# New laboratory criterion for the differential diagnosis of sarcoidosis and pulmonary tuberculosis

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**Research**

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# Abstract

In some cases there is a problem of differential diagnosis of sarcoidosis (SD) and tuberculosis (TB) because of the similarities in clinical, X-ray and laboratory features. The aim of this study was to search for new differential diagnostic criteria for sarcoidosis and tuberculosis by calculating the index, based on the level of autoantibodies to modified citrullinated vimentin (anti-MCV) and the level of B-cell subpopulations. These parameters were measured in patients with sarcoidosis (n = 93), tuberculosis (n = 28) and healthy donors (n = 40) using the ELISA and cytometry. The absence of a statistically significant difference when comparing the level of anti-MCV, the number of B-cells in SD and TB suggests that these changes may be characteristic of granulomatous diseases. The use of the formula Ds=([B-naïve%]\[B-memory%])*([B-CD38%]+[B-CD5%])/[anti-MCV] might allow to differentiate SD with an increase in the calculated index of more than 5 units with a sensitivity of 80.00% and specificity of 93.10% (AUC = 0.926).


# Take-home Message

This study provides a new diagnostic index based on the antibodies to mutated citrullinated vimentin concentration and the level of different B subsets. Determination of index over 5 Units proved to be characteristic of sarcoidosis, what might help to differentiate SD from TB.

# Background

Sarcoidosis and tuberculosis are granulomatous diseases and have many similarities in clinical symptoms, which makes differential diagnosis difficult and leads to the prescription of improper therapy. Tuberculosis is an infectious granulomatous disease caused by *Mycobacterium tuberculosis* and is characterized by the formation of caseous granulomas in the lungs, as well as in other internal organs and bones. Sarcoidosis is a granulomatous disease of unknown etiology, in which noncaseating granulomas in various organs and tissues are detected, most often in the lungs, mediastinal lymph nodes, and skin (1). At the moment, differential diagnosis is based on the detection of *M. tuberculosis* in the patient's biomaterials and on the data of histological examination, if the mycobacterium was not isolated by laboratory diagnostic methods (2). However, only in 40-60% of cases the infectious agent is diagnosed (3), and according to the results of histological examination, diagnostic errors are 40% (4). Because of the similarity of clinical and radiological signs of both diseases, making decisions about the correct diagnosis is significantly complicated.

It is assumed that the presence of common features in these diseases may be due to some similarities in their pathogenesis. Despite the fact that the etiology of sarcoidosis is not yet fully understood, an autoimmune theory is being actively discussed (5). Infectious agents, for example, *Mycobacterium tuberculosis, Propionibacterium acnes, Chlamydophila pneumoniae*, viruses (herpes viruses HHV6 and HHV8, cytomegalovirus, retroviruses), mold (6), and environmental hazards are considered to play an important role in the development of this disease (7-10). The greatest attention is given to the role of *M.*

*tuberculosis*, which relationship with the disease was defined when bacteria contents and their nucleic acids were detected in sarcoid granulomas (11, 12). The immunological studies shown the presence of antimycobacterial antibodies in patients' serum (13-15) and described Mycobacteria-specific cytotoxic T cells in patients with sarcoidosis (16, 17). The possible association of Mycobacteria infection and sarcoidosis development was revealed with animal models of sarcoidosis (18).

It is important to note that patients with tuberculosis often have autoimmune complications, such as granulomatosis polyangitis, arthritis, and uveitis (19, 20). The possibility of autoimmune reactions development during *M.tuberculosis* infections has been described in various experimental studies, showing that mycobacteria promoted an autospecific T-cell immune response (21-23). The presence of autoantibodies in patients with tuberculosis is widely described in the literature. The high titers of antibodies to cardiolipids, β2-glycoprotein, prothrombin, proteinase-3, neutrophil cytoplasm (24), and cyclic citrulline peptides (25) were found in patients, which allude the ability of mycobacteria to induce autoimmune processes.

Vimentin is considered to be one of the possible autoantigens for sarcoidosis and tuberculosis. Wahlström et al. identified vimentin autoantibodies and vimentin-specific T cells in the bronchoalveolar fluid of patients with sarcoidosis and also described the relationship of the HLA-DRB1*03 genotype with the production of these antibodies (26). According to a serological study elevated titers of autoantibodies to modified citrullinated vimentin were also found in blood serum of tuberculosis patients (27).

The development of autoimmune inflammation due to *M. tuberculosis* could be explained with the molecular mimicry and similarity of immunogenic epitopes of bacterial and autoantigens, resulting in a cross-reaction and activation of T-lymphocytes (28-30). Despite the list of possible mycobacterial antigens involved in the cross-reaction (heat shock proteins Mtb-HsP60, Mtb-HsP65, protein p36, ESAT-6 protein, catalase (mKatG) (31), the bacterial antigen with molecular similarity to vimentin is not described yet. Perhaps this reaction may be caused by vimentin expressed by macrophages infected with mycobacteria. It was shown that due to the oxidative stress and in the presence of pro-inflammatory factors, upregulation of vimentin expression activates the NKp46 receptor of natural killers, which leads, in turn, to the lysis of infected macrophages (32, 33).

In addition to the autoantibodies detection in patients with sarcoidosis, the typical changes in B-cell populations were observed (34, 35). An increase in the number of naive B cells and a decrease in the number of memory B cells can be described in granulomas (36, 37), blood, and bronchoalveolar fluid (34, 35). Similar changes have been revealed in some autoimmune diseases, such as rheumatoid arthritis and granulomatous polyangiitis (38, 39). One of the confirmations of the active role of B cells in the pathogenesis of sarcoidosis is the effectiveness of rituximab in the treatment of different clinical forms of the disease (40, 41).

In tuberculosis, signs of the B cells involvement in the pathogenesis of the disease were also found (42, 43). According to Willem J. du Plessis, a similar picture is observed in such patients, with a reduced level of memory B cells and an increased level of naive B cells in comparison with healthy individuals. During

the treatment of tuberculosis, not only the normalization of the ratio of naive B cells and memory cells is observed, but also the changing of prevailing memory B cells population from a switched class to a non-switched class (44).

