Improved sensitivity of a novel recombinant protein skin test for the diagnosis of TB

Badaró[1,2], R, MD; Machado[1], BAS, PhD; Casper[3], C, MD; Coler[3], RN, PhD; Araujo-Neto[2], CA, MD; Pedral-Sampaio[2], D, MD; Nakatani[2], M, MPH; Campos-Neto[2], A, MD; Reed[3], SG, PhD

[1]*Institute of Health Technologies, SENAI CIMATEC, Bahia, Brazil;* [2]*Department of Medicine, Federal University of Bahia, Bahia, Brazil;* [3]*Infectious Disease Research Institute, Seattle, Washington, USA.*

Poster 767

Roberto Badaró, MD
Institute of Health Technologies
SENAI CIMATEC
41650-010 Bahia – Brazil
Email: badaro@fieb.org.br

Background

- The tuberculin skin test (TST) or Mantoux reaction (MR) has been used worldwide in the diagnosis of *M. tuberculosis* infection for over 100 years.
- The accuracy of the TST with PPD is limited by prior BCG vaccination, infection by other non-tuberculous mycobacteria, reduced sensitivity in immunocompromised hosts with a high risk of tuberculosis (TB) such as HIV, and limited ability to diagnose active TB.
- We previously described a recombinant protein ("DPPD") derived from *M. tuberculosis* (MTB) that can replace the classical PPD antigen used in TST[1].
- **Study objective:** To determine the accuracy of DPPD in the diagnosis of TB infection in persons with and without HIV co-infection.
- **Hypothesis:** The DPPD antigen is sensitive and more specific than PPD antigen in the diagnosis of TB infections.

Methods

- ***Study Setting and Population:*** We compared DDPD to PPD using MR in a population of 672 individuals living in the state of Bahia, Brazil. Participants were divided among 5 specific groups.
  - ***Group 1:*** 232 BCG-vaccinated individuals with no signs or symptoms nor history of active TB;
  - ***Group 2:*** 40 HIV-negative patients with active TB as defined by a clinical isolate with a positive culture for MTB or positive acid fast stain;
  - ***Group 3:*** 38 HIV-positive individuals with active TB and MTB detected by Lowenstein Jensen positive culture or positive in the direct acid fast stain;
  - ***Group 4:*** 182 HIV-positive patients with signs and symptoms compatible with active TB, but without microbiologic confirmation, who responded to the specific drug treatment for TB;
  - ***Group 5:*** 115 HIV-negative patients with a clinical diagnosis of active TB, without microbiologic confirmation of MTB, who responded to the specific drug treatment for TB.
- ***Tuberculosis diagnostic criteria***: (a) radiological image compatible to TB lesions; (b) Positive direct smear BAAR (acid-fast bacilli on Ziehl–Neelsen stain); (c) positive culture in the Lowenstein-Jensen medium; (d) Treatment responses to the specific TB treatment (INH+RM+PZA+ETB).
- ***Definitions for Positive:*** Asymptomatic, without HIV, previous history of BCG, from TB endemic area: **10 mm induration**; Suspicion of active TB or HIV positive, BCG positive or negative: **5 mm induration**.
- PPD and DPPD were applied intradermal in contralateral arms using a standard Mantoux test technique.

Amino Acid Sequence of DPPD Antigen

The name DPPD represents the single letter code of the first four amino acids of the N-terminus sequence of the mature form



Results

- Among healthy people previously vaccinated with BCG (Group 1), 57% had positive PPD and 41% had positive DPPD with a good concordance index (Kappa = 0.90).
- In the context of active TB, DPPD was positive in 93% of patients with active co-infection HIV and TB (Groups 3 and 4), compared to 50% of PPD (p <0.001).
- All (100%) of HIV negative patients with active TB (Groups 2 and 5) were both DPPD and PPD positive.

