STUDY OF CORRELATION BETWEEN SEVERAL DIAGNOSTIC TESTS FOR LATENT TUBERCULOSIS

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Summary
Tuberculosis (TB) is a disease caused by bacteria named Mycobacterium tuberculosis (MT) and it has a global impact on health. Our aim was to evaluate the performance of several tests for latent TB diagnosis. We determined the accuracy of tuberculin skin test (TST) and QuantiFERON-TB Gold (QFT-G) for diagnosis of TB, by comparing data obtained from two groups: one with active TB and one without active TB, but with a high risk of TB infection. For active TB we calculated for TST a sensitivity of 88.2%, a specificity of 72.3%, a positive predictive value of 86.6% and a negative predictive value of 75%. For active TB we calculated for QFT-G a sensitivity of 82.3%, a specificity of 94.5%, a positive predictive value of 85% and a negative predictive value of 93.3%. Also, we found a fair agreement between QFT-G and TST (k=0.384; p=0.01). We demonstrated the high accuracy of QFT-G for immunologic diagnosis of TB in a geographic region with moderate incidence of disease.

Key words: latent tuberculosis, diagnostic accuracy, tuberculin skin test, QuantiFERON-TB Gold

Introduction
Tuberculosis (TB) is a disease that has a global scale impact on the health. Mycobacterium tuberculosis (MT) is an intracellular pathogenic agent that survives phagocytosis by macrophages in spite of an adaptive immune response. Infection with MT can cause TB. The remarkable adaptability of MT along the evolution of human species constitutes the base of its growing resistance to therapy (Dorhoi et al., 2011). Approximately one third of Earth’s population is infected with MT without having active disease. This type of TB is defined as latent TB (LTB). Every year about 8 million TB cases appear, and the mortality related to this disease is at least 2 million, fact that labels the infection with MT as pandemic (Lawn and Zumla, 2011).

Although in the last decades important progress regarding diagnosis and treatment of TB were made, and the efforts for controlling the spreading of the disease were intensified, the fight for the eradication of TB is very hard, especially because of some factors like poverty, HIV infection or resistance to anti-TB drugs (Dye and Williams, 2010). Until 10 years ago, the only diagnostic test for LTB was tuberculin skin test (TST). TST consists of an intradermic injection of purified protein derivative that contains a blend of bacterial antigens, which determines a late-phase hypersensitivity reaction with the emergence of an induration at the site of injection after 48-72 hours. The main inconvenient of TST is the inability of discerning between LTB and prior sensibilization to other mycobacteria. In this category are included subjects which were vaccinated with Bacillus Calmette-Guerin (BCG) vaccine. Therefore it was necessary to develop new tests that transcend these inconveniences. Identification of some genes from MT genome, which are not present in Mycobacterium bovis BCG and other nontuberculous mycobacteria, led to
development of more specific tests for diagnosis of LTB. These genes code specific MT proteins like ESAT-6 (early secretory antigen target-6), CFP-10 (culture filtrate protein 10) and TB7.7 antigen. Thus, tests which utilize interferon-gamma (IFN-\(\gamma\)) were created. There were designated as Interferon–Gamma Release Assays (IGRA). They measure the levels of IFN-\(\gamma\) from a blood samples stimulated.

The aim of our study was to evaluate the accuracy of QFT-G in diagnosis of LTB in subjects that were at high risk of MT infection and to compare the sensibility and specificity of QFT-G with other diagnostic tests.

**Material and methods**

The study was carried on at Clinical Hospital of Pneumology „Leon Daniello” from Cluj-Napoca, between February 2010 – February 2011. Patients were included after signing the consent form.

Subjects were divided into two groups. First group included 18 patients that were at high risk of MT infection, without specific TB images on radiography, with negative sputum smears and cultures. Some of these patients presented signs or symptoms that could be attributed to their comorbidities or to an active TB. Risk of infection was considered to be high in people that presented one or more of the following situations: extreme poverty, homeless persons, social assistants; HIV infection, drug users, chronic residents of psychiatric units; cancer, diabetes mellitus, HCV or HBV infection; immunosuppressive treatment; hospital workers; people with history of active TB; contacts of patients with TB. The second group comprised 17 patients with active TB (clinical, radiological, immunological and bacteriological criteria) (American Thoracic Society, 2000).

