Tuberculin Skin Test for the Screening of Latent Tuberculosis Infection Before Starting Treatment with Tumour Necrosis Factor- α Inhibitors In Systemic Immunoinflammatory Rheumatic Diseases in India - A High Burden Tuberculosis Region of the World

Anand N Malaviya¹, Vishal K Aggarwal², Alok Kalyani², Sadhana S Baghel³, Qamar Zaheer⁴, Shriram Garg⁵, Parth Sharma⁶, Vivek Anand⁷ and Sanjiv Kapoor⁵.

¹Consultant Rheumatologist and Head of the Department, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070

²Fellow in Rheumatology, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070

³Rheumatology Nurse Counsellor, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070

⁴Occupational therapist, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070

⁵Consultant Rheumatologist, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070

⁶Junior Resident, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070

⁷Physiotherapist, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070.

Received: May 19, 2018; Accepted: May 28, 2018; Published: June 15, 2018

***Corresponding author:** Anand N Malaviya, Consultant Rheumatologist and Head of the Department, Department of Rheumatology, ISIC Superspeciality Hospital, Vasant Kunj, New Delhi, INDIA - 110070. E-mail: anand_malaviya@yahoo.com

Abstract

Aim: India is a high burden tuberculosis country. Past BCG vaccination could cause sensitisation against TB antigens. Patients with systemic inflammatory rheumatic diseases (SIRDs) have inherent anergy. Also, they are often treated with glucocorticoids and other immunomodulatory drugs. These two confounders may affect TST, which is otherwise a robust screening method for studying TB epidemiology. Possibly for these reasons Indian Rheumatology Association did not recommend TST for the screening of TB infection (latent or disease) before initiating TNFi treatment. The present work examined TST results in SIRDs with the objective of whether it could be used for the screening of latent TB infection among SIRD patients in the Indian context.

Method: 60 adult rheumatoid arthritis (RA) and 191 axial Spondyloarthritis (axSpA) patients were Mantoux tested using a higher PPD strength of 10 TU to offset inherent disease anergy. The test was read after 48 to 72 hours. It was interpreted according to the nationally recommended cut-offs that takes into account sensitisation to BCG or environmental mycobacteria. Thus, in this study, an induration of >10 mm was considered as 'latent TB infection'.

Results: The positivity among RA and axSpA patients was 31.66% and 40.31% respectively, similar to that seen in adult Indians.

Conclusion: Based upon these results use of modified TST with 10 TUPPD using >10 mm induration as the cut-off point is recommended for diagnosing TB infection; the test may be appropriate for the screening of latent TB infection in the Indian setting. IRA may consider revising its policy for latent TB screening accordingly.

Keywords: Tuberculin Skin Test; India; Latent Tuberculosis Infection; High-Burden TB Country; Latent Tuberculosis Infection Screening; Tumour Necrosis Factor- α Inhibitors

Introduction

Infection with *Mycobacterium tuberculosis* causes tuberculosis (TB) that is further classified as 'latent TB infection' (LTBI) and 'TB disease' [1-3]. Tuberculin skin (Mantoux) test (TST) for the detection of TB infection is among the oldest tests in medicine, developed by Charles Mantoux between 1907-12 [4]. Despite problems and controversies, TST has been widely used around the world to estimate the prevalence of LTBI in the population [5, 6].

Soon after the approval and availability of tumour necrosis factor- α inhibitors (TNFi) for the treatment of rheumatoid arthritis (RA) it was reported that these agents increased the risk of flare of TB infection, the magnitude of which could vary with the agent, dose, dose schedule, and the background load (high, medium, or low burden regions) of TB [7]. RA is a chronic systemic inflammatory disease with some degree of immunocompromised state [8] in common with other chronic conditions [9]. Moreover, most patients are treated with immunomodulatory drugs, often with glucocorticoids (GC) that could interfere with TST response [10]. Further confounders to TST status could be previous BCG vaccination and possible high back-ground positivity in a high-burden TB country like India. Possibly for this reason Indian Rheumatology Association (IRA) did not recommend TST for LTBI screening before initiating TNFi treatment in RA patients [11].

The present study was conducted to systematically evaluate the status of TST among patients being considered for treatment with TNFi in a real-world situation. These patients were under the follow-up of the joint disease clinic of this hospital. The RA patients were being treated with conventional synthetic disease-modifying drugs (csDMARDs), mostly in combinations with once a week GC, a schedule that has been followed for the last several years [12]. Patients with axial Spondyloarthritis (axSpA) were being treated with continuous (not 'on-demand') non-steroidal anti-inflammatory drugs (NSAIDs) [13]. Those failing this treatment regimen were offered TNFi treatment. TST was performed as part of the standard LTBI screening procedure recommended in an earlier publication [14].

