PIDSP Journal, 2000 Vol, 4 No. 1 & 2

CLINICAL EVALUATION OF RAPID IMMUNOCHROMATOGRAPHIC ASSAY USING THE 38 KDA ANTIGEN FROM MYCOBACTERIUM TUBERCULOSIS

ARACELI L. BARRIENTOS, M.D.*, ROSALINDA B. SORIANO, M.D.*

Abstract: **Since diagnosing childhood is still a dilemma for most physician, a rapid immunochromatographic test was developed to circumvent this difficulty. It is a serological assay which detects the antibody against the 38 KDA antigen of mycobacterium tuberculosis in serum or plasma. Its clinical usefulness was evaluated in this study.**

One hundred nineteen patients aged 6 months to 17 years old, admitted at the service ward were categorized into Class I -IV as defined in the 1997 National Consensus of Childhood Tuberculosis were enrolled in the study. Both class I and II had 11 patients each; Class III PTB had 25 cases; Extrapulmonary TB had 28 cases and miliary TB had 10 cases; Class IV had 13 patients. Sixteen patients with diagnosis other than TB were included to serve as control group. Five patients were eliminated because of incomplete data and inability to complete the serial AFB smear.

Complete history and physical examination were done, exposure to adults with tuberculosis was also noted. All patients underwent tuberculin testing, chest radiography, sputum/gastric AFB smear for 3 consecutive days and ICT test.

The test detected 12 out of 25 cases (48%) of PTB, 14 out of 28 cases (50%) of extrapulmonary TB and 7 out of 10 patients (70%) with miliary TB. The sensitivity and specificity of this test in PTB patients were 48% and 94% respectively; in extrapulmonary TB 50% and 94% while in military TB 70% and 94% respectively. Positive predictive value in these groups range from 87%-92%. Interestingly, 2 more patients outside class III had (+) ICT test, 1 from the control group and 1 from class IV, however, statistically they were not significant (pval=>0.05).

The effect of the nutritional status was also evaluated and only patients who were well nourished correlated well with ICT test. In contrast, the role of previous primary booster BCG vaccination did not modify the result of this test.

In conclusion, ICT test had a low sensitivity and high specificity; thus a negative result will not rule out the possibility of TB but a positive test tells us the high probability of the disease. Therefore, this test can be used as an adjunctive laboratory method that can support the existing criteria accepted in our setting. Additional studies are recommended to further validate this test.

Keywords: mycobacterium tuberculosis, TB immunochromatographic test
*Philippine Childrens' Medical Center, Quezon City

INTRODUCTION:

Tuberculosis (TB) remains one of the major health problems in the world, with more than 8 million new cases and approximately 3 million deaths each year[1]. In the Western Pacific Region, the Philippines rank second among the countries with the highest notification of new cases infected with tuberculosis. Effective methods for its control are available and have been applied successfully for several decades. However, the prevalance of tuberculosis is still inordinately high.

The definitive diagnosis of TB continues to rely on microscopy and culture[2]. Refinements in digestion and decontamination methods, development of new tools for differential species identification, and application of numerical taxonomic methods have resulted in sharpened capabilities to isolate Mycobacteria from clinical specimens. However the long generation time of the Mycobacteria and multiplicity of time consuming differential tests needed for species identification and sometimes the falsely negative result are still some of the major limitations of the definitive test. Traditional culture methods often require 4-6 weeks for isolation of the organism and another 2-4 weeks for susceptability testing.

In children, this problem was even confounded because of the poor sensitivity of currently available diagnostic methods. AFB smears in the pediatric age group are positive in only 10 - 20% of cases meaning that 80 - 90% of cases will be missed. The gold standard remains to be the positive culture. For this method, gastric aspirate is the best specimen used in under 6 years old and above this age, sputum is the specimen obtained to isolate *Mycobacterium tuberculosis* (MTB). The yield from the culture of one early morning gastric aspirate from children with pulmonary tuberculosis is fewer than 10%. Hence, three consecutive morning gastric aspirates are recommended to have a higher yield of *M. tuberculosis*, that is, 30 - 50% of cases in children and in infants as high as 70%. In children with extrapulmonary tuberculosis, the yield is 30 - 50%. Because the low

rate of positive yield in these cultures, the diagnosis of tuberculosis in children is often supported only by epidimiologic, clinical and radiographic findings.

At present, improved diagnostic and rapid tests are being revolutionalized; an example is the polymerase chain reaction (PCR) in mycobacterial DNA. This test is highly sensitive but laborious and too expensive for the developing world[5]. Serodiagnostic test for TB was proven inexpensive, hence cultivated. Moreover the speedy procedure and its adherent advantage of obviating the need for a specimen from the site of the disease made this ideal. Recently, however, purity problems and cross-reactive epitopes has resulted in poor sensitivity and only moderate specificity. This later on led to further improvement in the purification and characterization method of a number of a immunodominant antigens specific for this organism. To date, the most sensitive and specific among these antigens has been the 38 kDA protein. The gene for this antigen has been cloned sequence and expressed in *Escherichia coli*. The 38 kDa antigen was reported to be an extracellular lipoprotein involved in phosphate metabolism which is specific for the tuberculosis complex *(M. tuberculosis, M. bovis, M. africanum and M. microti)*. When this protein was applied in an enzyme-linked immunosorbent assay (ELISA) competition assay it yielded a higher sensitivity and specificity as compared to the other serological assay. To further evaluate the significance of this antigen in patients with *M. tuberculosis* a rapid membrane assay system was invented

This is the first local study conducted in pediatric patients to evaluate the efficiency of the rapid immunochromatographic test in the diagnosis of TB infection and disease.

