

Tuberculin skin test response in patients with juvenile idiopathic arthritis on anti-TNF therapy

Kenan BARUT^{1*}, Sezgin ŞAHİN¹, Amra ADROVIC¹, Muhammet KÖŞKER², Ömer KILIÇ², Yıldız CAMCIOĞLU², Haluk ÇOKUĞRAŞ², Necla AKÇAKAYA², Betül SÖZERİ³, Özgür KASAPÇOPUR¹

¹Department of Pediatric Rheumatology, Cerrahpaşa Medical School, İstanbul University, İstanbul, Turkey

²Department of Pediatric Infectious Disease, Cerrahpaşa Medical School, İstanbul University, İstanbul, Turkey

³Health Sciences University, Ümraniye Education and Research Hospital, İstanbul, Turkey

Received: 27.10.2017 • Accepted/Published Online: 27.05.2018 • Final Version: 12.12.2018

Background/aim: The aim of this study is to evaluate the effect of biologic drugs on the tuberculin skin test in patients with juvenile idiopathic arthritis.

Materials and methods: A total of 234 biologic drug-using juvenile idiopathic arthritis patients and 45 healthy controls were enrolled in the study. The tuberculin skin test results of the patients, which had been routinely provided during follow-up, were obtained from the patient files. Tuberculin skin test values of ≥ 5 mm were considered to be positive.

Results: The mean diameter of tuberculin skin test induration was 4.99 ± 6.84 mm (IQR: 0–10 mm) and 7.83 ± 3.47 mm (IQR: 0–16 mm) in patients and controls, respectively ($P < 0.05$). Tuberculin skin test positivity (≥ 5 mm) was found in 96 (41%) and 38 (84.4%) of patients and controls, respectively ($P < 0.001$). There was no induration in 125 (53.4%) patients and 3 (6.6%) healthy controls, respectively ($P < 0.001$).

Conclusion: In the patients with juvenile idiopathic arthritis who were using biologic drugs, tuberculin skin test induration was significantly lower compared to the control group. Tuberculin skin tests alone seem inadequate for recognition of latent tuberculosis in juvenile idiopathic arthritis patients on anti-TNF therapy.

Key words: Juvenile idiopathic arthritis, tuberculin test, biologic therapy

1. Introduction

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in children that presents with peripheral arthritis. The pathogenesis of the disease is unknown, although it is thought to be triggered by factors such as stress, infections, autoimmunity, trauma, and genetic tendency (1).

Patients with inflammatory diseases such as JIA are more prone to severe systemic or localized infections. This increased risk is due to a combination of dysfunctional immune system and secondary immune deficiency as a consequence of immunosuppressive drugs. Tuberculosis (TB) is one of the serious infections, especially in developing countries. A latent form of this disease could be detected by the tuberculin skin test (TST) (2). Factors that can facilitate the development of TB are increased autoimmunity leading to immune dysfunction, immunosuppressive agents, Bacillus Calmette–Guérin

(BCG) vaccination status, previous TB infection, and the burden of TB disease in the community (3). TB has varying degrees of clinical phenotypes, ranging from asymptomatic cases to severe disseminated infection. After the primary infection has been acquired, the disease course differs.

Latent TB infection is a condition in which a child is infected with *Mycobacterium tuberculosis* bacteria but does not have apparent clinical or radiological signs of the disease. Patients with latent TB do not transmit the infection to others. However, they are at risk of developing an active and contagious infection in the case of immune system dysfunction (4).