Patients and Methods

This hospital is a private sector enterprise where the patients have to pay for health-care. Because most of the patients in India do not have health insurance coverage, they themselves have to bear the cost. Thus, the patients included in this study belonged to the middle- and upper-middle class well-to-do strata of the society. Financially low-income class of patients usually attends government hospitals or charity hospitals where the health-care is either free or highly subsidized.

Patients aged 18 years or more with RA or axSpA under the follow-up of the Joint Disease Clinic (JDC) of this hospital were included in the study. Approval of the Institutional Ethics Committee was obtained for extracting information retrospectively from an electronic database of the JDC under strict confidentiality. RA and axSpA patients who had failed the

standard csDMARDs (in RA) and NSAID treatment of axSpA respectively, and were under consideration for the initiation of TNFi treatment, seen from 22 October 2010 to 10 January 2015, were included in this study.

The standard screening procedure for LTBI [14] with modifications described in the subsequent publication [15], were followed. Mantoux test was performed and read exactly as recommended by the experts [4,16,17] with the difference that 10TU/0.1ml PPD (I.P. with Tween 80 as stabilizer corresponding to 0.005% and phenol $\leq$ 0.5%; Span Diagnostics, Surat, Gujarat, India; web: www.span.in) was used for the test. The reading was taken any time between 48 to 72 hours. The exclusion criteria were mainly those that are contraindications for TNFi treatment, i.e. patients presently on cancer treatment or having received such treatment in the past, severe heart failure, within a year of joint replacement, pregnancy or lactation. Also, patients who could not return to this clinic for Mantoux reading and the results were read and reported by their local physicians were excluded from the analysis of the results.

In India Bacillus Calmette-Guérin (BCG) vaccination programme was abandoned in 1987 when it was proven to be ineffective in preventing TB infection [18]. Moreover, several studies have shown that the impact of BCG vaccination wanes off over time, when it is given to children and it does not interfere with the Mantoux reading [19]. However, as a routine left deltoid region of the patients being administered Mantoux test was examined routinely.

Screening for active TB infection

All the patients offered TNFi treatment were first screened for clinically active TB infection (TBI) using the standard 'Four-symptom complex for TB screening' ('4S' strategy) questionnaire for adults i.e. Current 1. Cough, 2. Fever, 3. Weight loss and 4. Night sweats [20] supplemented with a standard chest radiograph. The sensitivity of '4S complex' questionnaire is 85%, with a negative predictive value for cases of pulmonary TB (NPV 97.7% [95% CI 97.4-98.0]).

Interpretation of Mantoux test results

For the interpretation of Mantoux test, recommendations of National Tuberculosis Institute, Bangalore (now Bengaluru), were followed [4, 16, 17].

1. An induration of **< 5 mm** was considered to be indicative of lack of tuberculin sensitization or having severe immunosuppression [4, 16, 17]. We used this result as a surrogate for 'never having contracted TB'.
2. An induration from **5 to 9 mm** was considered due to cross-sensitivity to environmental mycobacteria or due to BCG-induced sensitization.
3. An induration from **10 to 14 mm** that could be attributable to 3 different causes namely (i) Infection with tubercle bacilli; (ii) Due to cross-sensitivity to environmental mycobacteria; (iii) BCG-induced sensitization. Because TB flare is considered a serious adverse effect of TNFi therapy, we wanted to be over

cautious and considered all the patients in this category as having an infection with tubercle bacilli.

4. An induration of **>15mm** was considered TB infection.

Mantoux test-based Intervention

Category 1. Patients with induration from 10 to 14 mm were initiated on isoniazide hydrochloride 300mg (5 mg/Kg body weight (body weight) daily with pyridoxine 10 mg daily treatment simultaneously with the initiation of TNFi treatment.

Category 2. Patients with induration of >15 mm were initiated on the nationally recommended standard anti-tuberculosis treatment (4 drugs for 2 months, 2 drugs for 4 months) under the supervision of the pulmonologist. At least 1 month of this treatment was given to the patients before initiating the TNFi treatment. The above TB prophylaxis schedule was based upon local TB specialists; where category 1 was considered as having LTBI while category 2 patients the possibility of active TB disease

was a consideration.

