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Abstract

Clinical and radiological features of tuberculosis and sarcoidosis are quite overlapping, and therefore, a diagnostic dilemma often persists. There are no commonly accepted criteria for the diagnosis of sarcoidosis due to the lack of data on the etiology of the disease. The exclusion of tuberculosis in every patient with suspected sarcoidosis is a mandatory stage of diagnosis, especially in countries with a high burden of tuberculosis. A prospective study was conducted with two groups of patients: group I ($n = 50$)—patients with pulmonary sarcoidosis established according to standard criteria; group II ($n = 28$)—patients with pulmonary tuberculosis with bacterial excretion. The control group ($n = 24$) was presented by healthy subjects. The examination complex included x-ray, bacteriological, immunological (Mantoux test with 2 TE, TB.SPOT test), and histological methods. All patients and healthy subjects were assessed for immune complexes with the use of the dynamic light scattering (DLS) method and adding of "healthy lung tissue extract" antigens and specific tuberculosis antigens ESAT-6 and SFP-10 in vitro. Significant differences were found in determining specific immune complexes in patients with pulmonary sarcoidosis and pulmonary tuberculosis. Registration of specific immune complex formation with "healthy lung tissue extract" in 100% cases may indicate the autoimmune nature of sarcoidosis. The absence of the immune complex formation in response to ESAT-6/SFP-10 antigens can be used for the differential diagnosis of two diseases. The diagnostic significance of the DLS method was 100% for sarcoidosis and 92.2% for tuberculosis. The data obtained in the study allows not only understanding the etiology of sarcoidosis, but also obtaining new criteria for the differential diagnosis of tuberculosis and pulmonary sarcoidosis.

Keywords separated by ' - '
Sarcoidosis - Tuberculosis - Allergen test with tuberculosis recombinant - Immunological tests - Autoimmune diseases - Dynamic light scattering method

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ORIGINAL ARTICLE

Specific features of immune complexes in patients with sarcoidosis and pulmonary tuberculosis

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Abstract

Clinical and radiological features of tuberculosis and sarcoidosis are quite overlapping, and therefore, a diagnostic dilemma often persists. There are no commonly accepted criteria for the diagnosis of sarcoidosis due to the lack of data on the etiology of the disease. The exclusion of tuberculosis in every patient with suspected sarcoidosis is a mandatory stage of diagnosis, especially in countries with a high burden of tuberculosis. A prospective study was conducted with two groups of patients: group I ($n = 50$)—patients with pulmonary sarcoidosis established according to standard criteria; group II ($n = 28$)—patients with pulmonary tuberculosis with bacterial excretion. The control group ($n = 24$) was presented by healthy subjects. The examination complex included x-ray, bacteriological, immunological (Mantoux test with 2 TE, TB.SPOT test), and histological methods. All patients and healthy subjects were assessed for immune complexes with the use of the dynamic light scattering (DLS) method and adding of "healthy lung tissue extract" antigens and specific tuberculosis antigens ESAT-6 and SFP-10 in vitro. Significant differences were found in determining specific immune complexes in patients with pulmonary sarcoidosis and pulmonary tuberculosis. Registration of specific immune complex formation with "healthy lung tissue extract" in 100% cases may indicate the autoimmune nature of sarcoidosis. The absence of the immune complex formation in response to ESAT-6/SFP-10 antigens can be used for the differential diagnosis of two diseases. The diagnostic significance of the DLS method was 100% for sarcoidosis and 92.2% for tuberculosis. The data obtained in the study allows not only understanding the etiology of sarcoidosis, but also obtaining new criteria for the differential diagnosis of tuberculosis and pulmonary sarcoidosis.

Keywords Sarcoidosis · Tuberculosis · Allergen test with tuberculosis recombinant · Immunological tests · Autoimmune diseases · Dynamic light scattering method

Introduction

Sarcoidosis and tuberculosis belong to the group of granulomatosis—diseases similar to each other not only in clinical and radiological manifestations, but also in the histological picture. Unlike tuberculosis, the etiology of sarcoidosis is currently unknown that makes the differential diagnosis of these diseases in the absence of bacteriological verification of tuberculosis quite difficult [1–5].

