

Decreased Risk for Atopic Disorder Associated With Highly Hyperreactive Tuberculin Skin Test Reaction in Children and Adolescents

Marija Maćešić, BSc,* Mirjana Turkalj, MD, PhD, Žaneta Jelčić, MD, Slavica Dodig, PhD, Branka Kristić-Kirin, MD, Boro Nogalo, MD, MSc, and Davor Plavec, MD, PhD

Summary. Background: It is speculated that the exposure to *Mycobacterium tuberculosis*, either by infection or by *Bacillus Calmette-Guérin* vaccination, may inhibit the onset of atopy by the modification of immune profiles leading to a shift of T_H1/T_H2 balance to the T_H1 side. Objective: One hundred eighty-six patients hyperreactive at tuberculin skin test (TST) were examined in order to investigate the prevalence of atopic disorder, particularly referring to the association between the size of the TST induration and the prevalence of sensitization and manifest atopic disorder. Methods: The study consisted of a family history record, patients' medical history assessment and clinical examination, skin prick test (SPT), serum total and allergen-specific IgE (sIgE) measurement and eosinophil count. Results: Atopic disorder was present in 49 (26.3%) patients tested. No significant difference between the groups based on the TST induration size (15–24 mm vs. ≥ 25 mm) was found for gender distribution, family atopy history, total IgE measurement, eosinophil count, positive SPT, and the presence of sIgE. A significant difference was found for the age median (14.0 years vs. 13.0 years), childhood atopy record, and manifest atopic disorder. No association between the size of the TST induration and the incidence of allergic sensitization was demonstrated. However, a significant inverse association between the size of the TST induration and manifest atopic disorder was demonstrated. Conclusion: In patients highly hyperreactive at TST, the size of the induration is inversely associated with manifest atopic disorder. **Pediatr Pulmonol.** © 2009 Wiley-Liss, Inc.

Key words: atopy; atopic disorder; hyperreactive tuberculin skin test; allergic sensitization; hygiene hypothesis.

INTRODUCTION

Epidemiological and clinical evidence suggests that the prevalence of atopic diseases—hay fever, asthma, and eczema—has increased over the past decades.^{1,2} This is thought to be associated with the industrialization and westernized life style. Since one of the most dramatic changes in the general health of westernized societies during the mentioned time period has been a marked reduction in the prevalence of modifiers of infectious diseases, exposure to respiratory infections in childhood—tuberculosis (TB) in particular—has been highlighted as a potential modifier of the onset and course of the allergic diseases and atopy.^{3,4}

The hypothesis that infections may inhibit the development of atopy postulates that infectious agents, such as viruses and intracellular bacteria, preferentially stimulate the development of T-helper type 1 (T_H1) immunity. Mycobacteria, especially *Mycobacterium tuberculosis* (MTB), are known to be potent inducers of the T_H1 immune response.⁵

The association between atopy and MTB infection or *Bacillus Calmette-Guérin* (BCG) vaccination in childhood still remains controversial.⁶ Many authors speculate

that the exposure and response to MTB may inhibit the onset of atopy by the modification of immune profiles.⁷ Chronic infections such as TB may down-regulate atopic response through the induction of T-regulatory cells and production of anti-inflammatory cytokines.

In order to contribute to the knowledge on the association of a strong T_H1 immune response and atopic disorder, this study was conducted with the aim to investigate the prevalence of manifest atopic disorder in children, adolescents, and young adults with hyperreactive tuberculin skin test (TST) reaction, particularly referring to the association between the size of the TST

Srebrnjak Children's Hospital, Zagreb, Croatia.

*Correspondence to: Marija Maćešić, Srebrnjak Children's Hospital, Srebrnjak 100, 10000 Zagreb, Croatia. E-mail: macesic@bolnica-srebrnjak.hr

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induration and the prevalence of sensitization and manifest atopic disorder.

