VIA MEDICA

ORIGINAL RESEARCH

Anna Kempisty[1], Beata Białas-Chromiec[2], Dagmara Borkowska[3], Jan Kuś[1]

[1]First Department of Lung Diseases, Institute of Tuberculosis and Lung Diseases
[2]Department of Laboratory Diagnostics, Institute of Tuberculosis and Lung Diseases
[3]Department of Microbiology, Institute of Tuberculosis and Lung Diseases

Interferon gamma release assays based on *M. tuberculosis*-specific antigens in sarcoidosis patients

Testy oparte na wydzielaniu interferonu gamma pod wpływem antygenów swoistych dla *M. tuberculosis* u chorych na sarkoidozę

The authors declare no finacial disclosure

Abstract

Introduction: This study is a part of the project on interferon gamma release assays performed in the group of untreated sarcoidosis patients formerly BCG vaccinated. The aim of the study was to assess the rate of positive commercial interferon γ release assays in sarcoidosis patients. We discussed the results in the context of hypothesis that *M. tuberculosis* antigens may play a role in the pathogenesis of sarcoidosis.
Material and methods: 151 patients, mean age 38 $\pm$ 10.3, treatment naive, with newly diagnosed pulmonary sarcoidosis were enrolled into the study. All participants underwent QFT-GIT assay. A subgroup of 81 patients underwent also T-SPOT.TB assay.
Results: QFT-GIT was positive in 7/151. T-SPOT.TB was positive in 3/81. There were no indeterminate results in both IGRAs. There was no statistically significant relationship between IGRAs results and sarcoidosis parameters such as the radiologic stage, disease duration and the presence of Löfgren's syndrome.
Conclusions: In sarcoidosis patients formerly BCG vaccinated, positive rate of IGRAs was 4.6% for QFT-GIT and 3.7% for T-SPOT.TB. We did not find the influence of the selected parameters of sarcoidosis on IGRAs results.

Key words: sarcoidosis, interferon gamma release assays, latent tuberculosis infection

Pneumonol Alergol Pol 2015; 83: 126–134

Streszczenie

Wstęp: Prezentowana praca jest częścią projektu dotyczącego testów opartych na wydzielaniu interferonu gamma pod wpływem antygenów swoistych dla prątka gruźlicy przeprowadzonego na dużej grupie nieleczonych chorych na sarkoidozę w populacji powszechnie i wielokrotnie szczepionej przeciwko gruźlicy. Celem pracy była ocena wyników testów IGRA w tej grupie chorych. W pracy dyskutowany jest problem przydatności testów IGRA w wykrywaniu zakażenia prątkiem gruźlicy w kontekście współcześnie prezentowanych poglądów dotyczących roli antygenów prątka gruźlicy w etiopatogenezie sarkoidozy.
Materiał i metody: W badaniu uczestniczyło 151 chorych na sarkoidozę, w wieku 38 $\pm$ 10,3 roku, nigdy nieleczonych. Wszyscy uczestnicy badania mieli wykonany test QFT-GIT. Z grupy badanej utworzono podgrupę 81 chorych na sarkoidozę, którym wykonano w tym samym czasie drugi test IGRA: T-SPOT.TB.
Wyniki: Dodatni wynik testu QFT-GIT stwierdzono u 7/151 badanych, a wynik testu T-SPOT-TB u 3/81 badanych. Nie stwierdzono wyników nieokreślonych. Nie stwierdzono statystycznie istotnej zależności pomiędzy wynikiem testu IGRA a wybranymi parametrami klinicznymi sarkoidozy.
Wnioski: U chorych na sarkoidozę szczepionych w przeszłości przeciwko gruźlicy szczepionką BCG odsetek dodatnich wyników IGRA wynosił 4,6% dla QFT-GIT oraz 3,7% dla T-SPOT.TB. Nie wykazano wpływu wybranych parametrów klinicznych sarkoidozy na wynik testów IGRA.

Słowa kluczowe: sarkoidoza, testy oparte na wydzielaniu interferonu gamma, utajone zakażenie prątkiem gruźlicy

Pneumonol Alergol Pol 2015; 83: 126–134

Address for correspondence: dr n. med. Anna Kempisty, I Klinika Chorób Płuc IGiChP, ul. Płocka 26, 01–138 Warszawa, tel.: 22 431 21 47, faks: 22 431 24 43, e-mail: a.kempisty@igichp.edu.pl
DOI: 10.5603/PiAP.2015.0020
Received: 9.10.2014

ISSN 0867–7077

Introduction

Sarcoidosis is a rare multiorgan granulomatous disease, diagnosis of which often relies on properly conducted differential diagnosis of disorders with similar clinical and histopathological features. On the top of a long list of granulomatous diseases that need to be differentiated from sarcoidosis is tuberculosis. The necessity of exclusion of tuberculosis as granulomatous disease of known origin is a *sine qua non* of diagnostic procedure in sarcoidosis, and it is included in a major pathologic differential diagnosis criteria for the disease [1, 2].

