

RELATIONSHIP BETWEEN CD4 LYMPHOCYTE LEVELS AND PPD SKIN TEST MEASUREMENT IN ADULT AIDS PATIENTS WITH PULMONARY TUBERCULOSIS

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SUMMARY

A simple and inexpensive procedure like the Purified protein derivative (PPD) skin test may be alternative marker of disease progression in human immunodeficiency virus (HIV) infected individuals. The correlation between the PPD induced skin induration and the CD4 T lymphocyte levels (absolute CD4 counts and percentages) was evaluated in acquired immunodeficiency syndrome (AIDS) patients with pulmonary tuberculosis. This pilot study was carried out at the Chonburi General hospital and the zonal tuberculosis (TB) center in Chonburi, Thailand. A total of 22 AIDS patients with pulmonary tuberculosis and 20 HIV seronegative pulmonary tuberculosis patients with (controls) were skin tested with 5 tuberculin unit (TU) of PPD. Anergy was observed in 82% of AIDS cases, all with very low CD4 counts. The remaining 18% showed skin reactions ranging between 1mm and 9mm. The parsons correlation coefficient test showed a good correlation between the PPD induration measurement and the absolute CD4 counts. The coefficient $r = 0.79$ ($p < 0.001$) with the absolute CD4 counts, and $r = 0.60$ ($p < 0.001$) with the percentage of CD4 lymphocytes. In the control group, no such correlation was found and anergy was not observed in any of the patients. With a larger sample size, the association may be better established and may predict the level of CD4 T lymphocytes and hence disease progression, especially in areas of high case load of AIDS and tuberculosis.

INTRODUCTION

The AIDS pandemic is spreading rapidly, particularly in the poorer developing nations. HIV being the most significant risk factor for progression of latent mycobacterium infection to active tuberculosis, tuberculosis had become the leading opportunistic infection in AIDS in parts of Asia and Africa.

The CD4 T lymphocyte counts have been shown to be the best marker of disease progression to AIDS.^{1,2} The concept of a decrease in CD4 counts and progression of HIV infection to AIDS has therefore been incorporated in the new CDC case definition of AIDS.³ Counts below 200 cell/ul are known to be an important landmark. Below this level patients are prone to develop a host of opportunistic infections, tuberculosis

TABLE - I
 BASELINE CHARACTERISTICS OF STUDY PATIENTS

Characteristic	AIDS group <i>n</i> = 22	Control group <i>n</i> = 20	<i>p</i> value
Age: years			
Mean ± SD	30 ± 7.2	31 ± 9.4	0.16
Range	20 - 45	20 - 49	
CD count: cells/μl			
Median	20	435	<0.001
Count (Range)	(0-490)	(220-1070)	
0-50	14(63%)	0	
51-200	4(18.5%)	0	
201-500	4(18.5%)	13(65%)	<0.001
>500	0	7(35%)	
CD4 percentage			
>14%	4(14%)	19(95%)	
<14%	18(86%)	1(5%)	
Median	3	28	<0.001
Percentage (Range)	(0 - 27)	(13 - 45)	
PPD Response:			
Reactors	4(18%)	20(100%)	<0.001
Non Reactors	18(82%)	0	
BCG vaccination	15(68%)	10(50%)	0.37

being the first and most important.⁴ But CD4 counts is and elaborate procedure, is expensive and subject to certain limitations. Diurnal variation, laboratory to laboratory variation and variation in pregnancy have been reported.^{3,5} There is a need to identify alternative and cheaper markers of disease progression.

The delayed type of hypersensitivity (DTH) response has been shown to be impaired in HIV infection.^{4,6,9} A study in the US, using PPD and four control DTH antigens, mumps, candida, tetanus toxoid and trichophyton concluded that DTH skin response, a measure of cellular immunity, is an independent predictor of progression to AIDS in HIV infection.¹⁰ Another study found a strong inverse relationship between

CD4 lymphocyte counts and anergy.¹¹ Our study was done exclusively on AIDS patients and patients with pulmonary tuberculosis, using PPD as a single DTH antigen. The aim was to evaluate the correlation between CD4 T lymphocyte levels, both the absolute counts and percentages and the size of PPD induced skin test measurements in AIDS patients with pulmonary tuberculosis and to estimate the cut off level of CD4 counts below which anergy is observed.