METHODS

The study was conducted on 186 children, adolescents, and young adults in Srebrnjak Children's Hospital, Zagreb, Croatia. The patients were evaluated for TB because of a hyperreactive TST reaction. Among this population there were 86 male and 100 female patients and their mean age [SD] was 13.6 [3.7] years, median 14.0 (range 1.0–24.0).

Children in Croatia are neonatally vaccinated with BCG vaccine SSI—0.0375 mg *Mycobacterium bovis*, Danish strain 1331 (attenuated) dissolved in diluted Sauton SSI injected intradermally.

TST was performed by injecting 2 tuberculin units (TU) of purified protein derivative (PPD) RT 23 SSI (Statens Serum Institut, Copenhagen, Denmark) on the volar surface of the left forearm. The transverse diameter of the indurated area was measured after 72 hr always by the same trained individual.

World Allergy Organization (WAO) and European Academy of Allergology and Clinical Immunology (EAACI) definitions for allergy and atopy^{8,9} were used when assessing patients' status.

Patients with a hyperreactive TST reaction (defined as induration ≥ 15 mm) were evaluated for atopic disorder on the basis of their own and family medical history, clinical examination, results of skin prick test (SPT), and additionally eosinophil count and serum total and specific IgE if indicated (positive SPT and/or suggestive history of atopy). Patients were classified as having atopic disorder if they exhibited a positive family atopy history, a positive SPT, and/or elevated total IgE or sIgE level. Patients were classified as having a manifest atopic disorder if they were diagnosed asthma (with or without conjunctivitis) and/or

atopic dermatitis and had symptoms during the period of 1 year prior to enrolment in the study.

To assess the family atopy history and childhood atopy record the parents and/or patients themselves where possible (young adults) were asked to answer on several aspects of the family health status and allergy history—allergic diseases, BCG vaccination, tuberculin tests, contact with TB, and the presence of pets in the household.

A detailed clinical examination was undertaken by hospital specialists. In order to diagnose TB, patients known to have been in contact with TB-positive subjects were subjected to chest X-ray, bacteriological examination of sputum, and broncho-alveolar lavage (BAL). In patients with positive chest X-ray result and/or confirmed MTB presence in sputum and/or BAL, TB was diagnosed. Patients with diagnosed TB were subsequently involved in the standard tuberculostatic therapy including four tuberculostatic drugs. The rest of hyperreactive patients underwent the standard prophylactic treatment—isoniazid (eutizon) during a 6-month period.

SPT was performed on the volar surface of the right forearm with extracts of 11 inhalation allergens: house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), animal dander (dog and cat), cocksfoot (*Dactylis glomerata*), mugwort (*Artemisia vulgaris*), ragweed (*Ambrosia elatior*), birch (*Betula alba*), hazel (*Corylus avellana*), *Alternaria alternata*, and *Cladosporium mix* (*Cladosporium cladosporioides* and *C. herbarum*) (all from Stallergenes, Marcy l'Etoile, France). Histamine hydrochloride (10 mg/ml) and phenolated glycerol-saline (both from Stallergenes) were used as positive and negative controls. A mean wheel diameter of ≥ 3 mm in excess of the negative control was defined as a positive SPT reaction.

For the patients with positive SPT result and/or suggestive history for atopic disorder, serum total IgE and allergen-specific IgE (sIgE) were measured and the eosinophils counted from the blood samples obtained by venipuncture. To obtain serum samples, serum was separated from blood samples after 10-min centrifugation and stored at $+4^{\circ}\text{C}$ until analysis, done within 3 days.

The concentration of total IgE was determined in 88 patients by the microparticle enzyme immunoassay (MEIA) method and reagents (Abbott, Abbott Park, IL) using mouse monoclonal anti-IgE antibodies. Fluorescence was measured on IMx autoanalyzer (Abbott).

The concentration of sIgE was measured in 65 patients by the UniCAP method (Pharmacia, Uppsala, Sweden) on a selective UniCAP 100 autoanalyzer (Pharmacia-LKB-Kabi).

