01.11.2024

Bronchial Hyperresponsiveness in Sarcoidosis

Unique Protocol Id: 6/2024/KB

4. METHODOLOGY

4.1. Study Characteristics

4.1.1. General Information

The study was observational, analytical, cross-sectional, with a retrospective component. The prospective part of the study involved performing a methacholine bronchial challenge test in 2024–2025. The remaining data included in the analysis were obtained retrospectively from medical records maintained at the Department of Pulmonology, Allergology and Pulmonary Oncology of the 10th Military Clinical Hospital with Polyclinic in Bydgoszcz between 2014 and 2025.

4.1.2. Study Population

The study included patients diagnosed with sarcoidosis who were hospitalized at the Department of Pulmonology, Allergology and Pulmonary Oncology of the 10th Military Clinical Hospital with Polyclinic in Bydgoszcz in 2024–2025. The data analyzed from medical records included information on the diagnosis of sarcoidosis, comorbidities, demographic factors, and the results of imaging, pulmonary function, and laboratory tests.

The inclusion criteria comprised a confirmed diagnosis of sarcoidosis according to current ATS/ERS/WASOG guidelines, availability of the required medical documentation, the ability to perform technically acceptable spirometry, and provision of written informed consent to participate in the study. Tuberculosis and other causes of granulomatous inflammation were excluded in all patients.

The exclusion criteria included refusal to participate in the study; contraindications to methacholine bronchial challenge testing; use of medications that could modify respiratory function without an adequate washout period; systemic glucocorticosteroid use within the preceding 3 months; a previous diagnosis of asthma; a respiratory tract infection within 8 weeks before the planned bronchial challenge test; age below 18 years; pregnancy; and breastfeeding. Patients with advanced pulmonary parenchymal fibrosis (stage IV) and limited ability to perform spirometric maneuvers were not enrolled because of the reduced quality of bronchial challenge tests in this population.

Of the 81 patients with confirmed sarcoidosis hospitalized at the Department of Pulmonology, Allergology and Pulmonary Oncology of the 10th Military Clinical Hospital with Polyclinic

in Bydgoszcz in 2024–2025, a total of 70 met all inclusion criteria and none of the exclusion criteria and were ultimately enrolled in the study.

During the selection process, 2 patients who did not consent to participate, 3 patients with a previous diagnosis of asthma (made many years before the diagnosis of sarcoidosis), and 6 patients with contraindications to methacholine bronchial challenge testing due to reduced pulmonary function parameters on spirometry were excluded.

4.1.3. Variables, Operational Definitions, and Measurement Methods

Bronchial hyperresponsiveness (BHR) was assessed using a methacholine bronchial challenge test in accordance with ATS/ERS guidelines. The tests were performed during follow-up hospitalizations at the Department of Pulmonology, Allergology and Pulmonary Oncology. The test result was determined based on the methacholine concentration required to induce a decrease in FEV1 of at least 20% relative to the baseline spirometric value. On this basis, four degrees of bronchial hyperresponsiveness were distinguished: borderline, mild, moderate, and marked. Patients who did not exhibit a decrease in FEV1 of ≥ 20% at a methacholine concentration of > 16 mg/mL were classified as having normal bronchial responsiveness. The detailed protocol for the methacholine bronchial challenge test is described in Section 4.2.

Radiological phenotypes of sarcoidosis were determined based on the predominant pattern of abnormalities observed on high-resolution computed tomography (HRCT), according to the seven-category Delphi classification (1–7).

Sarcoidosis stages were determined using the Scadding classification (0–IV), both at the time of diagnosis and at the time of methacholine bronchial challenge testing. To improve the accuracy of assessment, HRCT was used instead of conventional chest radiography.

Changes in pulmonary parenchymal abnormalities over time and the time elapsed since diagnosis were classified as radiological progression, regression, stability, or de novo diagnosis. The assessment was based on chest HRCT scans obtained at the time of bronchial challenge testing, which were compared with examinations performed 6–12 months earlier during follow-up hospitalizations at the Department. Patients with newly diagnosed sarcoidosis (less than 1 week before testing) were classified as de novo cases, whereas diagnoses established at least 6 months earlier were classified as historical cases. Apart from participants with a de novo diagnosis, no patients diagnosed with sarcoidosis less than 6 months before testing were included in the study.

