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ORIGINAL PAPER

Interferon Gamma Release Assays in Patients with Respiratory Isolates of Non-Tuberculous Mycobacteria – a Preliminary Study

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Abstract

Interferon gamma releasing assays (IGRAs) are extensively used in the diagnosis of latent tuberculosis infections. Comparing to tuberculin skin test (TST) they lack false positive results in the populations vaccinated with BCG, and in most non-tuberculous mycobacteria (NTM) infections. Nevertheless, *Mycobacterium kansasii, Mycobacterium marinum,* and *Mycobacterium szulgai* may induce positive IGRAs due to RD1 homology with *Mycobacterium tuberculosis*. The aim of the study was to investigate the possible influence of NTM respiratory isolates on the results of IGRAs. 39 patients (23 females and 16 males) of median age 61 years, with negative medical history concerning tuberculosis, entered the study. Identification of NTM was performed using the niacin test and molecular method GenoType CM test (Hain Lifescience). QFT-Plus was performed in 17 patients, T-SPOT-Tb – in 23 patients. Chest X-rays and a high-resolution computed tomography of the chest have been reviewed by the experienced radiologist blinded to the results of IGRAs, in search of past tuberculosis signs. Positive IGRAs results were obtained in three out of 39 patients (8%): 22% of patients with *M. kansasii* isolates and 18% of patients with radiological signs on HRCT that might be suggestive of past tuberculosis. Positive IGRAs correlated with radiological signs suggestive of past tuberculosis ($r = 0.32$, $p = 0.04$), and on the borderline with isolation of *M. kansasii* ($r = 0.29$, $p = 0.06$). These findings may suggest that a positive IGRAs result, in our material, could depend mostly on asymptomatic past Tb infection. The cross-reactivity of *M. kansasii* isolates with IGRAs was less probable; nevertheless, it requires further investigations.

Key words: interferon gamma release, non-tuberculous mycobacteria, *Mycobacterium kansasii*, latent tuberculosis infection

Introduction

The introduction of IGRAs to clinical practice enabled to improve the diagnostic accuracy of latent tuberculosis infection (LTBI). The assessment of LTBI by IGRAs is based on response to specific antigens: early secreted antigenic target 6 kDa (ESAT-6) and culture filtrate protein 10 kDa (CFP-10), localized in a specific genomic area of *Mycobacterium tuberculosis*, called the region of difference (RD1) (Borkowska 2011; Demkow 2011).

Subsequently, IGRAs have been extensively used to diagnose LTBI in susceptible populations, among others, persons after active tuberculosis contact, immunocompromised hosts, and the candidates to immunosuppressive therapy, especially to anti-TNF alfa treatment (Borkowska et al. 2011; Demkow 2011).

The overall prevalence of LTBI in different countries is closely related to tuberculosis (Tb) burden; thus, the countries with high Tb burden would have more LTBI cases diagnosed with IGRAs and those with low Tb burden – less such cases (Kuś et al. 2011).

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The superiority of IGRAs over tuberculin skin test (TST) in LTBI diagnostic pathway, is related to its higher specificity, e.g. lack of false positive results in the populations vaccinated against *M. tuberculosis* and those infected with most of non-tuberculous mycobacteria (NTM) (Demkow 2011; Kuś et al. 2011; Mancuso et al. 2012). Nevertheless, some NTM, such as *Mycobacterium kansasii*, *Mycobacterium marinum* and *Mycobacterium szulgai* share RD1 with *M. tuberculosis* and could induce false positive immunological response assessed by IGRAs (Demkow 2011). This observation may be important, especially in the countries with low Tb burden, defined by European Centre for Disease Prevention and Control experts as notification rate lower than 20 per 100 000 population (ECDC/WHO 2018), and with increased incidence of diseases caused by NTM (Prevots et al. 2010). In Poland, as well as in Slovakia and in the United Kingdom, a large proportion of NTM infections have been caused by *M. kansasii,* possibly influencing the specificity of IGRAs in LTBI diagnostics (Słupek et al. 1997; Hoefsloot et al. 2013; van der Werf et al. 2014; Wilińska et al. 2014; Bakuła et al. 2018).

Thus, the aim of the present study was to investigate the influence of NTM isolation from respiratory specimens on the results of IGRAs in patients with no medical history of tuberculosis.

