Low prevalence of reactive PPD prior to infliximab use: comparative study on a population sample of Hospital Geral de Fortaleza

Maria Roseli Monteiro Callado[1], José Rubens Costa Lima[2], Christiane Aguiar Nobre[3], Walber Pinto Vieira[4]

ABSTRACT

Objective: To identify tuberculosis infection in rheumatic patients on infliximab by use of PPD testing prior to immunobiologic therapy. **Methods:** This study comprised 157 patients undergoing infliximab treatment and 734 other patients undergoing laboratory screening for tuberculosis infection originating from several services. The Mantoux technique was used for PPD testing, and an induration of at least 5 mm was considered reactive status. **Results:** In the infliximab group, 13% of the patients reacted to PPD, while, in the other group, 27% of the patients reacted to PPD ($\chi^2 = 13$; P = 0.0003). These patients were divided into categories: adults with chronic diseases, PPD reactivity of 22%; and other controls, PPD reactivity of 31%. This shows the heterogeneous response of that population ($\chi^2 = 7$; P < 0.009). In the infliximab group, subdivided according to pathologies [rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriatic arthritis (PA)], different reactivity rates were observed, the lowest value occurring among RA patients: (RA x AS: OR = 0.13; CI: 0.03-0.47; $\chi^2 = 12$; P = 0.0004) and (RA x PA: OR = 0.16; CI: 0.02-1.04; $\chi^2_{\text{Yates corrected}} = 3.6$; P = 0.05). The PPD reactivity in the RA subgroup (4%) was also lower as compared with that of the chronic patients group (22%) (OR = 0.16; CI: 0.05-0.49; $\chi^2 = 14$; P = 0.0002), even when reclassified into four subgroups: rheumatology (OR = 0.19; CI: 0.04-0.72), kidney transplantation (OR = 0.16; CI: 0.05-0.51), infectology (OR = 0.21; CI: 0.05-0.75), and other conditions (OR = 0.13; CI: 0.04-0.44). **Conclusion:** The low prevalence of PPD reaction in this Brazilian population, mainly in chronic patients, with the worst performance among RA patients, shwoed that the test has limited value for diagnosis of tuberculosis infection in candidates to infliximab therapy.

Keywords: biological therapy, rheumatic diseases, prevalence, tuberculin test.

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INTRODUCTION

Surveillance studies on the use of tumor necrosis factor inhibitors (anti-TNF) after their approval in clinical trials have shown an increase in the incidence of tuberculosis (TB) reactivation.[1,2] Thus the presence of latent TB infection (LTBI) should be assessed prior to beginning the anti-TNF therapy by use of the cutaneous response to the tuberculin test with purified protein derivative (PPD), chest X-ray, and history of contact with TB.[3] The usual cutoff point of 10 mm for reactive PPD reading was reduced in those patients to 5 mm, based on the recommendations published by the Centers for Disease Control and Prevention (CDC) of the United States in 2000[4] and 2005,[5] aiming at eradicating TB. That cutoff point was challenged at the Tuberculosis Diagnosis, Treatment, and Prevention Guide of Universidade de São Paulo (USP) in 2006,[6] because,

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Rheumatology Service of Hospital Geral de Fortaleza – HGF. Brazil.
1. Doctor in Sciences by the USP (Rheumatology); Preceptor of the Medical Residence in Rheumatology of HGF
2. Epidemiologist; Master in Collective Health by the UNICAMP
3. Resident physician of Rheumatology of Hospital Geral de Fortaleza
4. Collaborating professor of the Medical School of the UECE. Chief of the Rheumatology Service of HGF
Corresponding author: Maria Roseli Monteiro Callado, Rua Ávila Goulart, 900, Papicu, Fortaleza, CE, Brazil. CEP 60155-290. E-mail: roselicallado@gmail.com.

although Brazil has endemic TB, no epidemiological survey to support that recommendation was performed in Brazil. This study aimed at assessing the prevalence of the response to the PPD test in patients of the Rheumatology Service prior to the anti-TNF therapy, comparing their test performance with that of the users of the Brazilian Unified Public Health System (SUS) cared for at the *Hospital Geral de Fortaleza* (HGF), the greatest public hospital of the state of Ceará, with a mean of 15 thousand outpatient visits per month.