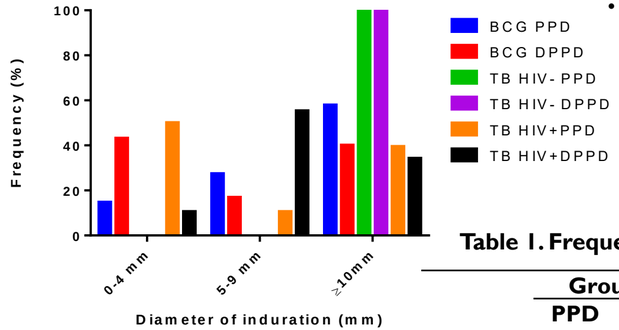


Figure 1. Frequency of PPD and DPPD positivity in the study (Groups 1, 2 and 3)

- Among HIV-positive patients with Active TB co-infected with HIV, DPPD was significantly more sensitive than PPD when stratified by CD4 cell counts (P=0.0001).

Table 1. Frequency of PPD and DPPD (%)

	Group 1		Group 2		Group 3	
	PPD	DPPD	PPD	DPPD	PPD	DPPD
0 - 4 mm	14.74	43.16	0.00	0.00	5.00	10.53*
5 - 9 mm	27.37	16.84	0.00	0.00	10.53	55.26
≥ 10 mm	57.89	40.00	100.00	100.00	39.47	34.21
Positive ≥ 5 mm Group 3					50.00	89.47

*CD4<100cells/mm^3

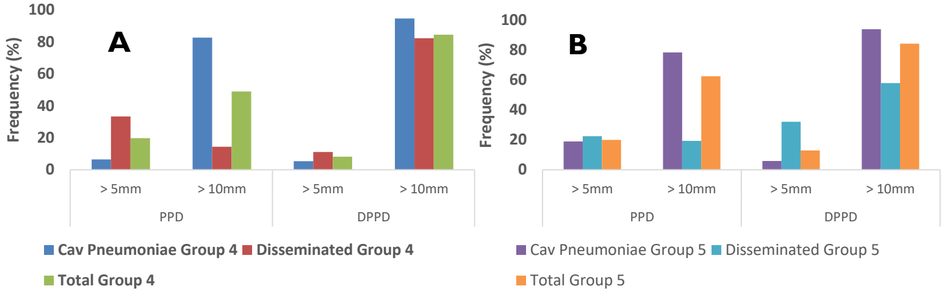
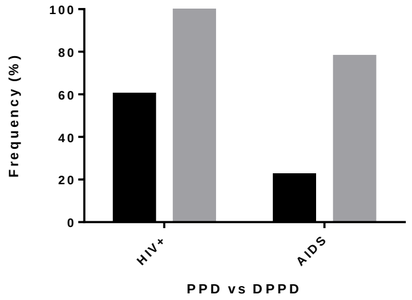


Figure 2. PPD and DPPD responses on Empirical treated patients for suspected of (A) TB/HIV+ (Group 4) and (B) TB/HIV- (Group 5).



	HIV+	AIDS
PPD	60%	22%
DPPD	100%	78%

Figure 3. Patients with HIV infected and MTB+ with response positive to PPD and DPPD tests considering induration ≥ 5 mm.

HIV+ (CD4<350cells/mm^3)
AIDS (CD4<200cells/mm^3)

Conclusion

- The recombinant DPPD antigen may be an important tool to replace the classical PPD antigen in the diagnosis and control of TB, as it may have improved sensitivity for the detection of active TB in immunocompromised individuals and a greater ability to differentiate BCG vaccination from latent infection by *M. tuberculosis*.

Acknowledgements

We thank the participants, IDRI, UFBA and SENAI CIMATEC.

[1]Campos-Neto et al. Evaluation of DPPD, a single recombinant *Mycobacterium tuberculosis* protein as an alternative antigen for the Mantoux test. Tuberculosis (Edinb). 2001;81(5-6):353-8.