Phenotypes, disease stages, and changes in pulmonary parenchymal abnormalities over time were assessed jointly by two pulmonologists and a radiologist, with consensus reached on all classifications presented in the study.

Additional variables analyzed included demographic factors (age and sex); comorbidities potentially affecting respiratory symptoms (gastroesophageal reflux disease, atrial fibrillation, ischemic heart disease, and obstructive sleep apnea); smoking status; laboratory parameters (C-reactive protein [CRP], total calcium, ionized calcium, and urinary calcium in a 24-hour urine collection); results of skin prick tests for inhalant allergens; and information on treatment, including oral glucocorticosteroids and inhaled medications.

4.1.4. Comparison Groups

All patients enrolled in the study who had no contraindications were eligible to undergo methacholine bronchial challenge testing. Following completion of the prospective part of the study, participants were divided into two comparison groups: patients with bronchial hyperresponsiveness and patients without bronchial hyperresponsiveness. Between-group comparisons included the distribution of disease stages and phenotypes, as well as the assessment of the remaining parameters, according to the presence and degree of bronchial hyperresponsiveness.

4.1.5. Statistical Analysis

The study results were entered into a database (Excel spreadsheet) and subjected to statistical analysis using a significance level of 0.05. Analyses were performed in R (version 4.5.2; R Core Team, 2025) under Windows 11 Pro 64-bit, using packages including *ggsignif*, *ggpubr*, *report*, *gtsummary*, *ggplot2*, *readxl*, *dplyr*, *ggstatsplot*, *rio*, and *stringr*.

Descriptive statistics included the mean, standard deviation, range, and sample size for continuous variables, as well as counts and percentages for categorical variables. 95% confidence intervals were calculated using the t-distribution method for numerical variables and the Wilson method for proportions. Continuous variables were compared between two groups using Welch's t-test. For comparisons involving more than two groups, Welch's one-way analysis of variance (ANOVA) followed by Games–Howell post hoc tests was used. Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test. Linear trends in the relationships between the severity of bronchial hyperresponsiveness and continuous variables were assessed using linear regression. P values for trend were calculated

based on the regression coefficient (beta). The false discovery rate (FDR) correction was applied to account for multiple comparisons.

4.1.6. Ethical Considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol received a positive opinion from the competent Bioethics Committee. All participants provided written informed consent to participate in the study. The data were analyzed in anonymized form.

4.2. Methodology of the Methacholine Bronchial Challenge Test

4.2.1. Eligibility Assessment

Potential contraindications to methacholine bronchial challenge testing were identified in all patients through a detailed medical history and physical examination. The test was not performed in cases of reduced baseline FEV1 (< 70% of the predicted value or < 1.5 L), clinically significant cardiovascular disorders, recent eye surgery, or conditions associated with an increased risk of elevated intracranial pressure (Table 1). The test was also not performed in patients who had experienced a respiratory tract infection within the 8 weeks preceding the examination.

Table 1. Contraindications to methacholine bronchial challenge testing.

Category	Contraindications to methacholine bronchial challenge testing
Pulmonary function impairment	Baseline FEV1 < 70% of the predicted value or < 1.5 L on spirometry
Spirometry quality	Inability to perform technically acceptable and repeatable spirometric maneuvers
Cardiovascular disorders	Uncontrolled hypertension (systolic blood pressure > 200 mmHg or diastolic blood pressure > 100 mmHg); myocardial infarction or stroke within the preceding 3 months; aortic aneurysm

Ophthalmic conditions	Recent eye surgery; conditions associated with an increased risk of elevated intracranial pressure
Medications	Use of cholinesterase inhibitors
General contraindications	Inability to cooperate with the procedure; respiratory tract infection within the preceding 8 weeks; pregnancy

A list of all medications taken by each participant was compiled. Patients were also advised to avoid substances such as alcohol and tobacco before testing. Patients using inhaled medications requiring temporary discontinuation were informed in advance. The required washout periods for individual medication classes are presented in Table 2.

Table 2. Required washout periods for inhaled medications before methacholine bronchial challenge testing.