Thus, in addition to clinical and radiological similarities in sarcoidosis and tuberculosis, similar pathogenetic mechanisms can be described in these diseases, as the appearance of autoantibodies to modified citrullinated vimentin and changes in B-cell subpopulations. Immunological similarities leave even more questions regarding the pathogenesis of both diseases, which requires a more detailed study of the characteristics of the immune response in sarcoidosis and tuberculosis.

**The aim of this study** was to reveal new differential diagnostic criteria for sarcoidosis and tuberculosis by to development the new immunologic index, based on the level of autoantibodies to modified citrullinated vimentin and the level of B-cell subpopulations.

# Materials Of The Study

A prospective comparative study was undertaken in 2015-2019. The patient selection was performed at St. Petersburg Research Institute of Phthisiopulmonology, at St.-Petersburg «City Tuberculosis Hospital №2» and at St.-Petersburg “City Hospital № 2”. The study was approved by the independent ethical committee of the St. Petersburg Scientific Research Institute of Phthisiopulmonology (extract from protocol No. 34.2 of 01/19/2017) and the Local Ethics Committee of St. Petersburg State University (protocol No. 01-126 06/30/17). The laboratory part of the study was performed in the Laboratory of autoimmune diseases diagnostics of Pavlov First Saint Petersburg State Medical University

Patients with lung sarcoidosis (n = 93), lung tuberculosis (n = 28) and healthy subjects (n = 40) were included. Healthy subjects did not have any chronic diseases, contacts with tuberculosis and positive immunologic test (ELISPOT). All study participants signed informed consent.  The clinical characteristics of patients are presented in Table 1.

Table 1. The clinical characteristics of patients with sarcoidosis and tuberculosis

| N (%) | Lung sarcoidosis (n = 93) | Lung tuberculosis (n = 28) |
|---|---|---|
| Men | 40 (43.9%) | 10 (57.5%) |
| Women | 53 (56.1%) | 18 (42.5%) |
| X-ray examination | | |
| Focal infiltrates in the lungs | 93 (100.0%) | 25 (100.0%) |
| Mediastinal lymphadenopathy | 76 (81.7%) | 11 (39.3%) |
| Complains | | |
| Cough | 36 (38.7%) | 10 (35.7%) |
| Shortness of breath | 24 (25.8%) | 12 (42.8%) |
| An increase in body temperature | 35 (37.7%) | 15 (53.6%) |
| Weight loss | 21 (22.6%) | 15 (53.6%) |
| Chest pain | 7 (7.5%) | 8 (28.5% ) |
| Immunologic tests ELISPOT | | |
| Positive | 9 (9.8%) | 24 (85.7%) |
| Negative | 84 (90.2%) | 4 (14.3%) |
| | | |
| Sputum + biopsy specimen microscopy for MBT (positive) | 0 | 28 (100.0) |

The groups were comparable by gender and age. Exclusion criteria were: more than 2 years after the diagnosis evaluation, immunosuppressive and anti-tuberculosis therapy, plasmapheresis less than 2 months from the date of inclusion, HIV infection, syphilis, tumor diseases, decompensated diabetes mellitus.

## Methods of the study.

All patients underwent a complex of examinations, including a clinical examination, multislice computed tomography (MSCT) of the chest, laboratory blood tests, a standard set of tests for tuberculosis, histological verification of the lungs lesions and intrathoracic lymph nodes (obtained with transbronchial and video thoracoscopic biopsy).

The diagnosis of pulmonary sarcoidosis was made according to standard criteria of the American Thoracic Society (ATS), European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Diseases (WASOG). Criteria included typical radiological changes (mediastinal lymphadenopathy, disseminated foci in lung tissue); histological verification of lung lesions or intrathoracic lymph nodes (detection of granulomas of epithelioid cells without caseous necrosis and acid-resistant mycobacteria); the exclusion of other causes of granulomatous changes, especially tuberculosis.

The diagnosis of pulmonary tuberculosis was made using typical radiological changes (mediastinal lymphadenopathy, focal and infiltrative changes with or without destruction); positive results of a tuberculosis tests (detection of *M. tuberculosis* (MBT) and/or MTB DNA in sputum with molecular genetic and bacteriological methods).

*Determination of the antibodies level.*

In the serum of patients with sarcoidosis (n = 93), tuberculosis (n = 28), and the control group (n = 40), the level of antibodies to the modified citrullinated vimentin (anti-MCV) was determined using ELISA (ORGENTEC, Germany). All measurements were performed using a BIO-TEK ELx800 ELISA spectrophotometer. A positive result is the detection of the level of these antibodies was considered to be more than 19.5 U/ml.

*Immunotyping.*

The preparation of peripheral blood samples and tuning of the flow cytofluorimeter was performed in accordance with the recommendations of S. Khaidukov et al. (45). The selection of optimal antibody-fluorochrome pairs was performed in accordance with the principles described in the literature (46). To identify main B cell subsets, 200 µl of whole EDTA-stabilized peripheral blood was stained for surface antigens using the following combination of monoclonal antibodies conjugated with fluorochromes: Anti-IgD Alexa Fluor 488 (clone IA6-2, isotype - Mouse IgG2a, κ), anti-CD38 PE (clone LS198-4-3, isotype Mouse IgG1), anti-CD27 PC7 (clone 1A4CD27, isotype Mouse IgG1), anti-CD24 APC (clone J3-119, isotype Mouse IgG1), anti-CD19 APC / Cy7 (clone HIB19, isotype Mouse IgG1, κ), anti-CD5 Pacific Blue (clone BL1a , isotype Mouse IgG2a) and anti-CD45 Krome Orange (clone J33, isotype Mouse IgG1). IgD and CD19 were from BioLegend, Inc. (USA). Antibodies to CD38, CD27, CD24, CD5, and CD45 were from Beckman Coulter, Inc (USA).