The eosinophil count was performed in peripheral blood samples of 88 patients by fluorescence flow cytometry (Sysmex XT 1800i, Kobe, Japan).

ABBREVIATIONS

BCG	<i>Bacillus Calmette-Guérin</i>
CI	Confidence interval
EAACI	European Academy of Allergology and Clinical Immunology
GM	Geometric mean
IFN- γ	Interferon- γ
MEIA	Microparticle enzyme immunoassay
MTB	<i>Mycobacterium tuberculosis</i>
PPD	Purified protein derivative
RR	Risk ratio
SD	Standard deviation
sIgE	Allergen-specific IgE
SPT	Skin prick test
TB	Tuberculosis
TST	Tuberculin skin test
TU	Tuberculin units
T _H 1	T-helper type 1
T _H 2	T-helper type 2
WAO	World Allergy Organization

Statistical Analysis

Descriptive statistics, with mean, standard deviation (SD), and median with range for normally distributed continuous variables and geometric mean (GM) and range for total IgE were used. Categorical variables were presented as number (%). Since TST results were not normally distributed, they were divided into four groups by quartiles. As the first analysis based on division by quartiles showed that there was no difference in risk ratio (RR) for sensitization or manifest atopic disorder between the first three groups, we present the subgroup analysis based on two subgroups (last group vs. first three groups combined). Subgroup comparisons were performed using Student's *t*-test (age, eosinophil count), Mann-Whitney *U*-test (positive SPT to at least one allergen, presence of sIgE to at least one allergen), one-tailed Fisher exact test (gender, childhood atopy record, family atopy record, atopic disorder), and Chi-square test (total IgE), depending on the variable. Multivariate logistic regression analysis was used to depict variables independently associated with the manifest atopic disorder in our sample. $P < 0.05$ was considered statistically significant. All tests were performed using STATISTICA version 6.0 (StatSoft, Inc., Tulsa, OK).

The study was approved by the Ethic Committee of Srebrnjak Children's Hospital.

RESULTS

Average wheal diameter [SD] for patients with positive TST reaction was 20.5 [4.1] mm, median 20.0 mm (range 15.0–35.0 mm). Using quartile distribution, the patients were divided into four groups according to the size of induration at TST: 15–19 mm ($N = 61$), 20 mm ($N = 53$), 21–24 mm ($N = 32$), and ≥ 25 mm ($N = 40$). For statistical analysis, two subgroups were formed: the first three groups combined (TST induration 15–24 mm,

$N = 146$) and the fourth group (TST induration ≥ 25 mm, $N = 40$). Average total IgE (GM) was 130.9 kIU/L and average eosinophil count [SD] $261.08 [248.48] \times 10^6/L$ (Table 1).

Seventy-two (38.7%) out of 186 patients had a positive SPT reaction for one or more inhalation allergens and for 49 (75.4%) out of 65 patients the sIgE for one or more allergens were detected (sIgE > 0.35 kIU/L).

On the basis of the clinical manifestation of allergy combined with a positive SPT result and/or elevated serum IgE level, it was concluded that manifest atopic disorder was present in 49 (26.3%) out of 186 tested patients.

In 21 (11.3%) out of 186 patients TB was diagnosed. Four (19.0%) of them, all with induration size 15–24 mm, were positive for manifest atopic disorder. This group was considered too small for further statistical analysis. Comparing the subgroups based on TST induration size, no significant difference was found for gender distribution, family atopy history, total IgE, eosinophil count, positive SPT, and the presence of sIgE (Table 1 and Fig. 1). A significant difference was found for the median age of patients (14.0 years vs. 13.0 years), childhood atopy record, and for manifest atopic disorder (Fig. 1).

Calculated risk difference for manifest atopic disorder between the two subgroups hyperreactive at TST (induration 15–24 mm [groups 1 + 2 + 3 combined, risk ratio = 1] vs. ≥ 25 mm [group 4]) was -14.45% (95% confidence interval [CI] -27.76 to -1.14) with the risk ratio of 0.422. The same pattern was present in the subgroup of patients with diagnosed TB (induration 15–24 mm, 4/14 with manifest atopic disorder; ≥ 25 mm, 0/7 with manifest atopic disorder).