Exclusion of active tuberculosis during differential diagnosis is not equal to exclusion of latent tuberculosis infection (LTBI). In sarcoidosis patients, this aspect is particularly important in those who are going to be treated with immunosuppressive drugs. The drugs used for treating sarcoidosis potentially increase the risk of LTBI progression to active disease. When prednisone at a dose of 15 mg (or other corticosteroid of equivalent dose) is used for at least 2–4 weeks, the risk of development of tuberculosis increases nearly 5-fold. In the case of TNF α antagonists, which are used in the treatment of refractory sarcoidosis, the risk of development of tuberculosis may grow even 17-fold [3].

The indirect evidence for the presence of infection with tubercle bacillus, without differentiation between latent infection and active disease is a positive tuberculin skin test (TST). TST is not a perfect tool for detection of infection with *M. tuberculosis* (MTB) as it has its limitations. In Poland, where the population is obligatory BCG vaccinated since the 50s of the 20th century, TST may give false positive results. Whereas, in some sarcoidosis patients infected with MTB, TST may give false negative results due to dominating tuberculin anergy.

At present, an alterative to TST are the tests based on the measurement of interferon gamma release by sensitized T lymphocytes in response to stimulation by antigens specific for *M. tuberculosis* [4]. These tests are: QuantiFERON–TB GOLD In-Tube (QFT-GIT) and T-SPOT.TB. In many studies, the advantage of interferon gamma release assays (IGRAs) over TST has been shown in respect of detection of MTB infection among the group of immunocompetent patients, particularly in BCG vaccinated populations [5, 6]. On the contrary in immunocompromised patients with dominant tuberculin anergy the value of IGRAs compared to TST are assessed equivocally, depending on the cause of immunosuppression [7–9].

In patients with sarcoidosis, the use of IGRAs in detection of LTBI may have a limited specifity, as has been shown in the studies based on molecular and immunological methods, which concerned participation of tubercle bacillus antigens in the ethiopathogenensis of sarcoidosis, with simultaneous lack of any evidence for the presence of an alive (metabolicaly active) microorganism in sarcoid granulomas [10–25].

The aim of the study was to assess the results of two commercial IGRAs and their value in detection of MTB infection in the group of treatment naive patients with sarcoidosis in the population repeatedly vaccinated against TB.

Material and methods

The study group

The study group included 151 patients hospitalised at the First Department of Lung Diseases, National Institute of Tuberculosis and Lung Diseases (IGiChP, Instytut Gruźlicy i Chorób Płuc), Warsaw between October 2005 and December 2011, in whom sarcoidosis at the stage I–IV was diagnosed. Sarcoidosis was diagnosed in accordance with the ATS/ERS/WASOG statement [1].

Active tuberculosis was excluded on the basis of the bacteriological investigation of the sputum, bronchial washing and biopsy specimens by Ziehl-Neelsen staining, culture on solid media (Löwenstein-Jensen medium) or by culture on liquid media (BACTEC — 460 TB system).

The following patients were excluded from the study: 1) patients without confirmed diagnosis of the sarcoidosis due to lack of consent to further invasive diagnosis, in whom sarcoidosis was diagnosed basing solely on clinical image; 2) patients who had sarcoidosis diagnosed outside the IGiChP and had no results of microbiological examinations for exclusion of tuberculosis; 3) patients treated with systemic or inhaled corticosteroids or other immunosuppressive drugs; 4) patients with the diseases that may affect immunity; 5) patients who had TST performed during the previous 6 months; 6) pregnant women; 7) patients with mental disorders that limit conscious participation in the study, 8) patients who were under the age of 18.

All study participants were informed about the objective of the study and they gave their written informed consent to the participation therein. The study was approved by the Bioethical Committee of the National Institute of Tuberculosis and Lung Diseases.

Methodology of the study

Venous blood samples were collected once from all 151 patients qualified for the study in order to perform at least one IGRA. All study participants underwent QFT-GIT assay. In the group of 81 patients the second IGRA, T-SPOT. TB was performed simultaneously, without using any additional qualification criteria.

In addition, a questionnaire was completed with clinical characteristics concerning demographic data (age, sex), information on tuberculosis exposure (history concerning past tuberculosis or a possible contact with a person with tuberculosis), and data regarding the course of the disease (stage of sarcoidosis, the presence of Löfgren's syndrome, duration of the disease).

QFT-GIT was performed by qualified staff of the Department of Laboratory Diagnostics (ZDL, Zakład Diagnostyki Laboratoryjnej), IGiChP using a commercial set produced by Cellestis Limited, Carnegie, Victoria, Australia, in accordance with the manufacturer's guidelines [26]. The results were divided in three categories: negative, positive and indeterminate. QFT-GIT ≥ 0.35 IU/ml was considered as a positive result.