After incubation with antibodies at room temperature in the dark for 15 min, red blood cells were lysed with 2 ml of VersaLyse Lysing Solution (Beckman Coulter, Inc., USA) supplemented with 50 µl of IOTest 3 Fixative Solution (Beckman Coulter, Inc., USA) for 15 minutes. Next, the samples were washed twice (7 min at 330g) with phosphate-buffered saline (PBS) supplied with 2% heat inactivated fetal bovine serum (Sigma-Aldrich, USA), the resulting cell pellet was resuspended in 0.5 ml of fresh PBS containing 2% neutral buffered formalin solution (Sigma-Aldrich, USA). Samples were analyzed using a Navios flow cytometer (Beckman Coulter, Inc., USA) equipped with 405, 488 and 638 nm lasers. For each of the samples, at least 10,000 CD19+ lymphocytes were analyzed. Gating strategy was described previously (45). The obtained data were analyzed using the Kaluza software (Beckman Coulter, Inc., USA). Finally, memory B cells (IgD−CD27+), naive (IgD+CD27−) and B-regulatory cells (CD24+++CD38+++ and CD5+CD27−) were identified.

*Statistical processing of results*

Statistical analysis was performed using GraphPad Prism 6 (Graph Pad Software, USA), Statistica 10 (Statsoft, USA) using Mann-Whitney criterion, Spearman statistical analysis. ROC analysis was used to determine the diagnostic significance of the results. The differences were considered statistically significant at a *p* level of less than 0.05, diagnostically significant at AUC> 0.80.

# Results

### *Testing the level of antibodies to modified citrullinated vimentin*

The level of anti-MCV was analyzed in 93 patients with lung sarcoidosis, 28 patients with lung tuberculosis and 40 healthy donors, the results are presented in Table 2.

Table 2. The results of the anti-MCV level testing in the study groups.

| Groups | Results of anti-MCV testing | | CI 95% |
|---|---|---|---|
| | High level anti-MCV in patients n/% | Absolute value Med (Q25; Q75) | |
| Lung sarcoidosis, n=93 | 42.2 (38/93) | 16.60 (10.73; 24.19) | 17.0-23.89 |
| Lung tuberculosis, n=28 | 60.7 (17/28) | 21.13* (16.50; 26.96) | 19.17-27.61 |
| Healthy subjects (control group), n=40 | 7.5 (3/40) | 6.47 (2.66; 11.25) | 5.82-10.65 |

* - p <0.05 - comparison between sarcoidosis and tuberculosis groups

According to the data described in Table 1, an increased anti-MCV level was found in 40.9% (38/93) cases in patients with sarcoidosis and in 60.7% (17/28) cases in patients with tuberculosis. The statistically significant difference was found between the two groups (Mann-Whitney criterion, p = 0.027). A high anti-MCV concentration was also determined in 7.5% (3/40) cases in the group of healthy donors. Mann-Whitney analysis revealed a statistically significant difference between the sarcoidosis and tuberculosis groups in comparison with the control group (p <0.0001 for both groups), figure 1.

In order to search for new criteria for determining the level of anti-MCV, ROC analysis was performed. According to the ROC analysis, for patients with sarcoidosis and tuberculosis in comparison to the control group, reference values of the anti-MCV level (AUC> 0.8, p <0.0001) can be determined: 12 units/ml with a diagnostic sensitivity of 77%, specificity of 70% for patients with sarcoidosis, 14 units/ml with a diagnostic sensitivity of 92%, specificity of 88% for patients with tuberculosis. However, no diagnostically significant difference between the two groups was found (AUC <0.8).

***Immunophenotyping results.***

An analysis of B-cell populations in the studied groups was performed and the most significant B cell subsets were revealed in comparison with the healthy control group. According to the classification of B cells by the presence of IgD and CD27 expression, B cells were divided into “naive” and memory B cells.

"Naive" IgD+CD27– B cells.

It was found that in patients with sarcoidosis and tuberculosis, the number of “naive” B cells were higher than in healthy donors. The results of “naive” B cells measurement are presented on Figure 2.

According to the Mann-Whitney test, the level of “naïve” B cells in patients with sarcoidosis was significantly higher when compared with healthy donors ($p < 0.0001$) and patients with tuberculosis ($p = 0.015$). The level of “naïve” B cells of patients with tuberculosis is also significantly higher than the number of “naïve” B cells in control group ($p = 0.007$), Figure 2.

According to the results of the ROC analysis, in comparison with the control group, diagnostically significant results were obtained only for patients with sarcoidosis (AUC = 0.819, $p<0.0001$). For this group, the reference value for “naïve” B cells number was 70% within total CD19+ cells (diagnostic sensitivity was 76%, while specificity – 70%). There was no diagnostically significant difference between the sarcoidosis and tuberculosis groups.

IgD–CD27+ memory B cells

The levels of memory B cells in patients with sarcoidosis and tuberculosis were lower than in the control group. The results of memory B cells analysis are presented on Figure 3.

According to Mann-Whitney U-test the levels of memory B cells in sarcoidosis and tuberculosis groups were significantly lower then in healthy control ($p < 0.0001$ and $p = 0.007$, respectively). Furthermore, the significant difference was also found when sarcoidosis and tuberculosis groups were compared ($p = 0.005$), figure 3.

Using the ROC analysis, diagnostically significant results were determined only for patients with sarcoidosis when compared with the control group (AUC = 0.819, $p < 0.0001$). In patients with sarcoidosis, the level of memory B cells was below 30% with a diagnostic sensitivity of 76% and a specificity of 70%. But significant difference between the sarcoidosis and tuberculosis groups was not shown.

By labeling the antigens CD24, CD38, CD5, CD27, group B of regulatory cells was determined, in which CD24+++CD38+++ B cells and CD5+CD27– cells were isolated.

CD24+++CD38+++ B-cells.

The level of CD24 +++ CD38 +++ B cells was elevated in patients with sarcoidosis and tuberculosis in comparison with the control group. The results of measuring the level of CD24 +++ CD38 +++ B-cells are

presented in Figure 4.

Mann-Whitney U-test showed a significant increase of CD24+++CD38+++ B cells patients with sarcoidosis and tuberculosis when compared with healthy donors ($p<0.001$ in both cares). But, no significant differences between the groups with sarcoidosis and tuberculosis were found, figure 4.

According to the ROC analysis, a diagnostically significant increase in CD24 +++ CD38 +++ B cells relative to healthy individuals was found only in patients with sarcoidosis (AUC = 0.904, $p <0.0001$), the reference value was 6.52% with a diagnostic sensitivity of 91%, specificity of 88%. There was no significant differenceы between the groups of sarcoidosis and tuberculosis.