OBJECTIVES

General Objective

To determine the diagnostic value of Rapid Immunochromatographic (ICT) test in childhood tuberculosis.

Specific Objectives

1. To determine and compare the sensitivity and specificity of the rapid immunochromatographic diagnostic test using 38 kD antigen in the detection of Childhood Tuberculosis in both pulmonary and extrapulmonary tuberculosis.
2. To determine the effect of nutritional status in the rapid ICT diagnostic test in children with tuberculosis.
3. To compare the diagnostic efficiency of the rapid ICT in patients who had previous BCG vaccination from those who were not immunized.

METHODS

Recruitment: All the pediatric patients who were admitted in the service ward from July 1,1997 to June 30, 1998 were evaluated for childhood tuberculosis. Patients were categorized into 4 classes based on the 1997 National Concensus on Chilhood Tuberculosis:

TB EXPOSURE (Class I): A child who was exposed to an adult/adolescent with active TB disease, has no signs and symptoms of TB, negative Mantoux tuberculin test and negative chest radiograph.

TB INFECTION (Class II): A child with or without history of exposure to an adult/adolescent with activeTB disease, has positive Mantoux tuberculin test, no signs and symptoms of TB and negative chest radiograph.

TB DISEASE (Class III): a child who has active TB has 3 or more of the following criteria:

1. Exposure to an adult/adolescent with active TB disease.
2. Positive Mantoux tuberculin test. A 5 'TU' PPD test was used. A Mantoux skin test was considered positive with induration of > 5mm for the high risk, non-BCG groups. For BCG-vaccinated children below 5 years old who were recently had contact with infectious TB cases, a >10mm was be considered positive. The tuberculin test was read after 48 - 72 hours.
3. Those with signs and symptoms suggestive of tuberculosis. The symptoms may be one or more of the following: Cough/wheezing > 2 weeks, fever > 2 weeks, painless cervical and /or other lymphodenopathy poor weight gain, failure to make quick return to normal health after therapy (pneumonia, otitis media). Infants (less than 2 years of age) who present with fever and cough,pallor, weight loss, with or without hepatomegaly, splenomegaly was also considered under this category. Diagnosis of extrapulmonary and miliary TB were based on histological and clinical evidence.

4. Abnormal chest radiograph suggestive of TB.
5. Laboratory findings suggestive of TB (histological, cytological, biochemical, immunological, molecular).

TB INACTIVE (Class IV): A child/adolescent with a history of previous TB, with or without previous chemotherapy, had a radiographic evidence of healed/ calcified TB, positive Mantoux tuberculin test, no signs and symptoms suggestive of TB, and negative smear/ culture for *M. tuberculosis*.

The criteria for the **Extrapulmonary Tuberculosis** as defined in the National Consensus, included the following organs: TB meningitis, pleural TB, TB lymphadenitis, endobronchial TB, hepatobiliary TB, renal TB and TB of the bone.

Miliary TB is the disseminated TB disease, including both pulmonary and extrapulmonary manifestation. Diagnosis is base on the multiple foci of infection. Symptoms ranged from occult signs to full-blown picture similar to sepsis depending on the infected site. Patient have fever, marked leococytosis, hepatomegaly, splenomegaly and general glandural enlargement, sometimes with metastatic evidence in the choroid, kidneys and skin. Calcifications may subsequently appear often in large numbers in the pulmonary apices (Simon foci) and the pleura. PPD is usally positive, and the chest x-ray shows millet seedings.

A group of patients with no TB exposure, infection nor disease were included to serve as control. This patients were admitted because of other disease condition like abscess, bronchopneumonia, meningitis, shigellosis, sepsis, Bartters syndrome, and rheumatic heart disease.

The exclusion criteria were as follows: those who were immunocompromised and those who were receiving immunosuppressive regimen.

Information such as the following were also obtained from each patient: age, sex history of present illness, BCG vaccination status, any anti-tuberculosis regimen being taken and the time of the start of therapy. These data were recorded in the case report form of each patient.

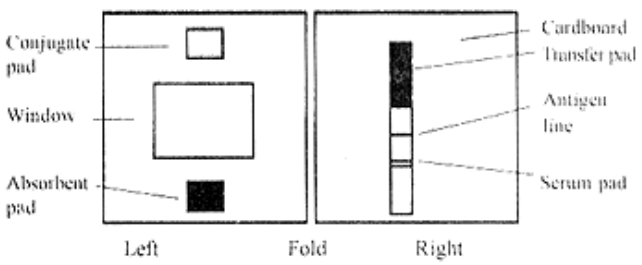
Consent was secured from the parents of each subject. Chest radiography, Mantoux test and gastric/sputum AFB, and culture were requested in all subjects. For the sputum AFB microscopy and culture, it was clarified that the specimen should come from the nasopharyngeal secretions after a productive cough and not mere saliva. AFB smear of the gastric aspirate was done early in the morning, after the patient has fasted for 8 - 10 hours and preferably still on bed. Rapid ICT test was done by collecting 1 cc of serum from each patient. The sample was coded, frozen at 30 to 80°C and sent to the laboratory where the rapid test was done. Information about the clinical diagnosis of enrolled patients was withheld by the principal investigator.