MATERIAL AND METHODS

The sample. This study sample comprised the following: Cases, group A - 1031 results of PPD tests of 157 rheumatologic patients using infliximab; and Controls, groups B and C - 874 patients undergoing TB screening referred to the clinical pathology laboratory of the HGF (Table 1). Group A comprised consecutive patients undergoing treatment with infliximab from May 2005 to October 2008, who had one of the following: rheumatoid arthritis (RA); ankylosing spondylitis (AS); or psoriatic arthritis (PA). Those patients met the criteria of the American College of Rheumatology (ACR) for their respective diseases. All patients changed to the biological medication after failure of the conventional therapy. Prior to beginning immunobiologic therapy, PPD testing was required. Patients with positive results from the PPD test received prophylaxis with isoniazid for six months, beginning 30 days before treatment. The characteristics of group A patients are shown in Table 2. Groups B and C comprised consecutive patients, men and women of all ages, from different medical services of the HGF, cared for at the HGF outpatient clinics and referred to the clinical pathology laboratory with a medical request for PPD testing, from January to October 2009. This PPD case series was substratified for analysis, meeting the criteria of group or subgroup specified in Table 1. Group stratification aimed at grouping individuals with equivalent cell immunity and epidemiological conditions.

Data collection. The database of the clinical pathology laboratory of the HGF was used for all patients of this study, with complementary review of the medical records of the rheumatological patients undergoing treatment with infliximab at the Immunobiologic Infusion Center of the HGF.

Study design. This is a retrospective cross-sectional descriptive study to assess the prevalence of PPD by use of a database.

Tuberculin skin test. Two units (0.1 mL) of the standard preparation of PPD RT-23 (Statens Serum Institut - Kopenhagen, Denmark) were injected in the intradermal region of the volar surface of the forearm (Mantoux method). After 72 hours, the reaction was read by measuring the

Table 1
Definition of the strata for analysis

Group		Strata	Definition	PPD request
Cases	**A**	**Infliximab case series**	Patients undergoing treatment with infliximab.	
	A1	Rheumatoid arthritis	Diagnoses meeting the criteria of the ACR for rheumatologic diseases.	Screening protocol
	A2	Ankylosing spondylitis		
	A3	Psoriatic arthritis		
Controls	**B**	**Chronic**	Control formed by adults with chronic diseases.	Clinical suspicion or screening protocol
	B1	Rheumato/2009	Subgroup of chronic patients of the rheumatology outpatient clinics.	Clinical suspicion
	B2	Kidney Tx	Subgroup of chronic patients of the kidney transplantation outpatient clinics.	Screening protocol
	B3	Other clinics	Subgroup of chronic patients of the services of Neurology, Endocrinology, Gastroenterology and Internal Medicine registered in specialized outpatient clinics (ex.: multiple sclerosis, diabetes, and hepatitis).	Clinical suspicion
	B4	Infectology	Subgroup of chronically infected patients of the HIV positive outpatient clinics.	Screening protocol
	C	**Other controls**	Patients of all ages cared for at the HGF outpatient clinics in different medical specialties (Pediatrics, Internal Medicine, Surgery, Gynecology, Obstetrics, etc.) not related to the above-cited special services.	Clinical suspicion

Table 2
Characterization of the patients using infliximab

Characteristics of the patients	Study group (n = 157), n(%)
Sex	
Male	58 (36.9)
Female	99 (63.1)
Age (years) *	47 ± 14.7
Male	41.1 ± 12.7
Female	49.1 ±15.1
Primary disease	
RA	90 (57.3)
AS	54 (34.4)
PA	13 (8.3)
PPD prior to treatment	
Reacting	21 (13.4)
Non-reacting	136 (86.6)

*: Mean ± standard deviation; RA: Rheumatoid arthritis; AS: Ankylosing spondylitis; PA: Psoriatic arthritis; PPD: purified protein derivative skin test for tuberculin sensitivity.

diameter of the induration in millimeters. The cutoff point for a positive skin test was aadopted as an induration area whose diameter was ≥ 5 mm.