CD5+CD27- B cells

An increase in CD5+CD27- B cells levels of relative to healthy individuals was found in the sarcoidosis and tuberculosis groups. The results of measuring the level of CD5 + CD27-B cells are presented in Figure 5.

According to the Mann-Whitney test, statistical significant increase of the CD5+CD27- B cells level was found only in patients with sarcoidosis (in comparison with healthy donors ($p <0.0001$), with tuberculosis patients ($p = 0.001$)), figure 5.

The ROC analysis revealed a diagnostically significant increase in CD5+CD27– B cells relative to the control group of more than 12.45% for patients with sarcoidosis (AUC = 0.795, $p <0.0001$) with a diagnostic sensitivity of 76%, specificity of 80%. No diagnostically significant difference was found between the sarcoidosis and tuberculosis groups.

Thus, according to the ROC analysis of a significant difference in the level of anti-MCV, the number of “naive” IgD + CD27-B cells, IgD-CD27 + B-cells memory, CD24 +++ CD38 +++ B-cells, CD5 + CD27-B cells were not found. In this connection, a comprehensive analysis of the data was performed in order to develop a diagnostic index.

A search for a comparative index showed that when using the formula (1) an obtained index of more than 5 units (from 3.8 or more for sarcoidosis; 0.2-18 for tuberculosis) shows a high probability of sarcoidosis with a sensitivity of 80.00% and a specificity of 93.10% (AUC = 0.926). This formula describes the dysregulation in the B-cell population, which may show the presence of autoimmune reactions.

$$Ds = \frac{Bnaive}{Bmemory} * \frac{(CD38-Bcells)+(CD5-Bcells)}{[anti-MCV]} \quad (1)$$

(B-naive \ B-memory) - reflects the activity of the humoral immune response; (CD38-Bcellls + CD5-Bcells) - the total number of regulatory B cells (Il-10 synthesis); [anti-MCV] - The concentration of anti-MCV, characterizing the presence of autoimmune reactions).

# Discussion And Conclusions

An increased level of autoantibodies to citrullinated modified vimentin is shown for both patients with sarcoidosis and tuberculosis ($p < 0.0001$), which reflects the presence of autoimmune inflammation in both diseases.

One explanation for the presence of high concentrations of autoantibodies to MCV may be the high immunogenicity of this antigen. Vimentin is a protein of connective tissue and is present in many cells (47). The development of autoimmune processes against citrullinated vimentin was described in rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune diseases (47). It is known that the processes of citrullination are necessary for the arginine-containing proteins elimination (vimentin, fibrin, and others) by macrophages (48, 49). However, in chronic inflammation, permanent tissue damage occurs, which leads to an increase in calcium concentration and hyperactivation of peptidyl-arginine deaminase, an enzyme involved in protein citrullination (50). For the carriers of the HLA-DRB1 genotypes, this process leads to the activation of autoimmune reactions against citrullinated peptides (51). A similar process has been described in the pathogenesis of rheumatoid arthritis and chronic lung diseases, in which patients find high titers of antibodies to citrulline proteins (48).

According to our previous work, high anti-MCV titers were not correlating with alveolitis and polyangiitis (27). Given that there was no diagnostically significant difference in the anti-MCV levels in sarcoidosis and tuberculosis, and that the reference level of anti-MCV for both groups was less than the recommended value for the diagnosis of rheumatoid arthritis (20 Me/ml), it can be assumed that in tuberculosis autoimmune inflammation against vimentin could exist, which, under certain conditions, can become systemic and lead to the formation of sarcoid granulomas. Determination of anti-MCV levels over 12 units/ml may indicate granulomatous inflammation in the lungs, but, the determination of this biomarker can`t be used for differentiation between tuberculosis and sarcoidosis.

Immunophenotyping showed that patients with tuberculosis and sarcoidosis had reduced level of memory B cells, an increased number of “naïve” B cells and CD24 +++ CD38 +++ B cells in comparison with healthy controls. Moreover, sarcoidosis patients had significantly lower frequencies of memory B cell, while their peripheral blood “naive" B cells were significantly up regulated when compared with tuberculosis group numbers. It is important to note that in patients with sarcoidosis, the levels of CD5+CD27– B cells were statistically higher then in blood samples from healthy individuals as well as patients with tuberculosis.

Alterations in B cell subsets may be closely related to the inflammatory reactions, that are the essential part of foreign antigen elimination by immune system. However, it remains unclear is there reference meanings of the analyzed cells reflecting the presence of an autoimmune process.

According to our data, sarcoidosis and tuberculosis patients showed an increase in “naive” B cells levels and a decrease in memory B cells numbers, though, the imbalance of memory B cells and “naive” in sarcoidosis patients was more pronounced, and it was confirmed by statistical analysis. Similar results

were obtained in the study of chronic sarcoidosis (35) and active forms of tuberculosis (44). According to the results of the Willem J. du Plessis, the described changes in B cell subsets were shown only for patients with tuberculosis in comparison with other lung diseases (viral or bacterial pneumonia, bronchiectasis, asthma, COPD), which did not statistically differ from the group of healthy individuals (44). According to O'Shea et al., during active tuberculosis, a decrease in memory B cells was detected, while the frequency of "naive" B cells was unaltered (52). Impaired memory and "naïve" B cells distribution have been described in some autoimmune diseases (38, 39).

Given the absence of changes in the ratio of memory B cells and "naive" in other pulmonary diseases, it can be assumed that such changes characterize diseases in which granulomatous inflammation plays an important role, which is shown in some infectious and autoimmune diseases.

