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Review Article

FUTURISTIC SCOPE OF BIOMARKERS IN TUBERCULOSIS

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ABSTRACT

One of the major causes of the mortality from single infectious agent, tuberculosis (TB) is prevalent worldwide. India has the highest number of TB cases in the world. It is the leading cause of death, because of its high mortality and morbidity because of the disease. Reason lies in the emergence of multidrug-resistant TB strains, and the HIV infection, which reactivates the latent TB making it more severe. Moreover, failure to diagnose TB early remains one of the primary hurdles in controlling of the disease. TB is a potentially hazardous infectious disease attacks various organs, mainly affecting lungs. The bacteria causing TB are spread from one person to another. Tiny droplets released into the air via coughs and sneezes lead to the passage of infection. The disease is generally diagnosed by its symptoms, radiographic methods, and sputum smear microscopy and by cultivation of the *Mycobacterium tuberculosis*, which is considered as gold standard. Current advances in molecular biology and molecular epidemiology and a better understanding of drug resistance in TB have given a new horizon to its rapid diagnosis. However, the cost-effective techniques, and their requirement for sophisticated equipment and skilled personals have excluded their implementation on a routine basis, especially in low-income countries.

Keywords: Biochemical markers, Tuberculosis, Microbiological tuberculosis.

INTRODUCTION

Once rare in developed countries, tuberculosis (TB) infections have become more prevalent 1985 onwards, partly because of the emergence of HIV. The virus weakens a person's immune system, and hence it becomes prone to TB infection. Despite so many studies reported over the past several years, biochemical markers have insignificant contributions in routine diagnosis of TB. It is not wrong to say that none of the markers available to look at, so far are entirely satisfactory. A wide variety of cytokines, markers of cell death and tissue remodeling on the host side, and a very large number of bacterial molecules on the pathogen side are very well known, but they all poorly predictive at patient level. Even though, they are rapid and does not require specimen from the site of the disease. Focusing on the various aspects of the biochemical markers, the scope of this article is to summarize the current state of knowledge about these markers both in pulmonary and extrapulmonary TB and their potential as prognostic and diagnostic tool and also present some of the future perspectives and challenges.

Over the last 100 years, one of the most important additions in the field of diagnostics has been the development of an assay based on the interferon-γ (IFN-γ) determination. So far the only diagnostic test available was tuberculin skin test (TST). The IFN-γ is based on the principle that T cells of the sensitized individuals produce IFN-γ when they reencounter the antigens of *Mycobacterium tuberculosis*. A high amount of IFN-γ production is then assumed to correlate with TB infection [1-4]. The very first IFN-γ assay made use of purified protein derivative (PPD) as the stimulating antigen [1]. The more advance assays exploiting the use of antigen that are specific to *M. tuberculosis*, such as early secretory antigen target 6 (ESAT6) and the culture filtrate protein 10 (CFP 10) [1]. These protein antigens are coded by the genes located on the region of difference 1 (RD 1) of *M. tuberculosis* genome and they are much more specific than PPD, since they are not shared with *Mycobacterium bovis*, Bacillus Calmette-Guerin (BCG) or various other non-tubercular *Mycobacterium*. Currently, commercially available INF-γ assays are QuantiFERON-TB assay and T-Spot TB test. These tests based on leveling of INF- γ by T-cells in response to TB antigen by enzyme-linked immunospot assay (ELISA) and enzyme-linked immunospot respectively. The QuantiFERON-TB a is whole blood assay and used PPD as antigen. Its current version QuantiFERON-TB Gold uses ESAT6 and CFP10 antigens, while T-Spot-TB assay uses peripheral mononuclear cells and ESAT 6 and CFP 10 as antigens to measure the number of T-cell producing INF-γ.

Various studies done apparently show that INF-γ assay using RD 1 antigen more reliable over TST as QuantiFERON-TB gold and ELIspot highly specific and they better correlate with previous exposure to *M. tuberculosis* and rarely cross react due to BCG vaccination or previous exposure to non-tubercular mycobacteria. Furthermore, this assay uses a variety of antigen rather than individual antigen, having better accuracy [5]. However, there is a need to assess the usefulness of these tests in the immunocompromized individual, in children and those with extrapulmonary TB.