PRINCIPLE AND PROCEDURE OF THE TEST

The ICT-TB detects antibodies to *Mycobacterium tuberculosis* in serum or plasma. Antigens secreted during an active TB infection are immobilized on the nitrocellulose strip. Serum or plasma is added to the blue pad or transfer pad (see below) and migrates down to the nitrocellulose strip past these antigen line. If human IgG antibodies against TB antigens are present in the serum or plasma they will bind to the antigen lines. Upon closure of the test card goat anti-human IgG attached to the colloidal gold, which has been impregnated unto the pink conjogate pad, migrates up to the nitrocellulose and binds to the TB antibodies immobilized on the antigen lines. Serum or plasma containing TB antibodies form one or more pink lines in the positive zone seen through the test card window. A negative serum sample shows no pink lines in the positive zone as no antibodies against TB antigens are present. Invalid test was considered if the control line does not appear. If this occurs the test was repeated using a new test card. The final result of the test was read 15-30 mins after the addition of the sera.

CONTENTS OF THE TEST:

1. 25 individually packed test cards.
2. 1 bottle of reagent A (pink capped bottle).



REAGENTS

The conjugate is a goat anti-human IgG (H+L) linked to 40 nm gold particles (British Biocell International) with an optical density of 3 at a wavelenght of 520 nm. The 38 kDA recombinant antigen was obtained from Omega Diagnostics, UK (lot number E14 1993).

STATISTICAL TEST

Chi-square and 2-tailed Fischer exact test were used in the analysis of the differences of the frequency. The latter was used in cases wherein the frequency were small numbers.

RESULTS

One hundred nineteen patients were enrolled in the study. Class I and II had eleven cases each; Class III PTB had 25 patients, Extrapulmonary TB had 28 patients, miliary TB had 10 cases; Class IV had 13 cases and the control group had 16 cases (fig.1). The breakdown of the involve organs in the extrapulmonary TB are seen below (fig. 2). The patients included in the control group were as follows: Pneumonia 6 cases, occipitoparietal abscess 1, foreign body aspiration 1, Bartter's syndrome 1, bacterial meningitis 3, shigellosis with HIE 1, sepsis 2, and bacterial lymphadenitis 1. Five were eliminated because of incomplete data: Two were discharged prior to PPD reading while 3 patients did not complete the serial AFB smear determination. Three patients were reentered in the study after 2 weeks - 2 months of anti-TB chemotherapy because of readmission. One had pulmonary TB, one with pleural TB and one with TB meningitis. Seven percent (54/114) were female while 53% (60/114) were male. The age of the patients range from 6 months to seventeen years old with mean age of 7 years old.

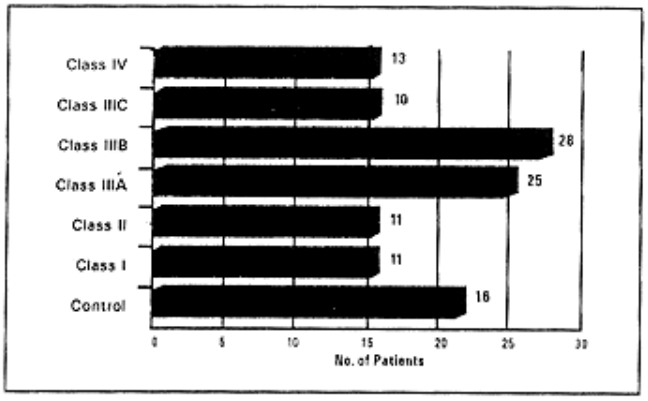


Figure 1. Distribution of Patients Based on TB
Class III A-pulmonary TB; Class III B- Extra pulmonary TB;
Class III C-Miliary TB

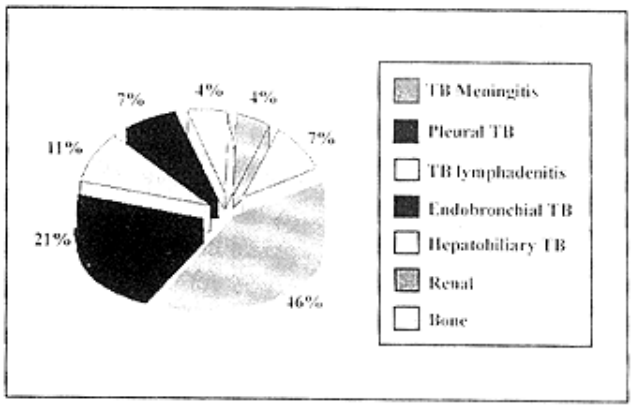


Figure 2. Distribution of involved sites in Extrapulmonary TB

The percentages of patients and control subjects with positive antibody responses to the 38 kDA antigen are shown in fig. 3. The test detected 12 of the 25 patients with pulmonary TB, 14 of the 28 patients with extrapulmonary TB, 7 or 7/10 of the 10 patients with miliary TB.

