, which manifests itself as sarcoidosis, develops as a result of combined effect of genetic factors, environmental exposure and infectious agents [7, 8].

Delayed-type hypersensitivity reaction is the basis of immunopathogenesis of sarcoidosis of respiratory organs. The development of immune-type granulomas in sarcoidosis (because of imbalance of immunoregulatory cell subpopulation and weakening of T-cellular component of immune system) says for the supposition that this pathology is caused by immunological disorders due to the exposure to various environmental factors which change the immune status or are caused by already changed immune status [1].

Accumulation of inflammatory and immune cells in organs and tissues is the initial stage that eventually leads to the damage of lung parenchyma. In lungs, the key role is given to alveolar macrophages which take part both in the inductive and effector phase of immune response [3].

Chemokines and cytokines are important components of pathogenesis in sarcoidosis, because these mediators lead to alveolitis, granuloma formation and tissue damage.

Alveolar macrophages are the major cellular source of TNF-α. They also produce growth factor which stimulates the proliferation of myofibroblasts, fibroblasts and B-lymphocytes. TNF-α is a non-specific, however, powerful pro-inflammatory cytokine that synthesizes a wide range of immune cells. In scientists’ opinion, TNF-α is a key cytokine which takes part in the formation of granuloma in sarcoidosis (Fehrenbach H. et al., 2003). It induces the synthesis of IL-1 and IL-6, contributes to the increase of leukocytes mobility, vasopermeability of microcirculatory bloodstream and intensification...
of apoptosis. Japanese scientists demonstrated that TNF-α is responsible for the overexpression of adhesive molecule-1 (Intracellular Adhesion Molecule-1 or ICAM-1) on alveolar macrophages, and the development of inflammatory granulomas is associated with the aggregation of macrophages. The inhibition of TNF-α synthesis reduces the ability to form granulomas [12].

In addition, IL-1 takes a major place among the products of macrophage secretion. It attracts T-lymphocytes to the inflammation site. Activation of T-helpers is an essential condition for granuloma formation, as they play the key role in pathophysiology of sarcoidosis.

Therefore, T-lymphocytes, namely Th1-cells, produce IL-2, which activates differentiation of effector cells, stimulates the release of factors for positive chemotaxis of monocytes and proliferation of T-cells, which consequently infiltrate the parenchyma of lungs, lymph nodes and other tissues affected by sarcoidosis [14].

IL-2 is a powerful inducer of T-cell proliferation and IFN-γ synthesis and plays the key role in immune response in sarcoidosis. Moreover, some scientists think that the increase of IL-2 and IFN-α level (cytokine, which promotes Th1 response) in bronchoalveolar lavage fluid may be the early predictor of inflammatory process and say for the sarcoidosis progression. World scientific literature represents a range of studies which demonstrate clear correlation between the concentration of soluble IL-2 receptor and T-helpers. The increase of soluble IL-2 receptor level in peripheral blood serum is an activity marker in patients with sarcoidosis of respiratory organs and extrathoracic damages (except Lofgren’s syndrome) [10, 11].

Increased release of cytokines, formed in macrophages (IL-1, IL-6, IL-8, IL-15, TNF-α), and chemokines (MIP-α, IL-16) contributes to the formation of sarcoïd granuloma and lung damage. Excessive production of fibrogenic cytokines (TGF-α and related cytokines, PDGF and IGF-1) by macrophages leads to the development of fibrosis [9, 13].

The process of sarcoïd granuloma development may be schematically divided into 3 stages:

1. Th-1 activation by antigen-presenting macrophages;
2. release of cytokines with diverse and cross functions;
3. accumulation of immunocompetent cells in sites of inflammation of affected organs.

Immune shifts in sarcoidosis on organ level lead to the development of three relative (though, not obligate for a specific patient) stages: alveolitis (lymphocytic infiltration) - granulomatosis (epithelioid-cellular granuloma) - fibrosis [4, 5].

Estimation of inflammatory process activity in sarcoidosis is of great practical consequence, as it enables the clinician to make personal prognosis for every patient, find adequate therapeutic approach and evaluate the effectiveness of administered treatment. Determining the regularities of TNF-α dynamics in bronchoalveolar lavage fluid and peripheral blood serum will make it possible to better analyze the management of patients. While its correlation with routine low-cost identification of C-reactive protein will allow to maximally bring the process closer to the primary medical care and make it cheaper.

**Objective.** The aim is to find additional criteria for inflammatory process activity in sarcoidosis of respiratory organs.

1. **Materials and methods**

We conducted in-depth instrumental examination of 68 patients with lung sarcoidosis before and after the three-month treatment. The mean age of patients was (35.7±6.6) years. All of them underwent in-patient treatment in the pulmonology unit of the regional clinical phthisiopneumologic centre (Ivano-Frankivsk).

