Immunodiagnostics of Latent Tuberculosis among Polish Armed Forces

JANUSZ KOCIK[1], ANNA BIELECKA[1], ANNA ROSZKOWIAK[2], LESZEK KUBIAK[1], KRZYSZTOF LASOCKI[1], MARCIN NIEMCEWICZ[2], TOMASZ TARGOWSKI[3]

[1]Department of Epidemiology, Military Institute of Hygiene and Epidemiology (MIHE), Warsaw, Poland
[2]Department of Microbiology, MIHE's Biological Threat Identification and Countermeasure Centre, Pulawy, Poland
[3]Clinic of Pneumonology, Military Institute of Medicine (WIM), Warsaw, Poland

Abstract

Tuberculosis (TB) is the second leading cause of death from an infectious disease worldwide [1-4]. Approximately 90% of people who get infected with TB will develop a latent TB (LTB) infection [5]. Due to specific character of military service, soldiers are a high exposure risk group [7, 8].

The main goal of our cross-sectional study was to determine the actual epidemiological situation of tuberculosis infection among Polish Armed Forces personnel. The difference in LTB incidence between the groups of Military Police – Civil Military Cooperation Group (MP-CIMIC) and soldiers not deployed abroad were assessed. The QFT-TB Gold assay was used for screening military personnel. Soldiers deployed frequently to international missions happened to have more frequently positive results of the test than the personnel located in Poland with no international deployment history. We conclude that TB-specific interferon-γ release assays may be useful as a screening tool in increased risk groups in otherwise healthy military personnel for identifying individuals with TB latent infections who are likely to develop an active form of the disease.

Key words: cellular immunity, latent tuberculosis infection (LTBI), interferon gamma release assay (IGRA), military personnel.

Introduction

Tuberculosis (TB) is a contagious airborne disease that spreads from person-to-person by direct contact. Different manifestations of TB infection reflect a balance between the bacterium and host defense mechanisms, in which quality of host defense determines outcome [4, 5].

Since the main route of entry of mycobacteria is the respiratory route, alveolar macrophages are the first line of defense. Destruction of the causative agent at this stage depends on both, intrinsic microbicidal ability of host phagocytes and mycobacteria virulence. Mycobacteria, which avoid the initial intracellular digestion will proliferate, pull into other macrophages and disrupt their response. Host circulating monocytes play important roles in the inflammatory response, which is crucial for the innate response to pathogens. In result, blood monocytes and inflammatory cells are recruited to the infection site. Unfortunately, monocytes that differentiate into macrophages cannot destroy the mycobacteria thus we observe logarithmical growth of tubercle bacilli and blood-derived macrophages accumulation. The third stage is specified by T-cell dependent immunity development, which occurs about 2-3 weeks after infection. Antigen-specific T lymphocytes accumulate and multiply within early tubercles. Simultaneously, macrophages capable of killing intracellular mycobacteria are activated. Following this, a logarithmic bacteria growth is inhibited. Necrotic processes in lesions and inhibition of extracellular mycobacteria growth cause stationary or dormant state of TB infection. There are two possible TB infection consequences. The disease may progress and manifest as an active tuberculosis with typical symptoms or stay at the latent stage for months/years and activate under conditions of immunodeficiency. The final outcome of mycobacterium infection depends on bal-

Correspondence: Col. Janusz Kocik MD, PhD, Kozielska 4, 01-163 Warsaw, Poland, phone +48 609 608 303, fax +48 22 838 10 69, e-mail: jkocik@wihe.waw.pl

ance between *M. tuberculosis* capability to exist inside the body and the innate immunity ability [9-11].

Many studies have confirmed that numerous types of cytokines are implicated in pathogenesis and control of Mtb infection. The recent molecular research of *M. tuberculosis* genome has revealed an unique region of difference in

Table 1. Reactivity of IGRA Test on Nontuberculosis Environmental Mycobacteria (NTM) [16]