The TST or purified protein derivative (PPD) test causes a delayed hypersensitivity reaction mediated by Th1 cells and monocytes/macrophages. It shows the person's previous exposure to the TB bacilli but does not give precise information of TB disease (5,6). The BCG vaccine

* Correspondence: kenan.barut@istanbul.edu.tr

may show cross-reactivity to the TST, but the reaction is usually smaller than 10 mm and gradually decreases. TST positivity is seen in 0%–18% of people vaccinated with BCG during infancy. Indurations larger than 10 mm are unlikely in the case of previous BCG vaccination. It was reported that there is a direct relationship between number of BCG vaccinations and TST reaction size (7). Tuberculin skin tests in biologic-naive JIA patients were assessed for the first time in a study conducted previously by our group (8). We found that neither the activity nor subtype of the disease or the use of corticosteroids and methotrexate affected the TST response. The results of the study showed that even the smaller indurations of TST should be considered as positive when screening JIA patients for latent TB (8). Another study from Turkey also confirmed the mentioned findings; TST response was smaller in JIA patients compared to healthy controls (9). The aim of the present study was to evaluate the effect of biological drugs on TST, which was not studied in our previous study.

2. Materials and methods

2.1. Study groups

We retrospectively evaluated the patient files of our 234 JIA patients (102 males, 132 females) who were on biologic therapy for at least 6 months. All the JIA patients were diagnosed and classified into subgroups according to the International League of Associations for Rheumatology criteria (10). All the data regarding age, sex, duration of disease, treatment options, and duration of therapy were recorded from patient files. The induration results of TST after the first 6 months of anti-TNF therapy were taken in a retrospective scope from patient records. Presence of any family member with TB and close contact with patients with infectious TB were noted. The subjects that had received INH prophylaxis were also recorded. TST induration results of 45 healthy children (23 males, 22 females) were obtained from a study previously performed by our group (8).

2.2. Tuberculin skin test

The TST is performed by an intradermal injection of 0.1 mL of PPD into the volar surface of the forearm. Hyperemia and induration occur at the site of injection after 2 to 3 days if the patient has been vaccinated with BCG or infected with TB bacilli (11). TST response in our clinic is evaluated according to the recommendations of the American Academy of Pediatrics, the US Centers for Disease Control and Prevention, the American Thoracic Society, and the Infectious Disease Society of America. TST induration of ≥ 5 mm is accepted as positive in children with a history of close contact with a known or suspected contagious TB patient, children suspected to have TB, those receiving immunosuppressive therapy, or those with immunosuppressive diseases. Induration of ≥ 10 mm is considered as positive in children with increased risk of

disseminated disease younger than 4 years of age or with chronic diseases/malignancy, or in children with increased exposure to TB. Induration of ≥ 15 mm is accepted as positive in children 4 years of age or older without any risk factors.

The TST is a routinely used test to screen latent tuberculosis in our JIA patients on biologic drugs. In our study, the diameter of induration was measured 48–72 h after injection by the same investigators (MK and ÖK). We evaluated TST results of the patients and controls in light of their BCG vaccination history. We compared our results with a previous historical JIA cohort on disease-modifying antirheumatic drug therapy to delineate the additional role of biologic drugs on the TST response other than the disease itself. The subjects with an induration equal to or larger than 5 mm were further evaluated by chest X-ray and QuantiFERON (QTF). The local ethics committee approved this study.

2.3. Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA). Distributional properties of continuous variables were expressed in terms of mean \pm standard deviation and median (min–max: minimum–maximum). Categorical variables were presented with frequency and percentage (%). The Mann–Whitney U and chi-square tests were used to determine the differences between continuous and categorical variables of the two groups. Since the design of the study was mainly based on the diameter of indurations, the TST variable was considered as the dependent variable of the study. According to the Shapiro–Wilk test, the TST result was a nonnormally distributed variable. The subjects were grouped into two categories according to whether the TST result was ≥ 5 mm or < 5 mm. The risk of the age variable that could result in a TST induration equal to or more than 5 mm was calculated. Since the mean ages of patients and healthy subjects were not similar, binary logistic regression analysis was used to adjust the groups for age ($P = 0.072$, Exp(B): 1.052, 95% CI for EXP(B) = 0.995–1.1129). As a result, we found that the age variable did not have any risk for TST results of > 5 mm. Statistical significance was obtained at $P \leq 0.05$.