The quality of the PPD test used at the HGF (non-published data) was satisfactory, with application, reading, and interpretation of the results in accordance with the guidelines of the Brazilian Ministry of Health, which also approved the reagent used, which is internationally accepted and referred in the literature as having a good performance.[7,8,9]

Statistical analysis of PPD *reactivity between the groups.* The statistical analyses were performed with the Epi Info 6, version 6.04d. For the comparison studies, the chi-square (χ^2) test was used for checking dissimilarity between strata, calculating the odds ratio (OR) with 95% confidence intervals (CI). The significance level of 5% ($P \leq 0.05$) was adopted. When necessary, the Yates correction for the χ^2 and Fisher's exact test was used for comparing groups with small-sized samples.

RESULTS

Of the initial sample of this study, losses corresponded to140 results (16%) referring to individuals of the control group, who missed the PPD reading. The PPD test was requested by an outpatient physician due to clinical suspicion of TB or through the screening protocol of the specialized outpatient clinics of infectology (HIV positive patients) and kidney transplantation. The remaining 734 results comprised groups B and C (Table 3). Group A, consisting of rheumatologic patients using infliximab, underwent PPD testing prior to beginning the immunobiologic therapy. The prevalence of reactive PPD was 24.5% in the entire study (Table 3), being lower among the cases (group A: 13%) than among the controls (sum of groups B and C: 27%) (χ^2: 13; P = 0.0003). The chances of PPD reactivity among the cases corresponded to only 42% of the chances of PPD reactivity among controls, ranging from 25% to 70% (OR: 0.42; CI: 0.25-0.70; χ^2: 13; P = 0.0003). The 27% PPD reactivity among controls (Table 3) was not homogeneous among the 'chronic controls' of the specialized outpatient clinics characterized in Table 1 (group B: 22%) and 'other controls' formed by patients at all ages cared for at the outpatient clinics of HGF in several medical specialties (pediatrics, internal medicine, surgery, gynecology, obstetrics, etc.), which were not related to special services for follow-up of chronic pathologies (group C: 31%) (χ^2: 7; P = 0.009). The chances of PPD reactivity in group B corresponded to 64% of the chances of reactivity in group C, ranging from 45% to 91% (OR: 0.64; CI: 0.45-0.91), characterizing the existence of a lower prevalence of PPD reactivity in chronic patients. The substratification of the chronic controls, comparing the prevalence between subgroups B1 to B4, did not manage to show dissimilarities between them (χ^2: 1.40; degree of freedom 3; P = 0.70; 9/36/28/97/21/60/12/54). In group A, PPD reactivity (Table 3) was not homogeneous when analyzed for the three subgroups, according to the pathologies involved (χ^2: 14; degree of freedom: 2; P = 0.0007). The PPD reactivity in group A was 4% among RA patients (subgroup A1), 23% among AS patients (subgroup A2), and 26% among PA patients (subgroup A3). The chances of PPD reactivity in subgroup A1 corresponded to 13% of the chances of reactivity in subgroup A2 (OR: 0.13; CI: 0.03-0.47; χ^2: 12; P = 0.0004), and to 16% of the chances of reactivity in subgroup A3. The latter showed borderline difference for the significance level of 5% (OR: 0.16; CI: 0.02-1.04; $\chi^2_{\text{Yates corrected}}$: 3.6; $P_{\text{Fisher exact}} = 0.05$).

The PPD reactivity of subgroup A1 (Table 3) was lower than that of group B (22%), corresponding to 16% of the chances of PPD reactivity in those controls (OR: 0.16; CI: 0.05-0.49; χ^2: 14; P = 0.0002). That relation was maintained even when the chronic controls were tested separately in the four services of chronic patients, and showed no dissimilarities amongst them (χ^2: 1.4; degree of freedom: 3; P = 0.70) (Table 3).