	ESAT-6	CFP-10	TB-7.7
Tuberculosis Complex			
M. tuberculosis	+	+	+
M. africanum	+	+	+
M. bovis	+	+	+
BCG Substrain			
Gothenberg	–	–	–
Moreau	–	–	–
Tice	–	–	–
Tokyo	–	–	–
Danish	–	–	–
Glaxo	–	–	–
Montréal	–	–	–
Pasteur	–	–	–
Environmental Strains			
M. abcessus	–	–	–
M. avium	–	–	–
M. branderi	–	–	–
M. celatum	–	–	–
M. chelonae	–	–	–
M. fortuitum	–	–	–
M. gordonii	–	–	–
M. intracellulare	–	–	–
M. kansasii	+	+	–
M. malmoense	–	–	–
M. marinum	+	+	–
M. oenavense	–	–	–
M. scrofulaceum	–	–	–
M. smegmatis	–	–	–
M. szulgai	+	+	–
M. terra	–	–	–
M. vaccae	–	–	–
M. xenopi	–	–	–

one section (RD-1), which is absent from both, all strains of *Mycobacterium bovis* using in BCG and from most nontuberculous environmental mycobacteria (NTM). Early Secretory Antigenic Target 6 (ESAT-6), Culture Filtrate Protein 10 (CFP-10) encoded by RD-1 genes as well as TB7.7-p4 peptide induces a strong T-cell response by producing IFN-g [12-14].

Identification of *M. tuberculosis* specific antigens has led to the development of a new generation of *M. tuberculosis* specific diagnostic tests detecting latent TB infection, namely, T-cell interferon-gamma release assays (IGRAs) [15]. Two test formats are used to detect *ex vivo* active disease and latent TB infection. The first one is based on the amount of IFN-γ released in whole blood in response to ESAT-6, CFP-10 antigens (QuantiFERON™-TB Gold, QFT-G, Cellestis, Carnegie, Australia) and to an additional antigen TB7.7-p4 encoded by a phage-inserted region, RD11 (QuantiFERON™-TB Gold In-Tube, QFT-IT, Cellestis, Carnegie, Australia). The second one, T-SPOT™.TB, is based on frequency of Mtb-specific T cells releasing IFN-γ isolated from peripheral blood mononuclear cells (Table 1).

Interferon Gamma Release Assays (IGRA) constitute a new and innovative approach in LTB diagnosis in comparison to Tuberculin Skin Test (TST) dating back 100 years. Technology of QFT is based on immunological response to TB infection. It is highly specific and unaffected by prior Bacille Calmette-Guérin vaccination or immune reactivity to most atypical mycobacteria. QuantiFERON®-TB Gold (Cellestis) has high sensitivity and specificity (respectively, 89-94% and 98%) for detecting early state of latent and active form of tuberculosis (Table 2) [18]. Table 3 shows the results of comparison of both: QuantiFERON®-TB Gold and TST [16, 17].

The studies have shown that soldiers on active duty represent one of the high-risk group for developing TB infection and progression to active TB disease, as well. Due to the fact TB spreads through the air, poorly ventilated and crowded spaces are one of the factors that most conducive the infection transmission. Sharing the same dormitory or staying at the same platoon favors the transmission of droplet-borne diseases. Moreover, deployment of troops in the endemic area of tuberculosis remains not without significance. It undoubtedly increases the risk of infection, due to increased likelihood of exposure of soldiers to the active form of tuberculosis [19, 20].

The main aim of our study was to determine the actual epidemiological situation of tuberculosis infection among Polish Armed Forces personnel. The difference in LTB incidence between Military Police – Civil Military Cooperation Group (MP-CIMIC) and soldiers never deployed abroad as controls was verified. We hypothesized that soldiers acting abroad are more exposed to TB infection than military personnel stationed in Poland. The QuantiFERON-TB Gold was used as a diagnostic tool to investigate the LTB incidence in Polish Army Forces. In this study, the first

TB screening results with this test of Polish military personnel stationed in country and deployed abroad has been presented.

Material and methods

The purpose of this cross-sectional observational study was to assess the efficacy of TB screening and compare the incidence of LTB in two groups of Polish soldiers. The first group represents soldiers stationed in the Polish contingent of Multinational Battle Group East acting as Military Police – Civil Military Cooperation Group (CIMIC) in Camp Bondsteel in KFOR Kosovo in 2009-2010. The blood samples were collected from 82 soldiers, 25-56 years old. The second group (control group) were recruits conscripted to Tadeusz Kościuszko 1st Warsaw Armoured Brigade, who had been never deployed abroad before. Blood samples were obtained from 53 military volunteers, aged 24 to 64. All 135 participants have got a valid Health Certificate and during sample collection have not reported any health disorders. Prior to specimen collection epidemiological investigation has been performed.

