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*Research Paper*

# Purified protein derivative skin testing on HIV/AIDS patients and logistic regression analysis of its risk factors

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## Abstract

**Objective**: To understand the reactivity of purified protein derivative skin test(PPD test) in HIV-infected persons and to determine the influential factors associated with PPD. **Methods**: 174 HIV/AIDS patients registered in the local center for disease control and prevention(CDC) participated this study from April to June in 2006. Questionnaire, CD4 count and thoracic roentgenogram were performed for all participants. **Results**: In this study, response rate of questionnaires was 83.65%. The majority of these participants had a different degree of immunodeficiency that accounted for 93.64%. Female patients had a higher CD4 count than that of males. The total positive rate of PPD was 38.15%. Analysis of single factor in our study indicated that CD4 count, previous tuberculosis history, tuberculosis contact history and thoracic roentgenogram manifestation of patients were related to their PPD diameters. Further analysis of multiple factors also supports the previous conclusion that CD4 count and previous tuberculosis history of patients were risk factors in the PPD test. **Conclusion**: The PPD test of HIV/AIDS patients could be affected by several factors. For persons infected with HIV, the confirmation of latent tuberculosis infection (LTBI) should be considered the combination effect of previous MTB infection and body cellular immune function.

*Keywords*: HIV/AIDS; TB-infection; PPD; CD4; analysis of influential factors; latent tuberculosis infection

## INTRODUCTION

In the last 20 years, HIV/TB co-infection has become an increasing public health epidemic, followed with the recrudescence of tuberculosis (TB) and the spread of HIV worldwide. In regions with high prevalence of HIV and TB, the epidemic of HIV, especially AIDS, prone to promoting patients to infect with Mycobacterium tuberculosis (MTB) [1-2], and TB infection may particularly favor early viral replication and dissemination, and therefore contribute to progression of HIV-1 disease[3]. Therefore, the control of latent tuberculosis infection in HIV-infected persons has become the priority in TB elimination strategy [4-7]. To understand the TB prevalence in HIV-infected persons and to determine the influential factors associated with PPD, we carried out a questionnaire test and PPD screening test among these patients.

## MATERIALS AND METHODS

One hundred and seventy-four persons from Suizhou city, Hubei province, (confirmed HIV-infected by Hubei CDC using Western blot) participated in the study with written consent, from April to June in 2006. The participants in the registry were tested with questionnaires and thoracic roentgenogram. Two ml peripheral blood sample was collected into an equal volume of EDTA vacuum blood vessel for CD4 count with FACScaliba flow

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cytometer (BD company, American), within 24 hours through matched fluorescently-labeled monoclonal antibody kits for each sample. Operations and results judgment were followed with illustration. Four ml peripheral blood samples were taken for a blood routine test and liver function test. DTH was performed by intradermal injection on the forearm of 5 TU (0.1 ml) of PPD (biological product company, Beijing). Induration was detected 48~72 hours after the injection, and was measured in millimeters (mm) at the average diameter.

The PPD test classifications: (a) negative reaction: energy to PPD or the average diameter was less 5 mm. (b) positive reaction: the average diameter was larger than 5 mm but less than 15 mm. (c) strong positive reaction: the average diameter was above 15 mm, or have necrosis, bubble, and lymphangitis. CD4 classification followed the diagnostic criteria of HIV/AIDS in China: (a) CD4 count above 500 cells/mm$^3$ which shows in patients of normal immunologic function. (b) CD4 count above 200 cells/mm$^3$ but less 500 cells/mm$^3$, which indicates damage of immunologic function. (c) CD4 count less 200 cells/mm$^3$ which indicates severe damage of immunologic function, and can be defined as AIDS present.

### Statistical analysis

Epidata software was used for data collection and management, and data were statistically analyzed with SPSS 13.0. Enumeration data was analyzed using $\chi^2$ test (tendency test), while the measurement data were deciphered using analysis of variance and correlation analysis. All tests of statistical significance were two-sided at the level of $P$ value < 0.05.

## RESULTS

### General state of health

There are 208 HIV-infected persons registered in Suizhou CDC. During the study period, all the 174 HIV-infected patients were given questionnaires except those out for work. The response rate of questionnaires was 83.65%. Among the participants, 106 were men and 63 were women. The age range for patients was 31 to 65 years (mean ± SD = 43.94 ± 6.61), No significant differences were found between male and female with respect to age ($P$ = 0.43), years of education ($P$ = 0.22), and marriage ($P$ = 0.31).