Results

The study included a total of 478 patients on whom the Mantoux test was carried out. Two hundred and twenty-seven out-station patients did not return between 48-72 hrs for the reading of the Mantoux test. Their Mantoux readings were carried out by their local physicians. Because of the questionable reliability of the reading technique, these patients were excluded from further analysis. The other 251 patients including 60 patients with RA and 191 patients with SpA made a return visit between 48 and 72 hours for Mantoux reading. For comparison, 151 patients with RA and 477 patients with SpA on whom Mantoux test was not carried out were included in the study.

The demographic characteristics of these patients are given in Table 1. (Table 1A, 1b)

Table 1A: Demographic characteristics of 211 patients with rheumatoid arthritis (RA)

Variables		Mean	Median	Range	Std dev.
Age at disease onset (yrs.)	Group I*	42.3	42	17-76	±13.2
	Group II**	40.9	40	18-71	±12.17
Age at the first clinic visit (yrs.)	Group I	45.3	44	18- 73	±11.9
	Group II	44.8	44	18-72	±12.3
Disease duration at first visit (mo.)	Group I	69.3	66	4-179	±51.7
	Group II	68.6	65	7-183	±50.3

Group I: 60 patients with RA on whom Mantoux test was performed.

**Group II: 151 Patients with RA on whom Mantoux test was not performed.

Table 1B: Demographic characteristics of 668 patients with Spondyloarthritis

Variables		Mean	Median	Range	Std dev.
Age at disease onset (yrs.)	Group I*	23.1	23	15-43	±9.1
	Group II**	23.8	24	16-42	±8.6
Age at the first clinic visit (yrs.)	Group I	32.4	33	20-49	±11.2
	Group II	32.9	34	18-51	±12.3
Disease duration at first visit (yrs.)	Group I	9.4	9	11-Apr	±8.6
	Group II	9.8	10	11-Apr	±8.2

*Group I: 191 patients with SpA on whom Mantoux test was performed.

**Group II: 477 Patients with SpA on whom Mantoux test was not performed

Of a total of 211 patients with RA there were 178 females with female: male ratio of 5.4: 1. Rheumatoid factor was positive in significant titers in 176 (83.4%), anti-CCP antibody in significant titer was present in 184(87.2%) patients, both of these antibodies were present in 149 (70.6%) patients. Only +ve RF was present in 27 (12.8%) patients and only anti-CCP was positive in 35 (16.6%) patients Sixty-nine (32.7%) patients had the disease duration

of < 6 months (early disease) while the other 141 (67.3%) had established or late disease at the first clinic visit. Using Clinical Disease Activity Index (CDAI)21 cut-off values 196 (93%) patients were in 'high' or 'moderate' disease activity state at the first presentation; majority were naïve to disease modifying anti-rheumatic drugs (DMARDs) or, treated with suboptimal doses of DMARDs.

Of a total of 668 patients with SpA there were 530 males with a male : female ratio of 4.8:1. HLA B27 was positive in 607 (90.8%). There were 459 (68.7%) with non-radiographic axial SpA. Peripheral arthritis of SpA pattern was present in 140 (20.9%) patients. Using ASDAS cut-off values 22, 499 (62%) patients had very high disease, 226(33.8%) had high disease,

80(12%) had low disease and none were in remission

The Mantoux test results are given in Table 2. As can be seen, using the augmented Mantoux test (i.e. using 10 TU tuberculin) 19 (31.6%) patients with RA and 77 (40.31%) patients with SpA showed a positive result (i.e. > 10 mm induration). (Table 2)

Table 2: Results of the Mantoux test on 251 patients performed with 10 TUPPD (RT23 with Tween 80).

Total patients - 251		
Mantoux reading (with 10 TUPPD)	Number of patients	Percentage
<5 mm	128	50.99%
5-9 mm	27	10.75%
10-14 mm	39	15.53%
>=15 mm	57	22.76%
Patients with RA - 60		
<5 mm	36	60%
5-9 mm	5	8.30%
10-14 mm	8	13.30%
>=15 mm	11	18.30%
Patients with SpA - 191		
<5 mm	92	48.16%
5-9 mm	22	11.51%
10-14 mm	31	16.23%
>=15 mm	46	24.08%

An important point to note in the present study was that during the (short) period of this study, none of the patients developed active TB infection while taking TNFi drugs.

