# Study The Presence of Fibronectin Binding Protein (FnBp) in Tuberculosis (TB) Patients by Elisa

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## Abstract

Fibronectin-binding protein (FnBp) antigens are a prominent secretory protein of short term culture supernatants of M. tuberculosis and M. bovis (BCG) and is conserved within the genus Mycobacterium. The 30/31 kDa antigen of M. tuberculosis is one of the major secretory molecules and is probably routinely recognised by the host immune system in the early stage of tuberculosis infection. Serum immune complexes, prepared from TB patients and normals, were analysed for the presence of FnBp by ELISA using an anti-30/31 kDa (FnBp) monoclonal antibody (CF8) and by western blotting using Fibronectin-HRP. A significant difference was seen between normals and TB patients ($p<0.05$). This test was found to have a specificity of 80% and a positive predictive value of 73%. This is a preliminary finding and the test needs tobe evaluated further for its performance on a larger number of confirmed TB patients and controls (JPMA 46:5, 1996).

## Introduction

Tuberculosis, one of the oldest recognised diseases, is still the main cause of death from a single infectious disease. It leads to 25% of all avoidable deaths worldwid[1]. Current methods for the diagnosis of tuberculosis depends upon available expertise and are based on clinical observations, radiological signs, microscopic exanunation of sputum and culturingthe organism[2]. The cultunng and typing ofmycobactena may take upto two months. Very few reports are available fordiagnosis of tuberculosis based on antigen detection[3,4]. The detection of mycobacterial antigens in clinical specimens may offer good diagnostic value and can be used for monitoring the effect of therapy in previously positive patients.

The 30/3 1 kDa antigens are secreted Fibronectin-binding proteins (FnBp) that correspond to antigens 85B and 85A of M. bovis BCG[5,6]. FnBp ma be important to allow the mycobacteria to avoid detection by the immune system or to facilitate interaction with the host cells[7]. These antigens are important targets for the human antibody response and have been successfully used as a serodiagnostic test in smear-positive pulmonary TB[8].

Unfortunately there is no sensitive and specific, cheap, rapid and reliable diagnostic test available which could help in identification of all cases of pulmonary and extrapulmonaiy tuberculosis at early stages of the infection. Such a diagnostic test is urgently needed, particularly in developing countries where the prevalence rate is very high.

## Materials and Methods

In order to study the presence of 30/31 kDA antigen in TB patients, senun immune complexes were prepared from TB patients and controls. These were analysed by ELISA using an anti-30/31 kDa (FnBp) m-Ab (CF8) and by western blotting using Fibronectin- HRP.

CF8: This is a monoclonal antibody which was raised in our laboratoiy by immunising mice with

MTSE. It reacts with the 30/3 1 kDa antigen of M. tuberculosis and the 85 complex of M.bovis.
Fibronectin-HRP: Human Fibronectin (Gibco) was conjugated with HRP in our Iabomtoiy and used to detect fibronectin-binding protein in the immune complexes.
A. Detection of FnBp by ELISA
Patients: 20 confirmed positive pulmonary TB patients serum samples selected for the test. Pàtiënts w re diagnosed on the basis of the presence of AFB in sputum and/or following sputum culture.
Normals: 20 healthy normal sera were provided by the Regional Blood Transfusion centre for the study.
Serum samples are precipitated with 2% (w/v) polyethy leneglycol 6000 (PEG) overnight and washed in PEG buffer at 4°C. They are dissolved in veronal buffer. Immune complexes were prepared from both the groups and applied onto 20 mg/mI anti-FnBp mouse monoclonal antibody-coated plates (CF8). The plates were incubated at 37°C for one hour and then washed with washing buffer. 100 ml of the rabbit anti-M. tuberculosis H37Rv - IgG. HRP conjugate (1:4000) in diluting buffer was added onto the plates and incubated at 37°C for one hour. Plates were then washed with washing buffer. The presence of bound antibody- conjugate was detected by adding 100 nil of orthophenylene diaminc substrate (OPD) (10 mg OPD/25 ml of the substrate buffer pH 5.0 with 25 ul of 30%H2O2) to well. The reaction was allowed toproceed at 37°C for 30 minutes inthe dark. The reactionwas stopped by adding 50 ml Of 20%(v/v) H2SO4 to each well. The plates were read on an ELISA reader (Labsystem MultiskanMCC) at 492 nm.
Results are recorded on the basis of OD levels. A value above the mean of the normal values±2SD was considered as positive.
B.Detection of FnBp by Western blotting
Patients: 13 bacteriologically-proven tuberçlosis patients sera, before therapy, were selected to prepare inununе complexes for the analysis of fibronectin-binding protein.
Normals: 5 healthy normal sera were provided by the Regional Blood Transfusion Centre for the study.
Immune complexes from tuberculosis patients and normals, together with MTSE, were run on SDS-polyacrylamide gels (10%) for analysis of fibronectin-binding protein (FnBp). Proteins were transferred to nitrocellulose membranes for analysis. Blots were stained with a 1:1000 fibronectin-HRP conjugate.