Experimental

Materials and Methods

Patients. Overall, 39 patients (23 females and 16 males) of median age 61 years (27–85 years), from whom NTM was cultured from respiratory specimens (sputum and/or bronchial washings) in the period of 2010–2017, and IGRA test was performed simultaneously, entered the study. Patients, who had been diagnosed and treated for tuberculosis, were excluded from the study.

Non-tuberculous mycobacterial lung disease (NTMLD) was recognized in 16 patients according to American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) and recent British Thoracic Society recommendations (Griffith et al. 2007; Haworth et al. 2017). In 23 patients, respiratory isolates did not cause the disease. The characteristic of the population is summarized in Table I.

Chest X-rays and high resolution computed tomography (HRCT) of the chest were reviewed by the experienced radiologist blinded to the results of IGRAs in search of fibrotic foci localized in the upper lobes or upper parts of lower lobes, as well as parenchymal and lymph nodes' calcifications.

Methods of NTM culture and identification. The specimens were digested with the sodium hydroxide and N-acetyl-L-cysteine (NaOH/NALC) method. After decontamination, the sample was neutralized with sterile phosphate buffer (pH 6.8) and centrifuged at 3000 × g for 15 min. The pellet was suspended in 2 ml of phosphate buffer. The strains were cultured on solid media: egg-based L-J medium, Stonebrink medium and in automated system MGIT (Becton Dickinson) (Klatt et al. 2015).

Identification of culture was performed using the niacin test and a molecular method GenoType CM test (Hain Lifescience). The GenoType CM test, using the DNA-STRIP method allowed the identification of *M. tuberculosis* complex strains and 14 clinically relevant NTM within a single procedure. The procedure for identifying strains consists of three steps: isolation of DNA, amplification using primers labeled with biotin and a reverse hybridization. This hybridization reaction includes the consecutive steps: chemical denaturation of the amplification products, hybridization of single-stranded amplicons labeled with biotin on a membrane coated with probes, washing, adding streptavidin/alkaline phosphatase conjugate, staining reaction using alkaline phosphatase (Zabost and Augustynowicz-Kopeć 2015).

Molecular detection of *M. tuberculosis*. Identification using the BD ProbeTec ET system (Becton Dickinson Diagnostic Instruments) was performed according to the manufacturer's instructions. The instrument

Table I
Characteristics of the population of patients, from whom NTM was isolated from respiratory specimens.

Sex	No of pts	Age Median (range)	BMI Median (range)	Number of patients with certain coexisting disease					
				COPD	ILD or GPA	CTD	CF	Npl	Others*
Males	16	58.5 (28–75)	24.9 (19.5–38.9)	5	3	0	0	3	5
Females	23	62 (29–85)	23.8 (16.2–37.3)	5	6	4	2	2	4
Total	39	61 (27–85)	24.3 (15–38.9)	10	9	4	2	5	9

COPD – chronic obstructive pulmonary disease; ILD – interstitial lung disease; GPA – granulomatosis with polyangiitis; CTD – connective tissue disease; CF – cystic fibrosis; npl – neoplasm; BMI – body mass index

* Diabetes (2), hypothyreosis (1), lung aspergilloma (1), renal insufficiency (1), bronchiectasis (2), actinomycosis (1), lung cirrhosis (1), lobar pulmonary artery agenesis (1), trombofilia (1)

reported amplification signals >3 500 method-other-than-acceleration (MOTA) units as positive (Klatt 2015). It was performed in all cases with a positive result of direct bacterioscopy.

Interferon gamma release assays. The IGRAs assays identified cellular immune responses to *M. tuberculosis* by measuring interferon-gamma (IFN-γ) after stimulation of T cells with *M. tuberculosis*-specific antigens. Two tests were available: T-SPOT.TB based on the Elispot-enzyme-linked immuno spot and Quanti-Feron TB Gold Plus (QFT-Plus) based on the enzyme-linked immunosorbent assays (ELISA) technique. The T-SPOT.TB test is based on measurement of the number of peripheral mononuclear cells that produce IFN-γ after stimulation with two antigens: ESAT-6 and CFP 10. The antigens used in QFT-Plus consisted of a peptide cocktail simulating the ESAT-6 and CFP 10 (Borkowska et al. 2017).

QFT-Plus was performed in 17 patients, T-SPOT-Tb – in 23 (including one patient, in whom both IGRAs have been performed).

Statistical analysis. The data were presented as medians and ranges or as number and percentage of positive cases. The differences between categorical variables were analyzed with the *chi*-square test, for quantitative variables – ANOVA test was used. The correlation was assessed with Spearman rank order test. $P<0.05$ was considered statistically significant.