Studies done predominantly in western countries [6] have reported an ELISA assay based on mycobacterial antigen 60 (A60) for estimation of specific immunoglobulins in serum, has been used successfully for rapid diagnosis of TB. Their findings showed very good specificity (92%) and good sensitivity (75%) when combined IgM and IgA antibody titer was considered in disease of childhood TB. In another report, the serum of the patients with active TB along with control serum group was assayed for IgA, IgG, IgM, IgE antibody activity to PPD using the ELISA. The patients with active TB clearly had a higher level of IgG antibody to PPD antigen. None other immunoglobulin was found in good correlation with the disease [7]. Now a days many commercial tests are available in the market for the diagnosis of TB. Most of these are based on the detection of IgA, IgG, IgM antibodies to specific Mycobacterium antigen or mixture of antigens. However, this also is not very reliable as many a times there is a lack of consistent rise of all three immunoglobulin classes even during the active phase of infection. Thereby there is a need to detect or determine an ideal antibody isotype assay for reliable diagnosis of TB, saving the unnecessary expenditure of the patients [8].

Among other markers of the TB serum adenosine deaminase (ADA) concentration is also well reported in the literature. The enzyme ADA belongs to purine salvage pathway and catalyzes the conversion of the adenosine and deoxyadenosine to inosine and deoxyinosine with the release of the ammonia. It is one of the important enzymes in

T-lymphocytes where it is 10 times higher in concentration. Its activity increases during reproduction and in response to antigenic stimulation of lymphocytes. Its increased concentration has been found in the region of tubercular serocities, and that can be used for the diagnosis [9-11]. ADA concentration in effusion has already been proved as TB marker of great importance.

Piras *et al.* for the first time reported high ADA level in tubercular pleural effusion [12]. Various studies done between 1966 and 1999 concluded the good performance of the test with significant sensitivity and specificity in cases of pleural effusion [13]. In 2007, a systematic review of ADA by NSG health technology assessment program gave conflicting conclusion that there is no evidence to support the use of ADA test for pulmonary TB diagnosis [14]. However, there is considerable evidence to support the use of ADA in pleural fluid samples for diagnosis of pulmonary TB, where sensitivity was very high and to a slightly lesser extent for tubercular meningitis. In both, the pleural and meningeal TB ADA test was proved to be highly sensitive than any other test [14].

There are reports to show the role of ADA in the differential diagnosis of pleural effusion [15].

The diagnostic value of the ADA activity was also studied to evaluate the differential diagnosis of tubercular meningitis by Rohani *et al*., and in all the patients [16], ADA activity was observed to be greater than the cut-off value of 9 IU/L. However, high ADA activity was also seen in 13% non-tubercular cases giving specificity of around 87%. Other researchers also say the importance and usefulness of ADA activity in the diagnosis of TB [17,18]. So, it is very apparent to state that even though the ADA activity determination is sensitive for TB, it is not specific enough to be used as a rapid diagnostic test. However, it is a useful adjunctive marker for TB when correlated with clinical signs, symptoms, and other laboratory tests.

In another published journal, the ratio of serum ferroxidase and albumin was established as a marker in pulmonary TB [19]. The ferroxisase is a serum ceruloplasmin, a copper transporting globulin protein and a very well known antioxidant. Ferroxidase is synthesized in the liver microsomes and acts in the serum by oxidizing ferric iron which could otherwise act as a catalyst in generating toxic free radicals via lipid peroxidation, etc. [20]. The high level of serum ceruloplasmin was observed with low albumin level in the patients of pulmonary TB. In this study, ferroxidase/albumin ratio was used in diagnosis and therapy of the pulmonary TB. Statistically more significant results were obtained. While using this ratio, its prognostic role on the prognosis of pulmonary TB could be assessed with follow-up studies.

Present knowledge for detection of *M. tuberculosis* biomarker does not have sufficient valid data. There is a need for understanding the interplay between the immune system of the host and *M. tuberculosis* [21] in order to look for some reliable biomarker. The current diagnostic methods available for TB are sputum for acid fast bacilli, histopathology, and radiological assessment of the chest. A reliable diagnosis of the TB depends upon assessing the level of *M. tuberculosis* by various well established microbiological, cytological, or histopathological methods but these classical methods have their own limitations [22]. Cultivation of the bacteria is very difficult and time taking. Also the material for polymerase chain reaction is every time is not available (except for cerebrospinal fluid and urine). For histopathological confirmation, there is a requirement of invasive biopsies [3,4,23]. Keeping in mind, increasing incidence of TB especially in the patients with HIV infection, there is a pressing need in todays scenario for effective immunological diagnostic test which is relatively easy to perform and economical too for diagnosis of the disease and its management. Since the TB bacillus generates antibodies against different antigens, a multiple immunoassay approach is needed. There are reports in the literature upon overexpression of six *M. tuerculosis* specific antigens, their purification and potential use in development of multiplex microbead immuneassay for the detection of *M. tuberculosis* infection in TB patients [24]. Such kind of studies will definitely provide base for the development of a multiple antibody based diagnostic test for detection of *M. tuberculosis* infection in reactivated TB patients and also in other categories like latent and active TB patients.