3. Results

3.1. Demographic features

Of the 234 JIA patients on biologic drug therapy, 102 (43.6%) were male and 132 (56.4%) were female. Mean ages of the patients and control group were 12.8 ± 4.7 years (3.2–19.8) and 8.1 ± 3.3 years (2.8–16), respectively. The mean duration of disease was 5.9 ± 4.1 years (range: 6 months to 17 years). Mean duration of biologic treatment was 2.5 ± 1.9 years, with a median of 2 years (IQR: 1–3.5). All demographic characteristics are summarized in Table 1.

Table 1. Demographic characteristics of the patients.

Patients	Parameters
Mean age of JIA group, mean \pm SD (years)	12.7 \pm 4.7
Mean age of control group, mean \pm SD (years)	8.1 \pm 3.3
JIA subtypes	
Oligoarticular, n (%)	66 (28)
Polyarticular, n (%)	101 (42.8)
Systemic, n (%)	26 (11)
Enthesitis-related arthritis, n (%)	37 (15.7)
Juvenile psoriatic arthritis, n (%)	4 (1.7)
Duration of disease, mean \pm SD (years)	5.8 \pm 4.1
Duration of methotrexate treatment, mean \pm SD (years)	5.5 \pm 4.3
Duration of steroid treatment, mean \pm SD (years)	5.6 \pm 4.5
Duration of anti-TNF drug treatment, mean \pm SD (years)	2.5 \pm 1.9
Type of anti-TNF drug	
Etanercept, n (%)	188 (80.4)
Adalimumab, n (%)	23 (9.8)
Infliximab, n (%)	23 (9.8)
Presence of TB in the family	22 (9.4)
History of contact with TB	6 (2.6)
Presence of isoniazid prophylaxis	28 (12)

JIA: Juvenile idiopathic arthritis, TB: tuberculosis, anti-TNF: tumor necrosis factor inhibitors.

3.2. Tuberculin skin test response

The mean diameter of TST induration was 4.9 ± 6.8 mm (IQR: 0–10 mm) in JIA patients and 7.8 ± 3.5 mm (IQR: 0–16 mm) in controls ($P < 0.05$). While only 96/234 (41%) of JIA patients had a TST induration equal to or more than 5 mm, 38/45 (84.4%) of the controls had an induration of ≥ 5 mm ($P < 0.001$). Fifty-nine patients (25.2%) and 19 controls (42.2%) had a TST induration of ≥ 10 mm ($P < 0.05$). The TST was nonreactive in 125 (53.4%) and 3 (6.6%) of the subjects in the JIA and control groups, respectively ($P < 0.001$) (Table 2).

3.3. BCG vaccination status

Of the 234 JIA patients, the BCG vaccination history was negative for 20 of them (6.5%). The mean size of induration was 2.7 ± 4.7 mm (IQR: 0–5) in this group and this response was significantly lower than in all of the JIA patients and healthy controls. The BCG vaccination history was positive in the rest of the JIA patients and control group. TST responses were compared regarding the number of BCG vaccinations between the control and the patient group after the exclusion of subjects with no history of vaccination. The mean TST induration was 5.0 ± 6.4 mm (IQR: 0–9) in 149 patients (63.7%) and 7.1 ± 3.2 mm in healthy subjects who had only been vaccinated once ($P > 0.05$). In the subjects who

had been vaccinated twice, the mean TST induration was 6.1 ± 8.03 mm (IQR: 0–10) in 65 (27.8%) of the JIA patients and 10.04 ± 4.1 mm in the control group ($P = 0.05$) (Table 3). Patients and healthy subjects were categorized according to their BCG vaccination status and then the two groups were compared with each other in terms of TST positivity (TST ≥ 5 mm). While 63/149 (42.2%) patients who had only one BCG vaccination in their history had TST positivity, 23/28 (82.1%) healthy controls had positivity ($P < 0.001$). In the subjects who had 2 BCG vaccinations in their history, 30 of 65 (46.1%) patients and 15 of 17 (88.2%) controls had TST positivity (≥ 5 mm) ($P < 0.001$). The two groups were also compared regarding TST induration of ≥ 10 mm. There were no significant differences between the JIA patients and the controls in TST positivity (≥ 10 mm) among those with a single BCG vaccine ($n = 35/149$, 23.5% versus $n = 8/28$, 28.5%; $P = 0.7$) and those with 2 BCG vaccines ($n = 23/65$, 35.4% versus $n = 11/17$, 64.7%; $P > 0.05$).