We noted the place of residence, the contact with relatives or people with active TB, history of active TB or treatment for suspicion of TB, prior vaccination with BCG, the presence of respiratory diseases with the described antigens (Pai et al, 2008). Presently there are two types of IGRAs: QuantiFERON-TB Gold (QFT-G) or QuantiFERON-TB Gold In-Tube (QFT-GIT, Cellestis, Australia) and T-SPOT TB (Oxford Immunotec Ltd, Marea Britanie). QFT-G, QFT-GIT and T-SPOT TB are considered to be tests with high specificity in diagnosis of TB (Mazurek et al, 2010; Chesca et al, 2012) (chronic obstructive pneumopathy (COPD), asthma, and pulmonary fibrosis), diabetes mellitus, psychiatric disorders, heart failure, and infectious diseases.

We noted the presence of fever, loss of appetite, fatigability, chills and nocturne diaphoresis, cough (for a period longer than 3 weeks), hemoptysis, pleuritic twinge, dyspnoea (American Thoracic Society, 2000: Global tuberculosis control; 2012).

Radiological exam was performed from two incidences: posteroanterior and lateral. We noted the imagistic signs that can appear in old and new TB: infiltrate, atelectasis, cavity, pulmonary nodules, pleural effusion and fibrosis (Grzybowski et al, 1971; Friedland, 2010).

For bacteriological exam we collected sputum from three consecutive days. The process was in conformity with standing regulations (Homorodean et al, 2005). Sputum smears were executed using Ziehl-Neelsen staining for acid-fast organisms. We counted acid-fast bacilli from 100 microscopic fields examined with a microscope with immersion. For cultures we used Löwenstein-Jensen medium.

For diagnosis of LTB we used two tests: TST and QFT-G. For QFT-G we collected 4 ml of blood in a container with heparin. We preserved the probes at a temperature of 22±5°C and we analysed them in an interval of maximum 12 hours after prelevation. After incubation, levels of IFN-\(\gamma\) were determined using enzyme-linked immunosorbent assay (ELISA) (Mazurek et al, 2005). Interpretation of result can be seen in table 1.
Table 1. Criteria for interpretation of QFT-G test

<table>
<thead>
<tr>
<th>NIL</th>
<th>ANTIGEN</th>
<th>MITOGEN</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>any value</td>
<td>≥ 0.35 UI/ml and</td>
<td>≥ 50% of Nil</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>≤ 0.7</td>
<td>≤ 0.35 UI/ml</td>
<td>negative</td>
</tr>
<tr>
<td>≤ 0.7</td>
<td>≤ 0.35 UI/ml</td>
<td>≥ 0.5</td>
<td>inconclusive</td>
</tr>
<tr>
<td>≥ 0.7</td>
<td>&lt; 50% of Nil</td>
<td>&lt; 0.5</td>
<td></td>
</tr>
</tbody>
</table>

TST was performed using Mantoux technique. We used 2 vials with concentration 10 UI/0.1 ml. We injected a dose of 10 UI in the upper layers of forearm skin producing a slight elevation of the skin (Howard et al., 1977). The test was interpreted after 48-72 hours from injection. The presence of an induration larger than 5 mm was considered as positive test in the following cases: HIV infection, recent contact with a person suffering from active TB, subjects with pulmonary fibrosis due to prior TB, subjects with immunosuppressive treatment. An induration larger than 10 mm was considered as positive test in the following subjects: recently immigrants (under 5 years) from geographic regions with high incidence of TB, health workers from laboratories which deal with MT, subjects with severe diseases. An induration larger than 15 mm was considered as positive test in any conditions (Mazurek et al., 2005).

Statistical analysis was carried on using Medcalc software version 12.3. Normality of distribution for continuous variables was tested using Kolmogorov-Smirnov test. Continuous normal distributed variables were analysed with T test for independent variables. For categorical data we used chi-square test. We calculated sensibility and specificity for TST and QFT-G for detection of TB infection, using as surrogate-standard the sputum cultures. We analysed agreement between tests by using Cohen kappa test. Binary logistic regression and ROC curves were used in order to examine the effect upon the accuracy of TB diagnosis of a combination between different tests. The level of statistical significance was set at p lower than 0.05.