T-SPOT.TB assay was performed by a qualified laboratory diagnostic technician of the Department of Microbiology, IGiChP using a commercial set produced by Immunotec Limited, Abingdon, Great Britain, precisely in accordance with the manufacturer's guidelines [27]. The results of T-SPOT.TB were grouped in the three categories: negative, positive and indeterminate. 6 spots or more were considered as a positive result.

Statistical analysis

The results of the study were analysed using the commercial programme for statistical analysis STATISTICA, version 9 produced by StatSoft Poland.

Descriptive results were presented, in the case of quantitative data, as means and standard deviations, and in the case of qualitative data — as numbers and percentages.

Depending on the selected demographic and clinical parameters, the results of IGRA were analysed as binary values. For qualitative and binary variables, depending on expected numbers, chi-square test or its variations (chi -square, V-square) were used. For the remaining variables the Mann-Withney test was used. $P < 0.05$ was assumed as statistically significant.

Results

Characteristic of the study group

The study group included 151 patients with sarcoidosis (97 men and 54 women). The mean age in the study group was 38 ± 10.3 years. Sarcoidosis was diagnosed in 32 cases on the basis of the presence of Löfgren's syndrome, in the remaining 119 patients it was confirmed histopathologically. 1 sarcoidosis patient, in whom histopathological confirmation was obtained, had also Löfgren's syndrome. The roentgenographic stages of thoracic changes were as follows: stage I was diagnosed in 71 patients, stage II — in 72 patients, stage III — in 4 patients, and stage IV — in 4 patients. The time from the onset of symptoms to diagnosis of the disease ranged from 0.25 to 60 months. All patients had a negative result of AFB smear and a negative results of sputum, bronchial washings or biopsy specimens cultures for tuberculosis. 16 patients reported a history of contact with a person with tuberculosis. In the remaining 135 cases, exposure to tuberculosis was not found. None of the subjects from the study group suffered from tuberculosis in the past. Observation time of patients amounted on average 14.8 months. During this time no single case of tuberculosis occured among the study participants. A detailed clinical characteristic of the study group is presented in Table 1.

The results of QFT-GIT

The range of the results of QFT-GIT in the study group was from 0 IU/ml to 3.217 IU/ml, on average 0.09 ± 0.35 IU/ml. Positive results of QFT-GIT made up 4.6%, whereas negative 95.4% of all results (Fig. 1). There were no indeterminate results.

The results of QFT-GIT were also analysed according to the selected demographic and clinical parameters (Table 2).

The group with a positive result of QFT-GIT was distinguished by older age, compared to the group with a negative result (44.4 ± 15.2 vs 37.7 ± 9.9). However, the difference was not statistically significant ($p = 0.1979$; Mann-Whitney test).

No statistically significant correlation was found between the result of QFT-GIT and sex ($p = 0.4209$, chi-square test with Yates' correction).

When the results of QFT-GIT were analysed depending on the stage of sarcoidosis, a positive result of QFT-GIT was found in 5/71 (7%) sarcoidosis patients at the stage I, and in 2/71 (2.8%) patients at the stage II. All patients with

Table 1. Baseline characteristics of study group

	n = 151 (%)
Age (yrs) range	22–69
mean ± standard deviation	38 ± 10.3
median	35
Age group (yrs)	
≤ 29	30 (19.9)
30–39	69 (45.7)
40–49	25 (16.5)
50–59	21 (13.9)
≥ 60	6 (4)
Gender:	
male	97 (64.2)
female	54 (35.8)
Stage of sarcoidosis:	
I	71 (47.0)
II	72 (47.8)
III	4 (2.6)
IV	4 (2.6)
Löfgren's syndrom	33 (21.9)
Duration of sarcoidosis (month):	3 (2.0)
< 1	71 (47.0)
1–3	43 (28.5)
4–6	22 (14.6)
7–12	12 (7.9)
> 12	
TB exposure	
yes	16 (10.6)
no	135 (89.4)

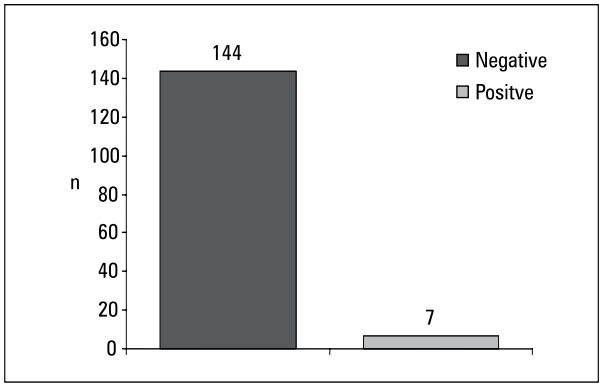

Figure 1. QFT-GIT results

sarcoidosis at the stage III and IV tested negatively for QFT. However, correlation between the result of QFT-GIT and the stage of sarcoidosis was not statistically significant (p = 0.2270, Mann-Whitney test).

