

Bronchial asthma and bronchial hyperresponsiveness and their characteristics in patients with Common Variable Immunodeficiency

Running title: Bronchial asthma and airway hyperresponsiveness in CVID

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Abstract

Background: Common variable immunodeficiency (CVID) is one of the most frequent primary immunodeficiencies characterized by disturbed immunoglobulin production and dysregulation of immune system. Results of previous studies suggest a higher prevalence of bronchial asthma (BA) in CVID patients compared to general population. Therefore, we initiated this study to evaluate lung functions and identify risk factors for BA and bronchial hyperresponsiveness (BHR) in patients with CVID.

Methods: Together 23 patients with CVID were included into this study. In all of them spirometry and metacholine bronchoprovocation test were performed. Furthermore, we investigated the role of atopy, eosinophilic inflammation and potential risk factors such as gender, age or immunoglobulin levels at the time of diagnosis.

Results: BHR was confirmed in 12 patients (52%), all of them had normal FEV1 and FEV1/FVC. However, BHR positive patients had significantly decreased MEF25. BHR positive patients had also more symptoms related to bronchial obstruction, 8 of them (35%) became suspected of having bronchial asthma at the end of the study. Higher prevalence of BHR was found in females with relative risk of 2.89.

Conclusions: An increased prevalence of BHR and BA was detected in CVID patients compared to general population. BA may develop despite the disturbed immunoglobulin production and in majority of the patients displays non-atopic and non-eosinophilic properties. These results suggest limited role of atopy and eosinophilic inflammation in pathogenesis of BA in CVID patients.

Key words: Bronchial Asthma, Bronchial Hyperresponsiveness, Common variable immunodeficiency, Bronchoprovocation, Risk factors

Introduction

Common Variable Immunodeficiency

Common Variable Immunodeficiency (CVID) is one of the most frequent humoral immunodeficiency with incidence of 1:25 000. [1] It is characterized by disturbed overall and antigen specific immunoglobulin production, reduced switched memory B cells count and broad variability of etiology and symptoms including infectious, autoimmune, lymphoproliferative and granulomatous manifestations. [2] These features are also reflected in diagnostic criteria. [3] Despite the effort to identify pathophysiological mechanisms and genetic background of the disease, it remains unknown in 85-90% of patients. Impaired immunoglobulin production results in increased susceptibility to infections, particularly recurrent or chronic upper (rhino/sinusitis, tonsillitis, otitis) and lower (bronchitis, pneumonia) respiratory tract infections. [4] Treatment is based on regular immunoglobulin substitution, ATB prophylaxis and other therapeutic measures, including management of non-infectious complications. In some patients, particularly those with recurrent respiratory tract infections, ATB prophylaxis is useful in preventing chronic lung disease. In particular, lower respiratory tract infections may result in permanent functional and structural alterations described as CVID-associated chronic lung disease (found in up to 68% of CVID patients) or bronchiectasis (found in up to 73%). [5] Chronic lung disease may be accompanied with obstructive or restrictive ventilatory disorder. The most common causes of lung restriction are interstitial lung diseases (incl. lymphocytic interstitial pneumonia or granulomatous-lymphocytic lung disease). [6, 7] The most common causes of obstructive lung disease are chronic obstructive pulmonary disease (COPD), which is frequent and well-documented in CVID patients [8-10], and bronchial asthma (BA), respectively asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS), which have been subjects of only few studies with CVID patients so far. [11, 12]

COPD is regarded as a multifactorial disease resulting from gene-environmental interaction (most prominently passive and active smoking, occupational exposures or air pollution). [13] It is characterized by persistent and usually progressive airflow restriction associated with chronic inflammatory response, which has specific features, e.g. increased number of cytotoxic T lymphocytes, macrophages and neutrophils. [14] The airflow restriction is usually accompanied with dyspnea, chronic cough and sputum production. [15, 16] Severe respiratory

infections have been associated with reduction of lung functions and increase of respiratory symptoms in adulthood, moreover infections are suspected to be a risk factor for disease exacerbation. [17, 18]