MATERIAL AND METHODS

This was a cross sectional study and was carried out from November 1995 to January 1996 in the Chonburi General Hospital and the zonal TB center, Chonburi central Thailand.

TABLE – II
PPD SKIN REACTION AND CD4 CELL
COUNT IN AIDS GROUP

PPD skin induration (mm)	n (%)	Median CD4 count (range)
Size of reaction: 0	18 (82%)	65 (0–360)
1–4	2 (9%)	332 (300–365)
5–9	2 (9%)	347 (200–495)

Subjects

A total of 42 consecutive adult male patients, between 20 to 49 years of age, with newly diagnosed pulmonary tuberculosis as suggested by positive acid fast bacilli in sputum smears (Zeil Nelson stain) and chest radiograph and who consented to HIV serological testing, were included in the study. Patients with miliary and extra pulmonary tuberculosis, patients on immunosuppressive and antitubercular and antiretroviral therapy, injectable drug users (IDU) and cancer patients were excluded from the study. Demographic and clinical information and a medical history were obtained and physical examination was performed.

Serum specimens were collected and tested for anti HIV antibody (two times with ELISA and once with particulate agglutination test). Whole blood samples were collected for CD4 lymphocyte phenotyping by flow cytometry (FAC Scan; Becton Dickinson, Erembodegem, Belgium). Specimens were consistently drawn between 7.00 AM and 9.00 AM to minimize the effects of diurnal variation on absolute CD4 counts.⁵ All patients received the standard, 0.1 ml (5TU) of PPD (Thai Red Cross) intradermally on the forearm by the Mantoux technique and the induration was measured transversely after 48 to 72 hours. Anergy was labeled as no skin reaction after 72 hours.

All the study patients were divided into two groups on the basis of their HIV status.

All HIV seropositive patients comprised the AIDS group and the HIV seronegative patients served as controls. Pre HIV and post HIV test counseling was given to all patients and confidentiality was maintained throughout the study.

Statistical Analysis

Data was entered in a database and analyzed with the EPI info 6 software program. The parsons correlation coefficient test was used to measure the association between the PPD induced skin induration measurement and the CD4 T lymphocyte levels (absolute counts and percentage). A p-value of less than 0.05 was considered as an evidence of statistical significant.

RESULTS

A total of 42 patients were included in the study. All participants were Thai nationals. There were 22 patients in the AIDS group and 20 patients in the controls. The mean age and range in both the group was comparable. The mean \pm SD age was 30 ± 7.2 (range 20–45 years) in the AIDS group and 31 ± 9.49 (range 20–49 years) in the control group ($p=0.16$)

Very low levels of CD4 lymphocyte counts were observed in the AIDS patients. The absolute CD4 count ranged between 0 and 490 cells/ul (median count 20 cells/ul) in the AIDS group, as compared to the counts in the range of 220–1070 cells/ul (median count of 435 cells/ul) in the control group ($p<0.001$). The cell counts were

TABLE – III
PPD SKIN REACTION AND CD4 CELL
COUNT IN CONTROL GROUP

PPD skin induration (mm)	n (%)	Median CD4 count (range)
Size of reaction: 1–4	0	
5–9	8 (40%)	455 (260-950)
>–10	12 (60%)	380 (220–1070)

stratified into different levels, and it was seen that, in the 22 AIDS patients, 14(63%) had counts between 0–50 cells/ul, 4(18.5%) between 51–200 cells/ul and the remaining 4 between 201–500 cells/ul. While in the controls, 13(65%) had counts of 201–500 cells/ul and 7(35%) were above 500 cells/ul. The percentage of CD4 lymphocytes was also different in the two groups. The median CD4 % (range was 3% (0–27) in the AIDS cases and 28% (13–45) in the controls ($p < 0.001$) table-I.

Regarding ranging between 1–9 mm and 18(82%) were anergic to PPD, while none of the patients in both the group had been skin tested with PPD in the past. The PPD positive patients were stratified according to the size of skin reaction and the median CD4 counts in that group. It was shown that the median CD4 counts in these groups increased with an increase in the size of the skin reaction. This was only seen in the AIDS cases. The median CD4 count among the anergic patients was 65 cell/ul. It was 332 cells/ul in the 1–4 mm group and 347 cell/ul in the 5–19 mm patients table-II. In the control group all patients

showed reactions in the range of 5–19 mm, with a median CD4 count of 455 in the 5–9 mm patients and 380 in the >10mm patients table-III.

