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Bronchial Asthma and Bronchial Hyperresponsiveness and Their Characteristics in Patients with Common Variable Immunodeficiency

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Keywords

Bronchial asthma · Bronchial hyperresponsiveness · Common variable immunodeficiency · Bronchoprovocation · Risk factors

Abstract

Background: Common variable immunodeficiency (CVID) is one of the most frequent primary immunodeficiencies and is characterized by disturbed immunoglobulin production and dysregulation of the immune system. Results of previous studies suggest a higher prevalence of bronchial asthma (BA) in CVID patients than in the general population. We initiated this study to evaluate lung functions and identify risk factors for BA and bronchial hyperresponsiveness (BHR) in patients with CVID. ***Methods:*** Twenty-three patients with CVID were included in this study. In all of them, spirometry and a metacholine bronchoprovocation test were performed. We also 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 whom had normal FEV_1 and FEV_1/FVC. However, BHR-positive patients had significantly decreased MEF25. BHR-positive patients had also more symptoms related to bronchial obstruction, with 8 of them (35%) being suspected of having BA at the end of the study. A higher prevalence of BHR was found in females, with a relative risk of 2.89. ***Conclusions:*** An increased prevalence of BHR and BA was detected in CVID patients compared to the general population. BA may develop despite the disturbed immunoglobulin production, and the majority of patients display nonatopic and noneosinophilic properties. These results suggest a limited role of atopy and eosinophilic inflammation in the pathogenesis of BA in CVID patients.

Common Variable Immunodeficiency

Common variable immunodeficiency (CVID) is one of the most frequent humoral immunodeficiencies with an incidence of 1:25,000 [1]. It is characterized by disturbed overall and antigen-specific immunoglobulin production, a reduced switched memory B cell count, and a

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broad variability of etiology and symptoms that include infectious, autoimmune, lymphoproliferative, and granulomatous manifestations [2]. These features are also reflected in the diagnostic criteria [3]. Despite the effort to identify the pathophysiological mechanisms and genetic background of this disease, these remain unknown in 85–90% of patients. Impaired immunoglobulin production results in increased susceptibility to infections, particularly recurrent or chronic upper (rhino/sinusitis, tonsillitis, and otitis) and lower (bronchitis and pneumonia) respiratory tract infections [4]. Treatment is based on regular immunoglobulin substitution, antibiotic (ATB) prophylaxis, and other therapeutic measures including the management of noninfectious complications. In some patients, particularly those with recurrent respiratory tract infections, ATB prophylaxis is useful in preventing chronic lung disease. Lower respiratory tract infections, in particular, 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 by an obstructive or restrictive ventilatory disorder. The most common causes of lung restriction are interstitial lung diseases (e.g., lymphocytic interstitial pneumonia and granulomatous-lymphocytic lung disease) [6, 7]. The most common causes of obstructive lung disease are chronic obstructive pulmonary disease (COPD), frequent and also well-documented in CVID patients [8–10], and bronchial asthma (BA) and asthma-COPD overlap syndrome (ACOS), respectively, which have been the subject of only a few studies on CVID patients to date [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 a persistent and usually progressive airflow restriction associated with a chronic inflammatory response and specific features, e.g. increased numbers 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 reduced lung functions and an increase of respiratory symptoms in adulthood; in addition, such infections are suspected to be a risk factor for disease exacerbation [17, 18].

BA also develops due to multiple factors. Respiratory infections due to exposure at an early age to respiratory syncytial virus, parainfluenza, or rhinovirus (the most common causes of bronchiolitis in childhood) [19], and also to allergens seem to be eminent [20]. Tobacco inhalation (passive and active) [21] and air pollution [22] are also potential risk factors but are not as clearly established. The hallmark of BA is chronic inflammation with skewing towards Th2, resulting in the activation of mast cells or eosinophils [23]. Additionally, epithelial cells produce IL-33 upon injury, which promotes an immune response bias towards Th2 [24] and an important source of nitrogen monoxide [25]. These responses lead to the functional and structural alteration of airways, including airway edema, mucous hypersecretion and, particularly, airway hyperresponsiveness with bronchoconstriction [26].

Bronchial hyperresponsiveness (BHR) is defined as bronchoconstriction with bronchial narrowing, triggered by various stimuli. BHR is found in 40–53% of patients with BA, compared to a 10–30% prevalence in children and adults in population-based studies [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 abovementioned immunopathologic mechanisms, several asthmatic phenotypes may be distinguished, such as atopic, eosinophilic, and nonesosinophilic BA [30].

ACOS represents a third obstructive lung disease phenotype, sharing the features of COPD and BA [31, 32]. In some cases, it may be difficult to distinguish between BA, COPD, and ACOS.

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

Study Design, Materials, and Methods

Inclusion and Exclusion Criteria

All patients in our noninterventional, prospective study fulfilled the diagnostic criteria for CVID as defined by the European Society for Immunodeficiency (ESID Registry Diagnostic Criteria, accessed 25 April 2017). They were included after written informed consents were obtained. An inability to undergo spirometric or fractional exhaled nitric oxide (FENO) examination, a history of smoking, pregnancy, an established bronchodilator or inhaled/systemic corticosteroid therapy in the preceding 3 months, restrictive ventilation disorder (FVC <80%), and a secondary

Table 1. Adjusted Asthma Control Test designed by the American Thoracic Society evaluating symptoms related to bronchial hyperresponsiveness in the past 4 weeks prior to the bronchoprovocation test

In the past 4 weeks, how frequently did your asthma disturb/restrict your daily activities at work, at school, or at home?								
All of the time	1	Most of the time	2	Some of the time	3	A few times	4	Never 5
During the past 4 weeks, how often did experience wheezing, coughing, shortness of breath, or chest tightness?								
More than once a day	1	Once a day	2	3–6 times a week	3	1–2 times a week	4	Never 5
During the past 4 weeks, how often did your symptoms (wheezing, coughing, shortness of breath, or chest tightness) wake you up at night or earlier than usual in the morning?								
More than 4 times a week	1	2–3 times a week	2	Once a week	3	1–2 times a month	4	Never 5

cause of bronchial obstruction or acute infection in the 4 weeks prior to the study 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 filled in questionnaires regarding their family and personal history and underwent an adjusted Asthma Control Test (ACT) designed by the American Thoracic Society (Table 1). The full achievable score in the test is 15 points (found in asymptomatic patients). A score of 10–14 points is related to moderate BA severity. Patients with a severe manifestation of BA attain <10 points.

Spirometry, Bronchodilator and Bronchoprovocation Tests, and FENO Measurement

Initially, spirometric and FENO examinations were performed on all included patients. Chest X-ray was performed to exclude secondary causes of airway obstruction, and the findings were compared to the available results of computed tomography (CT)/high-resolution (HR)CT.

Based on the results, patients were divided into groups with (FEV_1/FVC <70%) or without (FEV_1/FVC >70%) an obstructive ventilatory disorder. Patients with a restrictive ventilatory disorder (FVC <80% with a normal FEV_1/FVC ratio) were excluded. A 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 as having obstructive ventilatory disorder on initial spirometry, the bronchodilator test was not indicated in anyone. Initial spirometry, BPT with methacholine, and FENO measurement were performed and interpreted according to the recommendations of the European Respiratory Society and American Thoracic Society [33–35] using a MasterScope spirometer (ERT, Philadelphia, PA, USA) and Vero FENO analyzer (Niox, Solna, Sweden).

Skin Prick Tests and Serum Levels of Total and Allergen Specific IgE

Skin prick tests (SPTs) were performed and assessed according to the recommendations of the American Academy of Allergy, Asthma and Immunology (AAAAI). The test panel included allergen extracts of *Dermatophagoides pteronyssinus* and *D. 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 Co., Antony, France). Corresponding serum allergen-specific IgE antibodies, total serum IgE, and serum eosinophilic cationic protein (ECP) were detected by a chemiluminescence technique using the IMMULITE 2000 system (Siemens, Erlangen, Germany). The results were regarded as positive if the wheal diameter was >3 mm (together with unresponsiveness to a negative control) and the serum concentration of specific IgE >0.35 IU/mL.

IgM and IgG Levels and Lymphocyte Subpopulations

Serum levels of IgM and IgG were evaluated at the time of diagnosis by a nephelometry method using the IMMAGE 800 system (Beckman Coulter Inc., Brea, CA, USA).

Lymphocyte subpopulations were distinguished based on the expression of the specific cell surface membrane markers, CD3 and CD19, using fluorochrome-conjugated monoclonal antibodies (CD3-Alexa Fluor 700, CD19-APC; BioLegend, San Diego, CA, USA) by fluorescence-activated cell sorting (FACSAria II, BD Biosciences, San José, CA, USA). T cells were identified as CD3+ cells and B cells as CD19+ cells. Flow cytometric data were analyzed in FlowJo v10 (FlowJo LLC, Ashland, OR, USA).

Statistical Analysis

All data were statistically processed by GraphPad Prism, v6 (GraphPad Software, La Jolla, CA, USA). The unpaired nonparametric Mann-Whitney U test was used to compare independent samples, and mean values and 95% CI (confidence interval) were calculated. The χ^2 test was used to assess the differences in groups for gender and family history results. The statistical differences were regarded as positive with a p value ≤ 0.05. Sensitivity and specificity values were calculated for ECP and FENO.

Results

Characteristics of the Cohort

Twenty-three patients who fulfilled the inclusion criteria were enrolled in this study, 14 males (61%) and 9 females (39%). All patients were receiving immunoglob-

Table 2. Summary of pulmonary manifestations including the results of spirometry, ACT, chest X-ray, and chest CT/HRCT

Patient No.	FVC	FEV_1	FEV_1/FVC ratio	MEF25	ACT	Chest X-ray	Chest CT/HRCT
1	95	100	1.05	118	15	none	none
2	112	113	1.01	136	15	none	none
3	109	104	0.95	80	14	none	n.a.
4	93	93	1	70	5	BWT	BE, ATE
5	104	106	1.02	122	15	none	BE, NOD
6	86	91	1.06	88	13	none	none
7	89	93	1.04	77	15	BWT, NOD	BE
8	86	104	1.21	165	15	na.	n.a.
9	100	96	0.96	77	15	NOD	NOD
10	98	98	1	74	15	BWT, NOD	BE, NOD
11	96	98	1.02	73	15	none	none
12	95	87	0.92	63	13	none	none
13	102	87	0.85	72	11	none	none
14	104	92	0.88	52	5	none	BE
15	87	81	0.93	58	7	none	NOD
16	84	87	1.04	71	13	NOD	NOD
17	97	105	1.08	110	15	none	n.a.
18	105	107	1.02	79	15	none	none
19	109	92	0.84	51	15	none	none
20	95	100	1.05	97	15	none	none
21	101	103	1.02	103	15	none	none
22	87.9	97	1.1	107.8	15	none	BE
23	87	95	1.09	110	15	none	none

FVC, forced vital capacity; FEV_1, forced vital capacity in 1 s, MEF25, mean expiratory flow at 25%; ACT, Asthma Control Test; BWT, bronchial wall thickening; NOD, nodules; BE, bronchiectasis; ATE, atelectasis; n.a., not available.

ulin replacement therapy, 17 (74%) subcutaneously and 6 (26%) intravenously. Median age at recruitment was 33.7 years (±11.5). Median age at diagnosis of CVID was 23.7 years (±12.1). 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 (43%) patients. Chronic lung disease was diagnosed by CT/HRCT in 9 patients (39%): bronchiectasis in 4 (17%), lung nodules in 3 (13%), and both of these in 2 (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 anemia were diagnosed in 2 patients (9%) and type 1 diabetes mellitus in 1 (4%). Autoimmune duplicity was found in 3 patients (13%). Other noninfectious features were splenomegaly in 11 patients (48%) and lymphadenopathy in 6 (26%). Malignant lymphoproliferations including Hodgkin and Burkitt's lymphoma were revealed in 2 patients (9%). A summary of clinical manifestations is shown in Table 3. Neither concomitant noninfectious manifestations nor the presence of lung abnormalities on CT/HRCT were identified as BHR or BA risk factors (data not shown).

BHR and Its Characteristics

BHR was confirmed in 12 patients (52%) by BPT with metacholine. A mild degree of BHR (PC_{20}: 4–16 mg/mL) was found to be the most common finding, identified in 5 patients. Moderate (PC_{20}:1–4 mg/mL) and severe (PC_{20}: <1 mg/mL) BHR was diagnosed in 4 and 3 patients, respectively. This correlated with dyspnea, assessed by adjusted (a)ACT. A total of 8 patients with BHR (35%) also

Table 3. Summary of clinical manifestations

Patient No.	Chronic infection	Autoimmunity	Chronic lung disease	Allergic disease	Enteropathy	Lymph-adenopathy	Spleno-megaly	Malignancy	Other
1	yes (URTI)	no	no	no	no	no	yes	no	no
2	yes (URTI)	yes (AIT)	no	no	no	no	no	no	no
3	no	no	yes	no	no	no	no	no	no
4	yes (URTI)	yes (vitiligo)	no	no	no	yes	yes	no	chronic nephro-pathy, myomatosis
5	no	yes (ITP, AIT)	yes	no	no	yes	yes	yes (HL)	no
6	yes (URTI)	yes (AIT)	no	no	no	no	no	no	hepatopathy, myomatosis
7	no	yes (ITP, AIHA)	yes	no	no	yes	yes	no	CIHD
8	no	no	no	no	no	no	no	no	no
9	no	yes (ITP, AIHA)	yes	yes (AD)	yes (CRLD)	yes	yes	no	no
10	no	no	yes	no	yes (CRLD)	no	yes	no	no
11	yes (URTI)	no	no	no	no	no	no	no	GERD
12	no	no	no	no	yes (CELD)	no	yes	no	no
13	yes (URTI)	yes (AIT)	no	no	no	no	no	no	no
14	yes (URTI)	no	yes	no	yes (CRLD)	yes	yes	no	no
15	yes (URTI)	yes (ITP)	yes	no	no	no	yes	yes (BL)	no
16	yes (URTI)	no	yes	yes (AD)	no	no	yes	no	no
17	no	yes (ITP)	no	no	no	no	no	no	no
18	no	yes (AIT)	no	no	no	no	no	no	liver heman-gioma
19	no	yes (DMT1)	no	yes (AD)	no	yes	no	no	alopecia
20	no	no	no	no	no	no	no	no	no
21	no	no	no	yes (AD)	yes (CRLD)	no	no	no	no
22	yes (URTI)	yes (vitiligo)	yes	no	no	no	yes	no	GERD
23	no	no	no	no	no	no	no	no	no

URTI, upper respiratory tract infections; AIT, autoimmune thyreoiditis; AIHA, autoimmune hemolytic anemia; ITP, immune thrombocytopenic purpura; DMT1, diabetes mellitus type 1; AD, atopic dermatitis; CRLD, Crohn-like disease; CELD, celiac-like disease; HL, Hodgkin lymphoma; BL, Burkitt lymphoma; CIHD, chronic ischemic heart disease; GERD, gastroesophageal reflux disease.

reported symptoms related to bronchial obstruction such as wheeze, shortness of breath, chest tightness, and cough. The median value of aACT in the group with positive BPT was 11.8 points, compared to the group with negative BPT (14.9 points). This difference was significant ($p = 0.008$; Fig. 1). In 6 patients with 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 the results above. Both groups had a normal FEV_1 and FEV_1/FVC ratio (Fig. 2). However, in the BHR-positive group, a mild decrease in FEV_1 was noted (93.2 vs. 100%, $p = 0.021$) as well as in FEV_1/FVC ratio (82 vs. 88.9%, $p = 0.013$). In the BHR-positive group, the peripheral airways were the most prominently affected, with a significantly greater decrease in mean expiratory flow at 25% (MEF25) (76.7 vs. 106.5%, $p = 0.005$; Fig. 3).