**Results**

The serum immune complexes from TB and normals were analysed for the presence of FnBp by ELISA. The presence of the 30/31 kDa FnBp antigen was detected in 55% of the TB patients' serum immune complexes by using CF8 anti-FnBp rn-Ab. A significant difference was seen between normals and TB patients ($p<0.05$) as most of the control sera were negative for FnBp. This test has been found to have a specificity of 80% and a positive predictive value of 73% (Table).

Table. Analysis of the 30/31 kDa antigen test in TB patients and normal controls.

| Group | Positive N | Negative N | p value | Sensitivity | Specificity | Predictive value Positive | Predictive value Negative |
|---|---|---|---|---|---|---|---|
| Tuberculosis | 11 | 9 | <0.05 | 55% | 80% | 73% | 64% |
| Normals | 4 | 16 | | | | | |

FnBp was also detected in most of the TB patients immune complexes by western blotting using fibronectinHRP (Figure).

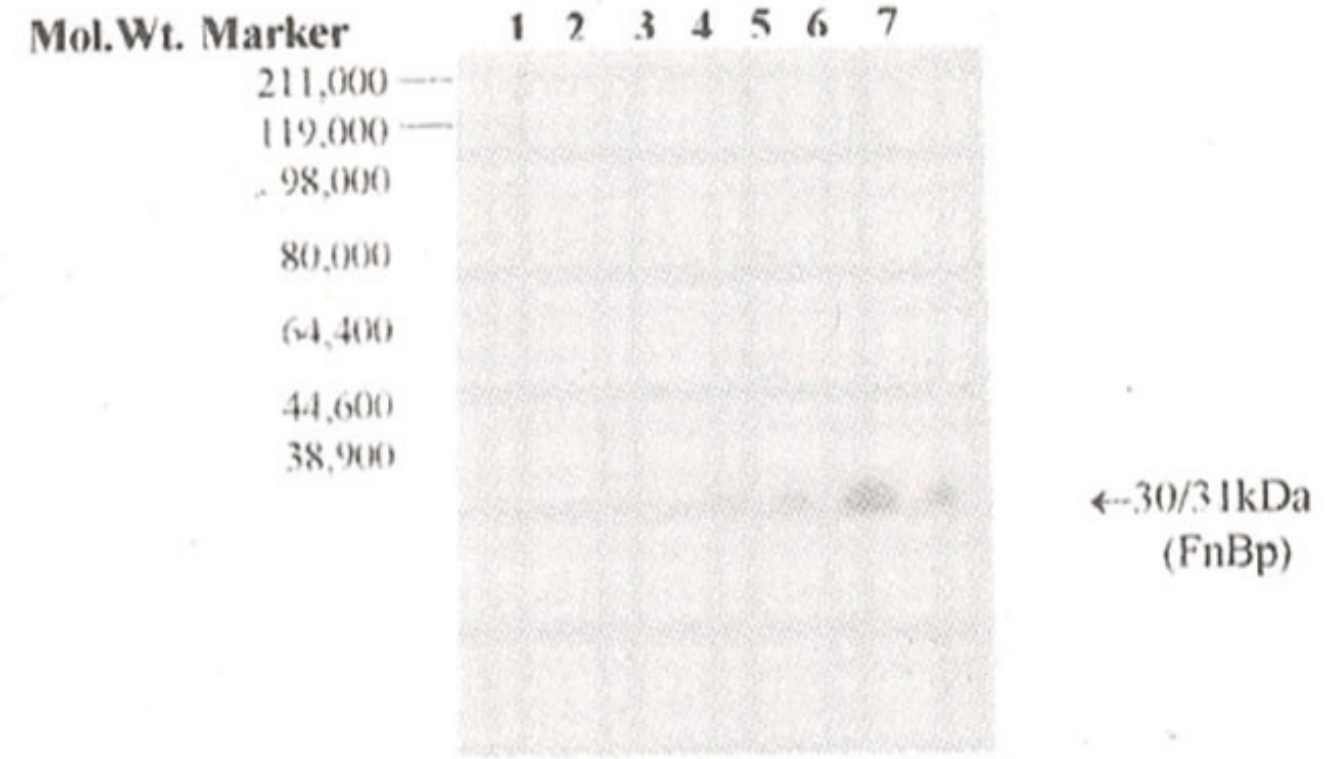


Figure. Western blot analysis of TB patients and normal immune complexes stained with fibronectin - HRP to demonstrate the presence of FnBp of 30/31 kDa in the TB patients samples. 10μg of TB patients and normals serum immune complexes were run on 10% SDS-PAGE. Proteins were transferred onto a nitrocellulose membrane and stained with Fibronectin-HRP (1:1000). Tracks 1-3: Normal serum immune complexes; 4-5 and 7: TB patients serum immune complexes; 6: Mycobacterium tuberculosis sonicate extract (MTSE). Note: Most patients immune complexes have substantial amount of FnBp. Only a few samples are shown in the Figure.

Nonnal serum complexes gave no reaction or very faint staining.

**Discussion**

The 30/31 kDa antigens are secreted FnBp of M. tuberculosis that correspond to antigens 85A and 85B of M. bovis. These antigens are important targets for the antibody response and have been used as a serodiagnostic test[5,6]. A serodiagnostic test has been developed for tuberculosis based upon the detection of anti-30 kDa IgG antibody in patients seia[8]. This test has 100% specificity and 70% sensitivity with pulmonary tuberculosis, but is much less sensitive with extmpulmonary tuberculosis. On the other hand, it was found that this test does not detect individuals with asymptomatic primary infection.