Using parsons correlation coefficient test an association was found between the size of the PPD skin induration and the absolute CD4 count in the AIDS case. Correlation coefficient, $r=0.97$ ($p < 0.001$) between PPD induration and absolute CD4 count and $r=0.60$ ($p < 0.001$) between PPD induration and percentage of CD4 lymphocytes Figure-1 and 2. No association was observed in the controls, with $r=0.01$ with the absolute CD4 count and $r=0.02$ with percentage of CD4 lymphocytes ($p < 0.001$) Figure 3 and 4.

DISCUSSION

Since cell mediated immunity is impaired in HIV infection, it has been seen that the DTH response to PPD and other skin test antigens is decreased and anergy is observed in advanced disease conditions.¹⁰ The association between CD4 levels and the PPD skin test anergy has been the subject of several studies. An inverse relationship

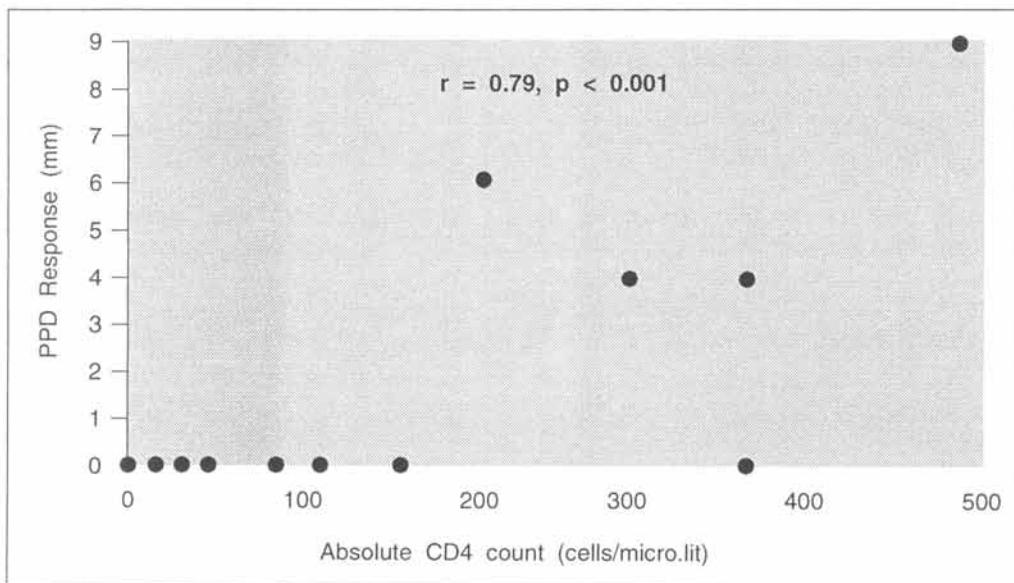


Fig. 1: Scatter plot between absolute CD4 counts and PPD skin response in AIDS group.