Some researchers associate the development of sarcoidosis with a complex of pathological autoimmune reactions caused by the interaction of various exogenous or endogenous adjuvants, which may result in the development of the so-called ASIA syndrome ("The Autoimmune/inflammatory Syndrome Induced by Adjuvants (ASIA) ~~Research Questionnaire~~") [6–8].

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The diagnosis of sarcoidosis takes a time which varies from 3 months to several years [9, 10]. There is often a necessity for histological verification of the diagnosis, which, in its turn, does not always lead to an unambiguous interpretation of pathomorphological changes in the biopsy material of the lymph node and lung tissue [11, 12]. However, morphological verification remains the "gold standard" in the diagnosis of sarcoidosis to this day [13, 14].

A number of studies have been dedicated to the search for immunological criteria that determine not only diagnostic but also prognostic value of various immunological parameters in the development of sarcoidosis and tuberculosis [10, 15, 16].

Recent studies have demonstrated diagnostic significance of new immunological methods (samples with tuberculosis recombinant allergen, TB.SPOT and QuantiFERON TB test) in the differential diagnosis of sarcoidosis and tuberculosis. The diagnostic significance of these tests is 84.7%, 79.2%, and 86.7%, respectively, that corresponds with the diagnostic significance of the morphological study (86.5%) [17–20]. These imply the production of false-positive results in patients with sarcoidosis and false-negative results in patients with tuberculosis, which means an incorrect diagnosis in 20–15% of cases. However, positive results in patients with sarcoidosis may be associated either with the role of *Mycobacterium tuberculosis* in the development of pulmonary sarcoidosis [21] or the presence of latent tuberculosis infection in the environment with high prevalence of tuberculosis.

Importantly, the diagnostic significance of immunoglobulins in granulomatous diseases has been studied with a view to their possible role in the formation of granulomas by attachment to immune complexes (IC) [9, 22]. It was determined that the circulating immune complex (CIC) level correlates with the disease activity and the degree of their accumulation in the affected tissues, but their diagnostic and prognostic role requires further investigation, which is also related to the resolving power of the utilized methods.

Currently, all existing methods for IR analysis (two-dimensional electrophoresis), immunological detection (Western blotting), and combined approaches based on the utilization of two-dimensional electrophoresis (EP) or high-performance liquid chromatography followed by mass spectrometry analysis are associated with releasing IR from blood plasma and other biological fluids. As a result, the analysis of the distribution of IR population by size and included components becomes impossible. A method effectively determining the immune complex components without isolating complexes from the plasma is the dynamic light scattering (DLS) method [23]. The unique features of the DLS method are in its possibility to detect the macromolecular complex formation in biological systems without fractionating or any other procedures that violate the native conditions of complex formation and, thus, allow to get more detailed information [24, 25].

A radical increase in dynamic light scattering while elevation of linear dimensions of macromolecular formations, combined with the possibility of estimating their real dimensions, allows detecting and identifying complexes' formation by various components of biological fluids, even when their amount is very small.

Thus, the study of the immune complexes formed after ~~stimulat~~ing immunoglobulins with specific antigens with the use of the dynamic light scattering method is carried out for the first time. The obtained data not only represents a significant step toward *understanding* the etiology of sarcoidosis, but also provides new criteria for the differential diagnosis of sarcoidosis and pulmonary tuberculosis.

The objective of this study was to determine the specific immune complexes formed after stimulating immunoglobulins with specific antigens in patients with sarcoidosis and pulmonary tuberculosis.

Material and methods

A cohort prospective study was conducted between December 2016 and July 2017, enrolled 78 patients at the FGBU "St. Petersburg Research Institute of Phthisiopulmonology" of the Ministry of Health of the Russian Federation and St. Petersburg GBUZ "City Hospital No. 2" and 24 healthy subjects (control group). The study was approved by the independent Ethics Committee of the FGBU "SPb NIIF" St. Petersburg Scientific Research Institute of the Ministry of Health of the Russian Federation (protocol no. 34.2 dated January 19, 2017); all participants of the study signed an approved form of informed consent.