No statistically significant correlation was found between the result of QFT-GIT and the presence of Löfgren's syndrome (p = 0.3635, chi-square test with Yates' correction).

The results of QFT-GIT were also analysed depending on the duration of the disease. Correlation between duration of sarcoidosis and the result of QFT-GIT was not found (p = 0.5592, Mann-Whitney test).

No correlation between the QFT-GIT results and exposure to tuberculosis was found (p = 0.6124; chi-square test with Yates' correction).

Table 2. Relationship between QFT-GIT results and demographic and clinical data in study group

	p
Age	0.1979
Gender	0.4209
Stage of sarcoidosis	0.2270
Duration of sarcoidosis	0.3635
Presence of Löfgren's syndrom	0.5592
TB exposure	0.6124

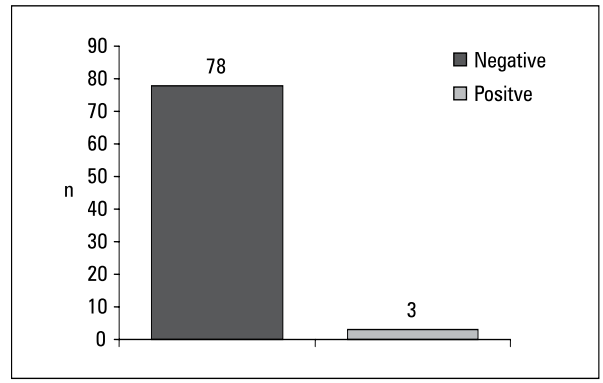

Figure 2. T-SPOT.TB results

The results of T-SPOT.TB assay

T-SPOT.TB results were obtained in a subgroup of 81 patients with sarcoidosis. 3 (3.7%) positive results of the T-SPOT.TB assay were found. The remaining (96.3%) results were negative. No indeterminate results were observed. Qualitative distribution of results is presented in Figure 2.

The age of sarcoidosis patients who tested negatively for T-SPOT.TB amounted on average to 38.3 ± 10.5 years, and of those who tested positively — to 37 ± 2.6. There were 2 men and 1 woman among the patients who tested positively. 1 out of 3 patients had a positive result of both IGRAs. A detailed clinical characteristic with a positive result of T-SPOT.TB was presented in Table 3.

No correlation between T-SPOT.TB assay result and age (p = 0.6526; Mann-Whitney test), or sex was found (p = 0.5875; chi-square test with Yates' correction).

Table 3. Patients characteristics with positive T-SPOT.TB result

	Patient no. 1	Patient no. 2	Patient no. 3
Age (year)	39	34	38
Gender	Male	Female	Male
Stage of sarcoidosis	IV	I	I
Presence of Löfgren's syndrom	No	Yes	No
Duration of sarcoidosis (months)	60	6	2
TBC exposure	No	No	No
TST result	10 mm	12 mm	6 mm
QFT result: quantitative qualitative	 0.22 IU/ml negative	 0 IU/ml negative	 1.32 IU/ml positive

T-SPOT.TB results were not dependent on the stage of sarcoidosis (p = 0.9005, Mann-Whitney test). The correlation between the test result and the presence of Löfgren's syndrome was not found either (p = 0.9329, chi-square test with Yates' correction). Duration of sarcoidosis did not have statisitically significant impact on the test result (p = 0.2252; Mann-Whitney test). No correlation was found between the test result and exposure to tuberculosis (p = 0.7550; chi-square test with Yates' correction).

The analysis of the influence of selected demographic and clinical parameters on the T-SPOT.TB assay results is presented in Table 4.

Table 4. Relationship between T-SPOT.TB results and demographic and clinical data in study group

	p
Age	0.6526
Gender	0.5875
Stage of sarcoidosis	0.9005
Duration of sarcoidosis	0.2252
Presence of Löfgren's syndrom	0.9329
TB exposure	0.7550

Discussion

For more than 10 years now, IGRAs are used in various countries to identify infection with tubercle bacillus. It is the only officially registered indication for the use of these assays. During this time thousands of studies were published concerning the application of IGRAs, both in immunocompetent as well as immunocompromised subjects. There are barely a few studies that concern sarcoidosis patients. Although there are many reasons for interest in this group of patients regarding the possibility of IGRAs appliacance, starting from LTBI identification, through differentiation between sarcoidosis and tuberculosis, and even searching for evidence of common ethiopathogenesis of the two diseases.

In 2008, Inui et al. published the results of the first study with the use of commercial IGRA in patients with sarcoidosis [28]. Positive result of QFT was found in 3.3% of the study participants. Similar results were obtained in our own material. In the study group, positive result of QFT-GIT was found in 4.6% of sarcoidosis patients. Whereas Gupta et al. found positive result of QFT-GIT in as many as 34.2% patients with sarcoidosis [29]. On the other hand, Milman et al. did not find any positive result of QFT-GIT in the examined group of sarcoidosis patients [30].