Multivariate logistic regression modeling depicted four variables associated independently with the manifest atopic disorder: gender, size of the TST induration, childhood atopy record, and a positive SPT reaction. Current odds ratios were: gender (male/female),

TABLE 1—Characteristics of Studied Subjects According to the TST Induration Size Subgroups ($N = 186$)

Characteristic	Total ($N = 186$)	TST induration size		<i>P</i> -value
		15–24 mm ($N = 146$)	≥ 25 mm ($N = 40$)	
Age, median [range], years	14.0 [1.0–24.0]	14.0 [1.0–24.0]	13.0 [2.0–21.0]	0.0433
Gender, male, No. (%)	86 (46.2%)	64 (43.8%)	22 (55.0%)	0.1410
Total IgE, GM, kIU/L ¹	130.94 (4.6–3088)	140.32 (4.6–3088)	103.47 (7–1095)	0.5569
Positive SPT result to at least 1 allergen, No. (%)	72 (38.7%)	58 (39.7%)	14 (35.0%)	0.8538
Eosinophil count, mean [SD] $\times 10^6/L$ ¹	261.08 [248.48]	257.20 [254.10]	284.36 [221.04]	0.7395
Presence of sIgE to at least 1 allergen (sIgE > 0.35 kIU/L), No. (%) ²	49 (75.4%)	42 (77.8%)	7 (63.6%)	0.4935
Childhood atopy record, No. (%)	45 (24.2%)	40 (27.4%)	5 (12.5%)	0.0361
Family history positive for atopy, No. (%)	54 (29.0%)	45 (30.8%)	9 (22.5%)	0.2046
Atopic disorder, No. (%)	49 (26.3%)	43 (29.5%)	6 (15.0%)	0.0469

TST, tuberculin skin test; SPT, skin-prick test; sIgE, allergen-specific IgE; GM, geometric mean.

¹Calculated based on $N = 88$; TST: 15–24 mm, $n = 68$; TST: ≥ 25 mm, $n = 20$.

²Calculated based on $N = 65$; TST: 15–24 mm, $n = 54$; TST: ≥ 25 mm, $n = 11$.

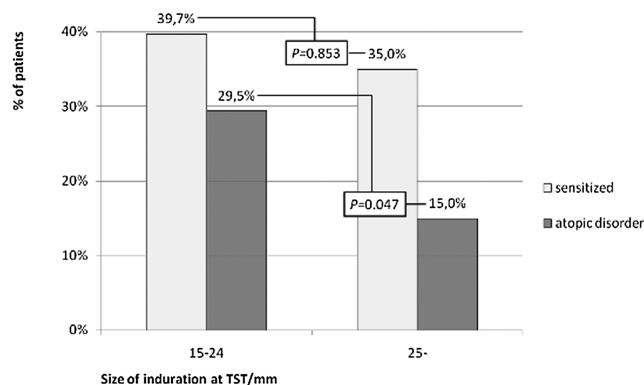


Fig. 1. Prevalence of sensitization to inhalatory allergens and manifest atopic disorder according to the TST induration size (N = 186).

4.14 (95% CI, 1.04–16.57, $P = 0.043$); size of the TST induration in mm, 0.81 (95% CI, 0.67–0.97, $P = 0.022$); childhood atopy record (positive/negative), 5.56 (95% CI, 1.61–19.27, $P = 0.006$); positive SPT, 37.9 (95% CI, 11.56–124.16, $P = 0.000$).

DISCUSSION

The results of the study demonstrate no association between the size of the TST induration and the prevalence of allergic sensitization in children, adolescents, and young adults hyperreactive at TST. They demonstrate, however, a significant inverse association between the size of the TST induration and manifest atopic disorder for the same group.