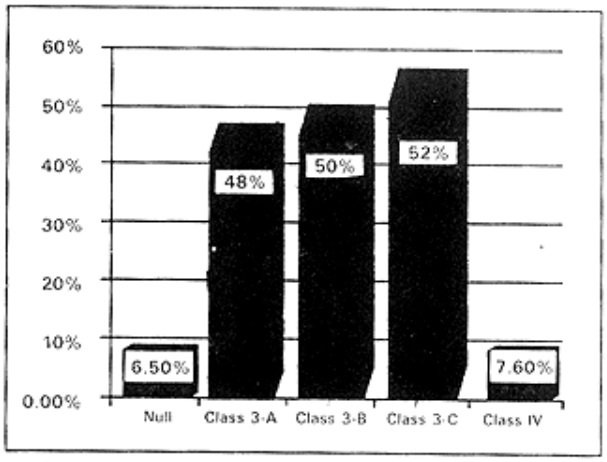


Figure 3. Percentage Distribution of Patients With Positive Immunochromatogrphic Test

Interestingly, 2 more patients had detectable levels of antibody. One (6.50%) from the 60 patients of the control group who had rheumatic heart disease and pneumonia and 1 (7.7%) from the treated cases who had cough and colds at the time of examination. The detection rates of the 38 kDa for these groups were not significantly different (P>0.05). The percentage of the Class III TB patients with positive responses were analyzed with the control goup. The sensitivity, specificity, positive predictive and negative predictive value of the test is shown in table 1.

Table 1. Analysis of the results of the Immunochromato graphy Test Among the Subjects.

	No. of Patients	Freq. of (+) ICT	Sensitivity	Specificity	PPV	NPV	Significance (p2 value <.05)
Null	16	1 (6%)					
Class III	63	33 (52%)	52%	94%	97%	33%	
a. Pulmo	25	12 (48%)	48%	94%	92%	53%	0.005*
b. Extrapul	28	14 (50%)	50%	94%	93%	52%	0.003*
c. Miliary	10	7 (70%)	70%	94%	87%	83%	0.0007*

*Chi-Square; Mantel-Haenszel test was used to compute the significance and the P-value <0.05 was considered significant

Analysis of each of the involved organs in the extrapulmonary TB patients were not possible because of the frequency of the cases.

Nutritional status of the general population was assesed and 44% (54/114) had wasting while 24% (27/114) had stunting. In particular, Class III PTB patients had 44% (11/25) and 16% (4/25) wasting and stunting, respectively. In correlation with the ICT, it did not show any significant difference, Likewise; in the extrapulmonary TB and miliary TB show the same results (Table 2). In contrast, however, when the analysis in the general population was stratified according to the degree of wasting and stunting and regardless of the involved organs, patients who were wasted or stunted show a good correlation with this test.

Table 2. Relationship of Nutritional Status and ICT in Class III Patients

	Wasting		Stunting	
	Frequency of Px with (+) ICT	Significance	Frequency of Px with (+) ICT	Significance
I. Pulmonary TB				
Normal	7/14 (50%)	p~0.0005*	10/21 (27%)	p~0.000018*
Mild	0/4 (0%)	NA	2/3 (66%)	NA
Moderate	4/5 (80%)	NA	0/0 (0%)	NA
Severe	1/2 (50%)	NA	0/1 (0%)	NA
Combined	5/11 (45%)	p~0.821	2/4 (50%)	p~>0.05
II. Extrapulmonary TB				
Normal	5/9 (55%)	p~0.0005*	9/17 (53%)	p~0.000018*
Mild	4/9 (44%)	NA	4/8 (50%)	NA
Moderate	4/8 (50%)	NA	1/2 (50%)	NA
Severe	1/2 (50%)	NA	0/1 (0%)	NA
Combined	9/19 (47%)	p~>0.709	5/11 (45%)	p~>0.05
III. Miliary TB				
Normal	1/1 (100%)	p~0.0005*	4/5 (80%)	p~0.000018*
Mild	0/0 (0%)	NA	3/4 (75%)	NA
Moderate	4/4 (100%)	NA	0/1 (0%)	NA
Severe	2/5 (40%)	NA	0/0 (0%)	NA
Combined	6/9 (67%)	p~1.0	3/5 (60%)	p~>0.05

NA - Not applicable because of the frequency of patients
* Stratified analysis with Chi-Square was employed to compute the significance among the general population

Primary BCG was seen in 75% (85/114) of the general population, while booster dose in patients above 7 years old was only 40% (23/58). In Class III PTB patients, statistical analysis did not show any association between primary BCG and (+) ICT (Table 3). Likewise, similar results were noted in extrapulmonary TB and miliary cases. Moreover, BCG booster dose did not modify the results of the ICT.

Table 3. Relationship of Previous BCG Immunization with Immunochromatographic Test

Classification	BCG 1		BCG 2	
	Frequency of Px with (+) ICT	Significance p-val	Frequency of Px with (+) ICT	Significance p-val
Null	1/15(7%)	3/10(30%)		
Class III				
A. Pulmonary	6/14(43%)	0.07	3/15(20%)	1.0
B. Extrapulmonary	5/9(83%)	0.38	1/7(14%)	0.41
C. Miliary	14/27(52%)	0.70	1/12(8.3%)	0.28
Class IV	1/8 (12%)	1.0	0(0%)	

Fischer exact test 2 -tailed P-Value was used to compute the significance P-Val=<0.05 is considered significant

The most qualifying criteria noted in diagnosing tuberculosis in this study were: signs and symptoms (100%) like chronic cough, prolonged fever and weight loss (Fig. 4B), positive radiology findings (94%) and positive mantoux test (81%). Exposure to adults with tuberculosis (75%) was variable while the (+) AFB smear (13%) was inconsistent (Fig. 4A).