Contrary to QFT, there has been only one study published in English that used the second commercial IGRA — T-SPOT.TB in sarcoidosis patients so far. German authors (Hörster et al.) found positive result of T-SPOT.TB in 5/17 (29.4%) of sarcoidosis patients [31]. In our study which was conducted in a significantly greater group, positive result of T-SPOT.TB was found merely in 3/81 (3.7%) sarcoidosis patients.

Apart from determinate (positive, negative) IGRAs results, there are also indeterminate results that are the effect of incorrect reaction in negative control or/and positive control with mitogen. The data of the in vitro reactivity of peripheral blood lymphocytes to phytohemagglutinin (PHA) in sarcoidosis are contradictory. According to them, too high number of indeterminate results may restrict the use of IGRA in this group of patients.

Earlier mentioned studies did not confirm these doubts. Solely in the study by Milman et al., in which sarcoidosis patients qualified for biological therapy were examined, indeterminate results of T-SPOT.TB were found. They constituted 7% of all results and concerned the patients on immunosuppressants, and in 1 case — the person infected with HIV [30]. The outcome has not been high. Helwig et al. found 28.9% of indeterminate results of QFT in the candidates for anti-TNFα treatment due to inflammatory bowel disease [32]. Cattamanchi et al. in their meta-analysis of the studies concerning HIV infected patients found indeterminate results of QFT-GIT in 2–11% of the subjects, and of T.SPOT.TB in 0–13% [8]. Diel et al., who analysed 116 studies, showed that the proportion of indeterminate results fluctuated between 0–40.96% (on average 2.14% for all studies and 4.42% in the subgroup of immunocompromised patients) for QFT, and 0 — 33.73% (on average 3.8% for all studies and 6.12% in the subgroup of immunocompromised patients) for T-SPOT.TB [33]. Whereas in the meta-analysis conducted by Sester et al, the proportion of indeterminate results of QFT amounted to 7%, and that of T.SPOT.TB — to 3.4% [34]. In our own material, we did not find indeterminate results in any case. As we mentioned above, the patients treated with immunossuppresive drugs or with other immunodificiency conditions were not included into our study. In 101/151 QFT-GIT and 81/81 T-SPOT.TB assays, the tests was performed with a positive control. INFγ release measured by QFT-GIT after stimulation with mitogen was in the normal range (0.86 IU/mL to > 10 IU/mL). In T-SPOT.TB, in all study participants (81/81), spots count in positive control exceeded 20.

However, from practical point of view, only determinate results are crucial for diagnosis. According to the intention of IGRAs' inventors, interpretation of the test result should be restricted to diagnosis of latent infection with MTB. However, in sarcoidosis patients, it is not so clear due to numerous reasons. Positive IGRAs should be treated as indirect proof of MTB infection, which confirms the presence of immunological response of the patient to mycobacterial antigens but which is not the evidence of the presence of alive microorganisms in his/her body.

Etiological factor of sarcoidosis remains still unknown. Due to clinical and histopathological similarity between sarcoidosis and tuberculosis, the majority of present studies focus on searching for the proof of the hypothesis that the mycobacteria are the etiological factor of sarcoidosis. In recent years, many studies appeared that identified mycobacterial antigens in sarcoidosis patients or T-cell and B-cell response to these antigens. Various mycobacterial antigens have been studied: early secreted antigenic target (ESAT-6), catalase-peroxidase (katG), superoxide dismutase (sodA), mycolyl transferase (Ag85A) and heat shock protein (hsp). ESAT-6 is the same antigen that is used in commercial IGRAs to stimulate T lymphocytes.

Using the ELISPOT method, Drake et al., detected in 8/26 (30.7%) sarcoidosis patients INF γ production from peripheral blood mononuclear cells (PBMC) after stimulation with ESAT-6 [16]. In the same year positive results of the immune response to 3 mycobacterial antigens, including ESAT-6, were published by Carlisle et al. They identified PBMC response to this antigen in 12/30 (40%) of sarcoidosis patients [17]. In both studies statistically significant difference was found between the study group and the control group with negative TST. Lymphocyte reactivity in bronchoalveolar lavage fluid (BALF) to various mycobacterial antigens have been also studied. Oswald-Richter et al. detected $CD4^+$ T-cell response to ESAT-6 or katG in BALF in 73% of sarcoidosis patients [18]. The same author in his subsequent paper reported that ESAT-6 induces the most frequent stimulation of Th1 immune response in these patients, as compared to katG, Ag85A, sodA and Hsp70 [19]. Recently, Oswald-Richter et al., using matrix-assisted laser desorption ionization imaging mass spectrometry localized signal consistent with antigen ESAT-6 in sarcoidosis granulomas [20].