3.4. Anti-TNF therapy and TST response

A total of 188 (80.4%) JIA patients were treated with etanercept, 23 (9.8%) with adalimumab, and 23 (9.8%) with infliximab. The mean durations of treatment with etanercept, adalimumab, and infliximab were 2.6 ± 1.8 years, 2.4 ± 1.2 years, and 3.7 ± 2.7 years, respectively.

Table 2. Comparison of tuberculin skin test values in study groups.

	Juvenile idiopathic arthritis (n: 234)	Control group (n: 45)	P
TST induration mm, mean ± SD	4.99 ± 6.84 mm	7.83 ± 3.47 mm	P < 0.05
TST induration 0–4 mm	138 (59%)	7 (15.5%)	P < 0.001
≥5 mm	37 (15.8%)	38 (84.4%)	P < 0.001
≥10 mm	59 (25.2%)	20 (44.5%)	P < 0.05

TST: Tuberculin skin test.

Table 3. Average tuberculin skin test width and positivity comparison according to the vaccination status.

	Juvenile idiopathic arthritis(n: 234)		Control group (n: 45)		P1	P2
	BCG = 1	BCG >1	BCG = 1	BCG > 1		
Vaccination status (No vaccination in JIA group, n = 20)	149 (63.7%)	65 (27.8%)	28 (62.2%)	17 (37.8%)		
TST induration, mm, mean ± SD	5 ± 6.4	6.1 ± 8.03	7.1 ± 3.2	10.04 ± 4.1	P > 0.05	P = 0.05
TST induration ≥5 mm	63(42.2%)	30(46.1%)	23(82.1%)	15 (88.2%)	P < 0.001	P < 0.001
≥10 mm	35(23.5%)	23(35.4%)	8 (28.5%)	11(64.7%)	P > 0.05	P > 0.05

TST: Tuberculin skin test, JIA: juvenile idiopathic arthritis, BCG: Bacillus Calmette–Guérin, P1: between JIA patients and healthy children who were vaccinated once, P2: between JIA patients and healthy children who were vaccinated >1 time.

The diameter of the TST induration was measured among the subjects using various biologic agents. The mean diameter of the TST induration was smaller in subjects using etanercept (4.6 ± 6.3 mm, P < 0.05) and adalimumab (4.9 ± 9.1 mm, P > 0.05) compared to the control group. However, there was no statistically significant difference between the patients using infliximab and the control group (8.3 ± 7.7 mm versus 7.8 ± 3.5 mm, P > 0.05). The etanercept group did not differ from adalimumab according to the diameter of the induration. The diameter of the TST induration was significantly larger in subjects using infliximab than the other biologic agents (P < 0.001).

3.5. Outcome

In our study, among 96 subjects with a TST induration of ≥5 mm, 28 patients (29.2 %) had a history of exposure to TB infection or a positive QTF test. Prophylactic INH treatment for 9 months was administered to these patients due to latent TB infection. Among 234 patients, active TB was detected in only one patient who had a history of close contact with a school friend with active tuberculosis.

4. Discussion

In this study, the TST responses of patients treated with biologic therapy were obtained from their medical records. Th1 lymphocytes play an important role both in the disease pathogenesis and in the reaction process of TST. Since anti-TNF agents inhibit this pathway, they are used in the treatment of JIA.

Anti-TNF agents seem safe in patients with JIA according to the majority of published studies. In two studies from Turkey and Brazil, where TB is common, none of the patients on anti-TNF treatment developed clinical or radiological active TB (12,13). Among the 144 JIA patients treated with anti-TNF, only one patient received anti-TB treatment due to QTF positivity (12). Similarly, only 3 of 69 JIA patients had TST of ≥5 mm in a recent study. Consequently, isoniazid prophylaxis was initiated in those 3 patients and none of them developed active TB during the follow-up (13).