Results

In table 2 we included demographic characteristics and diseases history of the study participants, as well as comparison between the two groups. Clinical and imagistic characteristic from the two groups can be seen in table 2.

Table 2. Demographic characteristics and diseases history of the study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with TB</th>
<th>Patients without TB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51.5±16.4</td>
<td>65.05±14.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Male</td>
<td>17 (100%)</td>
<td>13 (72.2%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>5 (27.2%)</td>
<td></td>
</tr>
<tr>
<td>Contacts with active TB</td>
<td>7 (41.2%)</td>
<td>0</td>
<td>0.003</td>
</tr>
<tr>
<td>History of TB</td>
<td>4 (23.5%)</td>
<td>3 (16.6%)</td>
<td>0.6</td>
</tr>
<tr>
<td>History of BCG vaccination</td>
<td>15 (88.2%)</td>
<td>11 (61.1%)</td>
<td>0.1</td>
</tr>
<tr>
<td>COPD</td>
<td>8 (47%)</td>
<td>10 (55.5%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Asthma</td>
<td>0</td>
<td>2 (11.1%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (5.8%)</td>
<td>3 (16.6%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>7 (41.7%)</td>
<td>10 (55.5%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>2 (11.7%)</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Cancer</td>
<td>4 (23.5%)</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1 (5.8%)</td>
<td>8 (44.4%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
For active TB we've calculated for sputum smears a sensitivity of 76.4%, a specificity of 100%, a positive predictive value of 81.8% and a negative predictive value of 100%. For active TB we’ve calculated for TST a sensitivity of 88.2%, a specificity of 72.3%, a positive predictive value of 86.6% and a negative predictive value of 75%. % For active TB we’ve calculated for QFT-G a sensitivity of 82.3%, a specificity of 94.5%, a positive predictive value of 85% and a negative predictive value of 93.3%.

We found a fair agreement between TST and sputum smears (k=0.394; p=0.01). We found a good agreement between QFT-G and sputum smears (k=0.763; p<0.001). We found a fair agreement between QFT-G and TST (k=0.384; p=0.01).

In order to study the effect of combining different test upon the efficiency of TB detection we constructed two logistic regressions for saving the predicted probabilities. The first regression included TST, sputum smear and QFT-G, and the second one included only TST and QFT-G.

We constructed ROC curves with predicted probabilities and we found an AUC of 0.977 for combination of TST + QFT-G + sputum smears and an AUC of 0.912 for TST + QFT-G.

**Discussions**

The study was conducted in order to compare the accuracy of QFT-G, as compared with TST, for diagnosis or exclusion of TB. We demonstrated high accuracy for QFT-G for immunologic diagnosis of TB in a geographic region with moderate incidence of disease.

We found a very good specificity for QFT-G regarding TB detection. When compared with TST, QFT-G excluded TB in about 20% patients more than the first test. This is the greatest advantage that IGRA have on TST. Although the sensitivity of TST was a bit higher that QFT-G (88.2% versus 82.8%), TST misclassified TB in about 25% subjects. There was a fair agreement between QFT-G and TST. The results were in accordance with medical literature.
Thus, Detjen et al. (2007), determined a sensitivity of 100% and a specificity of 58% for TST and a sensitivity of 93% and a specificity of 98-100 for IGRAs. The high specificity and sensitivity in their study was due to the more sensitive IGRAs used: QFT-GIT and T-SPOT TB.

A metaanalysis created by Sester et al (2011) found a sensitivity of 80-81% for IGRA for diagnosis of active TB, data that is in accordance with the results of our study. Another metaanalysis showed that IGRAs are suitable for diagnosis of TB in countries with low incidence of disease. They are not to be used in poor countries with high prevalence of TB, because in those areas they are not superior to TST and they cost more (Denkinger et al, 2011).

**Conclusions**

QFT-G could be useful in raising the specificity of diagnosis of suspected LTB cases and, by that, lowering the costs by reducing the number of cases treated for an infection that does not exists.

**References**


Homorodean, D., Moldovan, O., Diculescu, D., Chiriac, G., Muntean, I.: Îndrumar de tehnici de laborator de bacteriologie BK, 2005.