Patients were enrolled in the study after verification of the diagnosis of sarcoidosis and tuberculosis, according to internationally accepted criteria [26–28]; history of the diseases did not exceed 2 years, antituberculosis therapy—1 month.

According to inclusion and exclusion criteria, two main comparison groups were enrolled: group I ($n = 28$) with lung sarcoidosis; group II ($n = 50$) with pulmonary tuberculosis.

Pulmonary sarcoidosis was established by the following: complaints, radiologic changes, and histological verification (detection of epithelioid granulomas without caseous necrosis and acid-resisting mycobacteria); negative results of the laboratory examination for tuberculosis; and a reliable increase in the level of angiotensin-converting enzyme (ACE).

Pulmonary tuberculosis was diagnosed with typical complaints, radiologic changes, and positive results of laboratory examination (identification of MBT and/or MBT DNA according to molecular genetic and bacteriological methods in sputum analysis).

The exclusion criteria were as follows: history of immunosuppressive therapy, treatment with antituberculosis drugs, plasmapheresis course for less than 2 months before time of

inclusion, presence of HIV infection, syphilis, tumors, diabetes mellitus, and other granulomatous pulmonary diseases.

The inclusion criteria for healthy subjects were as follows: absence of acute and chronic diseases, no risk of tuberculosis, and negative results of immunological tests.

Study methods

All patients underwent a complex examination, including clinical assessment of the disease, multispiral computed chest tomography (MSCT), laboratory blood tests, a standard set of tests for tuberculosis.

Patients with negative results of sputum examination by microscopy, inoculation of culture in liquid and dense media, and polymerase chain reaction (PCR) on *M. tuberculosis* DNA underwent histological verification (biopsy of lung tissue obtained by fibrobronchoscopy or videothoracoscopy lung biopsy).

As part of TB examination, ELISPOT test (Oxford, UK) was carried out. The positive result was described as ≥ 6 spots in the ESAT-6 well or CFP-10 after subtracting the number of spots observed in the negative control well where negative control had 0–5 spots. If the negative control had ≥ 6 spots, then for the positive result, the ESAT-6 or CFP-10 panel should contain at least twice as many spots as the negative panel. The result was considered questionable if the negative control well contained more than 10 spots or control with the mitogen contained less than 20 spots (with a number of spots in the ESAT-6 and CFP-10 < 6 wells).

The plasma of all enrolled patients was studied at the B.P. Konstantinov FGBU "Saint-Petersburg Nuclear Physics Institute" to determine IR formed in vitro using DLS method with the proposed methodology (patent application no. 2015149694, publication date May 24, 2017, Filatov M.V., Landa S.B.). The measurements were based on the laser correlation spectrometer (certificate RU 39.003, A No. 5381) LC-03 (INTOS-MED, Russia).

The dynamic light scattering (DLS) method contributes to determine the components of the immune complex without isolating complexes from the plasma.

The DLS method detects large particles—the immune complexes. In addition, native samples are examined under physiological conditions, and a narrow range of necessary preanalytical procedures, i.e., dilution, centrifugation, and filtration, is available.

The blood plasma obtained from patients was diluted fourfold with phosphate buffer containing 10 mM ethylenediaminetetraacetic acid and processed to centrifugation for 15 min at 15,000 rpm and filtration with pore sizes of 100 nm to remove all particles and protein aggregates, exceeding this size. The dynamic light scattering (DLS) measurement of the obtained preparation should show the absence of any formations exceeding the size of 100 nm.

Ten microliters of the prepared antigen was added to the 400-μl obtained plasma samples. As a specific tuberculosis antigen, ESAT-6/SFP-10 (Generium, Russia) was used.

As a second antigenic material, the "healthy lung tissue extract" obtained at surgery was used in all studied cases. It was a prepared extract of peptide and other biological components. Different isotypes of specific immunoglobulins were detected after the in vitro addition of these antigens. All measurements were carried out by laser correlation spectrometer (certificate RU 39.003, A No. 5381) LC-03 (INTOS-MED, Russia).