Considering the outcome of these studies, one could expect high proportion of positive IGRA results in sarcoidosis patients. But what we found in the study material is not the case. Then how the differences can be explained? By different sensitivity due to various methodology of the studies? ESAT-6 antigen is a 6 kDa molecule. In experimental studies various amino acid sequences of peptide fragments of the ESAT-6 antigen are used. Although Drake et al. used 17 ESAT-6 peptides of various amino acid sequence, only one of them, NNALQNLARTISEAG was recognised by blood lymphocytes of sarcoidosis patients [16], whereas Carlisle et al. observed Th1 response to multiple peptides within ESAT-6 [17]. In commercial tests, ESAT-6 antigen provided by the manufacturer is used. But the reasons may be different, therefore, the study that would compare the two methods is needed.

Carlisle et al. put forward a hypothesis that lack of response to mycobacterial antigens in

some sarcoidosis patients may suggest that they had been wrongly diagnosed [17]. In our study material, sarcoidosis was diagnosed in accordance with the applicable quidelines [1].

According to another hypothesis, identification of immune response to mycobacterial antigens depends on whether the disease is in active phase or in remission. This theory has been confirmed in the study by Chen et al., who found statistically significant difference ($p = 0.027$) in response of T lymphocytes to katG antigen between the two groups [23]. In our study, the impact of chosen clinical parameters of sarcoidosis on IGRAs results was analysed. No statistically significant correlation was found between the results of QFT-GIT and T-SPOT.TB and sarcoidosis stage, features of acute course (Löfgren's syndrome) and duration of the disease.

Oswald-Richter et al. have proven that the presence of response to mycobacterial antigen is related to the occurrence of certain allele (HLA, human leukocyte antigen). In their research they found correlation between the presence of response to ESAT-6 and katG antigens and the occurrence of DRB1* 1101 HLA allele in sarcoidosis patients [35].

The results of commercial IGRAs in sarcoidosis patients are different in various studies. The majority of studies have shown that the prevalence of positive results of IGRAs in sarcoidosis patients reflects different epidemiological situation of tuberculosis in the countries where the studies were conducted. The study by Gupta et al. was carried out in the country of high incidence of tuberculosis — 178/100 000 (India), whereas the research by Milman et al. was conducted in Denmark, where incidence of tuberculosis is low (7.4/100 000) [36]. The exception is a German study which results have not correlated with a low incidence of tuberculosis in this country (5.6/ /100 000). Hörster et al. have specified that a large group of the study participants was from the countries of high incidence of tuberculosis [31].

Moreover, it cannot be excluded that IGRAs do not detect all persons infected with tubercle bacilli among sarcoidosis patients. It would be supported by the outcome obtained in our material, i.e. substantially lower percentage of positive results of IGRAs, compared to results obtained in a group of healthy volunteers who were examined by Kuś et al. [37]. However, this difference has a logical explanation. The study groups were different with respect to age, which influences the proportion of positive IGRAs results. Furthermore, the group examined by the author was exposed to infection with tubercle bacillus to a slight degree, which is reflected in small proportion of subjects reporting in history of tuberculosis contacts. The studies by other authors have shown that there is a significant correlation between positive result of IGRAs and the degree of tuberculosis exposure [38]. As in our study conducted among sarcoidosis patients, correlation between IGRAs results and exposure to tuberculosis was not found, it may be assumed that exposure to infection with MTB was minimal. The percentage of positive results in sarcoidosis patients in the study by Gupta et al. was also lower than in the healthy population [29]. Similar disproportion of IGRAs results can be found in the population with retained skin sensitivity to tuberculin. Kang et al. have found in the group of healthy individuals with no risk factors of tuberculosis, positive results in merely 4% of the subjects, whereas the proportion of people infected with tuberculosis in the general population was estimated by them at approximately 33% [39]. Lack of indeterminate results of IGRAs in sarcoidosis patients, particularly those that are caused by impaired peripheral blood lymphocytes reactivity after stimulation with mitogen (the so called positive control) implies that the occurrence of false negative results in the study group is little probable.

Of note is the precision with which IGRA result predicts the risk of occurrence of progression from LTBI to active tuberculosis. The follow-up observation of our study material, showed that there was no occurrence of tuberculosis among sarcoidosis patients who tested negatively for QFT-GIT or T-SPOT.TB. Milman et al., when selected sarcoidosis patients for TNF-α antagonists treatment have proved high negative predictive value of Quantiferon test for progression to active tuberculosis [30]. In the group that they examined, 2 persons with negative QFT result received treatment with etanercept and adalimumab. None of them came down with tuberculosis at follow-up. It is in accordance with the outcome of meta-analysis by Diel et al., in which negative predictive value for progression to active tuberculosis in the countries with low tuberculosis incidence was high and amounted to 99.8% for QFT-GIT test and 97.8% for T-SPOT.TB [40].