DISCUSSION

The introduction of immunobiologic therapy in clinical practice for treating rheumatic diseases caused the appearance of several

Table 3
PPD results in the population studied

Group	PPD	≥ 5 mm		< 5 mm		Total	χ^2	P	OR	95% confidence interval
		n	(%)	n	(%)	n				
A	Cases	21	13%	136	87%	157	-	-	-	-
B + C	Controls	198	27%	536	73%	734	13	0.0003	0.42	0.25 - 0.70
	Controls									
B	Chronic	70	22%	247	78%	317	-	-	-	-
C	Other controls	128	31%	289	69%	417	7	0.009	0.64	0.45 - 0.91
	Total	198	27%	536	73%	734				
	Cases									
A1	RA	4	4%	86	96%	90	-	-	-	-
A2	AS	14	26%	40	74%	54	12	0.0004	0.13	0.03 - 0.47
A3	PA	3	23%	10	77%	13	3.6**	0.05***	0.16	0.02 - 1.04
	Total	21	13%	136	87%	157				
A1	RA	4	4%	86	96%	90	-	-	-	-
B	Chronic	70	22%	247	78%	317	14	0.0002	0.16	0.05 - 0.49
B1	Rheumato/2009	9	20%	36	80%	45	7	0.009	0.19	0.04 - 0.72
B2	Kidney Tx	28	22%	97	78%	125	12	0.0005	0.16	0.05 - 0.51
B3	Other clinics	21	26%	60	74%	81	14	0.0001	0.13	0.04 - 0.44
B4	Infectology	12	18%	54	82%	66	6	0.01	0.21	0.05 - 0.75

mm: millimeter; χ^2: chi-square; P: statistical significance level; OR: odds ratio; n: number; *: Chronic adults with PPD requested by the services of Rheumatology (Rheumato/2009), Kidney Transplantation (Kidney Tx), and other clinics (Neurology, Endocrinology, Gastroenterology, and Internal Medicine), Infectology (HIV positive outpatient clinics); RA: Rheumatoid arthritis; AS: Ankylosing spondylitis; PA: Psoriatic arthritis; **: χ^2 Yates corrected; ***: Fisher exact test.

cases of active TB[10] in developed countries. Epidemiological surveillance studies have shown TB reactivation in previously infected individuals.[1,11,12] The World Health Organization (WHO) estimates that more than one third of the world population is infected with *Mycobacterium tuberculosis*, and 95% of the infected individuals live in the developing countries. Eighty percent of them are concentrated in 22 countries, among which Brazil is 15th in the rank,[13] with an incidence of 58 cases/100,000 inhabitants.[14] Façanha *et al*.[15] have reported a 18.5% TB undernotification in the city of Fortaleza, Ceará state, from 2000 to 2002. The incidence found in the municipality was 91.87/100,000 inhabitants, 58% greater than the national mean. Because of the high announced prevalence of TB among us, prevention of the primary infection or reactivation of LTBI in Brazilian candidates to the anti-TNF therapy followed the recommendations of the North-American CDC for immunosuppressed patients,[5] despite the lack of epidemiological studies indicating an ideal cutoff point for the size of the PPD induration in our population. Since then, it has been questioned that the prophylaxis instituted based on a 5-mm induration for Brazil[6] would lead to an excess of TB prophylactic treatment[16], increasing even more the treatment cost[17] of those patients, favoring their withdrawal and the appearance of multiresistant bacillary strains.[17,18]

This study was carried out after the discovery of a 13.4% prevalence of positive reaction to PPD prior to the use of the biological therapy (Table 2) among the 157 patients using infliximab at the HGF until October 2008. The possibility of the existence of technical flaws during test performance was considered, generating uncertainty about the effectiveness of the test for protecting against LTBI in patients with indication for biological therapy. That prevalence is lower than the one expected for an endemic area for TB, the Brazilian northeastern region, when the WHO statement, which projects a minimum value of TB of one third of the world population, is used as a parameter. Although there is no normality range for the frequency of positive PPD reaction in the Brazilian population, the prevalence of positive PPD reaction is expected to be greater than the 33% indicated by the WHO, especially among individuals of lower socioeconomic levels

and users of the SUS suspected of having TB. The search for an answer to the low frequency of positive PPD reaction found in this sample comprised a review of the laboratory quality of the test performed, with a careful evaluation of each technical step, in accordance with the guidelines of the Brazilian Ministry of Health. The test was performed with the national gold standard reagent,[19] which is internationally approved and reported as having good performance.[7,20,21] Thus, the low frequency of positive PPD reaction is believed not to be related to a deficit in the intrinsic quality of the test. The authors felt the need to know the actual performance of the PPD test being offered to the local population by conducting a survey about the PPD results in the patients of the HGF being tested during the year 2009.