Results

Positive IGRAs results were obtained in three out of 39 patients (8%): positive QFT Plus – in two cases, T-SPOT-TB – in one case. In one patient, in whom both tests were performed, T-SPOT-Tb was negative but QFT was positive.

IGRAs results according to the identified type of NTM were shown in Table II. Positive results were obtained in 2/9 (22%) of patients with *M. kansasii* and one patient with *M. fortuitum* isolate. IGRAs positivity was thus found in 2/10 (20%) of NTM sharing the RD1 region with *M. tuberculosis* (*M. kansasii, M. szulgai)* and 1/29 (3%) of those without RD1 sharing ($p=0.31$).

Positive IGRAs were obtained in 2/23 females (9%) and 1/16 males (6%), ($p=0.91$).

IGRAs results according to the patients' age (Table III) revealed that positive results were found only in the patients above 60 years of age; nevertheless, the age-related differences were not significant ($p=0.49$).

HRCT analysis revealed the presence of lesions suggesting the possibility of the previous infection with *M. tuberculosis* in 17/39 (44%) of patients.

The results of IGRAs according to the results of radiological analysis are shown in Table IV. Positive IGRAs were found in 3/17 (18%) patients with the above-mentioned radiological signs and none of the remaining patients ($p=0.15$).

Table II
IGRAs results according to the NTM species.

Species	IGRA (+)	IGRA (–)	Total
M. kansasii	2 (22%)	7	9
M. avium	0	7	7
M. gordonae	0	6	6
M. chimaera	0	5	5
M. xenopi	0	5	5
M. fortuitum	1 (33%)	2	3
M. szulgai	0	1	1
M. abscessus	0	1	1
M. mucogenicum	0	1	1
M. smegmatis	0	1	1
Total	3 (8%)	36	39

Table III
IGRAs result according to patients' age.

Age (years)	≤24	25–44	45–59	≥60	Total
IGRAs (+)	0	0	0	3 (14%)	3
IGRAs (–)	0	11	8	17	36
Total	0	11	8	20	39

Table IV
The IGRAs results according to radiologic signs of past tuberculosis.

Chest CT	IGRA (+)	IGRA (–)	Total
Past tb signs	3 (18%)	14 (82%)	17
No past tb signs	0 (0%)	22	22
Total	3	36	39

CT – computed tomography, tb – tuberculosis

Positive IGRAs results correlated with radiological signs suggestive of possibility of infection with *M. tuberculosis* in the past ($r=0.32$, $p=0.04$); the correlation of positive IGRA with the isolation of *M. kansasii* was borderline ($r=0.29$, $p=0.06$).

Discussion

Positive IGRAs have been found in three (8%) of patients with NTM cultured from respiratory specimens. Two positive IGRAs results concerned the patients with *M. kansasii* isolates (22%), the species sharing RD1 with *M. tuberculosis*, one – the patient with *M. fortuitum* isolate, the species not sharing RD1. The Japanese studies revealed IGRAs positivity in 19–52% of *M. kansasii* isolates (Kobashi et al. 2009; Sato et al.

2016). The European experience is scarce; nevertheless, Hermansen et al. (2014) found positive IGRAs results in 2/2 *M. kansasii* isolates (100%).

The discrepancies concerning IGRAs positivity were found also in *M. avium*/*M. intracellulare* (MAC) infection: from 0–8% positive results in the studies conducted in Northern Europe and Japan (Adams et al. 2008; Kobashi et al. 2009; Hermansen et al. 2014) to 34% in the study conducted in South Korea (Ra et al. 2011). In our study, all seven cases of *M. avium* isolates were IGRAs negative. As MAC doesn't share the RD1 region with *M. tuberculosis*, positive IGRAs obtained for the patients with MAC isolates by other authors might reflect the background influence of past tuberculosis, especially in countries with high Tb burden (Ra et al. 2011; Wang et al. 2016).

Poland belongs to low Tb burden countries, with the incidence rate of tuberculosis calculated as 19.7/100 000 in 2010 and 15.1/100 000 in 2017 (Korzeniewska-Koseła 2017, 2018). The analysis of LTBI prevalence assessed with QFT, performed in 2010 on 621 healthy subjects from Mazowieckie province, revealed the positive results in 23.3% of them, more frequently in older people compared to younger ones (Kuś et al. 2011). Positive IGRAs in the present study were found exclusively in patients >60 years of age, indicating possible influence of background non-recognized Tb infection in the past on the results obtained.