Next, we analyzed the frequency of CD24+++CD38+++ B cells and revealed their increased levels patients with sarcoidosis or tuberculosis versus healthy controls, but no significant differences were found between those two patients groups. It is known, that CD24+++CD38+++ B cells represent a population of immature transitional B cells that perform various regulatory functions (53). One of the main features is the ability to synthesize the anti-inflammatory cytokine IL-10 (54). Many studies have shown that B cells producing IL-10 could inhibit the activity of self-specific CD4+ T cells (55). A decrease of CD24+++CD38+++ B cell levels were found in patients with rheumatoid arthritis, which allowed researchers to confirm the role of CD24+++CD38+++ B cells in preventing autoimmune reactions (56). However, an increase in the level of these cells was noted in primary Sjogren's syndrome and systemic lupus erythematosus (57), as well as in patients in the active phase of sarcoidosis (34). In a Blair study in patients with systemic lupus erythematosus while the level of B cells with the CD24+++CD38+++ was increased, lower levels of CD38intCD24int B cells and CD24+++CD38– B cells were found (54). Perhaps an impairment of the distribution of those B cells indicates the activation of a compensatory anti-inflammatory response in infectious and autoimmune diseases. In autoimmune processes at some point an activation of immune system might be replaced by decompensation, characterized by a decrease in the level of these cells.

Interesting results were obtained when comparing CD5-expressing B cells. CD5-expressing B cells also belong to regulatory B cells capable of synthesizing IL-10 and can be found in various human tissues. Moreover, CD5+ B cells are capable of producing autoantibodies (including rheumatoid factor and antibodies against ssDNA), and the number of CD5+ B cells increases in autoimmune diseases such as rheumatoid arthritis and Sjogren's syndrome (58, 59). The ability of cells to produce autoantibodies was shown in mouse models; it was shown that CD5-expressing B cells belonging to a subpopulation of B1a, which is usually localized in the abdominal cavity, produce low-affinity IgM antibodies with autoreactive specificity (60). In addition, IL-10 synthesized by CD5+ B cells takes part in the control of autoimmune reactions in experimental encephalomyelitis in mice (61). In humans, CD5 is found on the cell membrane of transient CD24+++CD38++ T1 B cells (62), but according to recent studies, these cells produce a relatively low level of IL-10 compared to other transient B cell subsets (57). There is evidence that CD5 can be considered as a marker of activation of B cells in humans, and CD5 negative human B cells can

be activated in vitro by incubation with phorbol or thymoma EL4 cells, followed by the appearance of CD5 molecules on their membrane (63). According to Zhang et al., tuberculosis patients can also detect elevated levels of CD5-expressing B cells. At the same time, the ability of these cells to inhibit Th17 activity was shown (64).

Our study revealed a statistically significant increase in the number of B cells with the CD5+CD27– phenotype only in patients with sarcoidosis. At the moment, it is difficult to explain the absence of differences in the levels of CD5+CD27– B cells in patients with tuberculosis and healthy individuals, perhaps the results are associated with a small sample size.

According to the ROC analysis of immunophenotyping results, there were no diagnostically significant results for the differentiation of sarcoidosis and tuberculosis, however, a tendency to more pronounced changes in the level of B cells in patients with sarcoidosis is visible.

Despite the proposed laboratory parameters of the immune response (anti-MCV level, the number of “naive” IgD+CD27– B cells, IgD–CD27+ memory B cells, CD24+++CD38+++ B cells, CD5+CD27– B cells) have no diagnostic significance in the differential diagnosis of sarcoidosis and tuberculosis, some markers (increase in anti-MCV more than 14 U/ml, increase in “naive” B cells and decrease in memory B cells, increase in CD24+++CD38+++ B cells) may indicate the presence of granulomatous inflammation in the lungs.

At the moment, in clinical practice, the determination of the immune response is used in the diagnosis of tuberculosis. Immunological tests detect an enhanced T-cell immune response against *M. tuberculosis* antigens (65). The obtained diagnostic criterion allows us to differentiate sarcoidosis and tuberculosis and may indicate a predominance of autoimmune changes, characterizing the humoral immune response.

## Conclusions.

To date, there is no diagnostic criterion for the differential diagnosis of sarcoidosis and tuberculosis.

However, the use of a formula we developed with a sensitivity of 80.00% and a specificity of 93.10% suggests the presence of sarcoidosis with an increase in the calculated index of more than 5 units (AUC = 0.926).

This indicator may present that changes in B cell subsets, that typical for autoimmune diseases are more characteristic of sarcoidosis.

To confirm the diagnostic significance of the described criteria, studies are needed on the application of the Ds formula, determining an increase in the anti-MCV level of more than 14 U ml, changing the ratio of memory B cells and “naive”, increasing the level of CD24+++CD38+++ B cells in groups of patients with various autoimmune and granulomatous diseases, which will allow us to identify more accurate and specific values for differential diagnosis.

# Abbreviations

Anti-MCV – antibodies to mutated citrullinated vimentin

ATS - American Thoracic Society

AUC – area under ROC curve

COPD – chronic obstructive pulmonary disease

ERS - European Respiratory Society

MBT - M. tuberculosis

MSCT - multislice computed tomography

ROC – receiver operating characteristic

SD – sarcoidosis

TB – tuberculosis

Th – T-helpers

WASOG - the World Association of Sarcoidosis and Other Granulomatous Diseases

# Declarations

### Ethics approval and consent to participate

The study was approved by the independent ethical committee of the St. Petersburg Scientific Research Institute of Phthisiopulmonology (extract from protocol No. 34.2 of 01/19/2017) and the Local Ethics Committee of St. Petersburg State University (protocol No. 01-126 06/30/17).

### Consent for publication

“Not applicable”

### Availability of data and material

The data will not be shared with a reason.

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### Competing interests

“The authors declare that they have no competing interests.”

### Author contributions

AS: coordinator of the project, wrote the manuscript

AM: collected the laboratory data, the analytic calculation, analysis of the materials, wrote the manuscript

NB, YZ: collected the clinical data, wrote the manuscript

IK: collected the laboratory data, the analytic calculation; analysis of the materials

SL, AM, ES: collected the laboratory data, the analytic calculation

MP, EB, TS: collected the clinical data

PY: coordinator of the project.