Some authors have taken the controversial position that IGRAs may be used for differential diagnosis of sarcoidosis and tuberculosis [41]. They believe that if initial condition of tuberculosis is LTBI, the assessment of the presence of infection enables rapid exclusion of tuberculosis in the patient with suspected sarcoidosis. This

point of view is supported by lack of rapid and sensitive tests for differentiation between the two diseases. But this position has many weak points. It is known that only very few persons out of those infected with MTB come down with tuberculosis. According to the meta-analysis conducted by Diel et al., negative predictive value of IGRAs evaluated in patients with bacteriologically confirmed tuberculosis ranged between 70–100%, with a pooled value of 94% for T-SPOT.TB and 88% for QFT-GIT [40]. In the meta-analysis of the same author published one year earlier, sensitivity of QFT-GIT and T-SPOT.TB in patients with bacteriologically confirmed tuberculosis was 81% and 87.5%, respectively [33]. In another meta-analysis (Sester et al.) sensitivity of IGRAs in tuberculosis patients was 80–81% depending on the test. [42]. On the other hand, specificity of IGRAs in these patients was 79% and 59%, for QFT-GIT and T-SPOT.TB respectively, and was definitely lower than specificity evaluated in the earlier meta-analysis by Diel et al. [33] (99% and 86%). In practice, it means that sensitivity of IGRAs is insufficient to definitely exclude tuberculosis basing on their negative result. Whereas specificity of IGRAs is unsatisfactory to diagnose tuberculosis, as the positive result does not differentiate the active disease from LTBI.

Conclusions

In sarcoidosis patients formerly BCG vaccinated against tuberculosis, the rate of IGRAs was 4.6% for QFT-GIT and 3.7% for T-SPOT.TB. The influence of the selected clinical parameters of sarcoidosis on the results of IGRAs has not been found.

Conflict of interest

The author declare no conflict of interest.

References:

1. American Thoracic Society/European Respiratory Society/ Word Association of Sarcoidosis and Other Granulomatous Disorders: Statement on Sarcoidosis. Am J Respir Crit Care Med 1999; 160: 736–755.
2. Baughman R, Culver D, Judson A. Concise review of pulmonary sarcoidosis. Am J Respir Crit Care Med 2011; 183: 573–581.
3. Korzeniewska-Koseła M. Postępowanie wobec osób z kontaktu z chorym na gruźlicę — aktualne zalecenia dla krajów europejskich o małej częstości występowania gruźlicy i polska perspektywa. Med Prakt 2011; 2: 37–49.
4. Lalvani A. Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest 2007; 131: 1898–1906.
5. Mezies D, Pai M, Comstock G. Meta-analysis: new test for diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med 2007; 146: 340–354.
6. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med 2008; 149: 177–184.
7. Richeldi L, Losi M, D'Amico R et al. Performance of tests for latent tuberculosis in different groups of immunocompromised patients. Chest 2009; 136: 198–204.
8. Cattamanchi A, Smith R, Steigart K et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in hiv-infected individuals: a systemic review and meta-analysis. J Acquir Immune Defic Syndr 2011; 56: 230–238.
9. Smith R, Cattamanchi A, Steingart K et al. Interferon gamma release assays for diagnosis of latent tuberculosis: evidence in immune-mediated inflammatory disorders. Curr Opin Rheumatol 2011; 23: 377–384.
10. Mangiapan G, Hance A. Mycobacteria and sarcoidosis: an overview and summary of recent molecular biological data. Sarcoidosis 1995; 12: 20–37.
11. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis. Eur Respir J 2007; 30: 508–516.
12. Mootha V, Agarwal R, Aggarwal A, Gupta D, Ahmed J, Verma I, Bal A. The Sarcoid- Tuberculosis link: evidence from a high TB prevalence country. J Infect 2010; 60: 501–503.
13. Fité E, Fernandes-Figueras M, Prats R, Vaquero M, Morera J. High prevalence of Mycobacterium tuberculosis DNA in biopsies from sarcoidosis patients from Catalonia, Spain. Respiration 2006; 73: 20–26.
14. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Sarcoidosis and tuberculosis: the same disease with different manifestations or similar manifestations of different disorders. Curr Opin Pulm Med 2012; 18: 506–516.
15. Song Z, Marzilli L, Greenlee M et al. Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. J Exp Med 2005; 201: 755–767.
16. Drake WP, Dhason MS, Nadaf M et al. Cellular recognition of Mycobacterium tuberculosis ESAT-6 and KatG peptides in systemic sarcoidosis. Infect Immun 2007; 75: 527–539.
17. Carlisle J, Evans W, Hajizadeh R et al. Multiple Mycobacterium antigens induce interferon-γ production from sarcoidosis peripheral blood mononuclear cells. Clin Exp Immunol 2007; 150: 460–468.
18. Oswald-Richter KA, Culver DA, Hawkins C et al. Cellular responses to Mycobacterial antigens are present in bronchoalveolar lavage fluid used in the diagnosis of sarcoidosis. Infect Immun 2009; 77: 3740–3748.
19. Oswald-Richter K, Beachboard D, Zhan X et al. Multiple mycobacterial antigens are targets of the adaptive immune response in pulmonary sarcoidosis. Respir Res 2010; 11: 161.
20. Oswald-Richter KA, Beachboard DC, Seeley EH et al. Dual analysis for mycobacteria and propionibacteria in sarcoidosis BAL. J Clin Immunol 2012; 32: 1129–1140.
21. Dubaniewicz A, Dubaniewicz-Wybieralska M, Sternau A et al. *Mycobacterium tuberculosis* complex and mycobacterial heat shock proteins in lymh node tissue from patients with pulmonary sarcoidosis. J Clin Microbiol 2006; 44: 3448–3451.
22. Hajizadeh R, Sato H, Carlisle J et al. *Mycobacterium tuberculosis* antigen 85A induces Th-1 immune responses in systemic sarcoidosis. J Clin Immunol 2007; 27: 445–454.
23. Chen E, Walhström J, Song Z et al. T Cell responses to mycobacterial catalase-peroxidase profile a pathogenic antigen in systemic sarcoidosis. J Immunol 2008; 181: 8784–8796.
24. Brownell I, Ramirez-Valle F, Sanches M, Prystowsky S. Evidence for Mycobacteria in Sarcoidosis. Am J Respir Cell Mol Biol 2011; 45: 899–905.
25. Gupta D, Agarwal R, Aggarwal AN, Verma I. Immune responses to mycobacterial antigens in sarcoidosis: a systematic review. Indian J Chest Dis Allied Sci 2011; 53: 41–49.
26. http://www.cellestis.com/irm/content/PI/QFT/2PK/PL.pdf.
27. http://www.oxfordimmunotec.com/international/wp-content/uploads/sites/3/PI-TB-IVD-UK-V2. pdf.
28. Inui N, Suda T, Chida K. Use of the QuantiFERON-TB Gold test in Japanese patients with sarcoidosis. Respir Med 2008; 102: 313–315.