Discussion

In this study, 38.24% of a total of 251 patients were found to be Mantoux positive when the test was interpreted according to the recommendations of The National Tuberculosis Institute, Bangalore (now Bengaluru), India [4,16,17]. The positivity among RA patients was 31.6% and among SpA patients 40.3%. Thus, as can be seen in table 2, approximately 60% of RA and 48% of SpA patients were completely Mantoux negative with induration of < 5 mm. This would indicate lack of tuberculin sensitization, which could be considered as a surrogate for never having contracted TB [4, 16, 17]. The only other condition that may cause complete Mantoux negativity is severe immunodeficiency state [4,16,17] that these patients did not have. It needs to be mentioned here that in India, HIV screening is not mandatory in patients who are considered for biological treatment. In the experience of the senior author (ANM), in 18 years of practice only 1 patient was seen who had clinical HIV infection symptoms that was confirmed with laboratory testing.

There were 8.3 % RA and 11.51 % SpA patients who showed induration of 5 to 9 mm which could be attributable either to cross-sensitivity with environmental mycobacteria or to BCG vaccination [4,17]. There were 13.3 % RA and 16.23 % SpA

patients who showed induration from 10 to 14 mm, a range which needs to be interpreted very carefully [4]. This is because it could be attributable to 3 different causes namely 1. LTBI; 2. Due to cross sensitivity to environmental mycobacteria; 3.BCG-induced sensitivity [4, 17]. In clinical scenarios where it is imperative not to miss a TB infection, e.g. patients to be initiated on TNFi treatment, this category should be considered as 'LTBI'. There were 18.3 % RA and 24 % SpA patients with induration of >15 mm that is considered TB infection and treated as active TB infection [4,14,17]. Thus, a simple and cheap investigation namely, Mantoux test was able to provide robust information that 31.6 % of RA and 40.3 % SpA patients who underwent TST were candidates for TB prophylaxis. In a recent editorial, a similar opinion was expressed that it is too early to give-up TST, which happens to be a time-tested robust test for studying TB epidemiology [23].

TST positivity in general population of India, a high burden TB country of the world 24 has been reported to be 30 to 41% of the population [25, 26] the range being very similar to that found in the present study on the patient population that was considered for TNFi treatment. Thus, by using 10 TU PPD for Mantoux testing, any background disease-related anergy would seem to have been overcome.

There could be several objections and disagreements both to the method and the interpretation of TST in this study. Thus, the use of 10 TU PPD was only based upon one publication from India

where authors had used this dose to overcome a degree of anergy related to chronic diseases [27]. Similarly, the use of the cut-off values and the interpretation of the results were extrapolated from the results in the normal population. Whether this could be valid for chronic SIRDs like RA and SpA, remains an open question. The other lacunae in the study were that a comparator group of patients using the standard PPD dose for the Mantoux test, i.e. 1 or 2 TU [4] was not studied. Comparison of the results of the Mantoux test with standard dose and 10 TU PPD dose, could have given more reliable and robust information. Also, 10 TUPPD was commercially obtained. The reliability of its standardization could be questionable.

In recent years several interferon- γ release assays (IGRA) based tests for TB have been developed. These include QuantiFERON-TB Gold (QTGIT) [28,29] and ELISPOT/T. SPOT.TB test [30,31]. The present report has not compared TST with any of the IGRAs, and this could be a weakness of this study. We have initiated a study comparing QTGIT and TST but the results are still not available. However, the current status of the IGRA-based assays is discussed in a Morbidity and Mortality Weekly Report (MMWR) of The Centers for Disease Control (CDC) [32] and also by Pai and colleagues on their Indian experience [3]. Both of these publications state that in general, QFT-GIT and T. SPOT.TB test sensitivities are fairly comparable and similar to those for TST. The CDC report has further quoted 11 studies that compared QFT-GIT and TST in patients in whom active tuberculosis was diagnosed [32]. In 6 of these studies, there was no statistically significant difference between the two tests. The matter was further complicated by 5 other studies 3 of them demonstrated greater sensitivity for TST while the other 2 reported greater sensitivity for QFT-GIT. Similarly, a recent paper from Brazil (a high burden TB country comparable to India) has reported that T. SPOT.TB test is not superior to TST [33]. Reviewing these reports, it would appear that in a resource-constrained country like India IGRA-based assays cannot be recommended routinely for LTBI screening. Another point of note is that large-scale nation-wide TST surveys in India have also shown that prior BCG vaccination does not significantly affect the TST results [3]. Based on these studies Pai and colleagues have stated ‘... the TST is a useful test for LTBI in the Indian milieu, particularly because of the low cost, relatively easy accessibility, and because BCG does not significantly affect TST specificity’ [3]. Therefore, it can be confidently stated that TST can be used in India as a good test for the screening of LTBI. But, there is a caveat. Thus, if RA and SpA are considered separately it was observed that only ~ 31% of the patients with RA had a positive Mantoux reading. If the LTBI rate is ~ 40% in the general population, it would appear that a Mantoux test alone may miss some of the RA patients with LTBI. In one of the ongoing study, we have now supplemented the LTBI screening by adding QuantiFERON-TB Gold (manuscript in preparation).