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# Figures

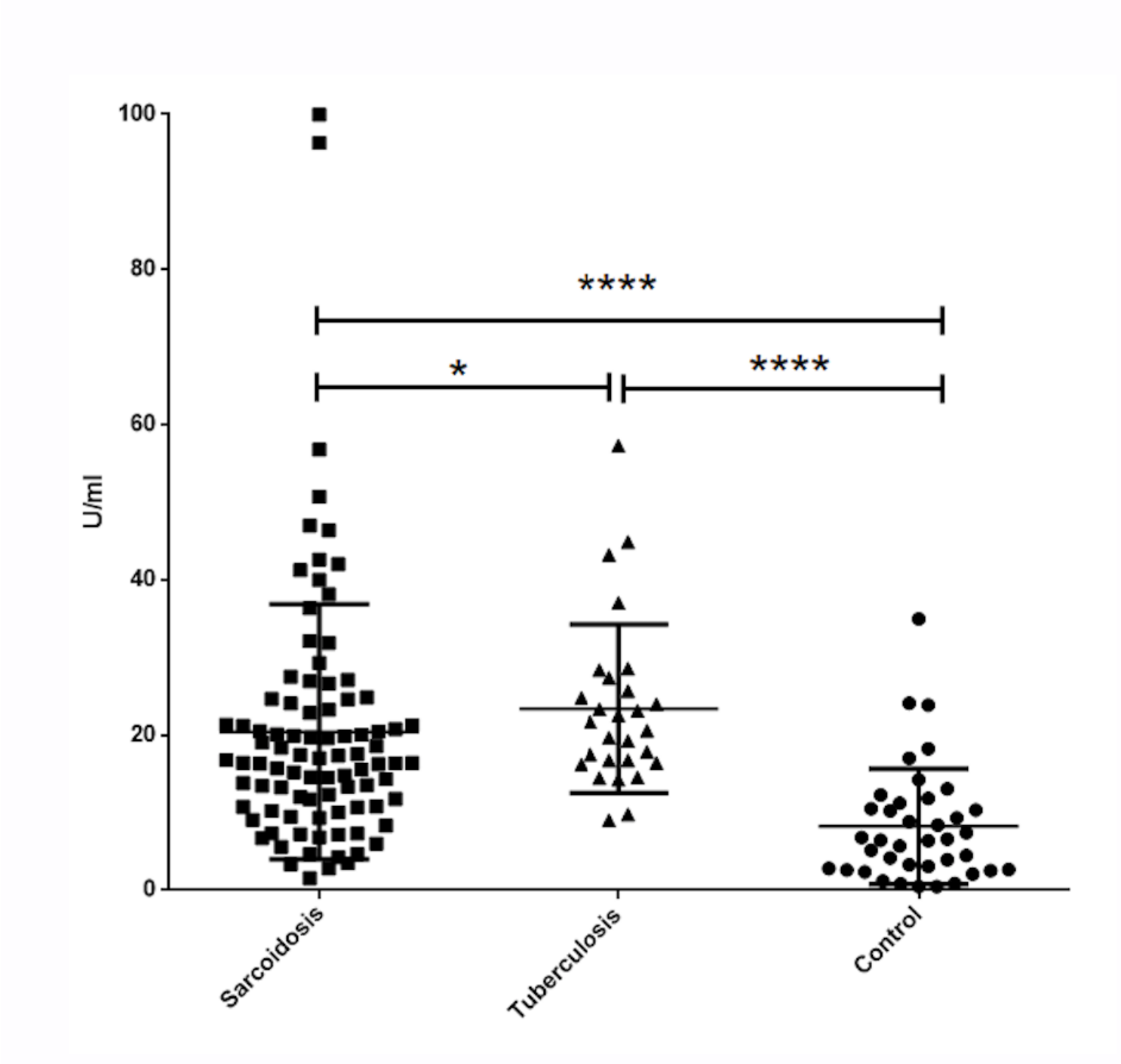
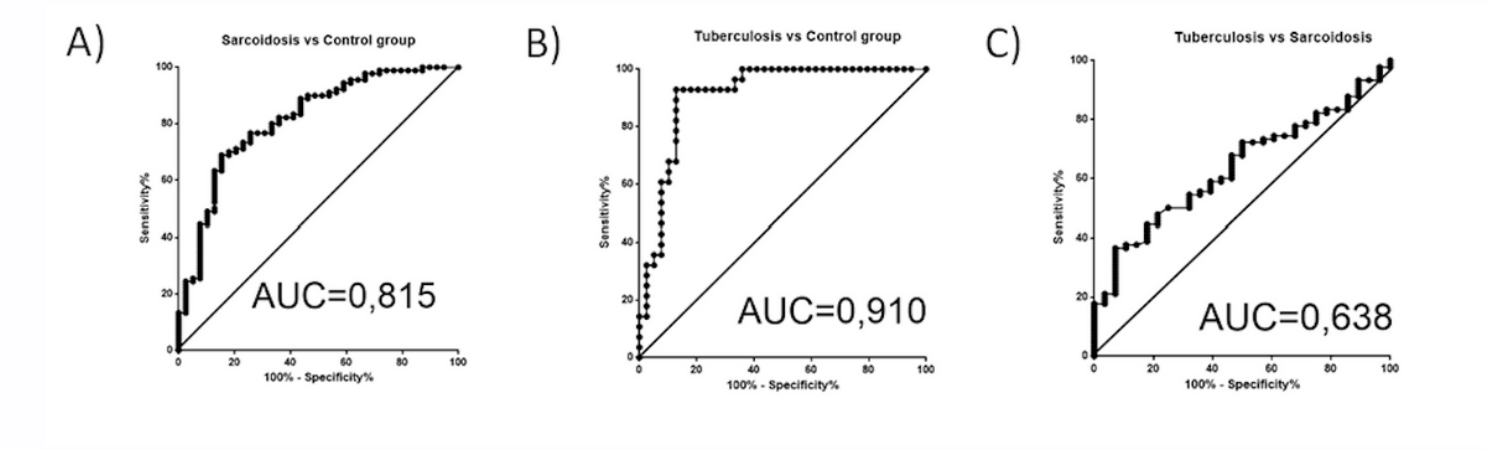