The control group involved 16 apparently healthy individuals.

All the patients underwent complete clinical examination in accordance with the 3rd level of specialized pulmonologic care in healthcare centres: general clinical and laboratory-instrumental examination, including multispiral computed tomography of thoracic organs and fibrobronchoscopy, as well as, determining the levels of C-reactive protein and TNF-α in bronchoalveolar lavage fluid and peripheral blood serum.

In the course of investigation, patients underwent fibrobronchoscopy with the help of "Olympus BF-20" fiber-optic bronchoscope (Japan) with simultaneous sampling of bronchoalveolar lavage fluid and estimation of inflammatory changes of tracheobronchial tree according to the generally established procedure by J. Lemoine in the modification of H.I. Lukomskyi.

The level of TNF-α was identified by means of enzyme immunoassay using "StatFax 303 Plus" analyser and "Human TNF-alpha ELISA" reagents produced by "Diaclone" (France). The level of C-reactive protein was determined by semi-quantitative method of latex particle agglutination test using “Dialab” reagents kit (Austria).

Database creation and statistical analysis of the material during the investigation period required implementation of authoring software programs on the basis of Microsoft Excel (computation of relative values, their errors, stratification analysis). Some investigation tasks were accomplished with the use of licensed software suites for statistical analysis Microsoft Excel and Statistica 5.5, involving descriptive statistic programs, geometrical mean values computation. (License number 76487 - DEM - 2241066 - 104/8).

Multispiral computed tomography of thoracic organs was performed using Toshiba Aquilion Prime scanner, with subsequent recording of findings on digital medium and assessing the density of lung tissue by Hounsfield unit (HU) scale: when the density of lung tissue in dynamics was less than 893.5 Hounsfield units the treatment was considered effective, but if the density of lung tissue, as compared to initial indices, exceeded 893.5 units the treatment was considered ineffective.

Complex therapy of patients with sarcoidosis of respiratory organs was carried out in accordance with the order of
Ministry of Health Care of Ukraine #634 from September 08, 2014 “On approval and implementation of medical and technological documents on medical care standardization in sarcoidosis”.

After three-month treatment the patients were divided into 2 subgroups according to their complaints, physical data and the results of multispiral computed tomography of thoracic organs:

- subgroup I - effective treatment (n=47) - patients with positive clinic-radiologic presentation;
- subgroup II - ineffective treatment (n=21) - patients whose symptoms either persisted or worsened, without any positive radiologic dynamics.

### 2. Results of the investigation and their discussion

The usability of CRP and TNF-α levels in bronchoalveolar lavage fluid and peripheral blood serum as potential additional markers for inflammatory process activity was evaluated by comparing them to the data of multispiral computed tomography of thoracic organs and clinical manifestations of the disease.

The most frequent subjective manifestations of the pathology were:

- cough - 52 (76.47%) patients, non-productive in most cases - 37 (71.15%), and in 15 cases (28.85%) with small quantity of mucoid sputum;
- dyspnea - 40 (58.82%) patients; in 26 (65%) cases it was caused by strenuous physical activity; in 13 (32.5%) cases it occurred with normal strenuous activity and in 1 case (2.5%) patient's physical activity was low;
- pain and chest discomfort - 6 (8.82%) patients;
- general weakness and rapid fatigability - 28 (41.17%) patients.

Inflammatory process activity was assessed by identifying the concentrations of C-reactive protein and TNF-α in bronchoalveolar lavage fluid and peripheral blood serum.

It has been established that the level of C-reactive protein in bronchoalveolar lavage fluid of patients with active lung sarcoidosis (n=68) made up (28.34±2.45) mg/l before treatment, that is 17.6 times higher as compared to the levels in the control group (n=16) - (1.61±0.17) mg/l (p<0.05) (Fig. 1).

The level of TNF-α in bronchoalveolar lavage fluid at the time of initial examination was (142.13±7.59) pg/ml and exceeded the given index by 4.98 times as compared to the control group of patients (Fig. 2) (p<0.05).

Simultaneously these parameters were identified in peripheral blood serum. The level of C-reactive protein in patients with active lung sarcoidosis (n=68) was 9.0 times higher than in apparently healthy individuals (n=16) (p<0.05), and made up (47.63±2.33) mg/l (see Fig. 1). The level of TNF-α was 3.2 times increased as compared to the control group (90.75±5.34) pg/ml (p<0.05), and made up (290.41±8.27) pg/ml (see Fig. 2).