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

Additionally, we investigated the role of the peripheral blood eosinophil and neutrophil counts as well as the CD3 and CD19 lymphocyte counts. No significant differences were observed between the BHR-positive and BHR-negative group. The summary of our results is shown in Table 4.

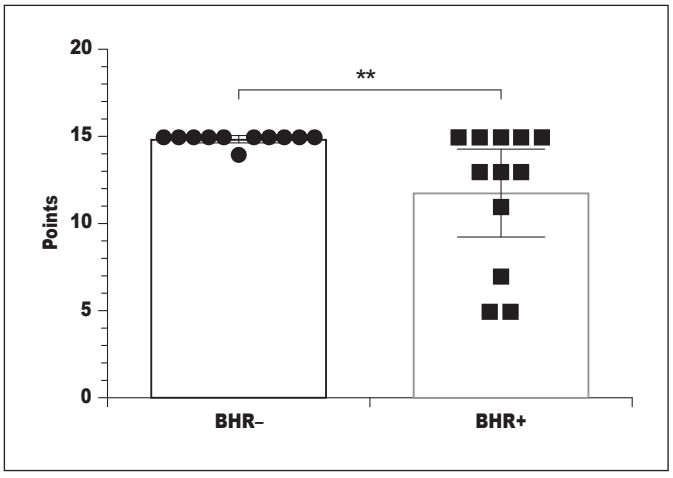

Fig. 1. Comparison of the results of the adjusted Asthma Control Test in the BHR-positive (mean: 11.8 points, 95% CI: 9.3–14.3 points) and BHR-negative (mean: 14.9 points, 95% CI 14.7–15 points) groups, with a significant difference ($p = 0.021$).

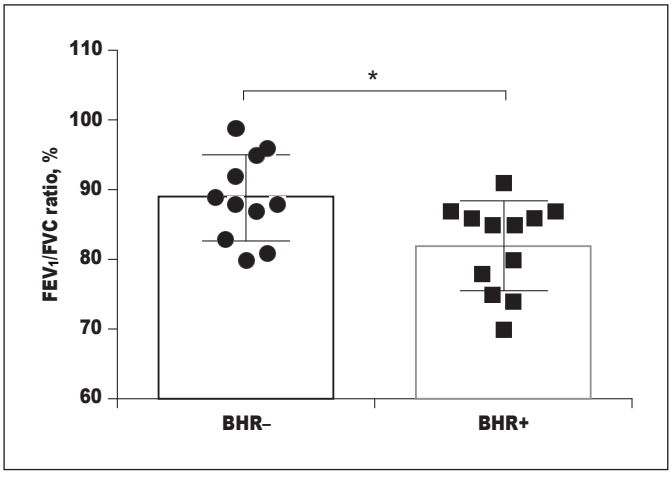

Fig. 2. Comparison of FEV_1/FVC ratio between the BHR-positive (mean: 82%, 95% CI: 77.9–86.1%) and BHR-negative (mean: 88.9%, 95% CI: 84.8–93.1%) groups, with a significant difference ($p = 0.013$).

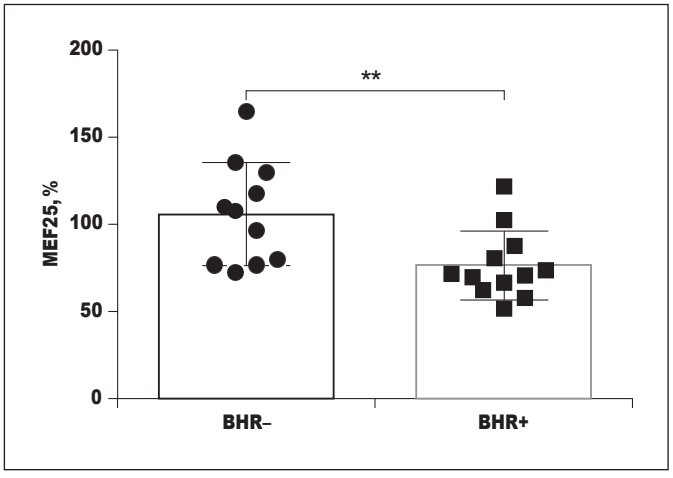

Fig. 3. Comparison of MEF25 between the BHR-positive (mean: 76.7%, 95% CI: 64.3–89.3%) and BHR-negative (mean: 106.5, 95% CI: 86.7–126.2%) groups, with a significant difference ($p = 0.0053$).

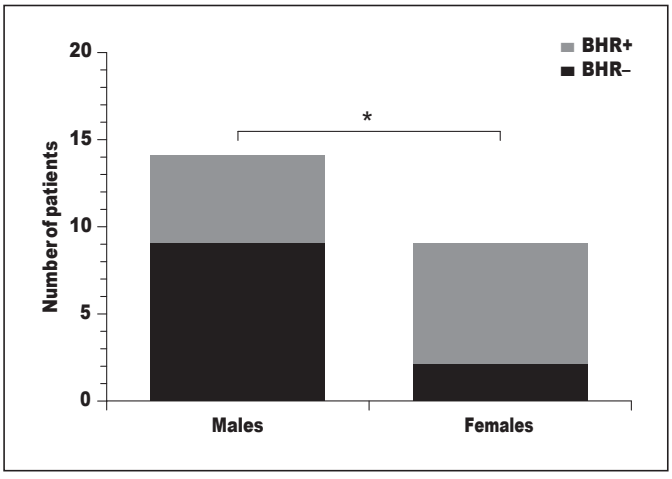

Fig. 4. Ratio of male and female patients in the BHR-positive (grey) and BHR-negative (black) groups, with a significant difference ($p = 0.048$) and a relative risk of 2.89 for females.

BHR-Associated Risk Factors

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

The role of serum total and allergen-specific IgE as a potential risk factor was evaluated. The IgE production was preserved in only 5 patients (22%); in the remaining patients (78%), IgE levels were undetectable (<0.1 U/mL). Three 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 positive SPTs. 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 pollen) displayed these symptoms.

Furthermore, serum IgG and IgM levels were analyzed at the time of diagnosis. The mean serum level of IgM was

Table 4. Summary of BHR characteristics and risk factors for all included CVID patients

	All patients	BHR-negative group	BHR-positive group	*p* value
BHR characteristics				
ACT, points	13.3 (11.2–14.7)	14.9 (14.7–15)	11.8 (9.3–14.3)	0.009**, [1]
FEV_1, %	96.4 (93.3–99.6)	100 (96.3–103.7)	93.2 (88.6–97.8)	0.021*, [1]
FEV_1/FVC ratio, %	85.3 (82.2–88.4)	88.9 (84.8–93.1)	82 (77.9–86.1)	0.013*, [1]
MEF25, %	91 (78.6–103.3)	106.5 (86.7–126.2)	76.7 (64.3–89.3)	0.0053**, [1]
FENO, ppb	25.9 (20.4–31.5)	22 (15–29)	29.5 (20.5–38.6)	0.205[1]
ECP, ng/mL	22.8 (16.6–29)	25.2 (13.4–37)	21.5 (15.4–27.6)	0.729[1]
Eosinophils, $\times 10^9$/L	0.175 (0.125–0.226)	0.169 (0.09–0.249)	0.181 (0.104–0.257)	0.94[1]
Neutrophils, $\times 10^9$/L	4.915 (4.183–5.647)	4.554 (3.448–5.660)	5.247 (4.143–6.350)	0.404[1]
CD3, $\times 10^9$/L	1.173 (0.94–1.41)	1.09 (0.74–1.43)	1.25 (0.89–1.61)	0.556[1]
CD19, $\times 10^9$/L	0.18 (0.12–0.25)	0.16 (0.09–0.23)	0.20 (0.09–0.31)	0.774[1]
Risk factors				
Family history (negative:positive)	12:11	7:4	5:7	0.292[2]
Gender (males:females)	14:9	9:2	5:7	0.048*,[2]
Age at diagnosis, years	23.7 (18.5–28.9)	20.7 (16.1–25.4)	27.2 (17.7–36.6)	0.496[1]
Disease duration, years	10 (7.4–12.5)	8.5 (4.1–12.8)	11 (7.3–14.7)	0.322[1]
IgM at diagnosis, g/L	0.31 (0.17–0.45)	0.21 (0.09–0.34)	0.40 (0.15–0.66)	0.151[1]
IgG at diagnosis, g/L	2.84 (2.11–3.57)	2.32 (1.27–3.37)	3.32 (2.22–4.42)	0.184[1]
IgE at diagnosis, IU/mL	6.93 (0.0–15.73)	9.4 (0.0–27.79)	4.69 (0.0–12.67)	0.854[1]

Values are expressed as *n* or mean (95% confidence interval). ACQ, Asthma Control Test; FVC, forced vital capacity; FEV_1, forced vital capacity in 1 s; MEF25, mean expiratory flow at 25%; FENO, fractional exhaled nitric oxide; ECP, eosinophilic cationic protein. * $p \leq 0.05$; ** $p \leq 0.009$; [1] Mann-Whitney U test; [2] χ^2 test.

0.31 g/L and that of IgG was 2.84 g/L. The serum levels of both IgM and IgG in the BHR-negative group were lower than in the BHR-positive group (0.21 vs. 0.40 g/L of IgM; 2.32 vs. 3.32 g/L of IgG), but the differences were not statistically significant.

Discussion

Of the cohort of 33 CVID patients followed up at our department, 6 (18%) had been diagnosed with BA, 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 so they were excluded. In the remaining 23 patients, BHR was diagnosed in a striking 52% (5 mild, 4 moderate, and 3 severe cases) compared to 10–16% of adults and 16–30% of 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 a prevalence of BA in the Czech general population of only 5% [36]. Compared to the CVID patients without BHR, those with BHR also displayed reduced values for FEV_1 (92 vs. 100%), FEV_1/FVC (100 vs. 105%) and, interestingly, MEF25 (71.5 vs. 107.8%), implying the predominant impairment of the peripheral airways. This observation corresponds well with findings in pediatric patients with allergic rhinitis and at the time of asthma onset [37, 38]. We have thus confirmed that a decrease in MEF25 is associated with BHR, also in patients with underlying humoral immunodeficiency.

To the best of our knowledge, only one similar study regarding BA or BHR in patients with CVID has been performed, by Agondi et al. [11]. In a cohort of 62 CVID patients, 48% were found to have an obstructive ventilatory disorder, 12% had a restrictive ventilatory disorder, and 40% had normal spirometry parameters. Abnormal SPTs and elevated specific IgE were found in 3% of the patients, a normal serum level of total IgE in 29%, and levels were undetectable in 68%. At the end of this study, the diagnosis of BA was confirmed in 15% and 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 the development of BA, which has also been shown in mouse models [39]. We therefore conclude that BA may develop, even in a setting of humoral immunodeficiencies, with disturbed IgE production.

Agondi et al. [11] did not assess any additional parameters which can help to determine the precise nature of BHR or BA, such as ECP, FENO, and neutrophilic or eosinophilic peripheral blood counts, all of which are useful for selecting an appropriate therapeutic approach. For instance, eosinophilic inflammation responds better to corticosteroids than neutrophilic inflammation does. Performing these tests in our cohort, we did not find any significant differences in FENO and ECP between the BHR-positive and BHR-negative groups. The cut-off points for FENO >25 ppb and serum ECP >25 ng/mL are highly predictive and support 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 the limited role of eosinophilic inflammation in the development of BA and BHR in CVID patients, favoring a nonallergic and noneosinophilic character and emphasizing 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 on chest X-ray. Chest X-ray thus seems to be less sensitive, and was particularly inadequate for detecting bronchiectasis, found in 26% of patients, and lung nodules, found in 22%. In other studies, chronic lung changes were identified in up to 96% patients, bronchiectasis in 64%, and nodules in 55%; these studies also suggest a predictive role of decreased levels of IgG and IgM as a potential risk factor for the development of structural lung damage [42]. However, we did not find such a correlation in our CVID cohort, nor have we detected any correlation between BHR and chronic upper respiratory tract infections, bronchiectasis, lung nodules, splenomegaly, or lymphadenopathy.

Gender was identified as a potential risk factor in our CVID cohort, with a higher prevalence of BHR in females (a relative risk of 2.89, similar to the 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. Decreased MEF25 may help to identify patients with BHR, in whom further investigation such as a metacholine BPT should be indicated. Initial MEF25 value seems to be more sensitive than FEV_1. Despite the low sensitivity and specificity of FENO, serum ECP and differential blood count, these tests should also be routinely performed in all CVID patients. Increased levels of FENO and ECP may be found in up to 25% patients, who would then benefit from a different therapeutical approach, e.g., the use of inhalant corticosteroids.

Despite the lack of a correlation between CVID-related complications (such as bronchiectasis or lung nodules) and the occurrence of BHR or BA, we recommend including regular chest CT/HRCT examinations into the management of CVID patients, as standard chest X-ray is insufficiently sensitive for detecting structural lung damage.

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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 the 2nd Faculty of Medicine Charles University in Prague.