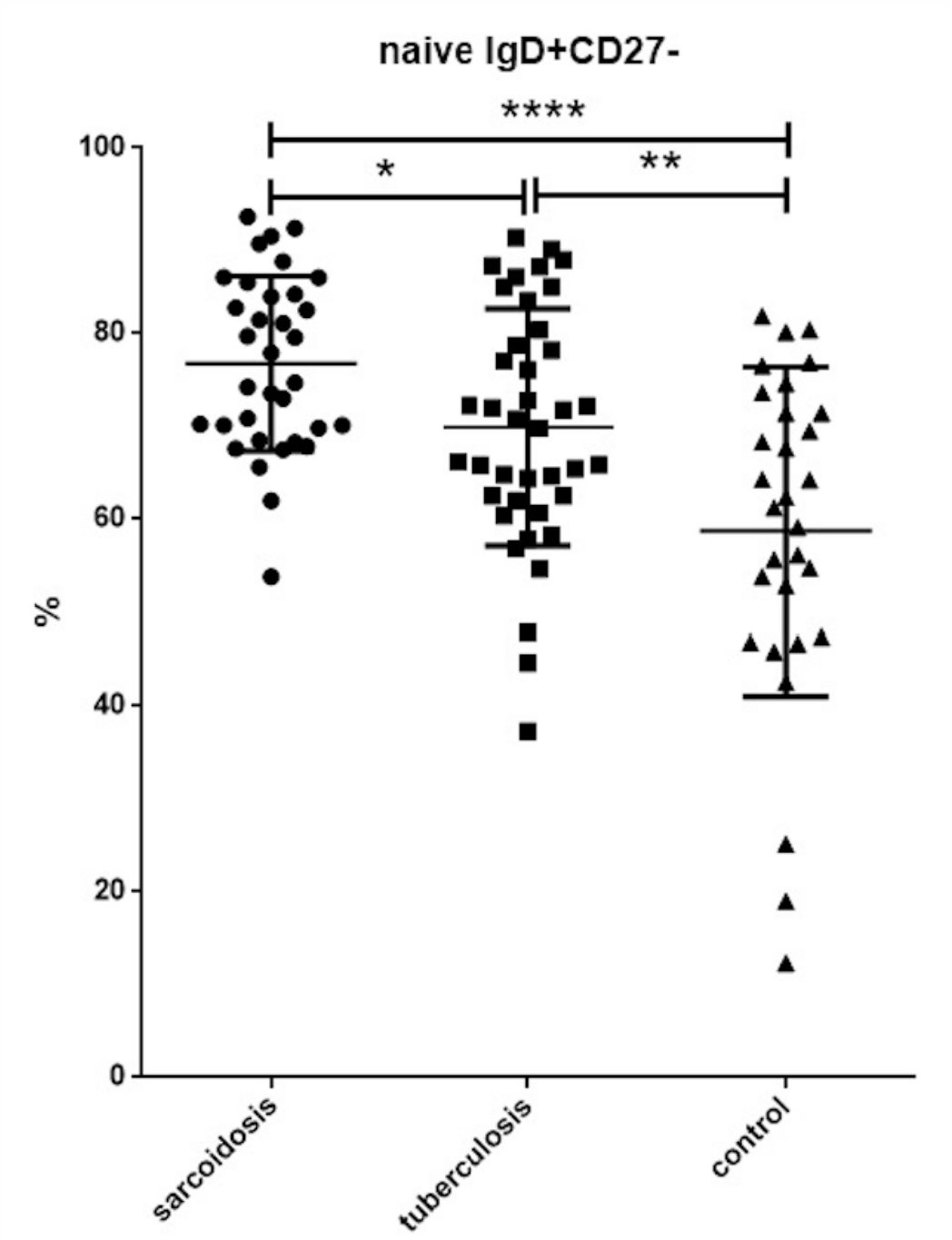

**Figure 1**

Anti-MCV concentrations in serum samples of patients with sarcoidosis (n=93) - 16.60 (10.73; 24.19) U/ml, with tuberculosis (n=28) - 21.13 (16.50; 26.96) U/ml, healthy subjects (n=40) - 6.47 (2.66; 11.25) U/ml. * - p <0.05 - comparison between sarcoidosis and tuberculosis groups, Mann-Whitney test. **** - p <0.0001 - comparison between sarcoidosis and the control group, between tuberculosis and the control group, Mann-Whitney test.