This study, performed with a sample size sufficient for obtaining conclusive results from the statistical tests, identified differentiated prevalence between cases and controls, and between their substratifications. Considering that the 16% loss of the initial control sample (140/874) is high, the occurrence of a selection bias is admitted, and could be introducing an upper bias in the final result, creating the dissimilarity found. Thus, considering the higher probability of negative results among the individuals who missed the test reading, because we believe that those with no local epidermal inflammatory reaction would tend not to return for test reading, the study simulation was performed. That simulation included the 140 missing individuals in the non-reactors category, confirming the dissimilarity found (21/136/198/676; OR: 0.53; CI: 0.31-0.88; $\chi^2 = 6.85$; P = 0.008). Thus, this bias did not jeopardize the study. Some researchers have reported losses depending on the group studied, such as the immigrants of the city of Toledo in Spain with 22.2% of individuals not completing the study,[22] and the health care workers in Mexico with 12.1% of losses.[23] Depending on the population, the active search of those missing individuals should be performed by the local surveillance in an attempt to decrease TB dissemination by the potential patients among individuals undergoing PPD testing.

The 27% prevalence of reacting PPD in the control group in 2009 was also below the expectations for an endemic region for *M. tuberculosis*. The controls in this study were assessed in specific subgroups after knowing that the population was not homogeneous. It comprised individuals of all ages, who either could have chronic diseases, or could have been tested due to a clinical suspicion of TB, or who followed a screen protocol, such as HIV positive patients or those with chronic kidney disease. Despite all efforts to separate and characterize that control sample, the highest PPD positivity index did not exceed 31% in higher-risk individuals, subgroup C, patients with clinical suspicion of TB. That subgroup showed a dissimilar prevalence as compared to subgroup B (chronic diseases). The dissimilarity tests in the chronic controls showed no dissimilar prevalence in patients suspected of having TB or not, and the four subgroups remained with reacting results around 20%.

Group A was also subdivided according to the pathologies, and subgroup A1 behaved differently from subgroups A2 and A3, both with positivity similar to that of group B. Subgroup A1, comprised by RA patients, showed a 4% prevalence of PPD reaction, much lower than that of any subgroup of chronic patients, including that of HIV positive patients and hemodialysis patients, whose response to late hypersensitivity tests, as already recognized in several studies, is jeopardized.[24,25,26,27,28,29]

Two recent Brazilian studies about response to PPD in rheumatologic patients candidate to immunobiologic therapy have revealed an attenuated response to PPD in RA patients,[16,30] similar to the international findings[31,32] that attribute that abnormality to deficiencies in cell immunity of rheumatologic patients. In the study developed in Recife in 2007, the prevalence of PPD reaction in the comparison group (33.3%) was greater than that in the RA group (14.6%), with a statistically significant difference (P = 0.034). The 48 healthy adults selected as controls had history of BCG vaccination during childhood, confirmed by the presence of the scar in their arm, some of whom were health care professionals working at the local rheumatology outpatient clinics.[16] Laurindo *et al.*[30] have reported that, in São Paulo, the frequency of positive PPD was significantly lower in RA patients (27%) as compared with the control group (58.6%), comprised by healthy individuals with a mean age of 34 years, working at the hospital administration office. However, those two national studies have factors that elevate the population frequency of the PPD reaction, such as previous vaccination[33] and/or health care professionals included in the control groups,[34,35] favoring the reported statistical differences between the groups compared. In a study developed in Peru,[36] where TB is endemic and the prevalence of the BCG scar is higher than 80%, the PPD test performed in a group of RA patients was compared with that of immunocompetent volunteers, paired by sex and age. A 71% PPD positivity (PPD > 5 mm) was identified in the immunocompetent group versus 29% in the RA group. Such discrepancies between results were associated with cell immunity abnormality in RA.[34] In Turkey, an area with a relatively high TB prevalence and BCG scar identified in 90% of the study sample, Köker *et al.*[37] have reported a PPD positivity in RA (29.8%) lower than that found in patients with

AS (65.9%), gouty arthritis (68.8%), and osteoarthritis (63%), with no correlation with the clinical symptoms of the disease.