Laboratory testing was carried out in Department of Microbiology, MIHE's Biological Threat Identification and Countermeasure Centre. The QuantiFERON®-TB Gold (Cellestis) was used to measure cellular immune response to TB-specific antigens (ESAT-6, CFP-10 and TB7.7(p4)). For each subject 1mL of blood were collected into Nil, Antigen and Mitogen tubes. All tubes were vigorously shaken to ensure properly coated entire inner surface of the tube with blood. Incubation of tubes were conducted at 37°C for 16 to 24 hours. Subsequent to incubation the tubes were centrifuged for 15 minutes at 2000 to 3000 RCF (g). Plasma from blood specimens were harvested and stored at 2°C to 8°C until their delivery to the MIHE's lab, where meas-

Table 2. TB Tests Sensitivity and Specificity based on Diel's meta-analysis [18]

	IGRA	TST
Sensitivity for Active TB		
All studies (*n* = 19; 988 patients)	81.0%	69.9%
Developed country studies (*n* = 13; 619 patients)	84.5%*	71.5%
Developing country studies (*n* = 6; 369 patients)	74.3%*	35.2%
Specificity for TB		
5 published studies involving 513 subjects	99.2%	
4 tested QFT positive		
Specificity (509/513)		
TST not evaluated by Diel *et al.*, but estimates relevant to France: Pai *et al.* 2008 (BCG vaccinated)		59%

* Significantly different, P < 0.001

urement by Enzyme-Linked Immunosorbent Assay (ELISA) were performed. Following the absorbance reading, test results were automatically calculated by PC software.

Specimens from Camp Bondsteel were incubated on site and then shipped to the MIHE. Blood samples from locations in near vicinity of MIHE were incubated and further processed at the Institute.

Results

The distribution of positive results of LTB among soldiers acting abroad mission and recruits were significantly different with higher percentage among soldiers acting in KFOR Kosovo. Positive QFT-TB Gold results were obtained in 13/135 (9.6%) participants. Among Polish sol-

Table 3. QuantiFERON®-TB Gold vs. TST [16, 17]

| QuantiFERON®-TB Gold	Tuberculin Skin Test
Totally unaffected by BCG vaccination	People without TB are often falsely positive because of BCG vaccination
Unaffected by nearly all non-tuberculous mycobacteria, absolutely specific for TB	People without TB are often falsely positive due to attendance of other common bacteria
Requires only a single patient visit	Requires at least two patient visits – to perfrom and read the test 2-3 days later - often people do not return for reading
Results obtained within 24 hours	TST requires 48-72 hours
Simple YES / NO answer	Subjective – two different readers: two answers
No possibility of adverse reactions in hypersensitive people	Allergic reaction may disturb reading of test result
Reliability in immunocompromised patients for immune system competence	Nonspecific local reactions may be observed
Specificity of ~99%	People with TB are often falsely negative
Not applicable	Booster effects
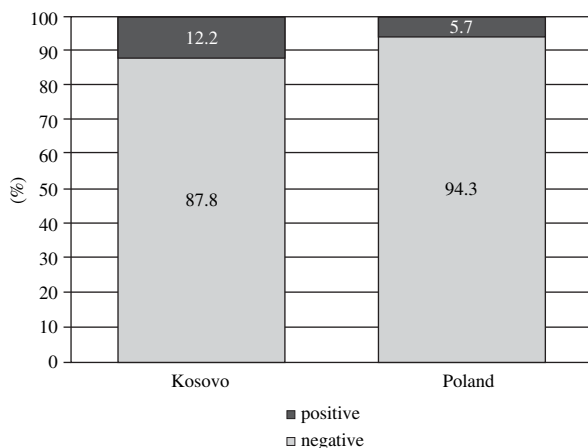
Fig. 1. Tuberculosis screening results among Polish Armed Soldiers

diers on mission in KFOR Kosovo, 10/82 (12.2%) had positive QFT- result. Among group of soldiers acting in country, 3/53 results were positive (5.7%). Perceptive epidemiological investigation among soldiers with positive results was undertaken, because of TB activation probability. None of the positive subjects was found to have symptoms or other clinical evidence of active tuberculosis in two year observation.

Discussion

The Republic of Poland (located in Central Europe) has a population of about 39 million and the prevalence of TB infection has been reported as 19.7 per 100,000 pop/yr . Its military personnel is deployed worldwide for NATO and United Nations led peace-keeping and support missions.