### CD4 count test

Among the 173 patients showing a CD4 count, the majority of these participants had differing degrees of immunodeficiency, which accounted for 93.64%. Among the 51 patients whose CD4 counts less 200 cells/mm$^3$ indicating AIDS, 37 were male and 14 were female. Female patients presented a significantly higher average CD4 count than that of male (314.00 ± 154.86 and 268.79 ± 139.05, respectively. $P$ = 0.047). Age, smoking, drinking, antiviral therapy, symptoms related to AIDS and other variables were independent to CD4 count.($P$ = 0.84, 0.12, 0.85, 0.35, and 0.07, respectively).

### Influential factor analysis of PPD test

### Analysis of single factor

Among the 173 patients tested, 36 were PPD-anergic, and 66 were PPD-positive(35 male and 31 female, respectively), of which 21 were PPD strong positive (14 male and 7 female). The total PPD-positive rate was 38.15%, and no significant difference was found between male and female($P$ = 0.11).

Among the patients PPD tested, 172 had information of previous tuberculosis histories. Patients with previous tuberculosis histories had a higher positive rate of PPD than that of patients without previous tuberculosis histories($P$ < 0.05). The relationship between the two groups is shown in ***Tab 1***.

***Tab 1*** **The relationship between PPD diameter and previous TB histories**

| TB histories | PPD diameter | | | Total ($n$) | Positive rate (%) |
|---|---|---|---|---|---|
| | < 5 mm | 5~14.9 mm | ≥15 mm | | |
| Yes | 2 | 3 | 4 | 9 | 77.78 |
| No | 105 | 41 | 17 | 163 | 35.58 |
| Total | 107 | 44 | 21 | 172 | 37.79 |

$\chi^2$ = 10.747, $P$ = 0.005.

Among the 171 patients who had TB contact information, the positive rate of PPD was higher in patients with confirmed TB contact histories than that of patients with vague histories and no history (66.67%, 53.33%, 33.33%, respectively. $P$ < 0.01).

Among the 169 patients who had thoracic roentgenogram and PPD results, PPD-positive rate of patients with typical TB roentgenogram results was higher than that of patients with atypical TB roentgenogram result (88.33%, 37.42%, respectively. $P$ = 0.03). Data about PPD results and TB contact histories, thoracic roentgenogram results are shown in ***Tab 2***.

Analysis of the 172 patients with both PPD results and CD4 counts indicated that PPD diameter changed following the CD4 counts variance. With the depletion of CD4, PPD diameters also decreased ($P$ = 0.001). Data on the relationship between PPD diameters and CD4 counts are shown in ***Tab 3***. Com-

***Tab 2*** **The effect of TB contact history and manifestation of thoracic roentgenogram on PPD results**

| Groups | | PPD results | | Total ($n$) | $P$ value |
|---|---|---|---|---|---|
| | | Positive | Negative | | |
| TB contact histories | Confirmed history | 10 | 5 | 15 | 0.009 |
| | Vague history | 8 | 6 | 15 | |
| | No history | 47 | 94 | 141 | |
| manifestation of | Typical manifestation | 5 | 1 | 6 | 0.034(exact) |
| thoracic roentgenogram | Atypical manifestation | 61 | 102 | 163 | |

***Tab 3*** **The relationship between CD4 counts and PPD diameters**

| PPD diameter(mm) | CD4 count (cells/mm$^3$) | | | Total ($n$) |
|---|---|---|---|---|
| | ≤200 | 201~ | ≥500 | |
| <5 | 38 | 64 | 6 | 108 |
| 5~ | 10 | 33 | 0 | 43 |
| ≥15 | 3 | 13 | 5 | 21 |
| Total | 51 | 110 | 11 | 172 |

$\chi^2 = 17.693$, $P = 0.001$; Kendall´s tau _ b= 0.174, $P = 0.018$.

pared to patients with CD4 counts less 200 cells/mm$^3$, the PPD-positive rate of patients with CD4 counts in the range of 201 to 499 and patients with CD4 counts above 500 were higher (OR = 2.10, 2.44, 95% CI = 1.01~4.38, 0.64~9.34, respectively). Tendency test indicated that there was a significant correlation between PPD diameters and CD4 counts (Kendall's tau_b = 0.17, $P = 0.02$). Correlation analysis also supported the relationship between PPD diameters and CD4 counts($r = 0.21$, $P = 0.01$).

Of the 51 AIDS patients, the positive rate of PPD was 25.49% (13/51), which was close to the rate of other study's result of HIV-negative persons(28.2%)[8].

Other variables such as sex, age, average income, average house areas, liver function, lencocyte count, symptoms associated to respirations and AIDS have no relation to PPD test ($P > 0.05$).

### Analysis of Multiple factors

According to the results of analysis of single factor, 4 variables were pooled for unconditional logistic regression analysis. Our findings indicated that previous TB histories and higher CD4 counts of patients were facilitative factors for a PPD-positive result, and the contribution of previous TB histories exceeded CD4 counts. Analysis results are shown in ***Tab 4***.