Another weakness of the present study is that it does not give prospective data on the efficacy of this approach in preventing TB among those receiving TNFi treatment. Work is in progress on both of these issues, and it is hoped that some conclusive results would become available shortly.

In conclusion, based on the present study we recommend the use of modified TST using 10 TU PPD for performing Mantoux test and using >10 mm induration as the cut-off point for diagnosing TB infection. Appropriate TB prophylaxis may then be begun to prevent TB flare on initiating TNFi treatment. We propose that IRA may like to revise its policy for TB screening accordingly before initiating TNFi treatment.

Acknowledgement

Authors wish to express their gratitude to Prof. Alladi Mohan, MD, FRCP (Edin), FCCP (USA), FAMS, FICP, FIACM; Chief, Division of Pulmonary and Critical Care Medicine, Professor and Head, Department of Medicine, Sri Venkateswara Institute of Medical Sciences, Tirupati, India, for his suggestions and help in getting several seminal publications on the subject that have been quoted in this paper

References

1. The Difference between Latent TB Infection and TB Disease. Centers for Disease Control and Prevention. 2011.
2. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med. 2000; 161(4 Pt 1):1376-1395. DOI:10.1164/ajrccm.161.4.16141
3. Madhukar Pai, Keertan Dheda, Jane Cunningham, Fabio Scano, Richard O.'Brien. Diagnosis of latent tuberculosis infection. 2007; 7(6): 428-438.
4. Surendra K. Sharma, Alladi Mohan. The tuberculin skin test. Indian J Med Res. 2013; 137(3): 455-493.
5. Daniello J, Zollner I. Experiences with the intracutaneous Mendel-Mantoux tuberculin test in routine screening in a public health office. Gesundheitswesen. 1997; 59(8-9): 519-522.
6. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA. 1999; 282(7): 677-686.
7. Joseph Keane, Sharon Gershon, P. Robert, P. Wise, Elizabeth Mirabile-Levens, John Kasznica, et al., Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med. 2001; 345: 1098-1104. DOI: 10.1056/NEJMoa011110
8. Palit J, Chattopadhyay C, Malaviya AN, Uberoi S, Kumar R, Sarika Bhalgat Ranade, Satish Doiphode. Some immunological parameters in rheumatoid arthritis from India. J Indian Soc Periodontol. 2012; 16(1): 22-27. DOI: 10.4103/0972-124X.94599
9. Malaviya AN, Kumar R, Kathpalia SC, Malhotra KK. Non-specific cellular and specific cell-mediated immunological responsiveness in chronic renal failure. Ind J Med Res. 1973; 1194-1199.
10. Agarwal S, Das SK, Agarwal GG, Srivastava R. Ragini Srivastava, Steroids Decrease Prevalence of Positive Tuberculin Skin Test in Rheumatoid Arthritis: Implications on Anti-TNF Therapies. Interdisciplinary Perspectives on Infectious Diseases Volume 2014; 1-5