Despite the low prevalence of PPD reaction observed when considering the entire study, the relative differences between the strata found in the literature are also identified,[16,30,31,32] with RA patients failing to react to PPD. The very low prevalence of PPD reaction in RA patients (4%) could be explained by the global attenuation of the response to PPD in this case series. Marques *et al*.[16], in a study carried out in another city of the northeastern region (Recife), have also reported a 14.6% prevalence of PPD reaction in RA, much lower than that reported by Laurindo *et al*.[30] in São Paulo (27%). Those results could lead to several questionings involving the socioeconomic, genetic, and epidemiological characteristics of those population samples. The prevalence of PPD reaction in the national studies was also lower than that found in Turkey and Peru, countries known to have epidemiological conditions for TB equivalent to those in Brazil.

The prevalence of an event in a population corresponds to the sum of its occurrences. Relating to PPD reaction, the prevalence corresponds to the sum of the percentages of individuals with TB and the percentages of individuals with vaccine sensitivity. The high prevalence of Turkey and Peru could be explained by the levels of local infection and influenced by intradermal BCG vaccination that induces persistent response to PPD.[31] Although this study does not provide data about vaccine history, one can state that, based on the predominance of adults in this population sample (age in group A over 30 years), such individuals were born before 1976, when the official replacement of oral BCG vaccine, which does not cause induration on PPD, by the intradermal BCG vaccine occurred in Brazil.[33] One can admit that the positive response to PPD of the HGF patients showed exclusively the existence of TB infection and not vaccine pre-sensitivity. Regarding the low frequency of TB infection among us evidenced by PPD reactivity, we recognize the need for population studies assessing the epidemiological situation projected by the WHO for Brazil. A study assessing the incidence of TB in the city of Vitória, with 178 nursing students with vaccine scar, from 1997 to 1999, showed a frequency of positive results (PPD $\geq$ 10 mm) of 20.3%[32] added to 21% of weak reacting students (PPD $\geq$ 5 mm and < 9 mm). The extension of that study to 441 medical students and 218 students of economics in 2002[38] showed PPD reactivity of 34.4% (strong, 18.4%; weak, 16%) among the medical students and only 13% (strong, 6%; weak, 7%) among the students of economics, suggesting an occupational risk for PPD reactivity, but with low prevalence, not compatible with the epidemiological situation projected by the WHO.

The follow-up of the presence of TB infection in patients using infliximab will be of paramount importance for assessing the PPD results. However, despite any local prevalence found in other studies, we understand that the response of RA patients will always be lower, confirming the limitation of PPD for diagnosing TB in such patients.

REFERENCES

REFERÊNCIAS

1. Keane J. TNF-Blocking agents and tuberculosis: new drugs illuminate an old topic. Rheumatology 2005; 44:714-20.
2. Wallis RS, Broder MS, Wong JY, Hanson ME, Beenhouwer DO. Granulomatosis infectious diseases associated with tumor necrosis factor antagonists. Clin Infect Dis 2004; 38:1261-5.
3. Sociedade Brasileira de Reumatologia. Projeto Diretrizes da Associação Médica Brasileira e Conselho Federal de Medicina. Artrite Reumatóide: diagnóstico e tratamento. Disponível em: http://www.projetodiretrizes.org.br/projeto_diretrizes/015.pdf. [Acessado em 23 de outubro de 2009].
4. Targeted Tuberculin Testing and Treatment of Latent Tuberculosis Infection. Disponível em: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr4906a1.htm. [Acessado em 23 de outubro de 2009].
5. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. Disponível em: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a4.htm. [Acessado em 23 de outubro de 2009].
6. Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. Guia para o diagnóstico, tratamento e prevenção da tuberculose. Disponível em: http://bvsms.saude.gov.br/bvs/publicacoes/manual_tuberculose_2006.pdf. [Acessado em 26 de outubro de 2009].
7. Rangel-Frausto MS, Ponce-De-León-Rosales S, Martinez-Abaroa C, Haslov K. Tuberculosis and tuberculin quality: best intentions, misleading results. Infect Control Hosp Epidemiol 2001; 22(8):481-4.
8. Teixeira L, Maciel E, Dutra ME, Perkins MD, Johnson JL, do Valle Dettoni V. Simultaneous comparison of reactivity to purified protein derivative RT-23 and Tubersol in health care workers in Vitória, Brazil. Int J Tuberc Lung Dis 2000; 4(11):1074-7.