Disclosure Statement

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

Author Contributions

Tomas Milota: main and corresponding author, initiated and designed the study, performed acquisition, analysis, and interpretation of data, and wrote the manuscript. Marketa Bloomfield: coauthor, contributed to the acquisition, analysis, and interpretation of data, and revised the manuscript. Zuzana Parackova: coauthor, contributed to the acquisition, analysis, and interpretation of data. Rudolf Horvath: coauthor, contributed to the acquisition, analysis, and interpretation of data. Anna Sediva: coauthor, contributed to the drafting of the study, and revised and approved the manuscript. Jirina Bartunkova: coauthor, contributed to the drafting of the study, and revised and approved the manuscript.

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CVID-Associated Tumors: Czech Nationwide Study Focused on Epidemiology, Immunology, and Genetic Background in a Cohort of Patients With CVID

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Background: Common variable immunodeficiency disorder (CVID) is one of the most frequent inborn errors of immunity, increased occurrence of malignancies, particularly lymphomas, and gastric cancers, has long been noted among CVID patients. Multifactorial etiology, including immune dysregulation, infections, chronic inflammation, or genetic background, is suggested to contribute to tumor development. Here, we present the results of the first Czech nationwide study focused on epidemiology, immunology and genetic background in a cohort of CVID patients who also developed tumors

Methods: The cohort consisted of 295 CVID patients followed for 3,070 patient/years. Standardized incidence ratio (SIR) was calculated to determine the risk of cancer, and Risk ratio (RR) was established to evaluate the significance of comorbidities. Moreover, immunophenotyping, including immunoglobulin levels and lymphocyte populations, was assessed. Finally, Whole exome sequencing (WES) was performed in all patients with lymphoma to investigate the genetic background.

Results: Twenty-five malignancies were diagnosed in 22 patients in a cohort of 295 CVID patients. SIR was more than 6 times greater in comparison to the general population. The most common neoplasias were gastric cancers and lymphomas. History of Immune thrombocytopenic purpura (ITP) was established as a potential risk factor, with over 3 times higher risk of cancer development. The B cell count at diagnosis of

lymphoma was reduced in the lymphoma group; moreover, post-treatment B and T cell lymphopenia, associated with poorer outcome, was found in a majority of the patients. Intriguingly, no NK cell depression was observed after the chemotherapy. WES revealed heterogeneous genetic background among CVID patients with tumors, identifying gene variants associated with primary immunodeficiencies (such as CTLA4, PIK3CD, PMS2) and/or increased cancer susceptibility (including BRCA1, RABEP1, EP300, KDM5A).

Conclusions: The incidence of malignancy in our CVID cohort was found to be more than 6 times greater compared to the general population. Gastric cancers and lymphomas were the most frequently diagnosed tumors. ITP was identified as a risk factor for malignancy in CVID patients. WES analysis confirmed a wide genetic heterogeneity among CVID patients. The identified causative or modifying gene variants pointed to errors in mechanisms contributing to both immunodeficiency and malignancy.

Keywords: common variable immunodeficiency, malignancy, lymphoma, gastric cancer, whole exome sequencing

INTRODUCTION

Immune control of tumor development and growth requires a functional immune system capable of complex immune responses necessary for recognition and elimination of malignant cells. As such, inborn errors resulting in immunodeficiencies may convey an increased risk of cancer. In general, the spectrum of malignancies in primary immunodeficiency (PID) patients is clearly biased when compared to malignant diseases in the general population. Malignancies in PIDs tend to be restricted to certain oncological entities, and their pathophysiology is often linked to the mechanism underlying the particular immunodeficiency (1). For example, immunodeficiencies associated with gain-of-function mutations in *PIK3* are associated with a high risk of lymphoma (2). The role of this signaling pathway in cancer genesis and immunodeficiency is validated by therapeutic success of targeted PI3K/mTOR pathway inhibition used in activating *PIK3* syndrome, as well as in malignant diseases (3). Similarly, immunodeficiencies arising from developmental defects of stem cells, myeloid cells or lymphocytes are associated with an increased incidence of leukemia or lymphomas, implying errors in the corresponding pathways (1). Recent advances in understanding of molecular mechanisms underlying primary immunodeficiencies, as well as tumors, has provided evidence for such associations. However, in immunodeficiencies that are not yet precisely defined by their molecular/genetic cause, the situation is more complex. Common variable immunodeficiency disorder (CVID) is one of the most frequent forms of antibody deficiencies; yet, its pathophysiology remains largely unknown. The hallmark of CVID is the impairment of the B cell compartment, typically manifesting as a reduction of mature forms of B cells and expansion of less differentiated stages of B lymphocytes (4). The T cell compartment is also usually skewed in CVID, specifically toward terminally differentiated forms, including senescent cells, typically affecting both CD4and CD8 T cells, which are crucial for anti-tumor immunity (5). The mechanisms of B cell involvement in anti-tumor immunity are largely unknown. B cells may promote both pro-tumourigenic responses (e.g., specific subsets of B cells may produce IL-10 or TGF-beta with immunosuppressive properties, B cells may promote tumor genesis and tumor progression by alteration of the angiogenic and proinflammatory microenvironment), as well as anti-tumourigenic responses (e.g., B cells may enhance cytotoxic T cell activity, indirectly mediate antibody dependent cytotoxic mechanisms or serve as professional antigen-presenting cells, initiating the T cell response) (6). Inborn impairment of the B cell lineage, along with T cell dysregulation, may facilitate the genesis of tumors in CVID patients. Furthermore, the immunologic defect is accentuated by recurrent and chronic infections. Chronic viral infections, particularly EBV, are strongly associated with lymphoproliferative diseases and lymphoma (7). Additionally, chronic inflammatory response, *per se*, represents a risk factor for tumor development, especially in patients genetically predisposed to malignancy.

Efforts made to establish the genetic etiology of CVID have thus far been successful in 2–10% of CVID patients (known CVID-associated gene variants are shown in **Table 1**) (8). Some of the CVID-associated genes represent a clear predisposition to cancer, as described in previously published CVID cohorts; most prominently, these mutations are in genes causing alterations in the NFkB or PI3k pathways or in genes affecting B cell receptors (3, 9, 10).

Overall, the factors contributing to increased incidence of malignancy in CVID are complex, encompassing genetics, immune response dysregulation, infections, inflammation, and perhaps other not yet elucidated mechanisms.

Here, we present the results of a complex study on a Czech national cohort of CVID patients who also presented with malignancy. National epidemiological data were collected, immune profiles were analyzed and, in a subgroup of CVID patients with lymphoma, Whole exome sequencing (WES) was performed.

TABLE 1 | List of genes associated with Common variable immunodeficiency (monogenic causes and modifier genes) and their prevalence, adjusted according to Bogaert et al. (8).

Gene	Prevalence (%)
MONOGENIC CAUSE OF CVID: 2–10%	
PIK3CD	26.74
LRBA	26.74
CTLA4	6.42
NFKB2	5.35
TNFRSF7 (CD27)	4.81
PIK3R1	4.81
ICOS	3.74
CD19	3.74
IL-21R	3.21
IKZF1 (IKAROS)	3.21
PRKCD	2.14
PLCG2	2.14
NFKB1	1.6
CR2 (CD21)	1.7
MODIFIER GENES: UNKNOWN PREVALENCE	
TNFRSF13B (TACI), TNFRSF13C (BAFF-R), MSH2, MSH5, CLECG1, MLH1, RAD50, ORC4L, FCGR2A.	

METHODS

This study was approved by local ethics committee of Motol University Hospital. Written informed consents were obtained from all enrolled patients.

Data Collection

Retrospective clinical and laboratory data of 295 enrolled patients were obtained from medical records of national referral centers for the treatment of adult patients with primary immunodeficiency diseases. The collected data covered the period from 1997 to 2016. They included a total amount and a length of surveillance of all CVID patients fulfilling ESID/PAGID criteria, a number of CVID cancer patients and patient-specific data: year of birth, age at first symptoms associated with CVID and their nature, clinical comorbidities, age at cancer diagnosis, type of cancer, therapeutic regimens, survival rates, cause of death (if applicable), and specific details of cancer diagnostics.

A more detailed set of clinical data was obtained from 11 CVID patients with cancer and from 160 randomly selected cancer-free CVID patients, who represented the reference group. This cohort included 95 females and 65 males with a median age of 48 years (range 19–88). Czech general population data on occurrence of malignancy were obtained from the Czech National Cancer Registry and covered the period from 1994 to 2014 (the last available reports).

Epidemiology

Prevalence and SIR were calculated to express the probability of cancer diagnosis in a CVID cohort compared to the general population. RR was used to assess the significance of comorbidities in CVID patients with cancer. Confidence intervals (95% CIs) were determined for both parameters. All results for which number 1 was beyond the 95% CI were accepted as statistically significant (11).

Immunophenotyping

Serum levels of IgM, IgG, IgA were evaluated by nephelometric method using Image 800 systems (Beckman-Coulter, Brea, CA, USA). Basic lymphocyte subpopulations, including T cells, T helper cells, T cytotoxic cells, B cells and NK cells, were distinguished by FACS based on the expression of specific cell surface membrane markers using fluorochrome-conjugated monoclonal antibodies CD3-FITC, CD4-PE/Cy, CD8-APC/Cy,CD19-APC, CD16-PE, CD56-PE; KOMBITEST, Exbio, Prague, Czech Republic. B cell subpopulations (including CD21low, naive, transitional, marginal zone-like, class-switched cells, and plasmablasts) and T cell subpopulations (recent thymic emigrants-RTE, naïve, central memory-CM, effector memory-EM, effector memory expressing CD45RA-TEMRA, activated T cells) were performed using antibody-fluorochrome conjugates: CD45-APC-H7, CD3-APC, CD4-PerCP-Cy5.5, CD16-PE, CD56-PE, TCRgd-PE-Cy7, CD38-FITC, CD21-APC, IgM-FITC, CD8-Horizon V-500, CD45RA-PE-Cy7 (BD Biosciences, San Jose, CA, USA), CD5-PE,CD8-FITC, CD27-Brilliant Violet 421, IgM-Brilliant Violet 510, IgD-PerCP-Cy5.5, CD4-Brilliant Violet 510, CD62L-Brilliant Violet 421, HLA-DR-PerCP-Cy5.5 (Biolegend, San Diego, CA), CD19-PE-Cy7, CD24-PE, CD24-APC-Alexa Fluor 750, CD8-APC-Alexa Fluor 750 (Beckman Coulter, Miami, FL, USA), CD27-Pacific Blue, CD38-Alexa Fluor 700, CD45RO-FITC, CD31-PE, CD4-Alexa Fluor 700 (Exbio, Vestec, Czech Republic), CCR7-PE (MiltenyiBiotec, BergischGladbach, Germany), CD3-PerCP-Cy5.5 (Affymetrix eBioscience, ThermoFisher Scientific, Waltham, MA, USA), and IgD biotin (SouthernBiotech, Birmingham, AL, USA) followed by Streptavidin-Qdot 605 (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA).

The absolute and relative counts were assessed for all subpopulations. Examinations of the basic subpopulations and immunoglobulin levels were performed at the time of diagnosis of CVID and at the time of diagnosis of lymphoma. The B and T cell subpopulations were measured prior to the genetic testing. All parameters were compared to the control cohort of 20 randomly selected CVID patients without lymphoma. All obtained data were statistically evaluated. A non-parametric Mann-Whitney test was used to compare independent samples, and a non-parametric Wilcoxon matched-pairs signed rank test was used to compare dependent samples; differences of $p < 0.05$ were regarded as significant. Median and 95% CIs were calculated for all analyzed parameters.

CTLA-4 Expression

CTLA-4 expression was assessed in patient with novel mutations using FACS. Intracellular CTLA-4 was detected 16 h following anti-CD3/CD28 stimulation (Dynabeads, Thermo Fisher Scientific, MA, USA) using CTLA4-APC antibody together with the FOXP3 Fix/Perm Buffer set (BioLegend, San Diego, CA, USA) in CD4+CD127dimCD25+ T regulatory cells (Tregs).

CD45-APC-H7, CD4-Brilliant Violet 510, and CD127-Brilliant Violet 421 (BD Biosciences, San Jose, CA, USA), CD25-PE-Cy7 and CD8-FITC (Exbio, Vestec, Czech Republic) antibodies were used for detection of Tregs.

Whole Exome Sequencing

Sequencing libraries were prepared using a SureSelectXT Human All Exon V6+UTR kit (Agilent Technologies, Santa Clara, CA) from DNA isolated from patients' peripheral blood with a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Sequencing was performed by our facility on the NextSeq 500 (Illumina, San Diego, CA) instrument according to the manufacturer's protocols. The reads in resulting Fastq files were aligned against the human reference genome hg19 with BWA (12). Genomic variants were called with samtools and VarScan (13). Variant annotation was performed using SnpEff (14). Variant filtering was performed with Ingenuity® Variant Analysis™ (IVA) software (www.qiagen.com/Ingenuity, QIAGEN). Only variants with read depths of at least 10 and allele frequencies of at least 0.3 were evaluated. Common variants with allele population frequencies of more than 0.1% or homozygous counts of 5 or more in the ExAC or gnomAD databases were filtered out unless reported as disease-causing in the HGMD® (BIOBASE GmbH) or dbSNP databases (15, 16). Variants predicted to have low impact by at least 2 out of 3 scores calculated by SIFT, PolyPhen2, or CADD and present in population databases were also discarded (17–19). Remaining variants were manually evaluated in Integrative Genomics Viewer (http://www.broadinstitute.org/igv) to exclude variants in reads with low mapping quality (20). The analysis was then focused on variants in genes reported as causative for inborn errors of immunity in the last International Union of Immunological Societies (IUIS) guidelines, cancer-predisposition genes in children and in-house lists of genes possibly leading to immune dysregulation based on recent publications and close interactions with causative genes reported by IUIS (21, 22).

RESULTS

Epidemiology and Clinical Manifestation

Our cohort of patients included 295 patients followed for 3,070 patient/years in total. The average ages at the first CVID-related symptoms and at the time of CVID diagnosis in a subgroup of CVID patients with malignancy were 34.2 and 38.3 years, respectively. A total of 25 malignancies were found in 22 patients (7.4% of all included patients) with SIR 6.3 (95% CI: 4.08–9.31). These cases included 6/25 (24.0%) gastric carcinoma (GC): SIR 5.7, 95% CI: 2.08–12.32, 4/25 (16.0%), B cell Non-Hodgkin lymphoma (B-NHL): SIR 5.5, 95% CI: 1.50–14.09, 5/25 (20.0%), Hodgkin lymphoma (HL):SIR 30.0, 95% CI: 9.73–69.93 and 10/24 (41.7%) other cancers (SIR 5.0, 95% CI: 2.40–9.16). These were two cases of spinocellular carcinoma, basocellular carcinoma, and T-cell lymphoma, and a single case each of tonsillar carcinoma, breast carcinoma, renal carcinoma and urine bladder cancer. Cancer duplicity was observed in 3 patients (gastric and tonsillar carcinoma, breast cancer and urothelial carcinoma, spinocellular, and gastric carcinoma). Malignancies were diagnosed in 16 males and 6 females; the average age at diagnosis was 52.3 years (15 years after the diagnosis of CVID and 19 years after the first symptoms). The average ages of manifestation of GC, B-NHL, and HL in CVID cancer group were 55–59, 35–39, and 40–44 years, respectively.