***Tab 4*** **Multiple analysis on PPD risk factors**

| variable | B | S.E. | $P$ | EXP(B) | 95% CI for EXP(B) |
|---|---|---|---|---|---|
| Previous TB history | 1.92 | 0.88 | 0.03 | 6.85 | 1.22~38.32 |
| CD4 count | 0.91 | 0.38 | 0.02 | 2.47 | 1.16~5.27 |

## DISCUSSION

Because the PPD test can predict active tuberculosis for patients and decrease the necessity of TB treatment [9-10], it is widely used for TB diagnose clinically. Data manifested that prophylactic treatment in PPD positive (PPD(+)) patients could decrease TB incidence [11-14]. Considering the characteristic of cellular immunity descending among HIV-infected patients, American CDC proposed to use a diameter of 5 mm as the judgment to maintain the sensitivity of PPD test [15], so as to diagnose LTBI patients, and then prevent TB development through prophylactic therapy[16].

Both MTB and HIV are intracellular pathogens and play an important role in cell-mediated immunity, thus cause HIV-infected patients to be susceptible to MTB infection, and then accelerate the progress of HIV clinically [17-18]. The major mechanism for HIV/TB co-infected patients to develop TB is that the decrease of cellular immune function can result in the recrudescence of LTBI. Some data suggests that HIV(-)PPD(+) persons have a chance of 10% to develop active TB during their lifetime, while HIV(+)PPD(+) patients could develop active TB at the percent of 5 to 15 in a year[19]. The mortality rate of HIV(+) TB patients is 4 times higher than that of HIV (-) TB patients, and more than 40% of deaths in AIDS sufferers can contributed to TB[20].

Our results indicate that 93.64% patients have a different degree of cellular immune deficiency among responders, of which 29.48% patients have a severe cellular immune deficiency meaning at the stage of AIDS. The average CD4 count of females are higher than that of males, which is accordance with the distribution of AIDS in the two groups, and it's also the main reason why the mortality is higher in males than that of females. Suizhou city (one of the high prevalence regions in China) has a TB in-

fection rate of 37.57% and a PPD-positive rate of 24.59% in HIV/AIDS patients, which is higher than that of other reports [8,11,21], indicating that the prevention and control of HIV/TB co-infection is urgent for the local government.

Similar to normal persons, with the increase of exposure to TB, the risk of infection with MTB is also increasing. The presence of antigenic-specific CD4 memory cells render patients with TB histories as having a higher chance of positive PPD result than that of patients with no TB history. Although the reliability of PPD test descends with the decrease of CD4 count in HIV-infected persons [22], it is reported that the result of PPD test among HIV/AIDS persons was closely related to the exposure degree of HIV and CD4 count [23]. Our findings also support that PPD diameters have a significant positive correlation to CD4 counts among HIV/AIDS patients. With the descending of CD4 counts, PPD diameters also decrease, suggesting that we should consider the effect of immunodeficiency on PPD results and pay more attention to the monitoring of PPD (-)-HIV/AIDS patients, and then diagnose and treat all MTB-infected patients, so as to improve their quality of life and control TB effectively.

Because of previous MTB and other environment Mycobacterium infection [24], BCG vaccination may affect PPD test in a certain degree, the specificity of PPD test always decreases in HIV-infected patients, while its specificity is cut down by their immunodeficiency likewise. Our study may give a new insight into considering the effect of PPD test in LTBI diagnosis among HIV-infected patients in regions where an epidemic of both TB and HIV is growing.

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### References

[1] Canwell MF,Binkin NJ. Tuberculosis in Sub-Saharan Africa:a regional assessment of the impact of the human immunodeficiency virus and National Tuberculosis Control Program Puality. *Tuber Lung Dis* 1996;77:220-5.

[2] Rieder HL, Cauthen GMm Cinstock GW & Sinder DE JR. Epidemiology of tuberculosis in the United States. *Epidemiologic Reviews* 11; 1989,79-98.

[3] Toossi, Z., Mayanja-Kizza, H., Hirsch, C. S., Edmonds, K. L., Spahlinger, T., Hom, D. L., *et al*. Impact of tuberculosis (TB) on HIV-1 activity in dually infected patients. *Clin Exp Immunol* 2001; 123:233-8.

[4] Centers for Disease Control and Prevention. Tuberculosis elimination revisited: obstacles, opportunities, and a renewed commitment. Advisory Council for the Elimination of Tuberculosis (ACET). *MMWR Morb Mortal Wkly Rep* 1999;48(RR-9):1-13.

[5] Institute of Medicine. Ending neglect: the elimination of tuberculosis in the United States. *Washington, DC: National Academy Press*; 2000:1-12.