Retrospective analysis of HRCT scans by an experienced radiologist blinded to IGRAs results, revealed the lesions suggestive of possibility of infection with *M. tuberculosis* in the past in 17 out of 39 patients (44%), despite lack of Tb in anamnesis. Positive IGRAs were noted in three out of 17 patients with radiological signs suggestive of past tuberculosis (18%) and none of the patients without such findings on chest CT scan.

These findings may suggest that positive IGRAs result, in our material, could depend mostly on asymptomatic past Tb infection. The cross-reactivity of *M. kansasii* isolates with IGRAs is less probable, because it was present only in two out of nine patients with *M. kansasii* isolates.

The same type of analysis has been performed by Sato et al. who found T-SPOT positivity in 33% of patients with *M. kansasii* isolates, but after exclusion of those with a history of tuberculosis (defined as either Tb diagnosis and treatment in the past or chest X-ray features suggesting previous tuberculosis), the percentage of T-SPOT positive cases decreased to 19%. They concluded that features of previous Tb are the only risk factor for positive IGRAs in the patients with *M. kansasii* respiratory isolates.

Since the group of patients was small in the current study, further studies are required to answer the question, whether the positive IGRAs in patients with *M. kansasii* isolates is caused by RD1 cross-reactivity or rather the background of asymptomatic tuberculosis in the past.

Conflict of interest

Author does not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Adams LV, Waddell RD, Fordham Von Reyn C. T-SPOT.*TB* Test® results in adults with *Mycobacterium avium* complex pulmonary disease. Scand J Infect Dis. 2008;40(3):196–203. doi:10.1080/00365540701642179 Medline

Adzic-Vukicevic T, Barac A, Blanka-Protic A, Laban-Lazovic M, Lukovic B, Skodric-Trifunovic V, Rubino S. Clinical features of infection caused by non-tuberculous mycobacteria: 7 years' experience. Infection. 2018;46(3):357–363. doi:10.1007/s15010-018-1128-2 Medline

Bakuła Z, Kościuch J, Safianowska A, Proboszcz M, Bielecki J, van Ingen J, Krenke R, Jagielski T. Clinical, radiological and molecular features of *Mycobacterium kansasii* pulmonary disease. Respir Med. 2018;139:91–100. doi:10.1016/j.rmed.2018.05.007 Medline

Borkowska D, Zwolska Z, Michałowska-Mitczuk D, Korzeniewska-Koseła M, Zabost A, Napiórkowska A, Kozińska M, Brzezińska S, Augustynowicz-Kopeć E. [Interferon-gamma assays T-SPOT.TB for the diagnosis of latent tuberculosis infection] (in Polish). Pneumonol Alergol Pol. 2011;79(4):264–271. Medline

Borkowska DI, Napiórkowska AM, Brzezińska SA, Kozińska M, Zabost AT, Augustynowicz-Kopeć EM. From latent tuberculosis infection to tuberculosis. News in diagnostics (QuantiFERON-Plus). Pol J Microbiol. 2017;66(1):5–8. doi:10.5604/17331331.1234987 Medline

Demkow U. [Interferon gamma based tests as a new tool in diagnosis of latent tuberculosis] (in Polish). Pneumonol Alergol Pol. 2011;79(4):261–263. Medline

European Centre for Disease Prevention and Control/WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2016–2018. Stockholm (Sweden): European Centre for Disease Prevention and Control. 2018.

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, et al.; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175(4):367–416. doi:10.1164/rccm.200604-571ST Medline

Korzeniewska-Koseła M (editor). Gruźlica i choroby układu oddechowego w Polsce w 2017 roku. Warszawa (Poland): Instytut Gruźlicy i Chorób Płuc. 2018.

Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF, Leitch A, Loebinger MR, Milburn HJ, Nightingale M, et al. British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax. 2017;72 Suppl 2:ii1–ii64. doi:10.1136/thoraxjnl-2017-210927 Medline

Hermansen TS, Thomsen VØ, Lillebaek T, Ravn P. Non-tuberculous mycobacteria and the performance of interferon gamma release assays in Denmark. PLoS One. 2014;9(4):e93986. doi:10.1371/journal.pone.0093986 Medline

Hoefsloot W, van Ingen J, Andrejak C, Ängeby K, Bauriaud R, Bemer P, Beylis N, Boeree MJ, Cacho J, Chihota V, et al.; Nontuberculous Mycobacteria Network European Trials Group. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. Eur Respir J. 2013;42(6):1604–1613. doi:10.1183/09031936.00149212 Medline

Klatt M, Zabost A, Augustynowcz-Kopeć E. Diagnostyka mikrobiologiczna gruźlicy z zastosowaniem testów genetycznych XPERT MTB/RIF. Zakażenia. 2015;15(6):85–92.

Kobashi Y, Mouri K, Yagi S, Obase Y, Miyashita N, Okimoto N, Matsushima T, Kageoka T, Oka M. Clinical evaluation of the Quanti FERON-TB Gold test in patients with non-tuberculous mycobacterial disease. Int J Tuberc Lung Dis. 2009;13(11):1422–1426. Medline

Korzeniewska-Koseła M. Tuberculosis in Poland in 2015. Przegl Epidemiol. 2017;71(3):391–403. Medline

Kuś J, Demkow U, Lewandowska K, Korzeniewska-Koseła M, Rabczenko D, Siemion-Szcześniak I, Białas-Chromiec B, Bychawska M, Sapigórski P, Maciejewski J. [Prevalence of latent infection with *Mycobacterium tuberculosis* in Mazovia Region using interferon gamma release assay after stimulation with specific antigens ESAT-6 and CFP-10]. Pneumonol Alergol Pol. 2011;79(6):407–418. Medline

Mancuso JD, Mazurek GH, Tribble D, Olsen C, Aronson NE, Geiter L, Goodwin D, Keep LW. Discordance among commercially available diagnostics for latent tuberculosis infection. Am J Respir Crit Care Med. 2012;185(4):427–434. doi:10.1164/rccm.201107-1244OC Medline

Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Blosky MA, Montes de Oca R, Shea YR, Seitz AE, Holland SM, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. Am J Respir Crit Care Med. 2010;182(7):970–976. doi:10.1164/rccm.201002-0310OC Medline

Ra SW, Lyu J, Choi C-M, Oh Y-M, Lee S-D, Kim WS, Kim DS, Shim TS. Distinguishing tuberculosis from *Mycobacterium avium* complex disease using an interferon-gamma release assay. Int J Tuberc Lung Dis. 2011;15(5):635–640. doi:10.5588/ijtld.10.0485 Medline

Sato R, Nagai H, Matsui H, Kawabe Y, Takeda K, Kawashima M, Suzuki J, Ohshima N, Masuda K, Yamane A, et al. Interferon-gamma release assays in patients with *Mycobacterium kansasii* pulmonary infection: A retrospective survey. J Infect. 2016;72(6):706–712. doi:10.1016/j.jinf.2016.03.011 Medline

Słupek A, Zwolska Z, Miller M, Rowińska-Zakrzewska E. [Pulmonary mycobacteriosis-diagnostic problem and prevalence in Poland (a retrospective study)]. (in Polish). Pneumonol Alergol Pol. 1997;65(5–6):326–332. Medline

van der Werf MJ, Ködmön C, Katalinić-Janković V, Kummik T, Soini H, Richter E, Papaventsis D, Tortoli E, Perrin M, van Soolingen D, et al. Inventory study of non-tuberculous mycobacteria in the European Union. BMC Infect Dis. 2014;14(1):62–70. doi:10.1186/1471-2334-14-62 Medline

Wang MS, Wang JL, Wang XF. The performance of interferon-gamma release assay in nontuberculous mycobacterial diseases: a retrospective study in China. BMC Pulm Med. 2016;16(1):163. doi:10.1186/s12890-016-0320-3 Medline

Wilińska E, Oniszh K, Augustynowicz-Kopeć E, Zabost A, Fijałkowska A, Kurzyna M, Wieteska M, Torbicki A, Kuś J, Sztrumowicz M. Non-tuberculous mycobacterial lung disease (NTMLD) in patients with chronic thromboembolic pulmonary hypertension and idiopathic pulmonary arterial hypertension. Pneumonol Alergol Pol. 2014;82(6):495–502. doi:10.5603/PiAP.2014.0066 Medline

Zabost A, Augustynowicz-Kopeć E. Use of GenoType MTBDR plus assay for the detection of mycobacteria molecular rifampicin and isoniazid resistance. Post Nauk Med. 2015;4:249–254.