Autoimmune cytopaenias, including Immune thrombocytopenic purpura (ITP) and Autoimmune hemolytic anemia (AIHA), were the most common complications in CVID both with and without malignancy. They were found in 8/22 (36.4%) and 27/160 (16.9%), respectively. Interestingly, a strikingly increased risk of malignancy was detected in a subgroup of CVID patients with a history of ITP (RR 3.52, 95% CI 1.42–7.26). Cumulative risk (RR 4.53, 95% CI 1.23–11.59) observed for B-NHL and HL together was similar. However, no risk increase was observed for isolated HL, B-NHL, and GC, probably due to the small number of cancer events and because the direct association with other documented CVID-related comorbidities was not significant (data not shown).

As mentioned above, lymphomas and GC represent the most frequent malignancies diagnosed in CVID patients in our cohort. While five out of six patients with GC (4 males, 2 females) underwent surgery, one received palliative chemotherapy with FLOX regimen (fluorouracil, leucovorin, oxaliplatin) due to highly progressed disease at the time of diagnosis. Five out of six patients had deceased before the study initiation, 2/6 (33.3%) due to disease progression, 2/6 (33.3%) passed away after achieving more than 10 years survival, and one patient died because of malnutrition due to severe enteropathy 2 years after the diagnosis of lymphoma. All patients suffered from various gastrointestinal complications related to CVID, and the majority of them also had splenomegaly.

All 5 patients (4 males, 1 female) with HL received chemotherapy. The following chemotherapeutic regimens were used: R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) in 2/5 (40%) of patients, BEACOPP (bleomycine, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone), eBEACOPP respectively, in 2/5 (40%) and DBVE-PC (adriamycin, bleomycine, vincristine, etoposide, prednisone, cyclophosphamide) in 1 patient (20%). No therapy-associated deaths or unexpected toxicities were noted. One patient died 3 years after treatment because of severe enteropathy, and 4/5 (80%) patients are still surviving today. All patients had previously described splenomegaly, and 3/5 (60%) had lymphadenopathy described prior to cancer diagnosis.

Similarly, all 4 patients (3 males, 1 female) with B-NHL received chemotherapy; no therapy-associated deaths or unexpected toxicities were noted. The following chemotherapeutic regimens were used: R-CHOP/CHOP [rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone in 3/4 (75%) patients−2 with DLBCL (Diffuse large B-cell lymphoma) and in 1 patient with MALT (Mucosa-associated lymphoid tissue] lymphoma. A GMALL (German multicenter ALL) regimen was used in 1 patient with Burkitt lymphoma. Two of these patients had been regularly followed even prior to the diagnosis of B-NHL for lymphadenopathy and previously reported splenomegaly. All patients are still

alive. T-NHL was diagnosed in 2 patients. The clinical features of CVID patients in whom lymphomas were diagnosed are summarized in **Tables 2**, **3**. In this cohort, WES and detailed immunophenotyping were performed as part of further investigation (results presented further).

Immunophenotype

Parameters of cellular immunity were investigated, including T cell (CD4 T helpers as well as CD8 T cytotoxic cells), B cell and NK cell counts. No significant differences were registered between the absolute counts of T cells, T helper cells and T cytotoxic cells at the time of diagnosis of CVID in a cohort of patients with lymphoma compared to those without lymphoma. The T cellcounts were also well within the normal reference ranges (T cells 0.8−2.10E9/l, T helper cells 0.3−2.8E9/l, T cytotoxic cells 0.2−1.0E9/l). Unsurprisingly, the chemotherapeutic regiments for lymphoma led to skewing of T cell numbers (median 0.65E9/l, 95% CI 0.46–0.75 vs. 1.22E9/l, 95% CI 1.07–1.47, $^{***}p = 0.0004$), specifically T helper cells (median 0.34E9/l, 95% CI 0.14–0.36 vs. 0.56E9/l, 95% CI0.53–0.76, $^{***}p = 0.0004$) and T cytotoxic cells (0.26E9/l, 95% CI 0.14–0.36 vs. 0.54E9/l, 95% CI 0.41–0.64, $^{**}p = 0.006$).

The number of total B cells at the diagnosis of CVID did not differ significantly from the control group of CVID patients without lymphoma and from normal ranges. No significant difference was noted in the serum levels of IgG in the group of CVID patients with lymphoma (median 2.88 g/l, 95% CI 1.83–3.91, normal values 7.65–13.6 g/l) compared to the CVID control group (median 2.02 g/l, 95% CI1.63–3.24). In contrast, the number of total B cells at the diagnosis of lymphoma was reduced in the lymphoma group (median 0.01E9/l, 95% CI 0–0.13 vs. 0.195E9/l, 95% CI 0.16–0.29, $^{**}p = 0.006$). Absolute B cell counts were further depleted by the chemotherapy (median 0.11E9/l, 95% CI 0–0.46 vs. 0.08E9/l, 95% CI 0.03–0.197, $^{*}p = 0.02$). Post-therapeutic B cell lymphopenia (B cells count ≤ 0.03E9/l) was found in 6 patients. A complete total B cell count reconstitution was achieved in only 3 patients (median 0.33E9/l, range 0.137–0.654); however, mature forms of B cells, including marginal zone-like, class-switched cells and plasmablasts, remained reduced in these subjects (mean interval after chemotherapy 102 months, range 6–204). The remaining 3 patients failed to re-establish their B cell populations and continued to maintain severely reduced B cell compartments (mean 0.02E9/l, range 0.001–0.08; mean interval after chemotherapy 39 months, range 4–145). Concerning NK cells, their absolute counts were similar to CVID patients without lymphoma and the general population (normal range 0.05–1.0 E9/l) and remained unchanged throughout the disease course. Curiously, no NK cell depression was observed after the chemotherapy. The immunophenotype profiles are summarized in **Figure 1**, and the B cell subpopulations are shown in detail in **Table 4** and **Figure 2**.

Whole Exome Sequencing

WES was performed in 10 out of 11 CVID patients with lymphoma in whom biological material for genetic testing was available. The WES results were divided into 5 groups.

TABLE 2 | Characteristics of the cohort of 11 CVID patients with lymphoma (M, Male; F, Female; LYM, Lymphadenopathy; SPLE, Splenomegaly; ITP, Idiopathic thrombocytopenic purpura; RTI, Respiratory tract infections; DBLCL lymphoma, Diffuse large B-cell lymphoma; MALT lymphoma, Mucosa-associated lymphoid tissue lymphoma; PTCL, Peripheral T-cell lymphoma; N/A, not applicable; Y, Yes; N, No; chemotherapeutic regimens are described in the results).

Patient Nr.	Gender	Age at diagnosis of CVID	Manifestation	IgG serum level	Age at diagnosis of lymphoma	Type of lymphoma	Cause of death	Survival interval	Staging	Therapy
1	M	35 years	RTI	1.9 g/l	57 years	T lymphoma	Infection	0 month	Died before staging	Died before treatment
2	M	53 years	LYM, SPLE	1.15 g/l	64 years	HL	Enteropathy	36 months	IVB	BEACOPP
3	F	41 years	ITP	3.89 g/l	58 years	HL	Alive	4 months	IIA	R-CHOP
4	M	18 years	RTI	2.43 g/l	45 years	DBLCL lymphoma	Alive	9 months	IIA	R-CHOP
5	M	39 years	LYM, SPLE	0.03 g/l	35 years	DBLCL lymphoma	Alive	96 months	IVB	R-CHOP
6	M	37 years	ITP	4.1 g/l	40 years	HL	Alive	12 months	IVA	R-CHOP
7	M	36 years	RTI	4.1 g/l	42 years	HL	Alive	6 months	IIIB	eBEACOPP
8	M	26 years	ITP	4.88 g/l	36 years	Burkitt lymphoma	Alive	25 months	IIIA	B-NHL GMALL
9	F	25 years	RTI	2.88 g/l	36 years	MALT lymphoma	Alive	145 months	IVA	R-CHOP
10	M	11 years	RTI	4.48 g/l	11 years	HL	Alive	204 months	IIIA	DBVE-PC
11	M	25 years	RTI	1.76 g/l	30 years	PTCL	Infection	9 months	IVA	CHOP

TABLE 3 | Characteristics of CVID-related complications in a cohort of 11 patients with lymphoma (ITP, Immune thrombocytopenic purpura; AIHA, Autoimmune hemolytic anemia; RA, Rheumatoid arthritis; LIPS, Lymphocytic interstitial pneumonia; BE, Bronchiectasis; ACOS, Asthma-COPD overlap syndrome; EAA, Exogenous allergic alveolitis; NLH, Nodular lymphoid hyperplasia; IBD, Intestinal bowel disease; CG, Chronic gstritis; Y, Yes; N, No).

Patient Nr.	Autoimmunity	Chronic lung disease	Enteropathy	Granulomatous complications	Lymph-adenopathy	Spleno-megaly
1	AI thyreoiditis	LIPS	NLH	N	N	Y
2	N	BE	Celiac-like disease	N	Y	Y
3	ITP	BE	N	N	Y	Y
4	ITP, AIHA	LIPS	Celiac-like disease	N	Y	Y
5	N	N	N	N	Y	Y
6	ITP	N	N	N	N	Y
7	RA	N	N	N	N	Y
8	ITP, psoriasis	ACOS	N	N	N	Y
9	N	EAA	IBD-like disease	N	Y	N
10	N	BE, NLH	NLH, CG	N	Y	Y
11	N	BE	NLH	N	Y	N

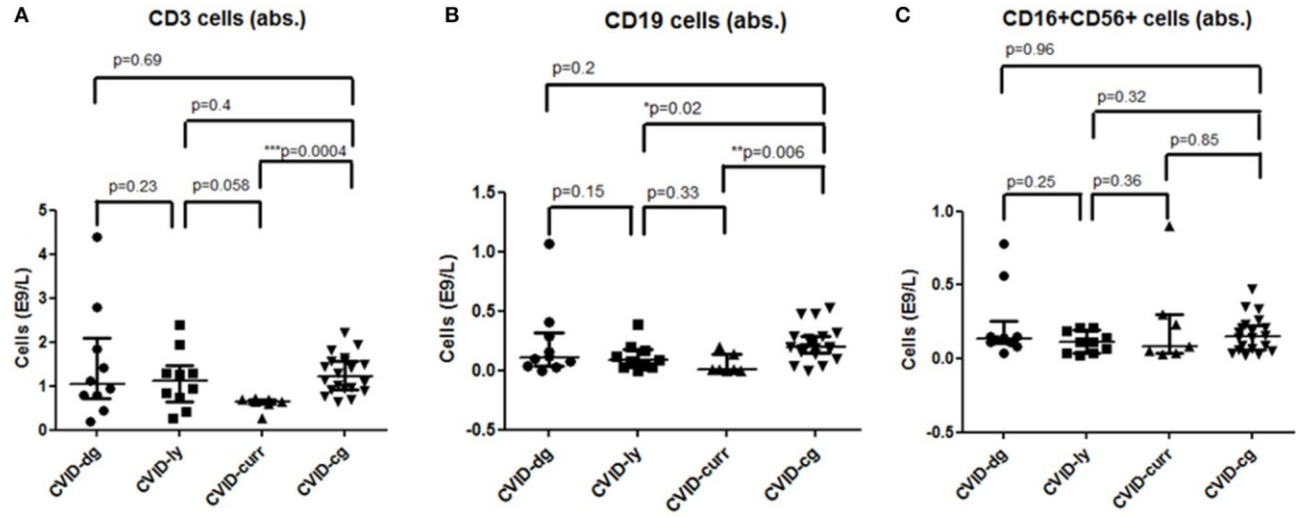

FIGURE 1 | Absolute counts of (A) T (CD3+) cells, (B) B (CD19+) cells, and (C) NK (CD16+, CD56+) cells in a cohort of CVID patients with lymphoma at the time of diagnosis of CVID (CVID-dg), at the time of diagnosis of lymphoma (CVID-ly) and current values (CVID-curr) compared to the control group of CVID patients without lymphoma (CVID-cg);median and 95% CI are shown.

Gene variants previously described in association with CVID or in patients with inborn error of immunity (Group 1) were found in 4 patients. A novel heterozygous missense variant in *CTLA4* was identified inpatient Nr. 9, who developed B—NHL (MALT) at the age of 36 years. She was also followed and treated for lymphadenopathy and enteropathy (with features of IBD-like disease), which is in concordance with the expected phenotype of CTLA4 deficiency. The deleterious effect of the mutation was verified by determination of decreased basal and stimulated (CD3/CD28) expression of CTLA4 protein in the patient's T regulatory cells (CD4+CD127dimCD25+) (**Supplementary Figure 1**).

Another type of B—NHL (DBLCL) was diagnosed in patient Nr. 4 at the age of 45 years. A genetic variant in *PMS2* was found, which has an important role in the mismatch repair system and class switch recombination (23, 24). In addition to the lymphoma, the patient also manifested with a broad spectrum of non-infectious complications, including autoimmune cytopaenias (both AIHA as well as ITP), celiac–like disease and generalized lymphoproliferation, including lymphadenopathy, splenomegaly, and lymphocytic interstitial pneumonia.

The clinical manifestation of patient Nr. 10, who was found to harbor a *PIK3CD* mutation, corresponded with the previously described APDS (activated PI3K-delta syndrome) phenotype due to an activating mutation (2). He has been followed for generalized lymphadenopathy, splenomegaly, and nodular lymphoid hyperplasia (NLH) of the lungs and gastrointestinal tract since his childhood. This patient developed Hodgkin lymphoma at the age of 11 years.

TABLE 4 | B cell subpopulations in CVID patients with lymphoma post-chemotherapy (absolute counts in E9/L; reference values for general population in brackets; (↓), decreased count; (↑), increased count; N/A, value not available).