In 112 adult patients with rheumatoid arthritis (RA), TST was statistically smaller than in the controls (4.5 mm

versus 11.5 mm) (6). Moreover, studies among adults reported that TST response is also poorer in RA patients receiving anti-TNF treatment (14). Similarly, both the mean diameter of TST induration and the percentage of TST positivity are shown to be significantly lower in patients treated with biologic drugs in our study. The TST response in the biologic-naive JIA patients was assessed for the first time in a previous study conducted by our group. According to this study, neither the activity of the disease nor the use of corticosteroids and methotrexate affect the TST response (8). It remains unclear whether the decreased TST response is related to the disease itself or biologic agents. Hence, we have compared our current results with the previous biologic-naive historical cohort (8). The diameter of the TST response and the frequency of TST positivity (≥ 5 mm) were not statistically different between our JIA patients using anti-TNF agents and the biologic-naive JIA patients of our previous cohort (4.9 mm versus 4.12 mm and 41% versus 39.1%, respectively, $P > 0.05$). However, the frequency of unresponsiveness in the TST was significantly higher in the biologic drug-using JIA patients compared to biologic-naive JIA patients and the healthy controls (53.4% versus 24.3% and 6.6%, respectively).

In another study from Turkey, agreement between TST response and the QTF test was evaluated among 39 JIA patients and 40 healthy controls. The median induration of the TST in the JIA patients was significantly smaller than in the control group (5.8 mm versus 10 mm). Since all of the QTF-positive patients ($n = 2$) were TST-negative, they found no agreement between the QTF and TST. In conclusion, a single TST test was shown to be incompetent to detect latent TB (9). There are some controversial results regarding the effect of JIA activity on TST response (6,8,9). While Kiray et al. (8) did not find any association between disease activity and the TST response, in another study 93% of the patients who were unresponsive to the TST had active disease (9).

In Turkey, the BCG vaccination is given according to the recommendation of the Turkish Ministry of Health. While a two-dose vaccination was performed in the past, only one dose at the end of the second postnatal month is given according to the current legislation. TST response is more prominent in patients who underwent BCG vaccination (15). In our study, the diameter of the induration was larger in patients with a BCG vaccination history. In addition, two doses of the vaccination led to a larger TST response than one dose, similar to the data from literature (8).

While only 6.6% ($n = 3/45$) of the healthy subjects and 24.3% ($n = 28/115$) of biologic-naive JIA patients were unresponsive to TST, more than half of our JIA patients ($n = 125/234$, 53.4%) showed no reaction. It is well understood that anti-TNF agents not only decrease the TST response but also completely prevent the TST reaction in more than half of patients. The unresponsiveness did not differ across the various anti-TNF agents. These results seem consistent with previous studies. The frequency of the lack of any reaction to the TST ranges from 24.3% to 65% among different studies (6,8,9).

Nonreactive or poor TST results were considered to be secondary to the disease activity and treatment in patients with RA (6). In our previous study, TST reaction was not observed in 24.3% of the biologic-naive JIA patients (8). However, in the current study the frequency of subjects with no reaction was found to be increased (53.4%). The disease itself causes a decline in the TST response. Anti-TNF agents also seem to further decrease and hinder the TST response in patients with JIA.

The major limitations of our study are the absence of biologic-naive JIA patients and its retrospective nature. However, we were able to compare the TST results of current anti-TNF-using JIA patients with the biologic-naive JIA patients of our previous study (8). Another limitation is the lack of correlation of the disease activity with TST response. We could not find any association between disease activity and TST induration previously (8). Since recruitment of a new control group to the study posed some ethical concerns, we used the same control group of our previous study. Hence, the mean age of the control and study group did not match.