Statistical methods

The statistical processing of the material was based on the program Statistica 7.0 with the use of variation statistics methods. The quantitative data was presented in the M ± SD form. The degrees of associations between proportions were estimated by confidence intervals, as well as the χ^2 criterion with Yates correction. For variable values less than 5, Fisher's exact test was used. Differences or communication rates were considered significant at $p < 0.05$ level. The diagnostic significance, including diagnostic sensitivity (DS), diagnostic specificity (DS), and diagnostic efficiency (DE), was assessed. The odds ratio (OR) was calculated with the formula $(a/c)/(b/d) = (a \times d)/(b \times c)$ (a, true-positive result; b, false-positive result; c, false-negative result; and d, truly negative result). A relative risk value of more than 1.0 was considered significant. In addition, the frequency of positive reactions was also determined based on the calculation of 95% confidence interval (95% CI).

Study results

At the first stage, the analysis of specific immune complexes in the blood plasma in patients in the compared groups (I and II), as well as in the control group (III) (Table 1), was carried out.

As Table 1 indicates, the specific immune complex formation with the antigenic material of *M. tuberculosis* (ESAT-6/SFP-10) was significantly more frequently detected in group II than in groups I (100% vs. 10.7%, $p < 0.001$) and III (control group) (100% vs. 11.1%, $p < 0.001$). A slightly positive result that is lower than the diagnostic value at the high TB infection spread level is permissible and may indicate weak manifestations of latent tuberculosis infection, as well as the high sensitivity of the method.

Q2 t1.1 **Table 1** Determining blood immune complexes after the addition of ESAT-6/SFP-10 antigenic material in groups

t1.2 Indicators	Norm	Group I (sarcoidosis) ($n = 28$)	Group II (tuberculosis) ($n = 50$)	Group III, healthy subjects (control group) ($n = 24$)
t1.3		M ± SD/*n* (%)		
t1.4 Total immune complexes	Less than 1.0	0.24 ± 0.63 3 (10.7)	7.25 ± 2.8 *, ** 50 (100.0)	0.25 ± 0.8 3 (11.0)
t1.5 IgG1	Less than 1.0	0.16 ± 0.6 2 (7.1)	4.25 ± 2.0 *, ** 50 (100.0)	0.06 ± 0.3 1 (3.7)
t1.6 IgG3	Less than 1.0	0	3.2 ± 1.2 *, ** 48 (96.0)	0.06 ± 0.3 1 (3.7)
t1.7 IgE	Less than 1.0	0.06 ± 0.2 2 (7.1)	3.48 ± 1.9 *, ** 46 (92.0)	0.02 ± 0.1 1 (3.7)
t1.8 IgG1 + IgG3	Less than 1.0	0	4.08 ± 2.1 *, ** 47 (94.0)	0
t1.9 IgG1 + IgE	Less than 1.0	0	7.72 ± 4.0 *, ** 13 (26.0)	0
t1.10 IgG3 + IgE	Less than 1.0	0	7.15 ± 3.6 *, ** 30 (57.0)	0

*Differences are significant ($p < 0.001$ for all indicators) compared to group II

**Differences are significant ($p < 0.001$ for all indicators) compared to the control group

Next, the results were analyzed to determine specific immune complexes after antigen stimulation of the "healthy lung tissue extract" (Table 2).

The ICs were determined more reliably in group I than in groups II (100% vs. 4.0%, $p < 0.0001$) and III (100% vs. 0%, $p < 0.0001$), which may indicate the development of an autoimmune reaction to pulmonary tissue under some external and internal factors.

The obtained data was used to calculate the indicators of the diagnostic significance of the methods presented.

The diagnostic significance of the specific immunoglobulins when stimulated with ESAT-6/SFP-10 is presented in Table 3.

The diagnostic significance of determining specific immunoglobulins when stimulated by "healthy lung tissue extract" is reflected in Table 4.