Medication	Minimum interval between the last dose and the test (hours)
Short-acting β2-adrenergic receptor agonist (e.g., salbutamol)	6
Long-acting β2-adrenergic receptor agonist (e.g., salmeterol)	36
Ultra-long-acting β2-adrenergic receptor agonist (e.g., indacaterol)	48
Short-acting muscarinic receptor antagonist (ipratropium)	12

Long-acting muscarinic receptor antagonist (e.g., tiotropium)	168
Methylxanthines (theophylline)	24

If no contraindications were identified and the patient was deemed eligible, the planned procedure was explained to each participant, along with potential adverse effects, including cough, chest tightness, and dyspnea. Patients were informed that there was a small risk of severe adverse reactions.

4.2.2. Testing Conditions and Protocol

Bronchial challenge tests were performed in the pulmonary function laboratory of the Department of Pulmonology, Allergology and Pulmonary Oncology in hospitalized patients under medical supervision. Technical accuracy was ensured by medical personnel with appropriate qualifications and extensive experience in performing pulmonary function tests.

A two-minute tidal breathing method involving continuous inhalation of progressively increasing methacholine concentrations was used. Methacholine solutions were prepared by a pharmacist at the hospital pharmacy of the 10th Military Clinical Hospital with Polyclinic in Bydgoszcz. The prescribed methacholine concentrations, diluted in 0.9% sodium chloride solution, were 0.25 mg/mL, 1 mg/mL, 4 mg/mL, 8 mg/mL, and 16 mg/mL. The preparation and dilution scheme for the methacholine solutions is presented in Table 3.

Table 3. Methacholine solution dilution scheme.

Starting material/solution	Volume of 0.9% sodium chloride added (mL)	Final concentration (mg/mL)
100 mg of methacholine	6.25	16 (Solution A)

3 mL of Solution A	3	8 (Solution B)
3 mL of Solution B	3	4 (Solution C)
3 mL of Solution C	9	1 (Solution D)
3 mL of Solution D	9	0.25 (Solution E)

The testing protocol, developed in accordance with ATS/ERS recommendations [1,2], included removing the vials containing the previously prepared solutions from the refrigerator approximately 30 minutes before the scheduled examination to allow them to reach room temperature.

For patients deemed eligible for testing, spirometry was performed first. After calibration of the Jaeger Vyntus APS system, nebulization with 0.9% sodium chloride solution was initiated. Standard tidal breathing was used during the two-minute nebulization period. If FEV1 decreased by at least 20% on spirometry performed after the first inhalation, the test was discontinued.

In subsequent stages, two-minute nebulization cycles were performed using progressively increasing methacholine concentrations (0.25 mg/mL, 1 mg/mL, 4 mg/mL, 8 mg/mL, and 16 mg/mL). Dynamic spirometry was performed 30 seconds after each inhalation. The interval between successive doses was approximately 5 minutes. If FEV1 decreased by ≥ 20% relative to baseline at any stage of the test, or after administration of the highest planned concentration, further dose escalation was discontinued.

After completion of the test, patients received inhaled salbutamol, and dynamic spirometry was repeated after 10 minutes. In cases where the decrease in FEV1 did not reach 20%, salbutamol was also administered if FEV1 had decreased by > 10%, followed by repeat spirometry.

4.2.3. Interpretation of Results

The results were interpreted according to the classification established in the ATS guidelines. A PC20 value between 4 and 16 mg/mL was considered indicative of borderline bronchial

hyperresponsiveness. Positive results were classified as follows: PC20 of 1–4 mg/mL indicated mild bronchial hyperresponsiveness, 0.25–1 mg/mL indicated moderate bronchial hyperresponsiveness, and < 0.25 mg/mL indicated marked bronchial hyperresponsiveness.

References

1. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for Methacholine and Exercise Challenge Testing - 1999. *Am J Respir Crit Care Med*. 2000;161(1). doi:10.1164/ajrccm.161.1.ats11-99

2. Coates AL, Wanger J, Cockcroft DW, et al. ERS technical standard on bronchial challenge testing: General considerations and performance of methacholine challenge tests. *Eur Respir J*. 2017;49(5). doi:10.1183/13993003.01526-2016