BA also develops as a result of multiple factors. An early-age exposure to respiratory infections with respiratory syncytial virus, parainfluenza or rhinovirus (the most common causes of bronchiolitis in childhood) [19], and to allergens seems to be eminent. [20] Tobacco inhaling (passive, as well as active) [21] and air pollution [22] are also potential risk factors, however not as clearly established. The hallmark of bronchial asthma is chronic inflammation with skewing towards Th2, resulting in activation of mast cells or eosinophils. [23] Additionally, epithelial cells produce IL-33 upon injury, which promotes immune response bias towards Th2 [24], and are important source of nitrogen monoxide (NO). [25] These responses lead to functional and structural alterations of airways, including airway edema, mucous hypersecretion and particularly airway hyperresponsiveness with bronchoconstriction. [26] BHR is defined as bronchoconstriction with bronchial narrowing triggered by various stimuli. BHR is found in 40-53% of patients with BA, compared to 10-16% prevalence in children and in adults. [27] The risk of BHR is higher in females. [28] Apart from gender, other BHR risk factors include atopy, increased levels of IgE, eosinophilia, positive skin test reactivity and smoking. [27] Respiratory tract infections also convey a significant risk for its development. [29] Reflecting the range of the above-mentioned immunopathologic mechanisms, several asthmatic phenotypes may be distinguished, such as atopic, eosinophilic and non-eosinophilic BA. [30] ACOS represents a third obstructive lung disease phenotype, sharing features of both COPD and BA. [31, 32] In some cases it may be difficult to distinguish between BA, COPD and ACOS.

Several studies documented the increased prevalence of obstructive lung diseases in COVID patients. While extensive data on COPD has been published, the higher prevalence of BHR and BA in COVID has only been suggested by few reports, which deliver limited information on the lung disease specifics. Therefore, we initiated this study in an attempt to determine prevalence and character of BHR and BA in a cohort of 23 COVID patients.

Study design, materials and methods

Inclusion and exclusion criteria

All patients in our non-interventional prospective study fulfilled diagnostic criteria of Common Variable Immunodeficiency as defined by European Society for Immunodeficiency (ESID Registry Diagnostic Criteria, version from 25th April 2017) and were included after written informed consents were obtained. Inability to undergo spirometric or FENO (fractional exhaled nitric oxide) examination, history of smoking, pregnancy, established bronchodilator or inhaled/systemic corticosteroid therapy in the preceding 3 months, restrictive ventilation disorder ($FVC < 80\%$), secondary cause of bronchial obstruction or acute infection in preceding 4 weeks were established as exclusion criteria. The study was performed between January and February 2016 and January and February 2017.

Patient Questionnaire

Before examination all patients had filled in questionnaires regarding patient's family and personal history and adjusted Asthma Control Test (ACT) designed by American Thoracic Society (Table 1). The full achievable score in the test is 15 points (found in asymptomatic patients). The gain of 10-14 points is related to moderate severity of BA. The patients with severe manifestation of BA reach less than 10 points.

Spirometry, bronchodilator and bronchoprovocation test, exhaled nitric oxide

An initial spirometry and exhaled nitric oxide measurements (FENO) were performed in all included patients. Chest X-ray was performed to exclude secondary causes of airway obstruction. The findings of performed chest X-ray were compared to available results of CT/HRCT.

Based on the results patients were divided into groups with ($FEV_1/FVC < 70\%$) or without ($FEV_1/FVC > 70\%$) obstructive ventilatory disorder. The patients with restrictive ventilatory disorder ($FVC < 80\%$ with normal FEV_1/FVC ratio) were excluded. Bronchoprovocation test (BPT) with metacholine was performed in the group without signs of airway obstruction. The degree of BHR was categorized according to metacholine concentration causing 20% decrease of FEV_1 (PC_{20}). Since no patients were identified to have obstructive ventilatory disorder on initial spirometry, bronchodilator test was not indicated in anyone. Initial spirometry, BPT with metacholine and FENO were performed and interpreted according to recommendation of European Respiratory Society and American Thoracic Society [33-35]

using spirometer MasterScope (ERT, Philadelphia, USA) and FENO analyzer Vero (Niox, Solna, Sweden).

Skin prick tests, serum levels of total and allergen specific IgE

Skin Prick tests were performed and assessed according to recommendation of American Academy of Allergy, Asthma and Immunology. Test panel included allergen extracts of *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alnus glutinosa*, *Betula pendula*, *Coryllus avellana*, *Carpinus betulus*, *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne*, *Anthoxanthum odoratum*, *Phleum pratense*, *Artemisia vulgaris*, *Ambrosia artemisiifolia*, *Alternaria alternata*, *Cladosporium herbarum*, *Canis familiaris*, *Felis domesticus* (Alyostal Prick, Stallergenes company, Antony, France) and corresponding serum allergen specific IgE antibodies, total serum IgE and serum eosinophilic cationic protein (ECP) were detected by chemiluminescent technique using Immulite 2000 systems (Siemens, Erlangen, Germany). The results were regarded as positive if skin reaction was >3mm (together with unresponsiveness to negative control) and with serum concentration of specific IgE >0,35 IU/ml

Immunoglobulin M, G levels and lymphocyte subpopulations

Serum levels of IgM and IgG were evaluated at the time of diagnosis by nephelometric method using Immage 800 systems (Beckman-Coulter, Brea, USA).