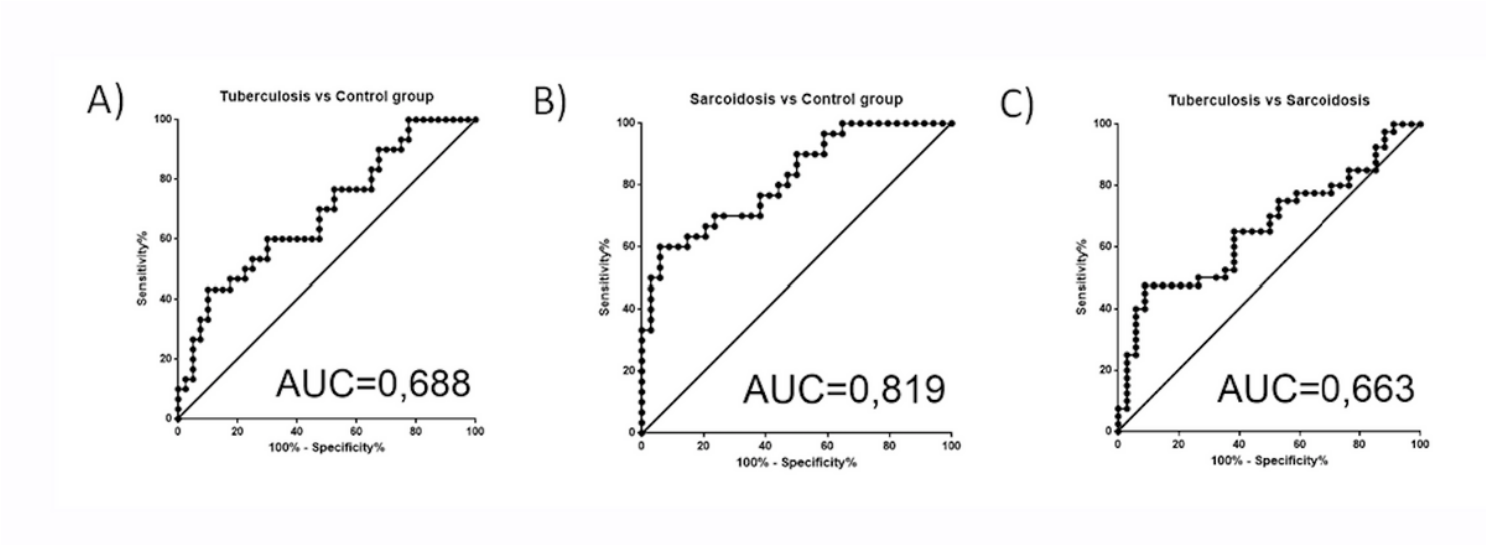

## Figure 2

Distribution of the level of “naive” IgD+CD27– B cells among the patients with sarcoidosis (n=30) – 76.31 (69.50; 84.57) %, with tuberculosis (n=28) – 70.31 (62.11; 79.98) %, healthy subjects (n=30) – 61.80 (47.25; 71.98) %. * - p <0.05: between sarcoidosis and tuberculosis ** - p <0.01: between tuberculosis and healthy subjects **** - p <0.0001: between sarcoidosis and healthy subjects



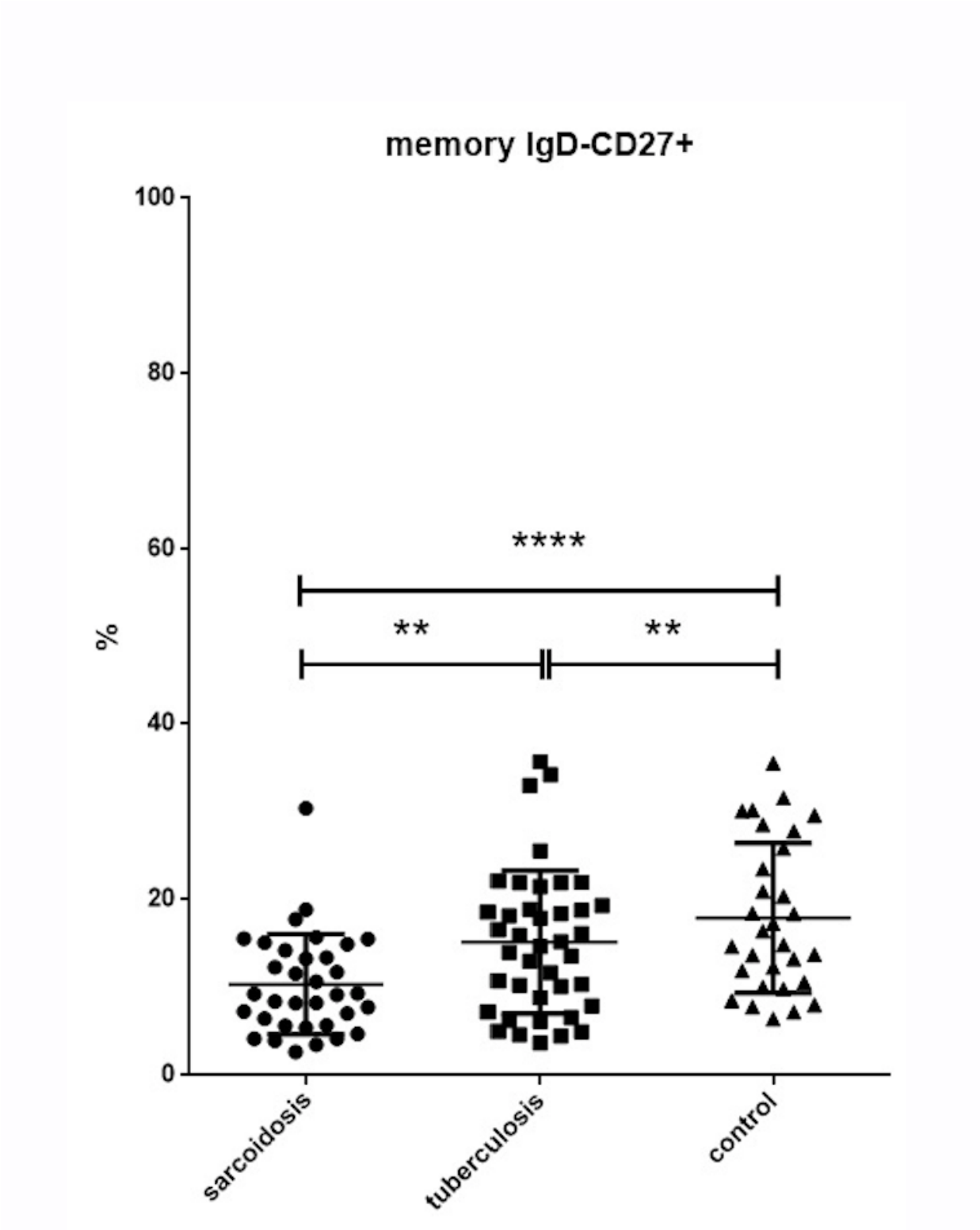

## Figure 3

Distribution of the level of IgD−CD27 + memory B-cells among the patients with sarcoidosis (n=30) – 23.69 (15.43; 30.51) %, with tuberculosis (n=28) – 29.69 (20.01; 37.89) %, healthy donors (n=30) – 38.21 (28.02; 52.75) %. ** - p <0.01: between sarcoidosis and tuberculosis, between tuberculosis and control **** - p <0.0001: between sarcoidosis and control




## Figure 4

Distribution of the level of CD24 +++ CD38 +++ in cells among the patients with sarcoidosis (n=30) – 9.79 (7.60; 15.49) %, with tuberculosis (n=28) – 7.95 (5.30; 14.62) %, healthy subjects (n=30) – 4.80 (3.05; 6.43) %. *** - p <0.005: between tuberculosis and control group **** - p <0.0001: between sarcoidosis and control group




## Figure 5

Distribution of the level of CD5+CD27– B cells among the patients with sarcoidosis (n=30) – 16.85 (11.76; 21.71) %, with tuberculosis (n=28) – 9.28 (4.50; 14.46) %, healthy subjects (n=30) – 7.95 (5.08; 11.43) %. ** - p <0.01: between sarcoidosis and tuberculosis **** - p <0.0001: between sarcoidosis and control group