9. Muñoz-Barret JM, Macías-Hernández AE, Hernández-Ramos I, Durán-Martínez E, Martínez-Magdaleno RM, Medina-Valdovinos H *et al*. Comparative tuberculin reactivity to two protein derivatives. Rev Invest Clin 1996; 48(5):377-81.
10. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD *et al*. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med. 2001; 345(15):1098-104.
11. Criscione LG, St. Clair EW. Tumor necrosis factor-alfa antagonists for the treatment of rheumatic diseases. Curr Opin Rheumatol 2002; 14:204-11.
12. Gomez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum 2003; 48:2122-7.
13. Ministério da Saúde - Programa Nacional de Controle da Tuberculose. Disponível em: http://portal.saude.gov.br/portal/saude/visualizar_texto.cfm?idtxt=28055. [Acessado em 07 de outubro de 2009].
14. Filho AC, Kritski AL, Barreto AW, Lemos ACM, Netto AR, Guimarães CA *et al*. II Consenso Brasileiro de Tuberculose. Diretrizes Brasileiras para Tuberculose. J Bras Pneumol 2004; 30(1):1-55.
15. Façanha MC, Guerreiro MFF, Pinheiro AC, Costa Lima JR, Vale RLV, Teixeira GFD. Resgate de casos subnotificados de tuberculose em Fortaleza-CE, 2000-2002. Bol Pneumol Sanit 2003; 11(2):13-6.
16. Marques CDL, Duarte ALBP, Lorena VMB, Souza JR, Souza W, Gomes YM *et al*. Resposta atenuada ao PPD no diagnóstico de infecção tuberculosa latente em pacientes com artrite reumatóide. Rev Bras Reumatol 2009; 49(2):121-5.
17. Cattamanchi A, Dantes RB, Metcalfe JZ, Jarlsberg LG, Grinsdale J, Kawamura LM *et al*. Clinical characteristics and treatment outcomes of patients with isoniazid-monoresistant tuberculosis. Clin Infect Dis 2009; 48(2):179-85.
18. Jain A, Dixit P. Multidrug-resistant to extensively drug resistant tuberculosis: what is next? J Biosci 2008; 33(4):605-16.
19. Braga, JU. Equivalência das Reações cutâneas do PPD entre dois produtos comercializados no Brasil. Informe Epidemiológico do SUS 2000. Disponível em: http://bvsms.saude.gov.br/bvs/periodicos/informe_epi_sus_v09_n3.pdf. [Acessado em 22 de outubro de 2009].
20. Teixeira L, Maciel E, Dutra ME, Perkins MD, Johnson JL, do Valle Dettoni V. Simultaneous comparison of reactivity to purified protein derivative RT-23 and Tubersol in health care workers in Vitória, Brazil. Int J Tuberc Lung Dis 2000; 4(11):1074-7.
21. Muñoz-Barret JM, Macías-Hernández AE, Hernández-Ramos I, Durán-Martínez E, Martínez-Magdaleno RM, Medina-Valdovinos H *et al*. Comparative tuberculin reactivity to two protein derivatives. Rev Invest Clin 1996; 48(5):377-81.
22. Alonso Moreno FJ, García Bajo MC, Lougedo Calderón MJ, Comas Samper JM, García Palencia M, López de Castro F *et al*. Prevalence of tuberculosis infection among immigrants in the Toledo Health District, Spain. Rev Esp Salud Publica 2004; 78(5):593-600.
23. Ostrosky-Zeichner L, Rangel-Frausto MS, García-Romero E, Vázquez A, Ibarra MJ, Ponce de León-Rosales S. Tuberculosis in health personnel: importance of surveillance and control programs. Salud Publica Mex 2000; 42(1):48-52.
24. Kunins HV, Howard AA, Klein RS, Arnsten JH, Litwin AH, Schoenbaum EE *et al*. Validity of a self-reported history of a positive tuberculin skin test. A prospective study of drug users. J Gen Intern Med 2004; 19(10):1039-44.