The obtained data and described approach were used when determining ICs during the stimulation of immunoglobulins by adding homologous antigens that are ingredients of various tissues in patients with sarcoidosis in various localizations of inflammation. The results of the blood plasma study by the

t2.1 **Table 2** Determining specific immune complexes in the study groups after stimulation by "healthy lung tissue extract" antigen

t2.2 Indicators	Norm	Groups		
t2.3		Group I (sarcoidosis) ($n = 28$)	Group II (tuberculosis) ($n = 25$)	Group III, healthy subjects (control group) ($n = 24$)
t2.4		M ± SD/*n* (%)		
t2.5 Total immune complexes	Less than 1.0	4.84 ± 0.84 *, ** 28 (100.0)	6.1 ± 0.3 1 (4.0)	0
t2.6 IgG1	Less than 1.0	3.72 ± 1.0 *, ** 28 (100.0)	2.7 1 (4.0)	0
t2.7 IgG3	Less than 1.0	2.9 ± 1.0 *, ** 27 (96.4)	0	0
t2.8 IgE	Less than 1.0	1.99 ± 0.6 *, ** 26 (92.8)	0	0
t2.9 IgG1 + IgG3	Less than 1.0	3.46 ± 0.9 *, ** 26 (92.8)	0	0
t2.10 IgG1 + IgE	Less than 1.0	5.69 ± 1.3 *, ** 25 (89.3)	0	0
t2.11 IgG3 + IgE	Less than 1.0	5.05 ± 1.2 *, ** 25 (89.3)	0	0

*Differences are significant ($p < 0.001$ for all indicators) compared to group II

**Differences are significant ($p < 0.001$ for all indicators) compared to the control group

Q3 **Table 3** Diagnostic significance of determining specific immunoglobulins when stimulated by ESAT-6/SFP-10 of ESAT-6/SFP-10

Indicators	Diagnostic sensitivity (%)	Diagnostic specificity	Diagnostic significance
Total immune complexes	94.3	87.5	*92.2*
IgG1	98.1	95.8	*97.3*
IgG3	97.9	95.8	*97.3*
IgE	97.8	95.8	*97.3*
IgG1 + IgG3	100.0	100.0	*100.0*
IgG1 + IgE	100.0	100.0	*100.0*
IgG3 + IgE	100.0	100.0	*100.0*

DLS method in one of the patients with generalized sarcoidosis were demonstrated in the clinical case.

Clinical example no. 1

Patient N., 67 years old, applied for consultancy to outpatient department in October 2016. Changes in lungs were firstly detected in August 2016 during preventive examination. The chest MSCT shows multiple perilymphatically located small foci and moderately enlarged paratracheal lymph nodes, 16 × 11 mm (upper) and 21 × 12 mm (lower). Based on the survey (threefold microscopy and PCR-sputum examination for acid-fast bacteria)—"negative." After repeated examination on the unchanged skin of the face and body, rounded, painless formations of red color and elastic density were described, slightly rising above the surface of the skin, measuring up to 1.0 × 1.5 cm. Skin biological material examination revealed granulomas; when performing lung biopsy by fibroscopy, epithelioid granulomas without caseous necrosis as well as acid-fast bacteria were detected, which indicate a granulomatous diseases like tuberculosis and sarcoidosis.

Table 4 Diagnostic significance of determining specific immunoglobulins when stimulated by "healthy lung tissue extract" antigen

Indicators	Diagnostic sensitivity (%)	Diagnostic specificity	Diagnostic significance
Total immune complexes	100.0	100.0	*100.0*
IgG1	100.0	100.0	*100.0*
IgG3	96.4	100.0	*98.1*
IgE	92.9	100.0	*96.2*
IgG1 + IgG3	92.9	100.0	*96.2*
IgG1 + IgE	89.3	100.0	*94.2*
IgG3 + IgE	89.3	100.0	*94.2*

Thus, further verification of the diagnosis was necessary. The patient's blood plasma was examined by DLS method. The antigenic material (extracts of healthy lung tissue, skin, pancreas, antigenic material of tuberculous mycobacteria—ESAT6/SFP-10) was added to the prepared blood plasma in vitro and purified from the preexisting immune complexes and other macromolecular aggregates.