Patient Nr.	CD21low (0.01–0.02)	Naïve (0.06–0.47)	Transitional (0.0–0.03)	MZ-like (0.01–0.08)	Class-switched (0.02–0.09)
1	N/A	N/A	N/A	N/A	N/A
2	0.0252 (↑)	0.069	0.004	0.009 (↓)	0.002 (↓)
3	N/A	N/A	N/A	N/A	N/A
4	0.001 (↓)	0.005 (↓)	0.002	0 (↓)	0 (↓)
5	0.216 (↑)	0.614 (↑)	0.137 (↑)	0.022	0.001 (↓)
6	0 (↓)	0 (↓)	0	0 (↓)	0 (↓)
7	0.005 (↓)	0.2	0.045 (↑)	0.003 (↓)	0 (↓)
8	0.004 (↓)	0.0076 (↓)	0	0.0013 (↓)	0 (↓)
9	0.0002 (↓)	0.002 (↓)	0	0 (↓)	0 (↓)
10	0.011	0.069 (↓)	0.059 (↑)	0.008 (↓)	0.009 (↓)

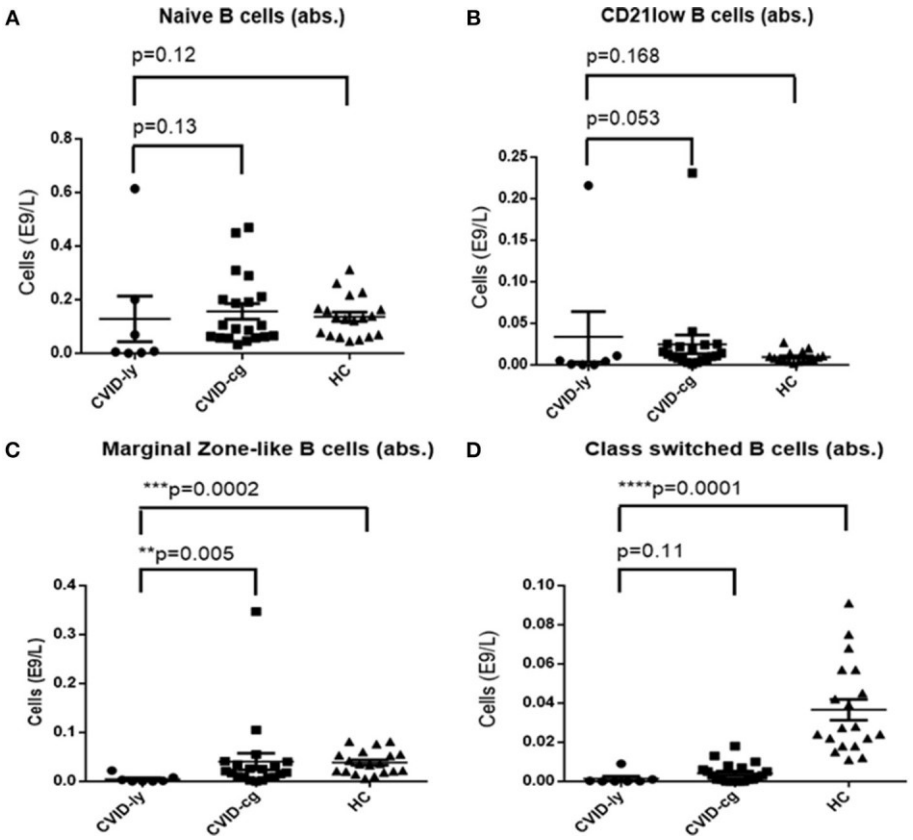

FIGURE 2 | Absolute counts of (A) Naïve B cells, (B) CD21low B cells, (C) Marginal Zone-like B cells, and (D) Class-switched B cells in CVID patients with lymphoma upon chemotherapy (CVID-ly) compared to the compared to the control group of CVID patients without lymphoma (CVID-cg) and healthy controls (HC); median and 95% are shown.

Nodular lymphoid hyperplasia and lymphocytic interstitial pneumonia were also noted in patient Nr. 1, who was followed and treated for splenomegaly and autoimmune thyroiditis before the diagnosis of T cell lymphoma, which developed at the age of 57. A gene variant in *TNFRSF13B (TACI)*, known to increase susceptibility to CVID, was found. *TACI* variants are not regarded as disease-causing but rather as modifying (Group 2) (8).

Furthermore, several heterozygous variants were identified in genes associated with known primary immunodeficiencies that are inherited in an autosomal recessive manner, such as *LYST, LRBA, RAG1, EXTL3, and STX11* (Group 4). The clinical phenotype of these patients did not match the respective disease; nevertheless, we report these variants because of their rarity in the healthy population and their potentially damaging character predicted by *in silico* tools. Functional assays, which would

elucidate the impact of these variants on protein function, were not performed, as they exceeded the scope of this study. The tumor DNA was not available for analysis of somatic "second-hit" mutations, which might explain the pathogenesis of some of the malignancies.

Variants in genes previously described in association with cancer susceptibility or as likely to increase the risk of cancer development, such as *BRCA1, RABEP1, EP300, KDM5A,* and others, were found in 6 out of 10 patients. They were divided into variants reported as pathogenic (Group 3) and variants of unknown significance and novel variants predicted as damaging *in-silico* (Group 5).The summary of WES results and a detailed description of the gene variants is presented in **Table 5** and in **Supplementary Table 1**.

DISCUSSION

Immune dysregulation associated with primary immunodeficiencies represents an increased risk of cancer development. We aimed to search for the occurrence of malignant diseases in a nationwide cohort of CVID patients, taking into account relevant epidemiology, immunophenotype, and the genetic background of the patients.

Similarly to published studies, we detected a higher incidence of malignancies among our CVID cohort (25–30). Also in alignment with previous reports, we noted a distinct spectrum of tumors in CVID patients, with Hodgkin and Non-Hodgkin lymphomas and gastric cancers being the most prevalent malignancies (**Table 6** and **Supplementary Table 2**). The overall risk of malignancy was more than 6 times greater in comparison to the general population, while the specific risk of HL was as much as 30 times greater. Curiously, an over 3 times greater risk of malignancy was determined in a subgroup of CVID patients with a history of ITP. Moreover, we noted that the diagnosis of GC (average age 55–59 years vs. 70–74 in general population) and B-NHL (35–39 years vs. 65–69) was established at a much younger age compared to the Czech general population, while HL developed later in life compared to the healthy population (40–44 years vs. 30–34).

Patients with CVID present with a characteristic immunophenotypic profile that is reflected in the diagnostic criteria of CVID. In this context, we specifically searched for potential differences between CVID patients with tumors and CVID patients who did not develop a malignant disease. Malignant hematologic diseases may, in general, reduce lymphocyte counts in up to 60% of patients, and lymphoma in particular may affect an entire spectrum of lymphocyte subpopulations, including CD4+, CD8+, CD19+, and CD56+ cells (31, 32). Nevertheless, in our cohort of CVID patients, we did not observe any significant differences between absolute or relative counts of CD3+, CD4+, CD8+, and CD56+ cells measured at the time of diagnosis of immunodeficiency and those measured at the time of diagnosis of lymphoma. Furthermore, the values of all T cell subpopulations and NK cells were similar to the control group of CVID patients without malignancy. In contrast, chemotherapy regimens had significant impacts on the

TABLE 5 | Summary of whole exome sequencing results performed in CVID patients with lymphoma.

Patient Nr.	Chromo-some	Gene symbol	Transcript variant	Protein variant	Geno-type	SIFT function prediction	SIFT score	Polyphen-2 function prediction	CADD score	ExAC Freq.	GnomAD Freq.	HGMD accession
GROUP 1: VARIANTS IN IUIS-CLASSIFIED GENES WITH LINKS TO CVID												
4	7	*PMS2*	c.1687C>T	p.R563*	Het				34.000	0.002	0.001	CM102799
9	2	*CTLA4*	c.515C>G	p.S172W	Het	Damaging	0.00	Possibly Damaging	28.700			
10	1	*PIK3CD*	c.3061G>A	p.E1021K	Het	Tolerated	0.07	Probably Damaging	31.000		0.000	CM067447
GROUP 2: MODIFIER GENES												
1	17	TACI	c.310T>C	p.C104R	Het	Damaging	0.00	Probably Damaging	25.900	0.321	0.346	CM052924
GROUP 3: CANCER SUSCEPTIBILITY GENES												
3	17	*BRCA1*	c.547+14delG		Het				0.424	0.012	0.009	CD176513
6	17	*BRCA1*	c.5263_5264insC	p.Q652fs*74	Het				35.000	0.016	0.016	CI941841

TABLE 6 | Summary of tumor prevalence and SIR (Standardized Incidence Ratio, median and 95% Confidence Intervals (CIs) shown) in a cohort of 295 CVID patients.

Tumor	Prevalence	SIR (95% CI)
All tumors	25/295 (8.5%)	6.3 (4.08–9.31)
B cell lymphoma (all types)	9/295 (3.0%)	10.1 (4.61–19.12)
B cell non-Hodgkin lymphoma	4/295 (1.4%)	5.50 (1.50–14.09)
Hodgkin lymphoma	5/295 (1.7%)	30.0 (9.73–69.93)
Gastric cancer	6/295 (2.0%)	5.70 (2.08–12.32)
Other types of cancer	10/295 (3.4%)	5.0 (2.40–9.16)

CD4+ and CD8+ cell counts. A similar observation has already been published in patients with lymphomas without underlying primary immunodeficiencies who underwent chemotherapy with an R-CHOP protocol. In the study, a reduction of CD4+ absolute counts to values $<0.343 \times 10^9$/l was declared an independent negative prognostic factor with a significant impact on 5-year progression-free survival and overall survival (33). Indeed, in our CVID cohort with lymphoma, the median level of post-chemotherapy absolute numbers of CD4+ was very low, 0.343×10^9/l, 95% CI 0.14–0.36 (R-CHOP being the regiment used in 5 out of 11 patient), which implies that a CVID population treated with chemotherapy should be prognostically regarded as a higher risk group. Quite unexpectedly, NK cell counts were not affected by the chemotherapy. However, the B cell compartment was profoundly depleted in all CVID patients who underwent chemotherapy. The total B cell count normalized in only 3/10 patients. However, even in those, selective reductions of mature forms (including class-switched, marginal zone-like B cells and plasmablasts) persisted. This observation was in striking contrast to patients with autoimmune diseases receiving anti-CD20 therapy (rituximab), in whom the reconstitution is achieved within 5–9 months in up to 90% of patients (34).

B cell deficiency seems to be the hallmark in CVID patients with lymphoma, as they presented with reduced B cell count even at the time of diagnosis of lymphoma, which decreased and remained persistently lower after chemotherapy. Despite this, neither B cell nor T cell detailed immunophenotyping provided a strong enough predictive tool for assessment of the cancerogenic predisposition of CVID patients. Therefore, we set out to search for possible genetic causes of malignancies in CVID using massive parallel sequencing. Out of 10 patients who were available for testing, we identified gene variants previously classified as associated with CVID in 4 patients, namely, *CTLR4, PIK3CD*, *PMS2,* and *TNFRSF13B*. It is noteworthy that mutations in *CTLA4* and *PIK3CD*, which account for the majority of currently known molecular causes of CVID (8), were both found among our small cohort of CVID patients.

CTLA4 heterozygous mutation was first described as a cause of CVID-like syndrome that displayed a significant overlap with CVID phenotype, including hypogammaglobulinemia, low B cell counts and immune dysregulation with variable organ involvement (35). The clinical and laboratory spectra of *CTLA4* haploinsufficiency were described in detail in a recently published cohort of 133 patients. In this cohort, 8 mutation carriers developed lymphoma, and 3 had gastric cancer. Thus, our finding of a single*CTLA4* mutation among our small cohort corresponds well with this report. Furthermore, this particular patient also presented with IBD-like gastrointestinal disease that was retrospectively reclassified as a CTLA4-related gastrointestinal presentation. Interestingly, the spectrum of tumors found in the above mentioned CTLA4 study was limited to lymphomas and gastric carcinomas, which also correlates with our findings.

PIK3CD is a well-established genetic cause of APDS, activated PI3K-delta syndrome. Similarly toCLTA4, the clinical presentation of APDS overlaps significantly with the CVID phenotype, and a number of patients originally diagnosed with CVID were found to harbor mutations in *PIK3CD*. A large study including 53 patients with APDS reported lymphoma occurrence in 13% of patients (36). Furthermore, somatic mutations of *PIK3* were found in several types of HL and NHL, thus suggesting the role of PI3K signaling in tumorigenesis.

Finally, the *PMS2* protein is involved in complex mechanisms of DNA repair. As such, mutations in *PMS2* are directly associated with an increased risk of cancer (37). At the same time, mutations in *PMS2* were also implicated in class-switch recombination defects and impaired immunoglobulin production (36). Therefore, our finding of *PMS2* mutation among CVID patients with lymphoma corresponds well with these reports.

Overall, we suggest that each of these CVID-associated genes may also convey a predisposition to tumor development.

The role of the *TNFSF13B* molecule, also known as *TACI***,** in CVID has long been discussed. *TACI* variants have been found to be associated with autoimmune complications in CVID (38). Moreover, given the involvement of *TACI* in B cell activation, its mutations might contribute to immune dysregulation and lymphoma development (39). However, *TACI* variants are regarded as modifier genes rather than a monogenic cause of CVID.

Apart of the above mentioned genetic findings, we identified several variants in genes involved in lymphogenesis and immune system regulation. Although sufficient data to postulate their role in immune deficiency or tumorigenesis are lacking, we report them along with our results for the sake of completeness, reflecting the previously described roles of heterozygous mutations in PID and possible epistatic roles of various genes in immune dysregulation (40–42).

Finally, we also detected several variants in genes involved in tumor surveillance in our cohort, such as BRCA1 and others listed in **Supplementary Table 1**. These variants were previously reported in patients with a broad spectrum of solid tumors (including breast, ovarian, colorectal cancer, and others) and may therefore represent another contributory mechanism of malignant susceptibility.

CONCLUSION

Malignancies belong to the most severe non-infectious complications of common variable immunodeficiency disorder. The prevalence of malignancy in our CVID cohort was found to be more than 6 times greater than in the general population. The spectrum of cancers was characteristically narrow, involving mostly gastric cancers and lymphomas. Moreover, ITP was elucidated as a novel risk factor for malignancy in CVID patients. Post-treatment T and B cell lymphopaenias, associated with poorer prognosis, were found in a majority of CVID patients who received chemotherapy. Surprisingly, NK cells remained unaffected. WES analysis illustrated a wide heterogeneity of potential genetic background of the oncogenic predisposition among CVID patients and identified several causative or contributing gene variants, pointing toward immune system dysregulation. In the future, modern genetic analytic approaches applied on larger cohorts of CVID patients, along with the use of oncogenomic tools, will undoubtedly enable the identification of other CVID-associated genes with increased risk of cancer and elucidate their roles in tumorigenesis.