In our opinion, the established correlations between the increase in TNF-α and CRP levels in bronchoalveolar lavage fluid (r=0.88; p<0.05) and between the increase of CRP levels in bronchoalveolar lavage fluid and peripheral blood serum (r=0.96; p<0.05) in activation/progression of the inflammatory process in patients with sarcoidosis of respiratory organs are especially valuable. These findings correspond to the data obtained by multispiral computed tomography of thoracic organs and obtained lung tissue density in Hounsfield units (HU).

We have noticed the normalization/improvement of general state and decrease/absence of cough, dyspnea and chest pain in patients from subgroup I after the three-month treatment.
While in subgroup II dyspnea persisted/progressed in 17 patients (85.71%), cough appeared or worsened in 14 (66.67%) patients, chest pain persisted in 1 (4.76%) case and 6 patients (28.57%) still complained of general weakness and rapid fatigability.

In subgroup I (n=47) the level of C-reactive protein in bronchoalveolar lavage fluid was (10.27±1.18) mg/l, that is 2.76 times lower as compared to the initial data (p<0.05). And what is more, this index has also decreased in peripheral blood serum (r=0.94; p<0.05) to (18.47±1.05) mg/l (p<0.05), that was 2.58 times lower than before treatment (Fig. 1).

On the background of three-month treatment, the level of TNF-α in bronchoalveolar lavage fluid has decreased by 3.87 times and made up (36.75±4.39) pg/ml (p<0.05). These positive results correlated (r=0.89; p<0.05) with the changes in peripheral blood serum, where the concentration of this cytokine was (141.22±6.97) pg/ml, that was 2.06 times lower than the initial data (p<0.05) (Fig. 1). At the same time the decrease in the level of TNF-α in peripheral blood serum correlated (r=0.82; p<0.05) with the decrease of CRP level in peripheral blood serum of patients with sarcoidosis of respiratory organs.

Treatment was considered to be inadequate in subgroup II (n=21), and the levels of C-reactive protein remained increased in both bronchoalveolar lavage fluid and peripheral blood serum and made up (31.86±2.64) mg/l (p<0.05), (55.15±2.68) mg/l (p<0.05), respectively.

TNF-α, in patients with ineffective treatment, tended to even increase: its concentration was 189.24±5.22 pg/ml (p<0.05) in bronchoalveolar lavage fluid and 382.13±15.44 pg/ml (p<0.05) in peripheral blood serum (Table 1). The dynamics of C-reactive protein and TNF-α with both effective and ineffective treatment corresponded to the data of general clinical examinations, and was associated with clinical symptoms and changes in multispiral computed tomography of thoracic organs and lung tissue density in Hounsfield units.

3. Conclusions

1. The characteristic symptoms of lung sarcoidosis activation requiring treatment are: onset of dyspnea; cough that is accompanied by changes in lung tissue density (according to the findings of MSCT of thoracic organs), as compared to the initial data - 893.5 HU: increase of CRP level in bronchoalveolar lavage fluid by 17.6 times (p<0.05) and by 9.0 times (p<0.05) in peripheral blood serum; increase of TNF-α level in bronchoalveolar lavage fluid by 4.98 times (p<0.05) and by 3.2 times (p<0.05) in peripheral blood serum as compared to the control group data.

2. The characteristic features of failure of three-month treatment for lung sarcoidosis requiring escalation of medication treatment regimens are: persistent and progressive dyspnea and cough, maintenance of high CRP concentrations in bronchoalveolar lavage fluid (31.86±2.64) mg/l (p<0.05) and in peripheral blood serum (55.15±2.68) mg/l (p<0.05), as well as the levels of TNF-α in bronchoalveolar lavage fluid and peripheral blood serum which made up (189.24±5.22) pg/ml (p<0.05) and 382.13±15.44 pg/ml (p<0.05) respectively.

3. Three-month treatment for lung sarcoidosis was considered to be effective when dyspnea and cough decreased, and the condition was associated with the decrease of lung tissue density below -893.5 HU (based on MSCT of thoracic organs), and decrease of CRP level by 2.76 times (p<0.05) in bronchoalveolar lavage fluid and by 2.58 times (p<0.05) in peripheral blood serum, decrease of TNF-α concentration by 3.87 times (p<0.05) in bronchoalveolar lavage fluid and by 2.06 times in peripheral blood serum, as compared to the initial data.

4. The decrease of TNF-α level in bronchoalveolar lavage fluid, on the background of three-month treatment, correlated (r=0.89; p<0.05) with the changes in peripheral blood serum, while, at the same time the decrease of TNF-α level in peripheral blood serum correlated (r=0.82; p<0.05) with the decrease of CRP level in peripheral blood serum of patients with sarcoidosis of respiratory organs.