As is depicted in Table 5, the immune complex formation was detected in response to antigenic "healthy lung tissue extract" and the skin biopsy material. The immune complex formation was not observed when the antigenic material of tuberculosis bacilli was added which can serve as an indirect confirmation of the negative examination data for tuberculosis in this patient. Moreover, a mixture of immunoglobulins of G1 + E and G3 + E classes contributes significantly to the immune complex formation on the tissues involved in the pathological process.

Based on the obtained data, the patient was diagnosed with "generalized sarcoidosis with lesions of lungs, intrathoracic lymph nodes, and skin."

Discussion

The obtained results allowed to conclude that during the pathological process in tissues afflicted with sarcoid granulomas, an autoimmune reaction to one's own tissue occurs likely provoked by the influence of some factors.

Taking into account the proven immunogenetic predisposition to the development of sarcoidosis (HLA-DRB1 * 0301 in acute sarcoidosis, HLA-DQB1 * 0201, DRB1 * 0301 with recurrent disease HLA-DQB1 * 0602, DRB1 * 150101 in chronic active sarcoidosis, HLA-DRB1 * 11, HLA-DR3 in extrapulmonary forms) [25], this reaction can be genetically conditioned.

Table 5 Determining immune complexes and immunoglobulin isotypes with antigens of various tissues and antigens ESAT-6 and SFP-10 to the patient's blood plasma added

	Antigens (%)			
	Extract of healthy lung tissue	Skin extract	Extract of a healthy pancreas	ESAT6/SFP10
Total IR	5.3	4.0	0	0
IgG1	4.0	2.7	–	–
IgG3	4.5	2.8	–	–
IgE	2.6	1.3	–	–
IgG1 + IgG3	4.8	3.2	–	–
IgG1 + IgE	6.9	4.5	–	–
IgG3 + IgE	7.0	4.7	–	–

In favor of this theory, there is also a significant difference in the IR formation in response to the antigenic material of the lung tissue in patients with sarcoidosis and tuberculosis. These changes may be an indirect evidence of the autoimmune mechanism of pulmonary sarcoidosis, which is realized in the IC formation on its own lung tissue. This phenomenon seems to be an essential part of the ongoing pathological process, but the immediate molecular cause that triggers this pathology remains unclear.

The absence of specific IC formation in response to antigenic material of *M. tuberculosis* in most patients with sarcoidosis indirectly denies the role of this microorganism as a causative agent of the disease.

The high diagnostic significance of this method with the use of specific antigens, which was 92.2% when determining specific ICs in the diagnosis of tuberculosis and 100% in the diagnosis of sarcoidosis, evidences for advisability of its use in differential diagnosis of the two similar diseases—tuberculosis and sarcoidosis—in the absence of bacterial excretion.

Conclusion

The formation of specific immune complexes in response to "healthy lung tissue extract" antigens in patients with sarcoidosis and the absence of such a reaction in tuberculosis may indirectly evidence for autoimmune nature of sarcoidosis. The significant difference in the groups when determining the immune complexes for the ESAT6/ESF10 antigen in vitro in patients with tuberculosis and respiratory sarcoidosis confirms the infectious nature of tuberculosis caused by a specific pathogen (*M. tuberculosis*) and denies it as an immediate cause of sarcoidosis. Taking into account the 100% diagnostic significance of the methodology for determining ICs after adding the "lung tissue extract" with the use of the dynamic light scattering method, we may conclude that currently it is the only significant method for differential diagnosis of tuberculosis and pulmonary sarcoidosis in the absence of bacterial excretion. The obtained results require further study and comparison with other granulomatous diseases.

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Compliance with ethical standards

The study was approved by the independent Ethics Committee of the FGBU "SPb NIIF" St. Petersburg Scientific Research Institute of the Ministry of Health of the Russian Federation (protocol no. 34.2 dated January 19, 2017)

Conflict of interest The authors declare that they have no conflict of interest.

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AUTHOR QUERIES

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