There are conflicting results about protective effects of infection with MTB and BCG vaccination on the incidence of atopic disorder published in literature.^{4,7,10–16}

Some of the conflicting reports in literature concerning the association between a T_H1 -biased immune response and atopy may be due to the use of different definitions of atopy and questionable objective measures of atopic status. Our study shows that patients positive only at SPT should be considered sensitized but not atopic.

Due to the lack of standardization on exact composition and dose of antigens for SPT and TST (2, 5, or 10 TU reported in literature), the results of our study could only be partially compared to previously undertaken studies at other centers and laboratories. This also refers to the age of administration of BCG vaccine, and the strain and the dose of BCG. Since all Croatian children are routinely neonatally vaccinated with the same strain of BCG our group however was uniform in that respect. Another issue to be considered is a different definition of hyperreactivity, that is, different size of the TST induration (≥ 10 or ≥ 15 mm reported in literature).

The age difference between our two groups of patients which were hyperreactive at TST might support the

hypothesis that the protection against atopic diseases provided by intradermal BCG vaccination in infants may be more active in younger patients, when a substantial memory of cellular immune modulation still exists.¹⁷ Our multivariate models however do not confirm this.

Multivariate logistic regression analysis showed that each of the four variables—gender, size of the TST induration, childhood atopy record, and positive SPT result—can be an independent predictor of atopy. Female patients were suggested more prone to atopy, which is not in accordance with the results published in literature.¹⁸

To our knowledge this is the first study involving a significant number of patients highly hyperreactive at TST. We believe a group as large as this was necessary to show that the size of the TST induration is inversely associated independent predictor of manifest atopic disorder although there is no difference in the rate of sensitization. An inverse association between TST reaction size and manifest atopic disorder was present for the hyperreactive patients with extremely large indurations (≥ 25 mm) only. This trend progressed in patients diagnosed with TB (not significant because of the small sub-sample size).

It is important to distinguish between atopy and manifest atopic disorder. While atopy denotes a tendency for exaggerated IgE response, that is, a predisposition to develop allergy, manifest atopic disorder refers to the clinical expression of atopic disease. Manifest atopic disorder is therefore caused by the combination of genetic predisposition and certain environmental triggers. It has been demonstrated that only sensitization, that is, the presence of sIgE or a positive SPT result without clinical symptoms are not sufficient to diagnose manifest atopic disorder.¹⁹

There are two possible explanations for the results of our study. The so-called hygiene hypothesis proposes that microbial exposure and infections early in life reduce the expression of atopy and may suppress allergic sensitization by stimulating the development of T_H1 immunity.^{7,10,20} Another possible explanation for our results is that decreased TST reactions in atopics represent a marker of atopy rather than pointing towards BCG vaccine as a cause of being non-atopic.

In order to avoid misinterpretation, a couple of possible shortcomings should be kept in mind when viewing the results of this study. The group of patients involved in the study was too small to analyze the association of hyperreactive TST with different atopic disorders. It was also somewhat biased by the selection criteria since all of our patients were neonatally vaccinated with BCG. All the conclusions about the T_H1/T_H2 dichotomy could be made only indirectly since no cytokine analysis was undertaken.

In conclusion, we want to emphasize that to our knowledge this is the first study involving a significant number of patients highly hyperreactive at TST, thus

giving our study enough statistical power to show that the size of the TST induration is inversely associated as an independent predictor of manifest atopic disorder. We suggest that, while keeping in mind the diverse genetic aspect of PPD reactivity and the fact that the effects of BCG vaccination might not be applicable to all forms of allergic diseases, further studies should be undertaken to answer the still open questions on the mechanism of association between the induction of T_H1 response and the onset of atopy.

CLINICAL IMPLICATIONS

In children and adolescents, a highly hyperreactive TST reaction is associated with a decreased risk for manifest atopic disorder.

CAPSULE SUMMARY

In patients highly hyperreactive at TST, the size of the induration is inversely associated with manifest atopic disorder.

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