25. Linquist JA, Rosaia CM, Riemer B, Heckman K, Alvarez F. Tuberculosis exposure of patients and staff in an outpatient hemodialysis unit. Am J Infect Control 2002; 30(5):307-10.
26. Poduval RD, Hammes MD. Tuberculosis screening in dialysis patients - is the tuberculin test effective? Clin Nephrol 2003; 59(6):436-40.
27. Wauters A, Peetermans WE, Van den Brande P, De Moor B, Evenepoel P, Keuleers H *et al*. The value of tuberculin skin testing in haemodialysis patients. Nephrol Dial Transplant 2004; 19(2):433-8.
28. Sester M, Sester U, Clauer P, Heine G, Mack U, Moll T *et al*. Tuberculin skin testing underestimates a high prevalence of latent tuberculosis infection in hemodialysis patients. Kidney Int 2004; 65(5):1826-34.
29. Habesoglu MA, Torun D, Demiroglu YZ, Karatasli M, Sen N, Ermis H *et al*. Value of the tuberculin skin test in screening for tuberculosis in dialysis patients. Transplant Proc 2007; 39(4):883-6.
30. Laurindo IMM, Seiscento M, Bombardia S, Souza TNL, Eluf Neto J, Radu AS *et al*. Diagnosis of latent tuberculosis in rheumatoid arthritis (RA) patients: tuberculin test (PPD) assessment. EULAR 2004, Rheumatoid arthritis clinical aspects [THU0235]. Disponível em: http://www.abstracts2view.com/eular/sessionindex.php?session=2004374&day=2004. [Acessado em 27 de janeiro de 2010].
31. Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, Mauri C. Compromised Function of Regulatory T Cells in Rheumatoid Arthritis and Reversal by Anti-TNF-α Therapy. J Exp Med 2004; 200:277-85.
32. Martins M, Lima M, Duppre N, Matos H, Spencer J, Brennan P *et al*. The level of PPD-specific INF- γ-producing $CD4^+$ T cells in the blood predicts the in vivo response to PPD. Tuberculosis 2006; 87(3):202-11.
33. Andrade TCB, Maia RM, Cosgrove C, Castelo Branco LRR. BCG Moreau Rio de Janeiro - An oral vaccine aginst tuberculosis - Review. Men Inst Oswaldo Cruz 2005; 100(5):459-65.
34. Maciel ELN, Viana MC, Zeitoune RCG, Ferreira I, Fregona G, Dietze R. Prevalence and incidence of Mycobacterium tuberculosis infection in nursing students in Vitória, Espírito Santo. Rev Soc Bras Med Trop 2005; 38(6):469-72.
35. Pilsczek FH, Kaufmann SHE. Prevalence and predictors of positive tuberculin skin test results in a research laboratory. Rev Soc Bras Med Trop 2008; 41(4):416-8.
36. Ponce de León D, Acevedo-Vásquez E, Sánchez-Torres A, Cucho M, Alfaro J, Perich R *et al*. Attenuated response to purified protein derivate in patients with rheumatoid artritis: study in a population with high prevalence of tuberculosis. Ann Rheum Dis 2005; 64:1360-1.
37. Köker IH, Pamuk Ã–N, Karlikaya N, Tunçbilek N, Cakir N. A low prevalence of purified protein derivative test positivity in Turkish patients with rheumatoid arthritis. Association with clinical features and HRCT findings. Clin Exp Rheumatol 2007; 25(1):54-9.
38. Maciel ELN, Meireles W, Silva AP, Fiorotti K, Dietze R. Transmissão nosocomial por *Mycobacterium tuberculosis* nos estudantes da área de saúde em uma região de alta incidência de tuberculose, Vitória, Estado do Espírito Santo. Rev Soc Bras Med Trop 2007; 40(4): 397-9.