The main aim of the Polish Military Health Care System is surveillance and maintenance of high healthcare standard for professional Armed Forces. The infectious diseases should be concerned in a particular way due to a specific military duty distinctive character. Moreover, one should bear in mind not only soldiers stationed abroad, but also soldiers on duty in national military units.

Military forces are represented by juvenile, healthy and in good physical shape soldiers with low rates of TB. Unfortunately, military settings often mean close personal contact living under high psychophysical pressure. In such conditions TB infections can spread quickly, and in consequence rapidly disturb each unit's readiness and operational capacity.

The Military Police – Civil Military Cooperation Group (MP-CIMIC) is one of the military structures that a particular attention should be paid to. The MP-CIMIC activity is aimed at achieving and maintaining a minimum level of security and at seeking interaction with the neighboring population. The MP-CIMIC Group is responsible for informa-

tion collection and maintenance of the direct contact with the local civilian people in an enemy territory.

Deployment of soldiers to endemic areas of TB augments the risk of infection due to increased probability of being exposed to individuals with active tuberculosis. Tuberculosis prevention strategy for military personnel at risk has been implied as an employer's duty in occupational protection legislation in Germany [21].

Currently, the usage of QuantiFERON-TB Gold in military is recommended in numerous countries including the USA, the UK, Switzerland, Germany, Japan, Italy, Norway, France, Canada, the Czech Republic, the Slovak Republic, France, Korea, Canada and the Netherlands. In routine QuantiFERON-TB Gold is used in Swiss, Dutch, German and Slovak Army [16, 20]. Moreover, U.S. Army approved QFT for use in all service branches [22].

Large-scale studies have confirmed high QuantiFERON-TB Gold test sensitivity and specificity in detecting both, LTB infection and active form of tuberculosis, even in the case of coexisting diseases [23]. The QuantiFERON-TB Gold test has received favorable recommendations from, inter alia, the U.S. Centers for Disease Control and Prevention (CDC), the National Institutes of Health (NIH) and Clinical Excellence, Schweizer Lungenlige. The CDC advises QuantiFERON-TB Gold usage instead of the TST in all circumstances in which the TST is currently used, including for contact investigation, evaluation of recent immigrants and serial testing for infection control [24]. Moreover, QuantiFERON TB-Gold is approved by the US Food and Drug Administration (FDA) as an alternative to the TST in all situations, including travel medicine [24]. However, the National Institute for Health and Clinical Excellence (NICE) suggests a two-stage strategy of TST followed by IGRA tests to confirm a positive TST result [26].

According to European Union experts [16, 25] the optimal solution for surveillance of LTB infection is screening by TST and confirmation of positive results by QuantiFERON-TB Gold test, which is more specific. In case of individuals constantly exposed to tuberculosis the most optimal choice seems to be QuantiFERON-TB Gold or T-SPOT.TB testing.

The best screening test for TB surveillance in military should have high level of sensitivity and specificity and detect both, latent and active TB infection. Uncomplicated collection of potentially infectious samples is required. Moreover, the material should be stable at room temperature for as long as possible before laboratory processing. Test procedures should be fully automatable in order to process large quantities of samples in short time.

In summary, our research and the observations of other authors suggest that a positive association between MTB exposure and the TB disease exists [27, 28]. We would expect that the proportion in the exposed group in which the disease develops (incidence in the exposed group) would be greater than the proportion in nonexposed group in which

the disease develops (incidence in the nonexposed group). Serving military in countries of high endemicity for tuberculosis was predictive for a positive QFT-TB Gold result.

Our test results show a correlation with a risk of exposure suggesting that IGRAs may be useful for the assessment of TB infection in MTB contacts. Due to high mobility of recruits and reduced traceability of contacts, QFT-TB Gold allow for an efficient screening of contacts at a single time point. Furthermore, the design of QFT-TB Gold has met military logistic needs of high throughput and robust logistics. Transportation of material from distant locations to MIHE was possible due to good stability of the blood samples.

Verification of modern, fast and safe test for TB diagnosis following NATO countries was the first step of our pioneer pilot study regarding of QuantiFERON-TB Gold in routine usage in military. The next step should be implementation a long-term TB prevention program in Polish Armed Forces. Nevertheless, due to low prevalence and cost-benefits ratio recruits should not be routinely screened on contrary to soldiers deployed at high-endemic countries.

Acknowledgments

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