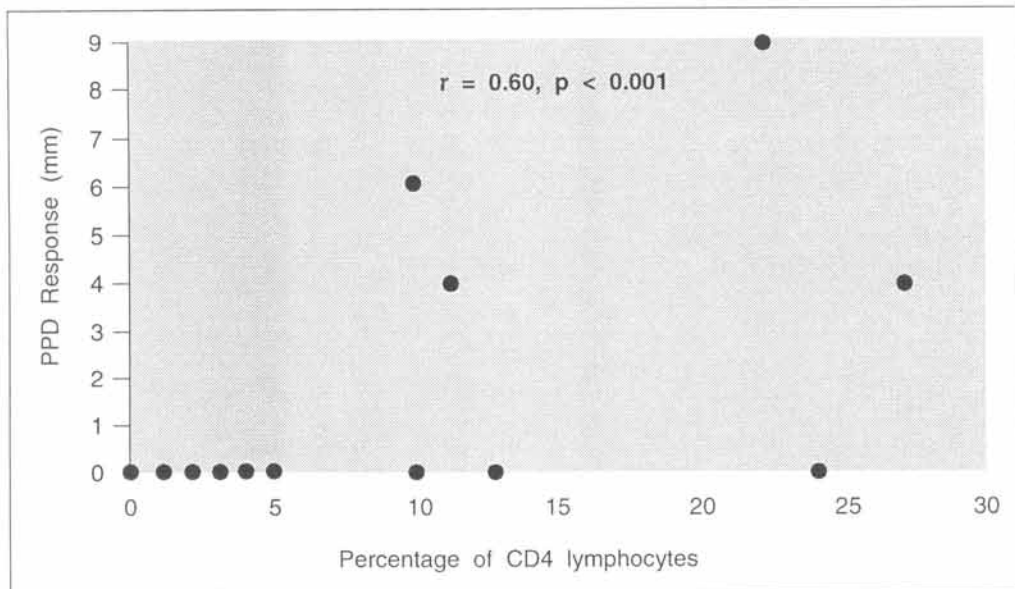


Fig. 2: Scatter plot between percentage of CD4 lymphocytes and PPD skin response in AIDS group.

between CD4 counts and anergy has been found, with concomitant decrease in PPD reactivity seen with lower CD4 counts.⁶ In our study, PPD was used alone since, DTH being antigen specific, patients of pulmo-

nary tuberculosis are expected to show a better response with PPD than with other antigens.¹² Tuberculin testing which has historically been used as a screening apparatus for tuberculosis, has limited

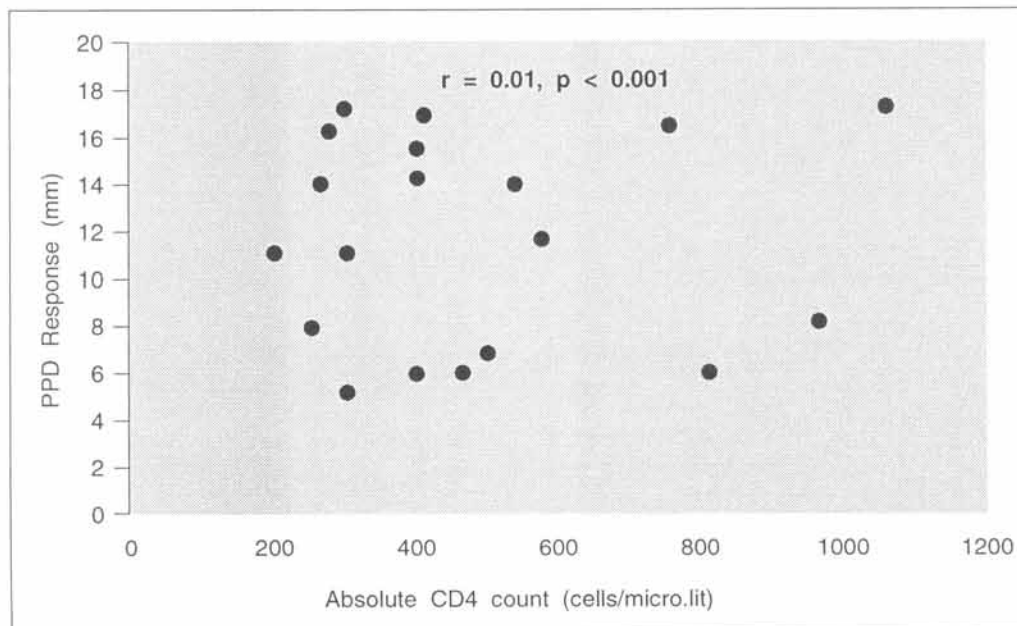


Fig. 3: Scatter plot between percentage of CD4 lymphocytes and PPD skin response in control group.