Biomarkers in the form of antibody profile can be potentially useful for sputum negative childhood TB and extra pulmonary TB which account for approximately 20-25% of TB patients in our country [1]. *M. tuberculosis* maleate synthase and MPT-51 are dominant antigens in the organism and antibodies to these antigens have been found to be important biomarkers in the diagnosis of incipient subclinical TB. Detection of these antigens is not been effected by concurrent HIV infection [25,26]. Volatile organic compounds (VOC) in breath have been identified as biomarkers for pulmonary TB. VOC include oxidative stress products like alkanes and alkane derivatives such as cyclohexane and benzene derivative [27].

Of great concern, for management and control of disease, in present time is the discovery of some more effective diagnostic tool which in economical too. Though most of the biochemical markers have high specificity but poor sensitivity. While the initial use of IFN-γ for detecting latent infection appear promising, but it still requires reconsideration upon its practical usefulness. IFN-γ tests are expensive tests and their high cost appears to limit their wider application, especially in developing and underdeveloped countries where the disease is prevalent at an alarming level. ELIS post-test cannot be performed in normal clinical laboratories as it requires isolation of mononuclear cells which is the very invasive procedure. Also, there is a requirement to assess the usefulness of these tests in immunocompromised patients and children. Other biochemical markers like ADA and ferroxidase/albumin ratio need comprehensive evaluation in well-designed and controlled clinical trials and tested in high endemic, low resource setting where the implementation and use of these methods are more needed to contribute to the improvement of TB control.

BARRIERS TO DEVELOPMENT OF NEW TB DIAGNOSTICS

Market failure has been an important factor hindering the development of new diagnostics for TB. Industry tends to avoid developing and marketing products that will be mainly used for poor patients in developing countries because such products will not generate profits when products are available, neither pricing nor performance is adapted for developing countries, and their benefits are unavailable for patients and health care provider who need them most. Health systems in developing countries are generally weak, making them unable to take advantage of TB diagnostics to achieve best possible performance, and to introduce new advances in diagnostic technologies. This emerging problem is due to poor management, insufficient financial resources and inadequate human resources [28-30].

OPTIMISM FOR THE FUTURE

The product pipeline for the future looks promising. In 2009 data work published on the first automated molecular test for TB, the Xpert *M. tuberculosis*/rifampicine (RIF), which was co-developed by the Foundation for Innovative New Diagnostics, Cephid (Sunnyvale, CA, USA), and the University of Medicine and Dentistry of New Jersey, NJ, USA. This assay, which was Conformite Europeenne marked in 2009, avoids most of the pitfalls of conventional nucleic acid amplification test (safety, contamination, ease of use, etc.), can be done by the staff with little training, and can be used for case detection or multidrug-resistant screening. Data from evaluation trials showed excellent performance in both smear-positive and smear-negative patients, and high accuracy for the determination of RIF resistance. Thus, this highly sensitive and simple-to-use system can detect *M. tuberculosis* directly from the sputum in <2 hrs [30,31].

For the diagnosis of latent *M. tuberculosis* infection, commercially available INF-γ release assays (IGRAs), have emerged as a strong alternative to the TST. These assays have very high speficity and have

specific logistical advantages compared with TST for a diagnosis. IGRAs, however, have no role as tests for diagnosing active TB in adult endemic settings [31,32].

The use if IGRAs is steadily increasing, with several countries with low and intermediate incidence opting to use them, mostly as follow-up tests in people with positive results from TST, especially in BCG - vaccinated populations.

A survey of IGRA guidelines showed much diverse IGRAs. The two-steps approach (initial TST, followed by confirmatory IGRA testing) seems to be the most common strategy, partly because of economic considerations. The optimum strategy for IGRA use is yet to established. Although targeted testing and preventive therapy for latent *M. tuberculosis* infection is well-established in low-incidence countries, the exact role of testing and treatment is disease - endemic countries remains controversial. However, testing latent *M. tuberculosis* infection is receiving increased attention in vulnerable subgroups, such as HIV - infected people and childhood contacts of active TB cases [32,33].

Some future developments are desirable and worthy of consideration:

- Improvement of the study design of clinical studies trying to identify some biochemical marker
- Increased international cooperation and pooling of the patient clinical data from different parts of the world
- Standardization and further development of reliable clinical tool for disease activity evaluation
- Reinforcement of the target lesion focused approach in order to identify the marker
- More and more research should be focused on existing biochemical markers that could of help to the diagnostic dilemma.

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