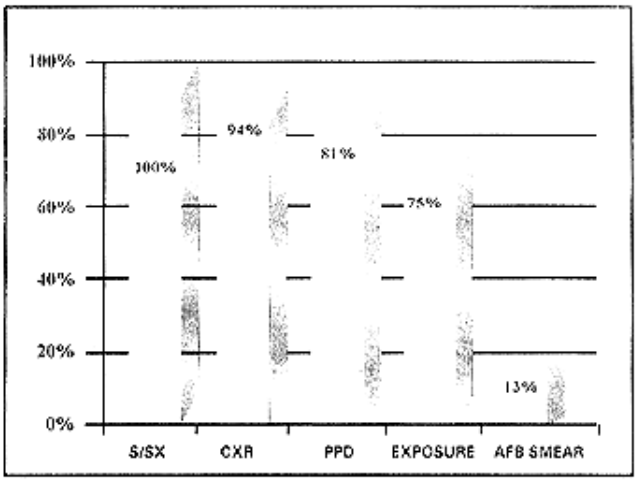


Fig 4A. Frequency of Qualifying Criteria for Class III Patients

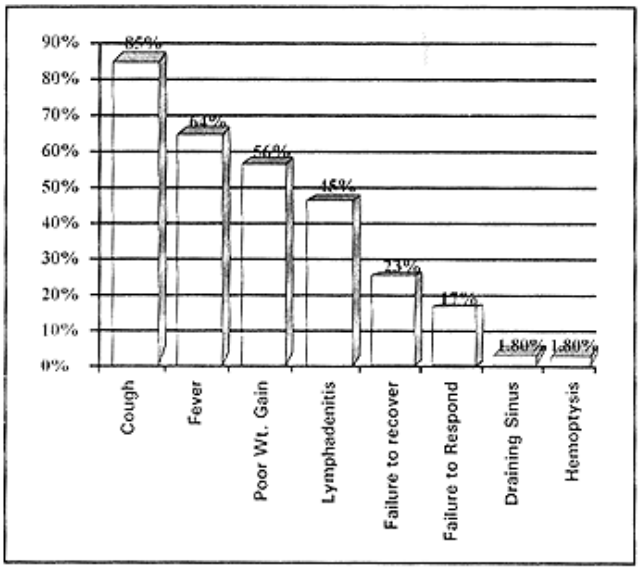


Figure 4B. Frequency of the Signs and Symptoms Present in the Subjects

DISCUSSION

The diagnosis of Class III TB cases in children is difficult. It is delayed in many cases and frequently made on circumstantial evidence alone. The clinical manifestations are varied and often mimic other diseases, the Ziehl-Neelsen smear is rarely positive, and culture is prohibitively slow.

Recent studies have indicated that the secreted proteins from actively growing *M. tuberculosis* plays a major role in the host immunity. It activates both the cellular and humoral immunity[13, 14] despite the questionable role of the latter in the host defense. The response to secreted antigens it produce, in particular the immunoglobulin IgG, IgA[15] and IgM[16] are mainly mediated by CD4+ Th-1-like lymphocytes and the gamma interferon release by these cells[17]. This hypothesis has led to renewed and intensive research on the isolation and characterization of many secreted proteins of *M. tuberculosis*. Verbon et al evaluated the different purified proteins: MW 10,000, 16,000, 24,000 and 38 kDAof *M. tuberculosis*. Among these proteins the 38 kDA antigen showed the highest sensitivity and specificity at 51% and 95%, respectively.

In this study, comparison between TB patients and the control group showed statistical significance however, their sensitivity was consistently low while the specificity remained high (table 1). The results of the test were lower as compared to the study done by Cole[19] and Zhou[20] who used the same test (Figure 5). The sensitivity and specificity in their study were higher at 72-74% and 92-93% for both pulmonary TB and extrapulmonary TB, respectively. The difference can be attributed to several factors like the setting and the study population. Their research was done in China, wherein the infectious rate is over 80-90%[21] and their study population was predominantly adult, in contrast to this study wherein the study population was purely pediatric. In several studies[22] TB serology validation is affected mainly by the endemicity of the disease in that setting, age and race. Moreover, the immunological response of a child to TB infection is different from adult patients[24].

Miliary and extrapulmonary TB likewise, exhibited good correlation with the ICT test (Table 1). However, in TB of the bone, TB meningitis the frequency of positive result appears to alter the trend of the results (Table 4). These cases can probably be explained by Sharma et al[25] wherein he suggested that lymphocytes were compartmentalized at the site of inflammation. He compared the cellular and humoral constituent on the peripheral and broncho alveolar lavage fluid in miliary and pleural TB. He noted, that there was an increase in helper lymphocytes in bronchial alveolar fluid in contrast to the depletion in peripheral blood in TB patients. Thus, limiting the humoral and cellular immunity activity to the said site and not on the peripheral blood. These observation may explain the state of relative energy encountered in patients and probably the explanation in some of the patients who exhibited a negative response in 38KDA antigen of the *Mycobacterium tuberculosis* in the ICT test.