Lymphocytes subpopulation were distinguished based on expression of specific cell surface membrane markers CD3 and CD19 using fluorochrome conjugated monoclonal antibodies (CD3-Alexa Fluor 700, CD19-APC, BioLegend, San Diego, USA) by fluorescence-activated cell sorting (FACS Aria II, BD Biosciences, San Jose, USA). T cells were identified as CD3+ cells and B cells as CD19+ cells. Flow cytometric data were analysed in FlowJo V10 (FlowJo LLC, Ashland, USA)

Statistical analysis

All data were statistically processed by GraphPad Prism, version 6 (GraphPad Software, La Jolla, USA). Unpaired nonparametric Mann-Whitney test was used to compare independent samples, mean values and 95%CI (confidence interval) were calculated. The Chi-square test was used to assess the differences in groups for gender and family history results. The statistical differences were regarded as positive when p value ≤ 0.05 . Sensitivity and specificity values were calculated for ECP and FENO.

Results

Characteristics of the cohort

Together 23 patients, who fulfilled inclusion criteria, were enrolled to this study; 14 males (61%) and 9 females (39%). All patients were receiving immunoglobulin replacement therapy - 17 patients (74%) subcutaneous (SCIG) and 6 patients (26%) intravenous (IVIG). Median age at recruitment was 33.7 years (± 11.5). Median age at diagnosis of CVID was 23.7 years (± 12.1) and median duration of disease was 10 years (± 5.9).

Clinical manifestation

Chronic upper respiratory tract infections, including chronic rhinitis or sinusitis, were the most common infectious complications found in 10 out of 23 (43%) included patients. Chronic lung disease was diagnosed by CT/HRCT in 9 patients (39%); bronchiectasis in 4 patients (17%) lung nodules in 3 patients (13%) and both findings in 2 patients (9%). In contrast, the standard chest X-ray revealed chronic changes in 5 patients only (22%). Chest X-ray and CT/HRCT findings are summarized in Table 2.

Autoimmune diseases were diagnosed in 12 patients (52%). The most frequent, Autoimmune thyroiditis and Immune Thrombocytopenic Purpura, manifested in 5 patients (22%). Vitiligo and Autoimmune Hemolytic Anaemia were diagnosed in 2 patients (9%) and type 1 Diabetes Mellitus in 1 patient (4%). Autoimmune duplicity was found in 3 patients (13%). Other non-infectious features were splenomegaly in 11 patients (48%) and lymphadenopathy in 6 patients (26%). Malignant lymphoproliferations including Hodgkin and Burkitt's lymphoma were revealed in 2 patients (9%). Summary of clinical manifestation is shown in Table 3. Neither the co-occurrence of non-infectious manifestations nor the presence of CT/HRCT-lung abnormalities were identified as BHR or BA risk factors (data not shown).

Bronchial hyperresponsiveness and its characteristics

Bronchial hyperresponsiveness was confirmed in 12 patients (52%) using BPT with metacholine. Mild degree of BHR (PC_{20} : 4-16mg/ml) was found to be the most common finding which was identified in 5 patients. Moderate (PC_{20} : 1-4mg/ml) and severe degree of BHR (PC_{20} : <1mg/ml) were diagnosed in 4, respectively in 3 patients. It correlated with dyspnea, which was assessed by adjusted Asthma Control Test (aACT). A total of 8 patients with positive BHR (35%) also reported symptoms related to bronchial obstruction such as

wheeze, shortness of breath, chest tightness and cough. The median value of aACQ in the group with positive BPT was 11.8 points, compared to the group with negative BPT, where median value was 14.9 points (a significant difference, $p=0.008$) (Figure 1). In six patients with positive BHR (50%) the severity of symptoms led to limitations in daily activities at least once or twice daily; 5 of them (42%) reported disturbed sleep.