29. Gupta D, Kumar S, Aggarwal AN, Verma I, Agarwal R. Interferon gamma release assay (QuantiFERON-TB Gold in Tube) in patients of sarcoidosis from a population with high prevalence of tuberculosis infection. Sarcoidosis Vasc Diffuse Lung Dis 2011; 28: 95–101.
30. Milman N, Soborg B, Svendsen C, Andersen AB. Quantiferon test for tuberculosis screening in sarcoidosis patients. Scand J Infect Dis 2011; 43: 728–738.
31. Hörster R, Kirsten D, Gaede KI et al. Antimycobacterial immune responses in patients with pulmonary sarcoidosis. Clin Respir J 2009; 3: 229–238.
32. Helwig U, Müller M, Hedderich J, Schreiber S. Corticosteroids and immunosupressive therapy influence the result of QuantiFERON TB Gold testing in inflammatory bowel disease patients. J Crohns Colitis 2012; 6: 419–424.
33. Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-γ release assays for detecting active TB: a metaanalysis. Chest 2010; 137: 552–968.
34. Sester M, Sotgiu G, Lange C et al. Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. Eur Respir J 2011; 37: 100–111.
35. Oswald-Richter K, Sato H, Hajizadeh R et al. Mycobacterial ESAT-6 and katG are recognized by sarcoidosis CD4+ T Cell when presented by the American sarcoidosis susceptibility allele, DRB1*1101. J Clin Immunol 2010; 30: 157–166.
36. World Health Organization. Global tuberculosis report 2013. World Health Organization 2013, http://www.who.int/tb.
37. Kuś J, Demkow U, Lewandowska K et al. Ocena częstości występowania zakażenia prątkiem gruźlicy w populacji województwa mazowieckiego na podstawie wyniku testu mierzącego uwalnianie interferonu gamma po stymulacji swoistymi antygenami ESAT-6 i CFP-10. Pneumonol Alergol Pol 2011; 79: 407–418.
38. Arend S, Thijsen S, Leyten E et al. Comparison of two interferon-γ assays and tuberculin skin test for tracing tuberculosis contacts. Am J Respir Crit Care Med 2007; 175: 618–627.
39. Kang Y, Lee H, Yoon H et al. Discrepancy between the tuberculin skin test and the whole-blood interferon-γ assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005; 29: 2756–2761.
40. Diel R, Goletti D, Ferrara G et al. Interferon-γ release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and meta-analysis. Eur Respir J 2011; 37: 88–99.
41. Thillai M, Lalvani A. IGRAs and sarcoidosis. In: Michell D (ed.). Sarcoidosis. Hodder Arnold, London 2012: 150-156.
42. Sester M, Sotgiu G, Lange C et al. Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. Eur Respir J 2011; 37: 100-111