The AFB smear of the study group was not significantly helpful in the early diagnosis of TB (12%). This is in agreement with several studies.[26,27] This is mainly because of the low tubercle bacilli density in the gastric aspirate and sputum. Moreover, in children the organism is contained in the regional lymph nodes where they replicate slowly and intermittently.[28]

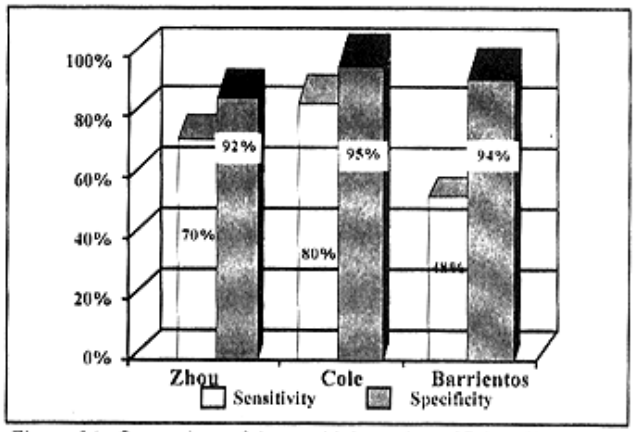


Figure 5A. Comparison of the sensitivity and specificity of ICT results in patients with Pulmonary TB

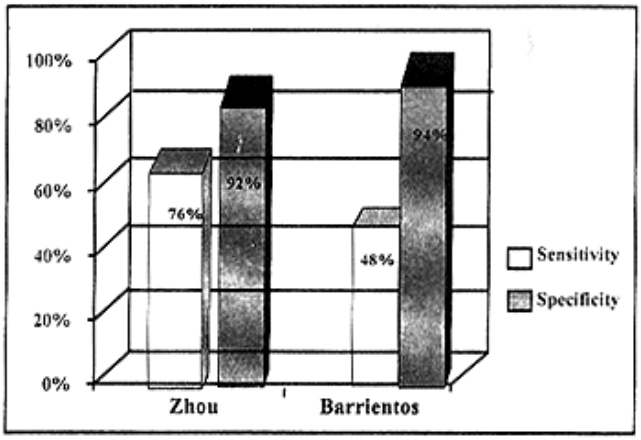


Figure 5B. Comparison of the sensitivity and specificity of ICT results in patients with Extrapulmonary TB

From the qualifying criteria signs and symptoms were consequently present in all patients and among them, chronic cough, prolonged fever and poor weight gain top the list (Fig 4B). This was not in agreement with the findings of Soriano R[29]. In her survey, wherein 3 most common symptoms noted by the practicing clinicians were failure to make a quick return to normal health after an infection, presence of painless cervical lymphadenopathy and non-responsive to appropriate drugs. Probably, this can be explained by the fact that the latter was limited in an outpatient setting, in contrast to this study.

The effect of the nutritional status was also evaluated in this study. The degree of wasting and stunting were stratified (table 2) and as shown, only those patients who were well nourished correlated significantly with the ICT. In the study of Chanda RK[30] he noted that malnutrition greatly affect the immunological parameters of an individual, in particular, the rosette-forming T-lymphocytes and cell-mediated immunity. It is a known fact that humoral immunity is dependent on the activity of the cellular immunity, thus, any defective cellular function may ultimately affect the production of immunoglobulins, therefore becomes the limitation of any serological test such as this test wherein the antibody level response is essential in its evaluation. In this study in particular, in miliary TB, 3 (3/10) exhibited negative results to ICT and interestingly, all were severely wasted but not necessarily stunted. Likewise, in extrapulmonary TB, out of 14 (50%) patients who were negative for ICT, 10 had wasting while 6 had stunting. In pulmonary TB, out of the 11 which is equivalent to (44%) of the patients who did not have any antibody response to the antigen, only 6 had wasting and 2 had stunting.

The effect of BCG vaccination in the detection of the antigen of TB has two point of views. In the study of Zhou et al[20] he assumed that BCG immunization in children affects the ICT test since they exhibited a higher percentage of positive result (9%) than the adult control group (2%). In contrast, Wilkins EGL and Ivanyi J[12] found no significant findings in the antibody titers between patients with diseases other than TB and BCG-immunized control patients. This finding is in agreement with the present study, more so with booster dose in relation to the ICT. This is rationalize by the fact that the antigen present in the BCG vaccine is only 1/10 of the concentration of *M. tuberculosis* antigen[19], therefore the antibody levels to this antigen from the *M. bovis* BCG vaccination would be expected to be very high or remain in circulation very long unless the organism is actively growing in the host causing clinical and subclinical infection.

Three of these patients were reentered in the study and 2 of them had an initial negative (-) result, however after taking the anti-TB regimen for 2 weeks to 2 months, the findings circumvented to a positive result . Although it is not within the scope of this study the possible rate of taking the anti-TB regimen prior to the ICT test is apparent in several of the studies[31,32] and is an interisting point that needs further investigation.