The initial spirometry results reflected these results. Both groups had normal FEV1 and FEV1/FVC ratio. (Figure 2) However, in BHR positive group a mild decrease of FEV1 was noted (93.2% vs. 100%, $p=0.021$) as well as of FEV1/FVC ratio (82% vs. 88.9%, $p=0.013$). In BHR positive group the peripheral airways were the most prominently affected, with significantly higher decrease of initial MEF25 (76.7% vs. 106.5%, $p=0.005$). (Figure 3)

In all patients FENO and serum ECP were measured to assess the specific character of bronchial hyperresponsiveness. The median value of FENO in both groups was 25.9 ppb, 22 ppb in BHR negative and 29.5 ppb in BHR positive group. The median value of serum ECP level in both groups was 22.8 ng/ml, 21.85 ng/ml in BHR negative and 20.45 ng/ml in BHR positive group. These differences were not significant. Together 8 of 12 patients with bronchial hyperresponsiveness (67%) had FENO level higher than 25 ppb, 5 of 12 (42%) had serum ECP higher than 22 ng/ml and only 3 patients (25%) had increased levels of both parameters.

Additionally, we investigated the role of eosinophilic and neutrophilic peripheral blood counts, as well as CD3 and CD19 lymphocytes counts. No significant differences were observed between the BHR positive and BHR negative group. Summary of our results is shown in Table 4.

BHR associated risk factors

In our cohort, the occurrence of BHR was significantly higher ($p=0.048$) in female patients compared to males, with relative risk of 2.89. (Figure 4) On the other hand, no significant correlation between BHR and the age at diagnosis and the disease duration was observed.

The roles of serum total and allergen specific IgE as potential risk factors were evaluated. The IgE production was preserved in only 5 patients (22%), in the remaining patients (78%) the IgE levels were undetectable (<0.1 U/ml). Three patients of those with detectable levels of IgE

also tested positive for allergen specific IgE, namely to birch and/or grass pollens, and the sensitization was confirmed by corresponding positivity of skin prick tests. No sensitization to perennial allergens was found. Two patients from the BHR positive group also suffered from atopic dermatitis and allergic rhinoconjunctivitis; in the BHR negative group only 1 patient (sensitized to birch pollens) displayed these symptoms (atopic dermatitis and allergic rhinoconjunctivitis).

Furthermore, serum IgG and IgM levels were analyzed at the time of diagnosis. The mean serum level of IgM was 0.31 g/l and of IgG was 2.84 g/l. The serum levels of both IgM and IgG in BHR negative group were lower compared to BHR positive group (0.21 g/l vs. 0.40 g/l of IgM; 2.32g/l vs. 3.32 g/l, respectively), but the differences were not statistically significant.

Discussion:

From our cohort of 33 CVID patients, who are followed up at our department, 6 (18%) had been diagnosed with bronchial asthma, 3 (9%) with COPD and 1 (3%) with idiopathic lung fibrosis before the initiation of this study. Appropriate therapy had been initiated in all of them prior to this study, and they were thus excluded. In the remaining 23 patients BHR was diagnosed in striking 52% (5 with mild, 4 with moderate and 3 with severe degree), compared to 10-16% in adults and 16-30% in children in population-based studies. BHR, being a cause of dyspnea, had a major impact on the patients' daily activities and quality of sleep. Based on the results, the diagnosis of BA was suggested in 8 patients (35%), contrasting with the prevalence of BA in the Czech general population being only 5% [36]. Compared to CVID patients without BHR, CVID patients with BHR also displayed reduction of FEV1 (92% vs. 100%), FEV1/FVC (100% vs. 105%) and, interestingly, MEF25 (71.5% vs. 107.8%), implying the predominant affection of peripheral airways. This observation corresponds well with findings in paediatric patients with allergic rhinitis and at the time of asthma onset. [37, 38] We have thus confirmed that the decrease in MEF25 is associated with BHR also in patients with underlying humoral immunodeficiency.

To our best knowledge, only one similar study regarding BA or BHR in patients with Common Variable Immunodeficiency has been previously performed by Agondi et al. [11] In a cohort of 62 CVID patients 48% were found to have obstructive ventilatory disorder, 12% had restrictive ventilatory disorder and 40% had normal spirometry parameters. Abnormal skin prick tests and elevated specific IgE were found in 3% of patients; normal serum level of total IgE was found in 29% patients and 68% of patients had undetectable level. At the end of this study the diagnosis of BA was confirmed in 15%, the allergic character of BA was detected in 6% of CVID patients. The results of both studies are consistent with the notion that IgE is not necessary for development of BA, which was also shown in mouse models. [39] Therefore, we conclude that BA may develop even in the settings of humoral immunodeficiencies with disturbed IgE production.