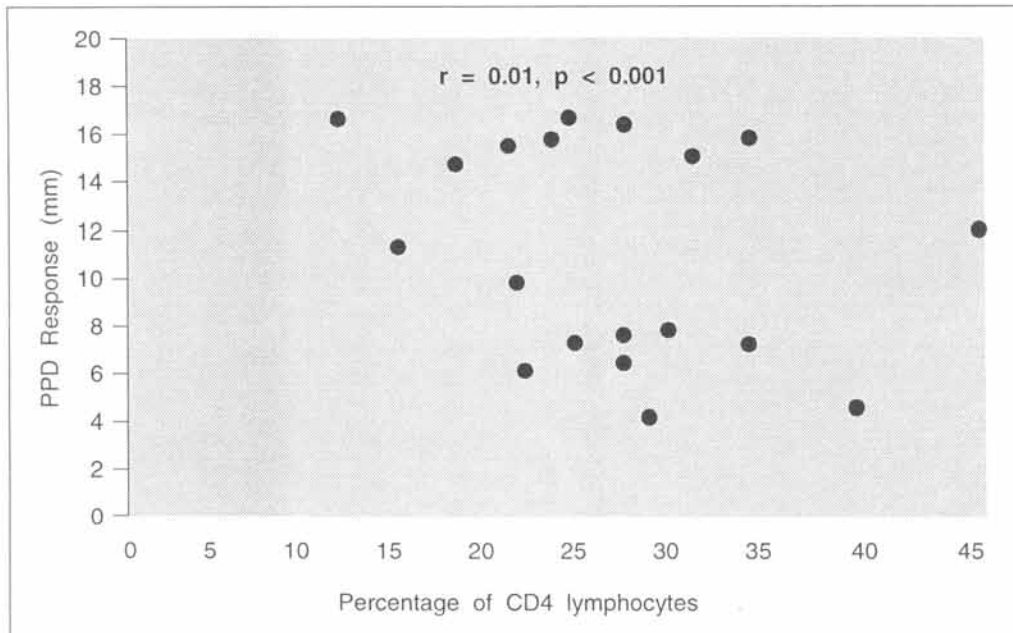


Fig. 4: Scatter plot between percentage of CD4 lymphocytes and PPD skin response in control group.

ability to identify latent mycobacterial infections in HIV infected persons.^{13,14,15} The use of tuberculin skin test needs to be redefined in this new condition, and now it may be used to predict decrease in the level of CD4 cell counts and hence the progression of the disease to AIDS in HIV infected people. We attempted to correlate the size of PPD skin reaction with the CD4 lymphocyte levels and found a good correlation between the absolute CD4 counts and the PPD skin test induration. The percentage of CD4 lymphocytes was also taken into consideration since it is thought by some to be a better predictor of disease progression, though a slightly lesser degree of association was observed between the percentage of CD4 and PPD response. No such association was found in the control group, which supports our idea that reaction sizes are associated with CD4 levels in the AIDS cases only and its clinical importance may be utilized only in this group.

Table-II shows an increase in the PPD reaction size with increased median CD4

counts in AIDS patients. We observed that the size of PPD skin reaction did not follow all or none law as reported by Moreno, et al, but their study was not done primarily on patients coinfecting with tuberculosis.¹⁵ A review of the literature failed to demonstrate any previous correlation with CD4 levels and size of PPD reaction in AIDS patients coinfecting with pulmonary tuberculosis. We do not know whether this correlation has never been sought or was sought but not found. With the increase in PPD reaction size, the median CD4 counts in the control group were not shown to be increased table-III. Anergy was seen among the patients with extremely low CD4 counts. This emphasizes past studies of the prevalence of anergy in patients with low CD4 counts and also proves the impairment of cell mediated immunity in advanced AIDS. In contrast, a hundred percent of the control patients showed a good DTH response to the PPD skin test. This is contrary to a previous study which suggested that anergy is seen in 10-15% of patients with pulmonary tuberculosis.¹⁶ No variation should be

expected from the PPD injection itself, since the samples used were from a single manufactured lot. Other possible conditions which might have caused anergy to PPD, like miliary tuberculosis, patients on immunosuppressive therapy, injectable drug users (IDU), cancer patients and also patients on antitubercular therapy were excluded from the study.^{6,17}

A cut off level of CD4 counts below which anergy may be seen could not be ascertained. This was due primarily to the fact that most of the patients presented in advanced disease conditions with very low CD4 counts and also due to a small sample size interestingly, a low range of CD4 counts (220–070 cells/ul) was observed the control patients. This can be attributed to the pulmonary tuberculosis itself or to the generally low CD4 levels, found in average healthy Thai adults (Webster et al, lympho-

cyte immunophenotype reference ranges in healthy Thai adults—unpublished). Infection with atypical mycobacterium (*Mycobacterium avium intracellulae*) *Nocardia* and previous exposure to PPD and BCG vaccination were potential confounders in the study.¹⁸ Prevalence of atypical mycobacterium and *Nocardia* in AIDS is known to be very low in Thailand. A study carried out on sputum culture in the same zonal TB center showed zero prevalence of atypical mycobacterium and *Nocardia* (personal communication, Director, Zonal TB center, Chonburi) Though none of the study patients had previous PPD skin test, 68% (15/22) of AIDS cases and 50% (10–20) of the controls were vaccinated in early childhood. No relationship could be established between BCG vaccination and PPD response, since prevalence of anergy in the AIDS cases was similar among the vaccinated and non vaccinated patients (Figure-5). Also the