11. Handa R, Misra R, Chaturvedi VP, Pispati PK, Rao URK, Joshi VR. Guidelines for tuberculosis prophylaxis during anti-tumour necrosis factor- γ treatment: Indian Rheumatology. *Jour Rheumatol*. 2006; 9:181–3.
12. Malaviya AN, Agarwal D, Sharma A, Gogia SB, Zaheer Q. Practising computer-aided objectified outcome-driven targeted treatment of rheumatoid arthritis in a resource constrained country: results from a single rheumatology clinic. *Ind J Rheumatol*. 2010; 5(1):3-10.
13. Kroon FPB, van der Burg LRA, Ramiro S, Landewe RBM, Buchbinder R, Falzon L, et al., D. Non-steroidal anti-inflammatory drugs (NSAIDs) for axial spondyloarthritis (axSpA). *Cochrane Database of Systematic Reviews*. 2015;7:
14. Malaviya AN, Kapoor S, Garg S, Rawat R, Shankar S, Nagpal S, Khanna D, Furst DE. Preventing tuberculosis flare in patients with inflammatory rheumatic diseases receiving tumor necrosis factor-alpha inhibitors in India -- An audit report. *J Rheumatol*. 2009; 36(7): 1414-1420. DOI: 10.3899
15. Anand Malaviya, Sanjiv Kapoor, Shriram Garg, Roopa Rawat. Recommended screening strategy for preventing tuberculosis flare in patients with inflammatory rheumatic diseases receiving tumor necrosis factor-alpha inhibitors in India - follow-up report. *J Rheumatol* 2010; 37(1): DOI: 10.3899/jrheum.090854
16. Chadha VK. Tuberculin test. *Indian J Pediatr*. 2001; 68(1): 53–58.
17. Aggarwal A, Guglani L, Faridi MM. Standardization of Mantoux test. *Indian Pediatrics*; 2002; 39:404-406.
18. Chandrakant Lahariya. A brief history of vaccines & vaccination in India. *Indian J Med Res*. 2014; 139(4): 491–511.
19. Seddon JA, Paton J, Nademi Z, Keane D, Williams B, Williams A, et al., The impact of BCG vaccination on tuberculin skin test responses in children is age dependent: evidence to be considered when screening children for tuberculosis infection *Thorax*. (2016); 71(10): 932-939. DOI:10.1136/thoraxjnl-2015-207687
20. Getahun H, Kittikraisak W, Heilig CM, Corbett EL, Ayles H, Cain KP, et al., Development of a standardised screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. *PLoS Med*. 2011; 8(1): DOI: 10.1371/journal.pmed.1000391
21. Aletaha D, Smolen J. The Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI): a review of their usefulness and validity in rheumatoid arthritis. *Clin Exp Rheumatol*. 2005; 23(5 Suppl 39):S100-8.
22. Pedro M Machado, Robert Landewe, Desiree van der Heijde. Ankylosing Spondylitis Disease Activity Score (ASDAS): 2018 update of the nomenclature for disease activity states. 2018; doi:10.1136/annrheumdis-2018-213184
23. Lauren F. Collins, Carolina Geadas, Jerrold J. Ellner. Diagnosis of latent tuberculosis infection: Too soon to pull the plug on the tuberculin skin test. *Ann Intern Med*. 2016; 164(2):122-124. DOI: 10.7326/M15-1522
24. Report of the Meeting of the Expert Committee to Estimate TB Burden in India. 2005;13-388.
25. National Tuberculosis Institute. Tuberculosis in a rural population of South India: a five-year epidemiological study. *Bull World Health Organ* 1974; 51:473-488.
26. Gothi GD, Chakraborty AK, Nair SS, Ganapathy KT, Banerjee GC. Prevalence of tuberculosis in a south Indian district - Twelve years after initial survey. 1979; *Ind Jour Tubercul* 26:121-135.
27. MA. Phadke, NA. Kshirsagar. Blood tests for diagnosis of tuberculosis. 2006;368(9532):282-322 DOI: 10.1016/S0140-6736(06)69063-6
28. Mazurek GH, Jereb J, LoBue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON®-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. Centers for Disease Control and Prevention. *MMWR Recomm*. 2005; 16;54(RR-15):49-55.
29. Centers for Disease Control. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. 2005; *MMWR* 54[No. RR-15]:49–55.
30. Lalvani A, Millington KA. Screening for tuberculosis infection prior to initiation of anti-TNF therapy. *Autoimmun Rev*. 2008; 8(2):147-52. DOI: 10.1016/j.autrev.2008.07.011
31. Lalvani A, Millington KA. Screening for tuberculosis infection prior to initiation of anti-TNF therapy. *Autoimmun Rev*. 2008; 8(2): 147-152. DOI:10.1016/j.autrev.2008.07.011
32. Gerald H. Mazurek, John Jereb, Andrew Vernon, Phillip LoBue, Stefan Goldberg, Kenneth Castro, et al., Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection — United States. *MMWR*. 2010; 59(RR05): 1-25.
33. Sztajn bok F, Boe chat NLF, Ribeiro SB, Oliveira SKF, Sztajn bok DCN, Clemax C et al., Tuberculin skin test and ELISPOT/T. SPOT.TB in children and adolescents with juvenile idiopathic arthritis.