Formerly, we demonstrated the decreased TST response in biologic-naive JIA patients. In this study, the TST response was found to be decreased in the JIA patients on anti-TNF agents compared to the controls. Although there was no difference between the JIA patients with and without use of biologic agents in terms of the mean TST response, the frequency of unresponsive patients among the JIA patients on biologic agents was substantially higher compared to the biologic-naive JIA patients and healthy controls.

In conclusion, TST alone seems inadequate to recognize latent TB both in biologic-naive JIA patients and in JIA patients on anti-TNF therapy. Further prospective studies evaluating the TST responses sequentially in JIA patients on anti-TNF treatment would provide better results about the effect of biologic drugs on the TST response.

References

1. Barut K, Adrovic A, Şahin S, Kasapçopur Ö. Juvenile idiopathic arthritis. *Balkan Med J* 2017; 34: 90-101.
2. Horneff G. Biologic-associated infections in pediatric rheumatology. *Curr Rheumatol Rep* 2015; 17: 66.
3. Young J, O'Connor ME. Risk factors associated with latent tuberculosis infection in Mexican American children. *Pediatrics* 2005; 115: e647-653.
4. Chapman HJ, Lauzardo M. Advances in diagnosis and treatment of latent tuberculosis infection. *J Am Board Fam Med* 2014; 27: 704-712.
5. Yıldırım C, Küçük AI, Öngüt G, Önguç D, Çolak D, Mutlu G. Evaluation of tuberculin reactivity in different age groups with and without BCG vaccination. *Mikrobiyol Bul* 2009; 43: 27-35 (in Turkish with abstract in English).
6. Ponce de Leon D, Acevedo-Vasquez E, Sanchez-Torres A, Cucho M, Alfaro J, Perich R, Pastor C, Harrison J, Sanchez-Schwartz C. Attenuated response to purified protein derivative in patients with rheumatoid arthritis: study in a population with a high prevalence of tuberculosis. *Ann Rheum Dis* 2005; 64: 1360-1361.
7. Araujo Z, de Waard JH, de Larrea CF, Borges R, Convit J. The effect of Bacille Calmette-Guerin vaccine on tuberculin reactivity in indigenous children from communities with high prevalence of tuberculosis. *Vaccine* 2008; 26: 5575-5581.
8. Kiray E, Kasapçopur O, Bas V, Kamburoglu-Goksel A, Midilli K, Arisoy N, Tastan Y. Purified protein derivative response in juvenile idiopathic arthritis. *J Rheumatol* 2009; 36: 2029-2032.
9. Camlar SA, Makay B, Appak O, Appak YC, Esen N, Gunay T, Anal O, Unsal E. Performance of tuberculin skin test and interferon gamma assay for the diagnosis of latent tuberculosis infection in juvenile idiopathic arthritis. *Clin Rheumatol* 2011; 30: 1189-1193.
10. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, He X, Maldonado-Cocco J, Orozco-Alcala J, Prieur AM et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol* 2004; 31: 390-392.
11. [No authors listed.] Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America (IDSA), September 1999, and the sections of this statement. *Am J Respir Crit Care Med* 2000; 161: 221-247.
12. Kilic O, Kasapçopur O, Camcioglu Y, Cokugras H, Arisoy N, Akcakaya N. Is it safe to use anti-TNF-alpha agents for tuberculosis in children suffering with chronic rheumatic disease? *Rheumatol Int* 2012; 32: 2675-2679.
13. Brunelli JB, Bonfiglioli KR, Silva CA, Kozu KT, Goldenstein-Schainberg C, Bonfa E, Aikawa NE. Latent tuberculosis infection screening in juvenile idiopathic arthritis patients preceding anti-TNF therapy in a tuberculosis high-risk country. *Rev Bras Reumatol* 2017; 57: 392-396.
14. Ringrose JS, Sanche SE, Taylor-Gjevre RM. Detecting latent tuberculosis infection during anti-tumor necrosis factor therapy. *Clin Exp Rheumatol* 2011; 29: 790-794.
15. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P, Monk P, Lalvani A. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. *Lancet* 2003; 361: 1168-1173.