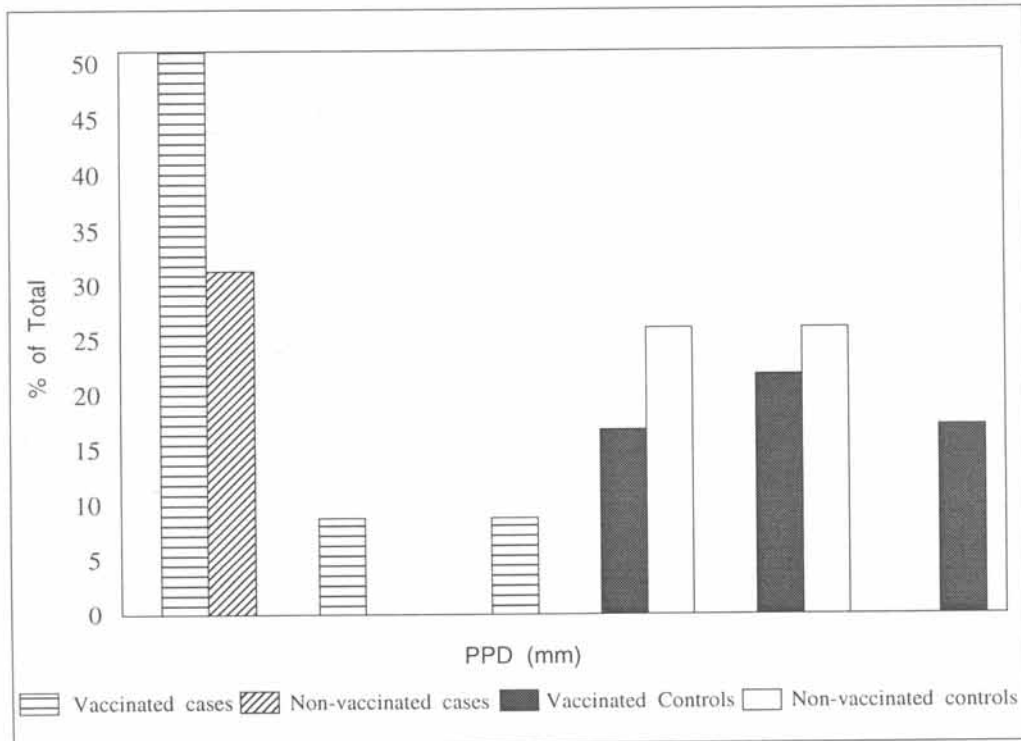


Fig. 5: BCG vaccination and PPD skin response in AIDS and control groups.

effected of BCG has been questionable and its effect is believed to be negligible after 10 to 15 years of the infection.¹⁸

CONCLUSION

HIV and TB coinfection is projected to rise in poor developing countries of Asia and Africa. Care of these patients most likely will be provided by facilities with limited budgets for laboratory tests and little access to the relatively sophisticated and expensive technique of CD4 counts. We therefore recommend that the relationship between PPD skin reaction size and CD4 counts be studied in larger sample size. If this relationship is confirmed, the PPD skin test may be incorporated in the clinical management of AIDS patients. This would have far reaching implications in crucial decision making regarding prophylactic therapy (e.g. *Pneumocystis carinii* pneumonia prophylaxis) and monitoring antiretroviral therapy. Further studies are needed relating DTH response and biological markers of HIV infection other than the CD4 counts since no comprehensive studies exist on this issue.¹⁹ Studies evaluating the effect of antiretroviral therapy on DTH response have shown that the DTH function may be restored in patients treated with antiretroviral agents, and in patients with miliary Tuberculosis after receiving anti TB drugs.²⁰ Further work is needed in this area to better define the characteristics of serial PPD skin testing in AIDS patients subsequent to anti retroviral and anti tubercular therapy.

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