AUTHOR CONTRIBUTIONS

PK and TM conceived and designed the study, collected data, and drafted manuscript. JL, IM, DJ, JP, JV, AZ, MB, ZP, and AK collected and provided primary patient data. EF and MS performed analysis and interpretation of data from genetic testing (Whole exome sequencing). VK performed analysis and interpretation of immunophenotyping data. JH provided statistical analysis of the obtained data and its interpretation. TK and AS provided critical revisions of the manuscript and final approval of the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2018.03135/full#supplementary-material

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Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: A large patient cohort study

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Background: Activated phosphoinositide 3-kinase δ syndrome (APDS) is a recently described combined immunodeficiency resulting from gain-of-function mutations in *PIK3CD*, the gene encoding the catalytic subunit of phosphoinositide 3-kinase δ (PI3Kδ).

Objective: We sought to review the clinical, immunologic, histopathologic, and radiologic features of APDS in a large genetically defined international cohort.

Methods: We applied a clinical questionnaire and performed review of medical notes, radiology, histopathology, and laboratory investigations of 53 patients with APDS.

Results: Recurrent sinopulmonary infections (98%) and nonneoplastic lymphoproliferation (75%) were common, often from childhood. Other significant complications included herpesvirus infections (49%), autoinflammatory disease (34%),

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and lymphoma (13%). Unexpectedly, neurodevelopmental delay occurred in 19% of the cohort, suggesting a role for PI3Kδ in the central nervous system; consistent with this, PI3Kδ is broadly expressed in the developing murine central nervous system. Thoracic imaging revealed high rates of mosaic attenuation (90%) and bronchiectasis (60%). Increased IgM levels (78%), IgG deficiency (43%), and CD4 lymphopenia (84%) were significant immunologic features. No immunologic marker reliably predicted clinical severity, which ranged from asymptomatic to death in early childhood. The majority of patients received immunoglobulin replacement and antibiotic prophylaxis, and 5 patients underwent hematopoietic stem cell transplantation. Five patients died from complications of APDS. Conclusion: APDS is a combined immunodeficiency with multiple clinical manifestations, many with incomplete penetrance and others with variable expressivity. The severity of complications in some patients supports consideration of hematopoietic stem cell transplantation for severe childhood disease. Clinical trials of selective PI3Kδ inhibitors offer new prospects for APDS treatment. (J Allergy Clin Immunol 2016;nnn:nnn-nnn.)

Key words: Activated phosphoinositide 3-kinase δ *syndrome, p110*δ*-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency, phosphoinositide 3-kinase* δ*,* PIK3CD *gene, bronchiectasis, immunodeficiency, hematopoietic stem cell transplantation, phosphoinositide 3-kinase inhibitor*

Activated phosphoinositide 3-kinase δ syndrome (APDS) is an autosomal dominant primary immunodeficiency caused by gain-of-function (GOF) mutations in *PIK3CD*,[1,2] which encodes the p110δ catalytic subunit of phosphoinositide 3-kinase δ (PI3Kδ). PI3Kδ, a class 1 PI3K isoform generating phosphatidylinositol 3,4,5-trisphosphate, is a heterodimer comprising p110δ and a p85 family regulatory subunit. PI3Kδ is expressed predominantly in leukocytes and plays an important role in their proliferation, survival, and activation.[3-5]

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Abbreviations used
APDS: Activated phosphoinositide-3 kinase *d* syndrome
BALF: Bronchoalveolar lavage fluid
CMV: Cytomegalovirus
CNS: Central nervous system
CT: Computed tomography
GOF: Gain of function
HSCT: Hematopoietic stem cell transplantation
HSV: Herpes simplex virus
OR: Odds ratio
PI3K: Phosphoinositide 3-kinase
PPV: Pneumococcal polysaccharide vaccine

Recently, we described 17 patients with a combined immunodeficiency disorder caused by the heterozygous *PIK3CD* GOF mutation E1021K.[1] Patients' lymphocytes displayed increased basal and poststimulation phosphatidylinositol 3,4,5-trisphosphate and enhanced downstream Akt–mammalian/mechanistic target of rapamycin signaling. This disorder was named APDS.[1] Lucas et al[2] independently reported 14 patients with a similar disease caused by E1021K and 2 other activating mutations in *PIK3CD*, designating it p110*d*-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI).[2] To date, 4 heterozygous GOF *PIK3CD* mutations (E1021K, N334K, E525K, and C416R) have been described, with E1021K the most common.[1,2,6-8] Patients in both cohorts experienced recurrent respiratory tract infections, bronchiectasis, herpesvirus infections, nonneoplastic lymphoproliferation, and lymphoma. However, possibly because of different case-finding strategies, we reported bronchiectasis in 75% of our cohort and herpesvirus infections in 24%, whereas Lucas et al[2] described bronchiectasis in 33%, but all patients had herpesvirus viremia. Recent reports have also underscored that patients with APDS have a high incidence of lymphoma[7,8] and possible autoimmune manifestations.[2,9]

In this study we describe the clinical, radiologic, histopathologic, and immunologic features of APDS in a genetically confirmed cohort of 53 patients, the largest to date. We demonstrated a wide spectrum of clinical findings and complications and unexpectedly noted an increased frequency of neurodevelopmental manifestations. These findings will aid clinical decision making in the diagnosis and treatment of APDS and facilitate patient counseling.

METHODS

Informed consent was obtained from patients, parents, or both. The study conformed to the Declaration of Helsinki and all local ethical requirements.

Mutations in *PIK3CD* were identified by means of Sanger sequencing.[1] Only patients heterozygous for an APDS-associated GOF *PIK3CD* mutation were included. Twenty-five patients from this cohort have been included in previous reports,[1,7] and 28 are reported for the first time.

Information on demographics, presentation, complications, laboratory parameters, management, and outcomes was compiled retrospectively by using patient/parent interview and medical note review. Pneumonia and bronchiectasis required radiologic confirmation. Chest computed tomographic (CT) scans from 31 patients were independently reviewed by 2 thoracic radiologists (J.B. and N.S.) for air-space opacity, atelectasis, nodules, bronchiectasis, mosaic attenuation, and lymphadenopathy.[10,11] Available histopathology specimens (29 specimens from 11 patients) were reviewed by 2 hematopathologists (C.M.B. and J.R.G.). Patients' most recent immunology results are described; postrituximab B-cell levels were excluded. All laboratory results were analyzed with reference to age-related normal ranges.[12-15] A poor pneumococcal polysaccharide vaccine (PPV) response was defined as a less than 4-fold increase in antipneumococcal IgG titer at 4 to 6 weeks after PPV vaccination.

Significant associations in clinical complications were determined by odds ratios (ORs) with 95% CIs and Fisher exact tests by using GraphPad Prism software (version 6; GraphPad Software, La Jolla, Calif). *P* values of less than .05 were considered significant.

TABLE I. Clinical manifestations of APDS

	Frequency, n/total studied (%)
Infectious complication	
Recurrent respiratory tract infections	51/53 (98)
Pneumonia†	39/46 (85)*
Bronchiectasis‡	32/53 (60)
Chronic rhinosinusitis	24/53 (45)
Recurrent otitis media	26/53 (49)
(with permanent hearing loss)	4/53 (8)
Severe or persistent herpesvirus infection	26/53 (49)
EBV	14/53 (26)
CMV	8/53 (15)
HSV and VZV	11/53 (21)
Tonsillitis	15/53 (28)
(with tonsillectomy)	7/53 (13)
Ocular infections	10/53 (19)
Noninfectious complication	
Lymphadenopathy§	34/53 (64)
Splenomegaly	31/53 (58)
Hepatomegaly	24/53 (45)
Autoimmune disease	22/53 (42)
Nodular mucosal lymphoid hyperplasia	17/53 (32)
Enteropathyk	13/53 (25)
Developmental delay	10/53 (19)
Lymphoma	7/53 (13)

Total studied 5 53 unless otherwise indicated.
VZV, Varicella zoster virus.
*N 5 46 because 7 patients had no chest radiology available.
†Pneumonia was defined as at least 1 clinically and radiologically diagnosed pneumonia episode.
‡Bronchiectasis diagnosed on thoracic CT imaging.
§Lymphadenopathy persistent for at least 3 months.
kNine of 13 patients with enteropathy had gastrointestinal nodular mucosal lymphoid hyperplasia confirmed on endoscopy.

RESULTS

Patients' characteristics

Fifty-three patients with APDS (34 male patients) from 30 unrelated families were included; 5 patients (4 male) were deceased. Living patients had a mean age of 17.2 years (age range, <1-65 years). Forty-two patients were of European descent, 4 were Afro-Caribbean, 3 were Middle Eastern, 2 were Indian, 1 was Chinese, and 1 was Japanese. Fifty patients were heterozygous for E1021K, and 3 related subjects were heterozygous for E525K.

Presentation

Recurrent respiratory tract infections occurred in 96% of patients, with onset from less than 1 to 7 years of age. Lymphadenopathy, hepatosplenomegaly, or both were common

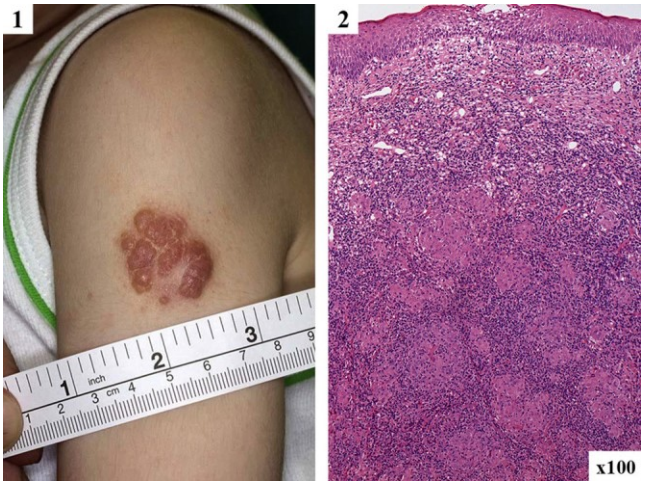

FIG 1. BCG-induced granulomatous inflammation in patients with APDS. *1*, Granulomatous skin lesion in a 4-year-old at the site of BCG vaccination administered at 4 months of age. *2*, Skin biopsy specimen showing granulomatous inflammation.

at presentation (42%). Five patients were identified in adulthood after their child received a diagnosis of APDS; 2 had bronchiectasis and recurrent respiratory tract infections, 1 experienced recurrent respiratory tract infections in childhood and a persistent granulomatous local skin reaction to BCG vaccination, 1 was under investigation for chronic cervical lymphadenopathy, and 1 had no reported health issues. The 4 symptomatic adults had abnormal immunoglobulin profiles, including increased IgM and reduced IgG_2 levels, although none had a low total IgG level.

Infective complications

Pneumonia (85%), bronchiectasis (60%), and upper respiratory tract infections were common, often with childhood onset (Table I). Only 2 patients did not report recurrent respiratory tract infections. The most common bacterial pathogens were *Streptococcus pneumoniae* and *Haemophilus influenzae*, with *Staphylococcus aureus*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Klebsiella* species also observed. The mean age at diagnosis of bronchiectasis was 8.6 years (range, 1.3-36 years). Four patients had permanent hearing loss from recurrent otitis media. Non–respiratory tract bacterial infections included ocular infections (21%: conjunctivitis [n 5 8], dacryocystitis [n 5 3], and orbital cellulitis [n 5 2]) and abscesses (17%: *S aureus* skin abscesses [n 5 4], salivary gland abscesses [n 5 3], dental abscesses [n 5 3], and *S pneumoniae* lymph node abscess [n 5 1]). No invasive bacterial infections were reported. Two unrelated patients had persistent granulomatous skin lesions at BCG vaccination injection sites (Fig 1); material from 1 lesion was culture positive for BCG. No other mycobacterial infections were reported.

Persistent, severe, or recurrent herpesvirus infections occurred in 49% of patients. EBV viremia was detected in 26%, with 6 (11%) patients having disseminated infection, including 1 case of EBV encephalitis. EBV was detected in lymph node (n 5 3), tonsillar (n 5 1), palatal (n 5 1), and gastrointestinal (n 5 1) biopsy specimens, as well as cerebrospinal fluid (n 5 1) and bronchoalveolar lavage fluid (BALF; n 5 1). Two patients had EBV-positive lymphoma. Eight patients had cytomegalovirus (CMV) viremia, 4 with systemic CMV infection successfully treated with ganciclovir. Four cases of EBV/CMV coinfection occurred. One patient with diffuse lymphadenopathy and hepatosplenomegaly had EBV, CMV, and human herpesvirus 6 identified by using PCR on lymph node biopsy. Two patients were hospitalized with severe primary varicella zoster virus infection, and 2 had recurrent shingles. A nongenotyped sibling reportedly died of varicella zoster virus pneumonitis at age 11 years. Recurrent herpes simplex virus (HSV) infections included oral ulceration (n 5 4), skin infections (n 5 2), and herpetic keratitis (n 5 1). HSV was identified in BALF of 2 symptomatic patients, 1 with severe pneumonitis. Adenovirus infections were reported in 9 (17%) patients, with positive isolates from blood, BALF, and stool. Warts (n 5 4) and *Molluscum contangiosum* (n 5 4) were extensive in those affected.

Cryptosporidium parvum was isolated from a patient with bloody diarrhea at age 6 to 18 months in whom cirrhosis was identified at age 8 years; the liver biopsy specimen was negative for *Cryptosporidium* species. A second patient had *C parvum*-positive diarrhea immediately after hematopoietic stem cell transplantation (HSCT). The only other parasitic infection identified was toxoplasmosis in a 9-month-old child. Oral mucocutaneous candidiasis requiring treatment was reported in 7 (13%) patients, including candida tracheitis (n 5 1) and esophageal candidiasis (n 5 1). No cases of *Aspergillus* species infection were identified.