Although the 30/31 kDa FnBp was detected in most of the semm immune complexes of TB patients by western blotting (Figure), we could not detect FnBp using CF8 anti-30/3 1 kDa m-Ab in the samples which were found positive with Fibronectin-HRP. It is possible that the 30/31 kDa molecules have been modified or denatured during SDS-PAGE and lose the epitopes recognised by the CF8 m-Ab. However,

55% of the TB patients were picked up by ELISA using CF8 anti-FnBp m-Ab. A significant difference was seen between normals and TB patients ($p<0.05$). This test had a specificity of 80% and a positive predictive value of 73%. The same result was encountered when a monoclonal antibody to the 30/31 kDa FnBp of M. tuberculosis was evaluated.

The 30/31 kDa antigens are secreted FnBp which may help mycobacteria to enter host cells through fibronectin attachment. This may allow the bacilli to be taken up by macrophages without host cell activation, These antigens have been used in serodiagnostie tests. However, a reliable test based on detection of mycobacterial 30/31 kDa FnBp has not been developed. This study used FnBp detection tests based both on western blotting using Fibronectin-HRP and ELISA using anti-30/3 1 kDa. Fibronectin itself may not be specific when applied in diagnosis of tuberculosis, as fibronectin can recognise fibronectin binding proteins from d.ifferentbacterial sources. However, the CF8 m-Ab is specific for mycobacterial FnBp, and both the anti-30/3 1 kDa and the Fibronectin could be used to sandwich FnBp. A few more FnBp specific rn-Abs could be produced to further develop the antigen-capture ELISA. or a solid phase antibody capture test, the design of which has been successful using other m-Ab specificilies.

The importance of mycobacterial secreted proteins in infection has been suggested by many investigators[9]. Three important antigens are secreted by M. tuberculosis, the 38 kDa, 30/31 kDa and SOD molecules. We developed different ELISA tests for the detection of 30/31 kDA antigens in the TB patients sera, with a specificity of 80% and a positive predictive value of 73% This test needs further evaluation on a larger number of confirmed TB patients and controls.

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