Agondi et al. did not assess additional parameters which may help to determine the precise nature of BHR or BA, such as ECP, FENO, neutrophilic or eosinophilic peripheral blood counts, which are useful for selecting the appropriate therapeutic approach. For instance, eosinophilic inflammation responds better to corticosteroids compared to neutrophilic

inflammation. Performing these tests in our cohort, we did not find any significant differences in FENO and ECP between BHR positive and BHR negative group. Cut-off points for FENO value >25 ppb and serum ECP level >25 ng/ml are highly predictive and supports the diagnosis of BA. [40, 41] However, only 62% patients with BHR had elevated levels of FENO, 42% had increased levels of ECP and 25% had both. These findings suggest limited role of eosinophilic inflammation in development of BA and BHR in CVID patients, favour non-allergic and non-eosinophilic character and emphasize the role of early-acquired and recurrent infections.

Structural lung abnormalities were revealed in 9 patients (39%) by CT/HRCT, but only in 5 patients by chest X-ray. Thus, chest X-ray seems to be less sensitive. It was particularly insufficient in detection of bronchiectasis, which were found in 26% patients, and lung nodules, found in 22% patients. In other studies chronic lung changes were identified in up to 96% patients, bronchiectasis in 64% and nodules in 55% patients. These studies also suggest a predictive role of decreased levels of IgG and IgM as a potential risk factor for development of structural lung damage. [42] However, we did not find such a correlation in our CVID cohort, neither have we detected any correlation between BHR and chronic upper respiratory tract infections, bronchiectasis, lung nodules, splenomegaly or lymphadenopathy.

On the other hand, gender was identified as a potential risk factor in our CVID cohort, with higher prevalence of BHR in female patients (relative risk 2.89 similar to general population). [28] On the other hand, no significant association of BHR and current age, age at diagnosis and disease duration was noted.

To conclude, based on our findings, screening CVID patients for signs of BHR/BA is warranted and spirometry should be performed in all patients. Decrease in MEF25 may help identify patients with BHR, in whom further investigation such as metacholine bronchoprovocation test should be indicated. Initial MEF25 seems to be more sensitive than FEV1. Despite the low sensitivity and specificity of FENO, serum ECP and differential blood count, these tests should be also routinely performed in all CVID patients. Increased levels of FENO and ECP may be found in up to 25% patients, who would benefit from a different therapeutical approach, e.g. the use of inhalant corticosteroids.

Despite the lack of correlation between CVID-related complications (such as bronchiectasis or lung nodules) and the occurrence of BHR or asthma, we recommend to include regular chest CT/HRCT examination into the management of CVID patient, as standard chest X-ray is insufficiently sensitive for detection of structural lung damage.

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None of the authors have any conflict of interest in relation to this work.

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Statement of Ethics

All subjects were included after written informed consents were obtained.

This study has been approved by local Ethics Committee of 2nd Faculty of Medicine Charles University in Prague.

Author contributions:

Tomas Milota – main and corresponding author, initiated and designed the study, performed acquisition, analysis, or interpretation of data, wrote the manuscript

Marketa Bloomfield – co-author, contributed to the acquisition, analysis and interpretation of the data, revised the manuscript

Zuzana Parackova - co-author, contributed to the the acquisition, analysis, or interpretation of data

Anna Sediva – co-author, contributed to drafting of the study, revised and approved the manuscript

Jirina Bartunkova – co-author, contributed to drafting of the study, revised and approved the manuscript

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Figure Legends

Fig. 1: Comparison of the results of adjusted Asthma Control Test between BHR positive (mean: 11.8 points, 95%CI: 9.3-14.3 points) and BHR negative group (mean: 14.9 points, 95%CI 14.7-15 points) with significant difference ($p=0.021$)

Fig. 2: Comparison of FEV1/FVC ratio between BHR positive (mean: 82%, 95%CI: 77.9-86.1%) and BHR negative group (mean: 88.9%, 95%CI: 84.8-93.1%) with significant difference ($p=0.013$)

Fig. 3: Comparison of MEF25 between BHR positive (mean: 76.7%, 95%CI: 64.3-89.3%) and BHR negative group (mean: 106.5, 95%CI (86.7-126.2%)) with significant difference ($p=0.0053$)

Fig. 4: Ratio of male and female patients in BHR positive (grey) and BHR negative group (black) with significant difference ($p=0.048$) and relative risk of 2.89 for females