Noninfective immune complications

Nonneoplastic lymphoproliferation. Chronic lymphadenopathy, splenomegaly, and/or hepatomegaly were observed in 75% of patients (Table I). Lymphadenopathy typically began in childhood, was persistent or recurrent, and was often localized to sites of infection. There were 14 cases of cervical lymphadenopathy; 8 of 10 patients with persistent intrathoracic lymphadenopathy had bronchiectasis and recurrent consolidation. Seven patients had diffuse lymphadenopathy, and EBV, CMV, or dual viremia was diagnosed in all 6 of these patients in whom viral PCR was performed. Lymphadenopathy was significantly associated with mucosal lymphoid hyperplasia (OR, 16; 95% CI, 1.9-133.8; P 5 .002), splenomegaly (OR, 9.1; 95% CI, 2.5-33.2; P 5 .0005), and herpesvirus infection (OR, 6.9; 95% CI, 1.9-25.2; P 5 .004).

Histologically (Fig 2), lymph nodes showed atypical follicular hyperplasia with absent or attenuated follicular mantle zones. Germinal centers were frequently disrupted and partially effaced by numerous T cells, many of which were programmed cell death protein 1 (PD1)[1], CD57[1], or both, which is consistent with follicular T_H cells. Parasinusoidal aggregates of monocytoid B cells were a recurrent feature. IgG[1] plasma cells were reduced in number. One lymph node showed features analogous to those of posttransplantation lymphoproliferative disorder, which is characterized by a polymorphic infiltrate of B cells, T cells, epithelioid macrophages, and light chain–restricted plasma cells; monocytoid B-cell hyperplasia; and equivocal immunoglobulin gene rearrangement assays. There was no progression to lymphoma on prolonged follow-up. Scattered EBV-positive cells, CMV-positive cells, or both were present in several lymph nodes, but florid infectious mononucleosis-like pathology was not encountered. Mucosal nodular lymphoid

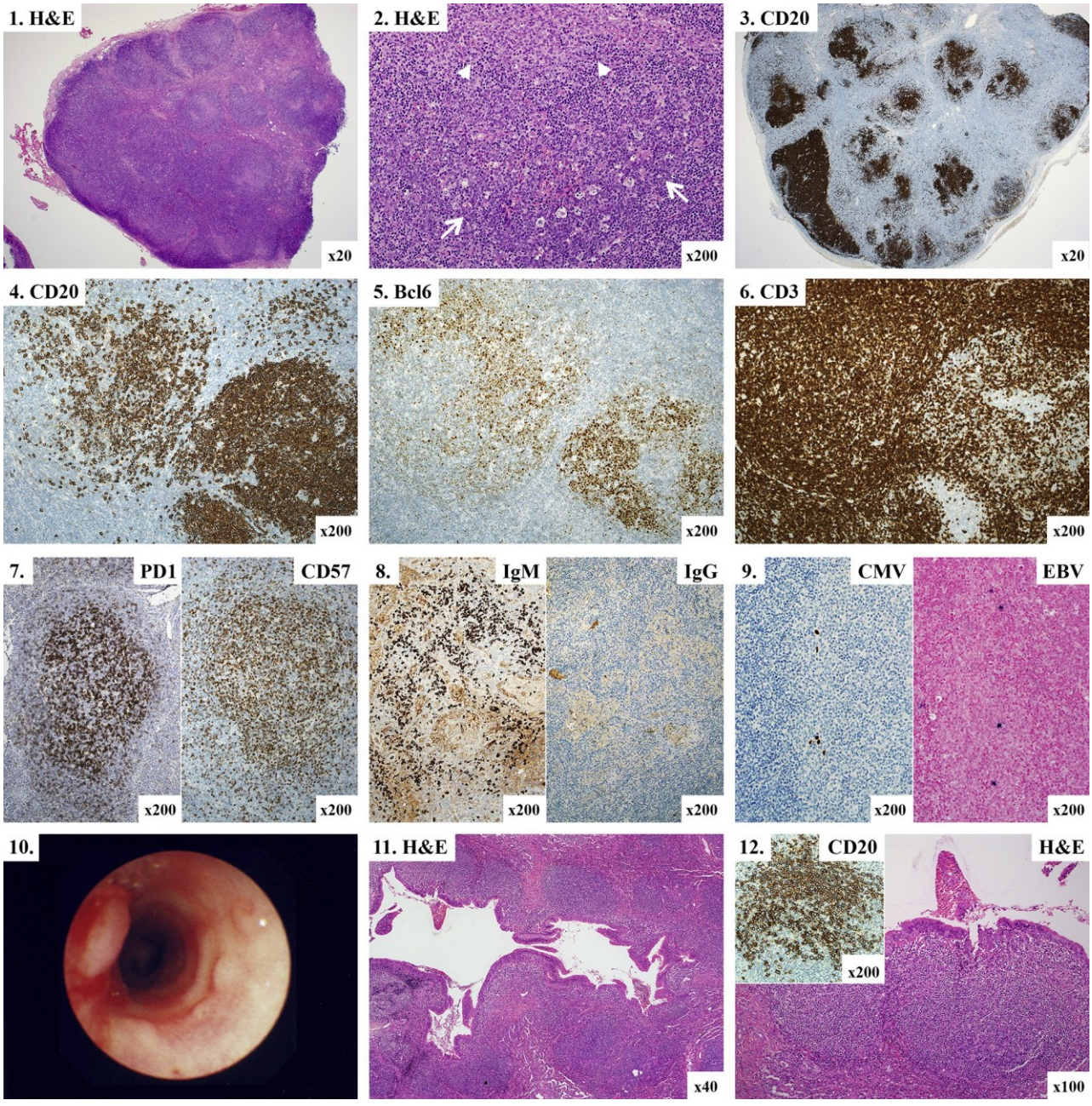

FIG 2. Lymphoid hyperplasia. *1* and *2*, Lymph node showing atypical follicular hyperplasia with disrupted follicles *(arrows)* and monocytoid B cells *(arrowheads)*. *3-5*, Disrupted germinal centers were highlighted by staining for CD20 (Fig 2, *3* and *4*) and Bcl6 (Fig 2, *5*). *6* and *7*, Follicles were infiltrated by T cells (Fig 2, *6*), many of which expressed PD1, CD57, or both (Fig 2, *7*). *8*, IgM-positive plasma cells were present, but IgG-positive plasma cells were reduced or absent. *9*, Several lymph nodes contained CMV or EBV (EBER). *10*, Tracheal endoscopy showing mucosal nodules. *11* and *12*, Lung showing peribronchiolar lymphoid hyperplasia (Fig 2, *11*) with disrupted follicles (Fig 2, *12*). *H&E*, Hematoxylin and eosin.

hyperplasia was visualized as cobblestone-like plaques or polyps in 17 (32%) patients. In the gastrointestinal tract mucosal lymphoid hyperplasia was identified endoscopically anywhere from the epiglottis to the rectum in 14 (26%) subjects and associated with diarrhea, bleeding, and rectal prolapse. Five patients had respiratory mucosal nodular lymphoid hyperplasia identified bronchoscopically (Fig 2). Biopsy specimens from mucosal lymphoid lesions showed follicular hyperplasia, often with features similar to those seen in lymph nodes (Fig 2), and were occasionally PCR positive for herpes viruses (EBV, n 5 1; HSV, n 5 1).

Autoimmune and inflammatory disease. Thirty-four percent of the cohort had clinical features suggestive of autoimmune or inflammatory disease. Cytopenias included Coombs-positive hemolytic anemia (n 5 7) and 2 cases of trilineage cytopenia responsive to steroids or rituximab. Glomerulonephritis affected 3 children, necessitating renal transplantation in 2 cases.

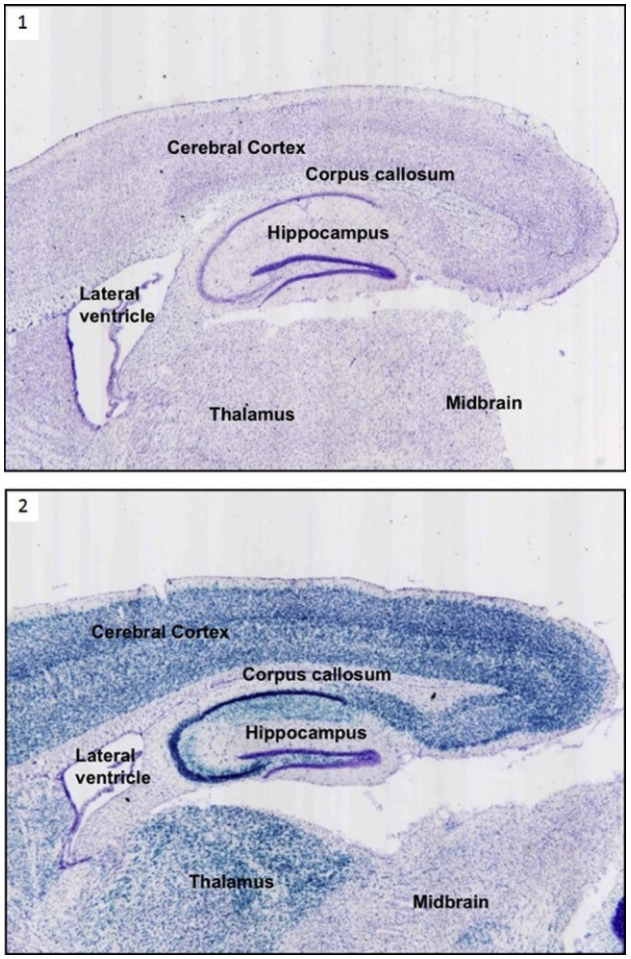

FIG 3. p110*d* expression in the mouse brain. Brain sections of adult wild-type (2lacZ cassette) mice *(1)* and p110 d kinase dead (1lacZ cassette) *b*-gal reporter mice16 *(2)* stained with the neuronal stain cresyl violet *(purple)* and X-gal *(blue)* representing p110*d* expression. Strong expression of p110*d* was observed in areas of the hippocampus, cerebral cortex, and thalamus.

Renal biopsy specimens showed proliferative, membranoproliferative, and focal and segmental changes. Two patients had exocrine pancreatic insufficiency. Autoantibody-positive thyroid disease was diagnosed in 3 patients in adulthood. Two patients had seronegative arthritis, and 1 had recurrent pericarditis.

Three patients had cirrhosis, of whom 1 also had sclerosing cholangitis in the setting of previous *Cryptosporidium* species–related diarrhea. Sclerosing cholangitis additionally affected a second noncirrhotic patient who had no evidence of *Cryptosporidium* species infection.[9] Thirteen (25%) patients had chronic diarrhea, 9 of whom had gastrointestinal nodular mucosal lymphoid hyperplasia confirmed on endoscopy.

Lymphoma and other malignancy. Seven (13%) patients had lymphoma at age 18 months to 27 years. There were 2 cases of diffuse large B-cell lymphoma, 1 EBV positive (see Fig E1 in this article's Online Repository at www.jacionline.org) and 1 EBV negative.[7] Single patients were reported as having nodular sclerosis classical Hodgkin lymphoma,[7] nodal marginal zone lymphoma,[1] and a lymphoplasmacytic lymphoma, the EBV status of which were unknown. An EBV-positive Hodgkin-type lymphoproliferative disorder was diagnosed in a child after renal transplantation. One child had a primary cutaneous anaplastic large cell lymphoma carrying t(6; 7) (p25; q23). This regressed from a 9 3 6–cm mass of tumor nodules to a 5 3 4–cm diameter flat erythematous plaque on 6 weeks of treatment with rapamycin (sirolimus, see Fig E2 in this article's Online Repository at www.jacionline.org). Three patients died of lymphoma-related complications, including both patients with EBV-associated lymphoma. No other malignancies have been identified within our cohort to date.

Neurological and other nonimmune features. Global developmental or isolated speech delay were diagnosed against standard criteria by specialist pediatric services in 10 (19%) patients. Three further patients were treated for anxiety disorders, 1 with a diagnosis of autism, and 3 children were reviewed by psychological services for behavioral issues. Of note, PI3K*d* is strongly expressed in the mature and developing murine central nervous system (CNS; Fig 3).[16]

Individual patients were born with macrocrania, unilateral hypoplastic kidney, and unilateral microphthalmia.

Thoracic radiology

Air-space opacity (Fig 4.1) was identified in 13 of 31 CT scans reviewed, and tree-in-bud opacities, bronchial wall thickening, or both were identified in 20 of 31 CT scans. Mosaic attenuation was present in 28 of 31 patients and classified as mild in 17, moderate in 7, and severe in 4 (Fig 4.2). Bronchiectasis was present in 21 of 31 scans, with an average of 3 lobes affected, and associated with atelectasis or lobar collapse in 12 patients. Sixteen patients had mediastinal lymphadenopathy, which was in a regional draining station to concurrent lobar consolidation in 4 instances. Follow-up imaging was available in 8 patients at a mean interval of 2.2 years. Four of the patients with air-space opacity, and regional lymphadenopathy showed resolution of presumed pneumonic changes but persistent volume loss, atelectasis, and development of bronchiectasis (Fig 4.1).

Immunology laboratory results

Lymphocyte immunophenotyping findings are summarized in Table II. Typical findings were reduced CD4 T-cell counts, increased CD8 T-cell counts of an effector/effector memory phenotype, and an expansion of transitional B cells. A history of herpesvirus infection was not associated with a deficiency in natural killer cells (P 5 .48), T_H cells (P 5 .47), or cytotoxic T cells (P 5 .35). Serial B-cell counts (n 5 19) suggest that patients' B-cell levels decrease more quickly over time than in age-matched control subjects (Fig 5).