[6] American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America. Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med* 2000;161:S221-S247.

[7] Lee B. Reichman, Alfred Lardizabal and Christopher H. Hayden. Considering the Role of Four Month of Rifampin in the Treatment of Latent Tuberculosis Infection. *American Journal of Respiratory and Critical Care Medicine* 2004; 170:832-5.

[8] Weiguo Cui, Lin Wang, Zhe Wang, Yuejin Yang, Changqing Zhang, Mingzhao Qin, et al. The relationship of PPD test and CD4 lymphocyte cells in HIV/AIDS patients. *Chin J Public Health* 2005; 21:677-8.

[9] AL Hersh, AN Tosteson, and CF von Reyn. Dual skin testing for latent tuberculosis infection: a decision analysis. *Am J Prev Med* 2003; 24:254-9.

[10] Scholten Jerod N,Driver Cynthia R., Munsiff? Sonal S., Kaye Katherine, Rubion Mary Ann, Gourevitch Marc N., *et al*. Effectiveness of isoniazid treatment for latent tuberculosis infection among human immunodeficiency virus (HIV)-infected and HIV-uninfected injection drug users in methadone programs. *Clin Infect Dis* 2003; 37:1686-92.

[11] Bucher HC, Griffith LE, Guyatt GH, Sudre P, Naef M, Sendi P, *et al*. Isoniazid prophylaxis for tuberculosis in HIV infection: a meta-analysis of randomized controlled trials. *AIDS* 1999;13:501-8.

[12] Neil W. Schluger, MD. Challenges of Treating Latent Tuberculosis Infection. *Chest* 2002; 121:1733-5.

[13] Alison D. Grant, MBBS, PhD; Salome Charalambous, MB-BCh, MSc; Katherine L. Fielding, PhD; John H. Day, MBBS; Elizabeth L. Cor bett, MBBS, PhD; Richard E. Chaisson, MD; Effect of Routine Isoniazid Preventive Therapy on Tuberculosis Incidence Among HIV-Infected Men in South Africa. *JAMA* 2005;293:2719-25.

[14] Henry M. Blumberg, MD; Michael K. Leonard, Jr, MD; Robert M. Jasmer, MD. Update on the Treatment of Tuberculosis and Latent Tuberculosis Infection. *JAMA* 2005;293:2776-84.

[15] Christopher C. Whalen,MD,MS. Diagnosis of Latent Tuberculosis Infection-Measure for Measure. *JAMA* 2005;293:2785-7.

[16] Johnson MP, Coberly JS, Clermont HC, Chaisson RE, Davis HL, Losikoff P, *et al*. Tuberculin skin test reactivity among adults infected with human immunodeficiency virus. *J Infect Dis* 1992; 166: 194-8.

[17] Wolday D, Hailu B, Girma M, Hailu E, Sanders E, Fontanet AL. Low $CD4^+$ T-cell count and high HIV viral load precede the development of tuberculosis disease in a cohort of HIV-positive Ethiopians. *Ethiop Med J* 2003; 41 Suppl 1:67-73.

[18] Aisu T, Raviglione MC, van Praag E, Eriki P, Narain JP, Barugahare L, *et al*. Preventive chemotherapy for HIV-associated tuberculosis in Uganda: an operational assessment at a voluntary counseling and testing centre. *AIDS* 1995; 9:267-73

[19] Shen, Y., Shen, L., Sehgal, P., Huang, D., Qiu, L., Du, G., Letvin, N. L., Chen, Z. W. (2004). Clinical Latency and Reactivation of AIDS-Related Mycobacterial Infections. *J Virol* 2004; 78: 14023-32.

[20] Jai P. Narain & Ying-Ku Lo. Epidemiology of HIV-TB in Asia. *India J Med Res* 2004;120:277-89.

[21] Santiago Moreno, Josu Baraia-Etxaburu, Emilio Bouza, Francisco Parras, Miguel Perez-Tascon, Pilar Miralles, *et al*. Risk for Developing Tuberculosis among Anergic Patients Infected

with HIV. *Annals of Internal Medicine* 1993;119:194-8.
[22] U.S. Public Health Service, Infections Diseases Society of America. 2002 Guidelines for preventing opportunistic infections among HIV-infected persons-2002. *MMWR Recomm Rep* 2002;51(RR08):1-46.
[23] Hiransuthikul N, Hanvanich M, Dore GJ, Mokkhawes T, Li Y, Perriens J, *et al*. Factors associated with tuberculosis skin test reactivity among HIV-infected people in Bangkok. *Southeast Asian J Trop Med Public Health* 2003;34:804-9.
[24] Jalba, Mihai-Sergiu. A starting point to a better detection of active and latent tuberculosis infection in HIV-positive individuals. *AIDS* 2003;17:1589.