Table 4. Distribution of Immunochromatographic test in Extrapulmonary TB

Organ Involved	Positive ICT (%)	Negative ICT (%)	Total
1. Meninges	3 (23%)	10 (77%)	13
2. Pleura	4 (66%)	2 (33%)	6
3. Bone	1 (33%)	2 (66%)	3
4. Endobronchus	2 (100%)	0	2
5. Lymphadenoid	2 (100%)	0	2
6. Hepatobiliary t.	1 (100%)	0	1
7. Renal	1 (100%)	0	1

CONCLUSION

In a disease with high morbidity and mortality rates as tuberculosis, whose confirmatory diagnosis is still a dilemna, rapid immunochromatography test is an optional tool that may assist clinicians in their decision whether to treat or not to treat patients with signs and symptoms highly suggestive of tuberculosis. It revealed a low sensitivity and high specificity, thus a negative result will not rule out the possibility of TB. However, a positive result would tell us the high probabilty of the disease. Limitations are realized, such

that the nutritional status, age and probably the site of the extrapulmonary TB (e.g. TB meningitis and TB of the bone) should be checked upon prior to the use of this test since it may modify the result, in contrast to the previous BCG vaccination status. The traditional mantoux test, chest radiography, AFB smear straining, culture and PCR are still the main focus of the diagnosis, but ICT can also help clinical practioners since it justifies that chemotherapy be started earlier than the conventional method. It is recommended that additional studies be conducted to validate further the use of this simple inexpensive test.

REFERENCES

1. WHO. Epidemiological Review of Tuberculosis in the Western Pacific Region (An Update). 1996.
2. Joklik w. et al. (eds): Zinsser Microbilogy. 20th Edition. Connecticut. U.S.A. 1992
3. Starke Jeffrey. New Concepts in the Diagnosis of Tuberculosis in Children. Proceedings: PIDSP 2nd Annual Convention. 1995; p. 10
4. Soriano R et al National Concensus on Childhood Tuberculosis 1997. p. 3
5. Beige J. Lokier J. Schaberg T et al. Clinical Evaluation of Mycobacterium tuberculosis PCR assay. J. Clin. Microbio. 1995: 33: 90-95.
6. Quadri SMH. Sith KK Nonspecificity of the Anda A60-tb ELISA Test for Serodiagnosis of Mycobacterial disease. Can J. Microbio. 1992: 38: 804-806
7. Sad E. Brenner PJ. Herrera T et al . Eval. Of Lipoarbinomannan of Serodiagnosis of Tuberculosis. J Clin. Microbio. 28: 2587-2590
8. Jackett PS. Bothamley GH. Batra HV et al. Specificity of Ab to immunodominant Mycobacterial Ag in Pulmonary tuberculosis. J. Clin. Microbiology. 1988. 26: 2313-2318.
9. Young D. Kent L. and Rees A et al. Immunological Activity of 38 kDA protein purified from tuberculosis. Infect Immune 1986; 54: 177-183.
10. Anderson AB. Hansen GB. Structure and Mapping of Antigen Domains of Protein Antigen b, a 38 000-Molecular wt. Protein of M. tubeculosis. Infec Imman. 1981; 57: 2481-2488.
11. Chang Z. Chondhory A. Lathigra R. et al. The immunodominant 38kDa Lipoprotein Ag of M. tuberculosis is a phospate-binding Protein. J. Biol. Chem. 1994; 262: 1956-1958.
12. Wilkins EG, and Ivanji J. Potential Value of Serology for the Diagnosis of Extrapulmonary TB. Lancet. 1990; 336: 641-644.
13. Bothamly GH, and Rudd RM. Clinical Evaluation of Serological Assay using a Monoclonal Antibody (TB72) to the 38 kDa of Mycobacterium tuberculosis. Eur. Respir. J. 1994; 7: 240-246
14. Starke JR. et al. Resurgence of Tuberculosis in Children. The J. of Pedia. 1992; 120 (6): 839-847.
15. Faulkner E. John M, Katilus J. et al. The Purification and Identification of Circulating Immune Complexes in Tuberculosis. Ame. Rev. Respir. Dis. 1983; 128: 920-925.
16. Wong CF and Saha N. Changes in Serum Protein (albumin, Immunoglobulin and Acute Phase Proteins) in Pulmonary TB during Therapy. Tubercle (1990) 71, 1993-97
17. Boesen H, Jensen BN, Wilke T and Anderson P. Human T-cell responses to Secreted antigen Fractions of M. tuberculosis. Infect. Immun. 1995; 63(4): 1491-7.
18. Verbon A. Wevering GJ and Kugiper S. et al. Evaluation of Different Tests for the Serodiagnosis of Tuberculosis and the use of Likelihood Ratios in [illegible] Ame. Rev. Respir. Dis. 1993; 148:378-84
19. Cole RA, Lu HM Shi, Wang J. et al. Clinical Evaluation of Rapid Immunochromatographic Test Assay Based on the 38 kDA Antigen of Mycobacterium tuberculosis on patients with Pulmonary Tb in China. Tubercle and Lung Disease.1996; 77: 363-368
20. Zhou AT, Ma WL, Zhang PY and Cole RA. Detection of Pulmonary and Extrapulmonary TB Patients with the 38 kDA Antigen from Mycobacterium TB in a Rapid membrane-based Assay. Clinical and Diagnostic Laboratory Immunology. 1996; 3(3): 337-341.
21. Gan GQ. Zhang LX. and Ma TQ. Evaluation and Tuberculosis Control Epidimiological parameters Bull. China. Antitubercle. Asso. 1990; 12: 49-50
22. Turner M, Van Nerom E and Devilde W. et al Immunoglobulin against Purified 32 and 64 kDa protein and Antigen 60 from Mycobacterium bovis BCG and against PPD in Active Tuberculosis Ame. J. Rev. of Respir. Dis. 1990; 141. A806.
23. Thomas DM, Debanne SM. The Serodiagnosis of Tuberculosis and other Mycobacterial Diseases by Enzyme-linked Dis. 1987; 135: 1137-1151.
24. Haas D. and Des Perez F. Mycobacterium Tuberculosis. In Mandell G. Bennet J. and Raphael D. (eds.) Principles And Practice of Infectious Diseases.. 4th. ed. Chuchill Livinstone, New York, 1995; 2213- 2241
25. Sharma SK, Pande JN Yadu NS et al. Pulmonary Function and Immunological Abnormality In Miliary tuberculosis. Tubercle. 1988; 69: 174-177.
26. Starke JR. Taylor-Watts KT. Tuberculosis in the