4. Prospects of future research

The study of dynamics of C-reactive protein and TNF-α in patients with sarcoidosis of respiratory organs will make it possible to assess the degree of local and systemic inflammation, modify the treatment options and make individual prognosis for every patient.

References

[1] Ahmadzai H, Loke WSJ, Huang S et al. Biomarkers in sarcoidosis: a review. Dove Medical Press. 2014; 4: 93-106. DOI: https://doi.org/10.2147/CBF.S46196
[2] ATS/ERS/WASOG Committee. Statement on Sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 1999; 16: 149-173. [PMid:10560120]
[3] Baughman RP, Nunes H, Sweiss NJ et al. Established and experimental medical therapy of pulmonary sarcoidosis. European Respiratory Journal. 2013; 41: 1424-1438. DOI: https://doi.org/10.2147/CBF.S46196
[4] Baughman RP, Culver DA, Judson MA. A concise review of pulmonary sarcoidosis. Am J Respir Crit Care Med. 2011; 183(5): 573-581. DOI: https://doi.org/10.1164/rccm.201006-0865CI [PMid:21037016 PMCid:PMC3081278]
Table 1. Indices of C-reactive protein and TNF-α in bronchoalveolar lavage fluid and peripheral blood serum in patients with lung sarcoidosis

| Indices                   | AHI, n=16 | Before treatment, n=68 | After treatment (Subgroup I) Effective treatment, n=47 | After treatment (Subgroup II) Ineffective treatment, n=21 |
|---------------------------|-----------|------------------------|--------------------------------------------------------|----------------------------------------------------------|
|                           |           | Levels in bronchoalveolar lavage fluid                      |                                                        |                                                          |
| CRP, mg/l                 | 1.61±0.17 | 28.34±2.45*           | 10.27±1.18*                                           | 31.86±2.64*                                              |
| TNF-α, pg/ml              | 28.53±5.44| 142.13±7.59*          | 36.75±4.39*                                           | 189.24±5.22*                                             |
|                           |           | Levels in peripheral blood serum                            |                                                        |                                                          |
| CRP, mg/l                 | 5.28±0.76 | 47.63±2.33*           | 18.47±1.05*                                           | 55.15±2.68*                                              |
| TNF-α, pg/ml              | 90.75±5.34| 290.41±8.27*          | 141.22±6.97*                                          | 382.13±15.44*                                            |

Notes:
Probability of indices variability between the control group and studied group of patients: *– p<0.05.

[5] Costabel U. Sarcoidosis: clinical update. European Respiratory Journal. 2001; 18: 56-68.
[6] Feshchenko YI, Protsyk LM, Cherednyk YO. Sarcoidosis of respiratory organs: present state of the problem: Ukr. Pulmonol. J. 2006; 3: 5-10.
[7] Gavrysyuk VK. Pulmonary Sarcoidosis. Health of Ukr. 2010; 2: 29-31.
[8] Gavrysyuk VK. Pulmonary sarcoidosis: epidemiology, clinical forms and stages, results of treatment: Health of Ukr. 2014; 1(25): 32-33.
[9] Mannino DM, Ford ES, Redd SC: Obstructive and restrictive lung disease and markers of inflammation: Data from the Third National Health and Nutrition Examination. Am J Med 2003; 114: 758-762. DOI: https://doi.org/10.1016/S0002-9343(03)00185-2
[10] Meyer KC, Raghu G. Bronchoalveolar lavage for the evaluation of interstitial lung disease: is it clinically useful? European Respiratory Journal. 2011; 38: 761-769. DOI: https://doi.org/10.1183/09031936.00069509 [PMid:21540304]
[11] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003;111: 1805-1812. DOI: https://doi.org/10.1172/JCI1200318921
[12] Sweiss NJ, Curran J, Baughman RP. Sarcoidosis, role of tumor necrosis factor inhibitors and other biologic agents, past, present, and future concepts. Clin Dermatol. 2007; 25: 341-346. DOI: https://doi.org/10.1016/j.cldermatol.2007.03.012 [PMid:17560312]
[13] Sahoo DH, Bandyopadhyay D, Xu M, Pearson K et al. Effectiveness and safety of leflunomide for pulmonary and extrapulmonary sarcoidosis. European Respiratory Journal. 2011; 38: 1145-1150. DOI: https://doi.org/10.1183/09031936.00195010 [PMid:21565914]
[14] Takahashi T, Azuma F, Abe S et al. Significance of lymphocytosis in bronchoalveolar lavage in suspected ocular sarcoidosis. European Respiratory Journal. 2001; 18: 515-521. DOI: https://doi.org/10.1183/09031936.01.99104501 [PMid:11589349]