Pediatric Population of Houston Texas. Pediatrics 1989; 84: 28-35.
27. Shimnick T and Good RC. Diagnostic Mycobacteriology Laboratory Practices./ Clinical Infectious Diseases. 1995; 21: 291-9.
28. Starke J and Smith M. Tuberculosis. In: Feigin R. Cherry J (eds.). Textbook of Pediatric Infectious Diseases. 2nd ed. WB Saunders Company, Philadepphia. 1998; 1196-1230.
29. Soriano R and Olazo R. A survey on the experience of Practicing Physicians on Clinical Recognition and Diagnosis of Childhood Tuberculosis. 1997 (Unpublished).
30. Chandra RK. Rosette-forming T-lymphocytes and cell-mediated Immunity in Malnutrition. Br. Med. J. 1974; 3: 608-609.
31. Bothamly G, Rudd R, Festenstein F and Ivanji J. Antibdoy Levels to Tuberculosis During treatment of Smear positive Tuberculosis. Ame. Rev. of Respir. Dis. 1990; 141: A805.
32. Pauw HF, Heyns A and Du P. The Effect of Oral INH on Human Lymphocyte Subpopulations. S. Afr. Med. J. 1979; 55: 86-88.
33. Zafran N, Heldal E, Pavlovic S, et al. Why do our patients Die of Active TB in the Era of Effective Therapy. Tuber. Lung Dis. 1994, Oct; 75(5): 329-333.
34. Anderson P. And Heron I. 1993. Specificity of a Protective Memory Immune Response Against M. tuberculosis. Infect. Immune. 61: 884-851.
35. Dunlap NE. and Briles DE. Immunology of Tuberculosis. Med. Clin. North Ame. 1993; Nov. 77(6): 1235-51.

NOSOCOMIAL INFECTIONS: A NEED FOR CONTINUED SURVEILLANCE

MARIA TERESA P. FABRE, M.D.*, MARIA CARMEN B. NIEVERA, M.D.*, THELMA M. LAO, M.D.*
MA. INES BETTINA L. SANTOS, M.D.*, LULU C. BRAVO, M.D.*

Abstract: **Whenever a nosocomial infection (NI) occurs, the hospital, instead of serving as an institution where patients get cured of their illnesses, becomes a threat to a patient's life. Thus, with the objective of determining the occurrence of NI's among paitents admitted to the pediatric wards, pediatric intensive care unit (PICU) and nursery intensive care unit (NICU) of a tertiary hospital, this one-year prospective epidemiologic study was conducted. Sensitivities of all isolates were done using the disk diffusion method. NI rates were as follows - 17% each for the two pediatric wards, 18% for PICU and 14% for NICU. Except for NICU where cases of necrotizing enterocolities (NEC) ranked second, the top three diseases were sepsis, pneumonia and urinary tract infection (UTI). Overall, the predominant organisms for sepsis were *Enterobacter spp' Candida* and *Pseudomonas spp.* For pneumonia, as much as 56% had no identifiable pathogen. The rest were gram-negtive organisms. *Candida* comprised a little more than half (51%) of UTI islates. In both sepsis and UTI, only about a quarter of *Candida* was identified as *C. albicans*. The following were the antibiotics which showed favorable sensitivity patters in general - amikacin, piperacillin-tazobactam, imipenem and ciprogloxacin. Strict adherence to infection control measures as well as more prudent and restrictive use of antibiotics should always be practiced if a reduction in NI is to be achieved.**

Keywords: nosocomial infection, nosocomial rate sepsis
*Department of Pediatrics, UPCM-PGH

INTRODUCTION

A hospital is supposed to be a place where patients get cured of their illnesses. There is one instance, however, when this very institution becomes a threat to a patient's life. This occurs whenever a NI sets in. A hospital-acquired or nosocomial infection is one that is neither present nor incubating at the time of a patient's admission [1].

As healthcare providers, we are tasked to give the utmost care to the patients, ensuring their safety while in the hospital and making sure they are well on discharge. Several risk factors, both inherent in each patient as well as environmental exposures, will have to be dealt with in order to meet that goal. Among those significantly associated with NI in two local studies were age, number of antibiotics prior to NI, nasogastric and orogastric tube insertions, use of steroids, underlying disease, ventilatory support and even blood transfusion [2,3].

With all these in mind, NI Surveillance in a tertiary care center which caters to a huge bulk of patients daily should be a top priority. Knowing where it occurs, what pathogens are likely to be encountered, and to which antimicrobials they are sensitive to will aid in the early recognition and management of such cases. Only then can the added physical and financial burden placed on the patients and their relatives be addressed.