Immunoglobulin levels (Table III) were variable, with 43% of patients having reduced total IgG levels. Fifty-eight percent of patients with normal IgG levels had IgG_2 subclass deficiency, and 89% who underwent testing exhibited a poor response to PPV. Reduced IgA (50%) and increased IgM (79%) levels were common. Two patients initially had marginally reduced IgM levels (age, 2 and 6 years), which over time became high (27 g/L) or normal (0.63 g/L), respectively. In 4 cases high IgM levels normalized after commencement of immunoglobulin replacement. One patient had a low IgG level after previous normal readings. Four patients with normal IgG and IgA levels responded poorly to PPV and had a previous diagnosis of specific antibody deficiency.[17]

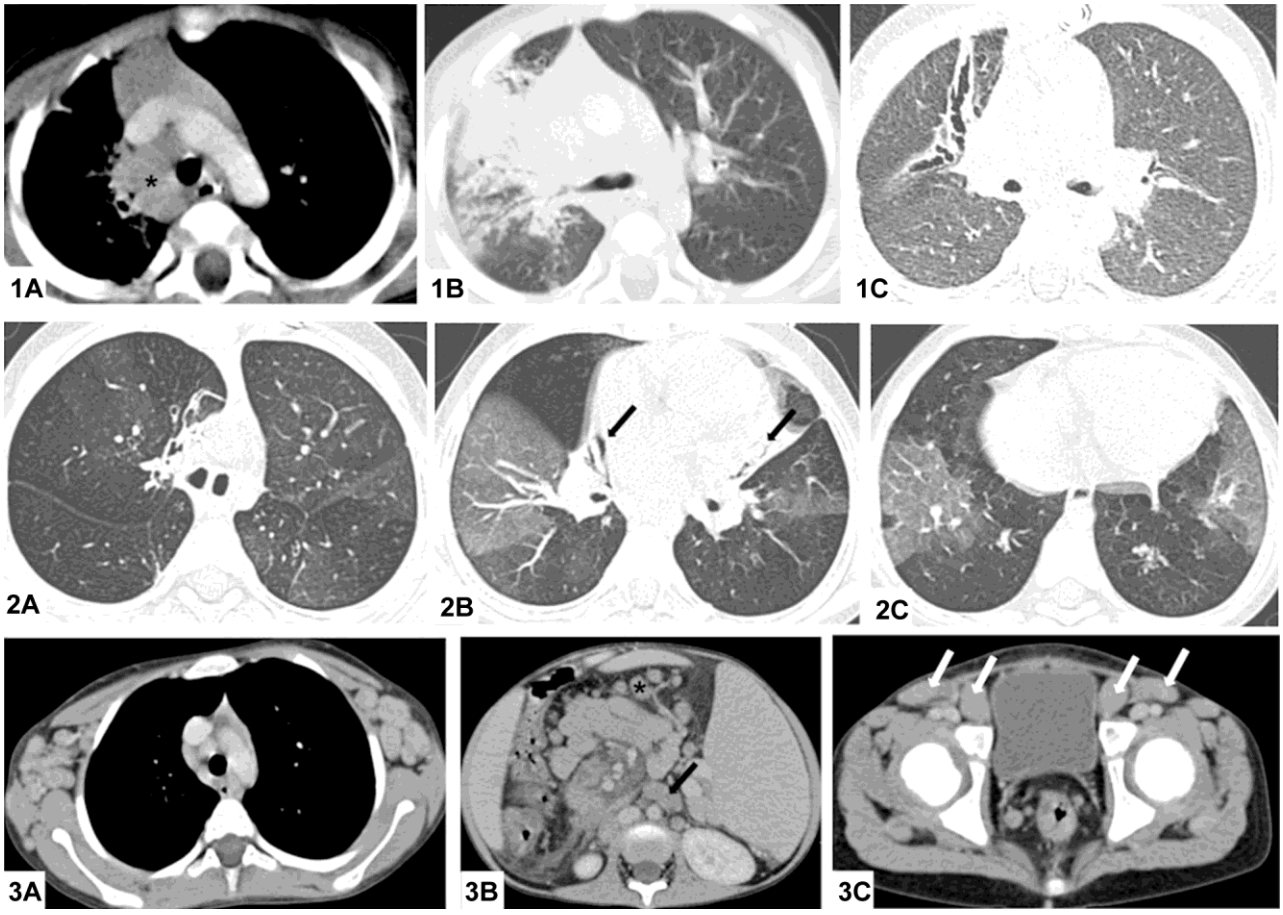

FIG 4. Radiology of patients with APDS. *1*, CT scan of the chest (2-year-old boy), demonstrating right paratracheal lymphadenopathy *(A)*, right upper lobe consolidation, and centrilobular nodules *(B)*, progressing 2 years later to severe right upper lobe bronchiectasis *(C)*. *2*, CT scan of the chest *(A-C)* in a 7-year-old boy reveals widespread mosaic attenuation (indicative of small airways disease), mild right upper lobe bronchiectasis *(A)* and atelectasis (*B*, *black arrows*). *3*, CT scan of the chest *(A)*, abdomen *(B)*, and pelvis *(C)* of an 8-year-old boy showing axillary, paratracheal, para-aortic *(black arrow)*, mesenteric and inguinal lymphadenopathy *(white arrows)*, and splenomegaly.

Treatment

Anti-infection prophylaxis. Sixty-two percent of the cohort currently receive and an additional 9% previously received antibiotic prophylaxis. Six (11%) patients are taking antiviral and 3 (6%) are taking antifungal prophylaxis.

Immunoglobulin replacement. Long-term immunoglobulin replacement was administered to 87% of the cohort, with reported benefit (reduction of infection) in the majority. In 3 patients aged 14 to 23 years, immunoglobulin replacement was switched to antibiotic prophylaxis (patient preference). The 7 patients who did not receive immunoglobulin replacement therapy included the 5 patients identified by genotyping relatives of patients with APDS.

HSCT. Five (9%) patients aged 5 to 14 years have undergone HSCT with medium- or reduced-intensity conditioning with a median follow-up after HSCT of 4.2 years (range, 1-14 years). Three transplantations (unrelated donors, one with 1A and 1B allelic mismatch) were successful, with minimal graft-versus-host disease, restoration of normal growth, and resolution of infection and nonneoplastic lymphoproliferation; chimerism in these patients ranged from 35% to 100%. A fourth procedure was complicated by poor engraftment (25% donor chimerism), resulting in long-term immunoglobulin therapy after transplantation. A fifth patient, who underwent splenectomy before transplantation, died of sepsis 2 years after HSCT.

Immunosuppression. Thirty percent of the cohort underwent at least 1 course of immunosuppressive therapy for lymphoproliferative, autoimmune, or inflammatory disease. Rituximab was of benefit in the management of autoimmune hemolytic anemia (n 5 8) and nonneoplastic lymphoproliferation (n 5 5) although often complicated by sustained B-cell lymphopenia. Six patients were treated with rapamycin; 5 experienced benefit, with a decrease in nonneoplastic or neoplastic lymphoproliferation, but therapy was stopped in the fifth patient because of side effects.

Fatal outcomes

Five patients with APDS died, 3 (aged 1, 19, and 27 years) from lymphoma, 1 (aged 14 years) from sepsis after splenectomy and HSCT, and 1 (aged 39 years) from respiratory failure and chronic lung infection. Additionally, infection-related deaths in childhood and early adult life (≤30 years old) were reported for 5 non-genotyped relatives of patients with APDS.

TABLE II. Summary of lymphocyte phenotypic characteristics of APDS

Lymphocyte subpopulation*	Frequency, n/total studied (%)
T cells	
Reduced T_H cell counts ($CD3^1CD4^1$)	43/51 (84)
Reduced recent thymic emigrant T-cell counts ($CD3^1CD4^1CD45RA^1CD31^1$)	14/22 (64)
Normal cytotoxic T-cell counts ($CD3^1CD8^1$)	34/51 (67)
Reduced cytotoxic T-cell counts ($CD3^1CD8^1$)	14/51 (27)
Increased effector-effector memory cytotoxic T-cell counts ($CD3^1CD8^1CCR7^2CD45RA^{1/2}$)	17/18 (94)
Reversed CD4/CD8 ratio	33/51 (65)
B cells	
Reduced B-cell counts ($CD19^1$)	32/48 (67)
Increased transitional B-cell counts ($CD19^1IgM^{11}CD38^{11}$)	24/32 (75)
Reduced nonswitched memory B cells ($CD19^1IgD^1CD27^1$)	15/30 (50)
Reduced class-switched memory B-cell counts ($CD19^1IgD^2CD27^1$)	17/30 (57)
NK cells	
Normal NK cell counts ($CD16^1CD56^1$)	28/43 (65)

NK, Natural killer.
*Results were deemed reduced, normal, or increased with reference to age-related normal ranges.[12-14] Most recent results available were used, and B-cell levels after rituximab were excluded.

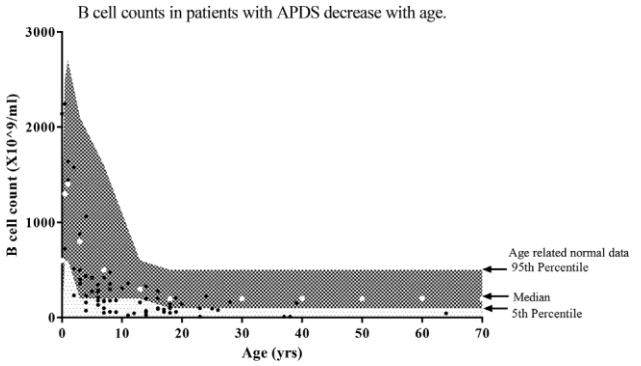

FIG 5. Age-related changes in B-cell counts in patients with APDS. Age-related median B-cell count *(white dots)*, B-cell count 5th to 95th percentile normal range *(checked area)*, and less than 5th percentile normal B-cell count *(spotted area)* were plotted.[12]

TABLE III. Summary of immunoglobulin characteristics of the APDS cohort

	Reduced, n/total (%)	Normal, n/total (%)	Increased, n/total (%)
IgG	21/49 (43)	26/49 (53)	2/49 (4)
IgA	25/50 (50)	24/50 (48)	1/50 (0.5)
IgM	0/50 (0)	12/50 (24)	38/50 (76)
Pneumococcal vaccine response*	25/28 (89)	3/28 (11)	

Immunoglobulin results were deemed reduced, normal, or increased with reference to age-related normal ranges.[15]
*A poor pneumococcal polysaccharide vaccine response was defined as less than 4-fold increase in anti-pneumococcal IgG titer at 4 to 6 weeks after PPV vaccination. Of the 25 patients in whom pneumococcal responses were not available, 15 had reduced IgG levels and received immunoglobulin replacement therapy.

DISCUSSION

We present an overview of the clinical course of APDS in the largest cohort to date with confirmed GOF *PIK3CD* mutations. The phenotype is highly variable (Fig 6), ranging from asymptomatic adults to profound immunodeficiency causing early death or necessitating HSCT in childhood; the clinical features overlap those of other primary immunodeficiencies, such as cytotoxic T lymphocyte–associated antigen 4 (CTLA4) and LPS-responsive beige-like anchor protein (LRBA) deficiency. Interestingly, 3 recent publications[18-20] describe heterozygous mutations in the *PIK3R1* gene (encoding the PI3K regulatory subunit), leading to hyperactivation of PI3K*d* and a clinical syndrome (APDS2 or PASLI-R1) highly reminiscent of that described herein. Conversely, a recessive mutation in *PIK3R1*, resulting in loss of p85*a* expression, was reported in a patient with agammaglobulinemia and absent B-cell lineage.[21] Together with the aberrant lymphocyte function in mice lacking PI3K*d* activity,[22] these findings indicate that balanced signaling in the PI3K*d* pathway is critical for normal immune function.

Recurrent respiratory tract infection is almost universally found in patients with APDS. Bacterial isolates were typical for antibody deficiency, and the incidence of bronchiectasis was similar or higher than in previously described common variable immune deficiency cohorts (see Table E1 in this article's Online Repository at www.jacionline.org).[23-26] Notably, 63% (20/32) of patients with bronchiectasis had normal total IgG levels, suggesting that patients with early-onset bronchiectasis and even minor immunoglobulin abnormalities should be screened for APDS mutations. Increased IgM levels were seen in 82% of the cohort, reminiscent of a class-switch recombination defect.[7,8] Thus we propose that patients presenting with reduced IgG and IgA levels and normal or increased IgM levels,[17] particularly those with normal CD40 ligand expression, should be screened for activating PI3K*d* mutations.

Almost half of our cohort had difficulty in resolving herpesvirus infections, particularly EBV and CMV. There was no association between herpesvirus infections and decreased T_H, cytotoxic T-cell, or natural killer cell counts, suggesting a functional defect underlies this susceptibility. Diffuse lymphadenopathy was associated with systemic herpes infections, with consistent features on lymph node histology. Other opportunistic infections were uncommon, and patients did not experience *Pneumocystis jirovecii* pneumonia. *Cryptosporidium* species was identified in only 2 cases, one of whom had cholangitis and liver disease, which is normally associated with MHC class II or IL-21/IL-21 receptor deficiencies but also described in CD40 ligand and CD40 deficiency.[27] Persistent granulomatous skin lesions after BCG vaccination occurred in 2 patients, but no other mycobacterial infections were reported. Although there was a moderate excess of skin infections and abscesses, there were no cases of invasive staphylococcal or *Aspergillus* species infections to suggest major neutrophil dysfunction.

Although APDS can present as a common variable immune deficiency–like disease, it is also characterized by viral infections; lymphocyte immunophenotyping confirms APDS is a combined immunodeficiency. The typical T-cell profile was of reduced T_H cells and recent thymic emigrants, whereas cytotoxic T cells had a predominantly effector or activated phenotype.

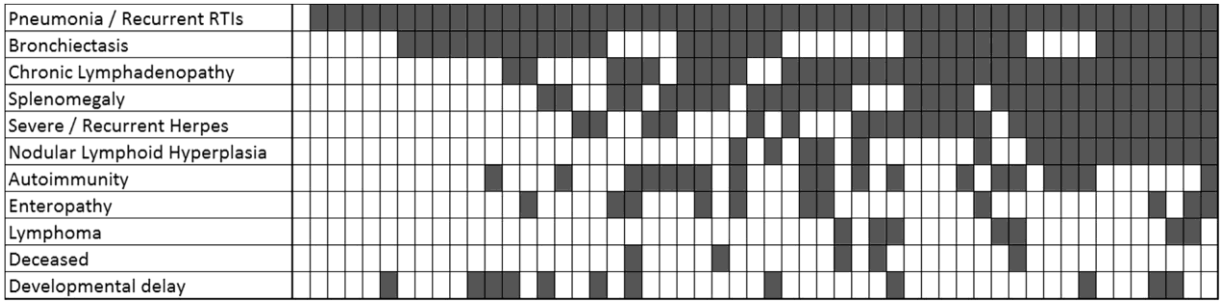

FIG 6. Variation in clinical phenotypes of APDS. Each *column* represents a patient with APDS. Each *row* represents a frequent or serious complication of APDS. *White boxes* and *gray boxes* depict the absence or presence of a complication, respectively.

B-cell numbers were often normal in early life but decreased with time. The reduction in B-cell counts, including class-switched memory B cells and expansion of transitional B cells, suggests defects in B-cell maturation or enhanced mature B-cell death.[22]

The development of focal bronchiectasis observed after consolidative changes strengthens the suspected causal link between infection and airway damage. Consistent with a role for infection in the florid nonneoplastic lymphoproliferation characteristic of patients with APDS, lymphadenopathy was often associated with regional (mediastinal lymphadenopathy in bronchiectatic patients) or systemic infection (herpesviral infections) and tended to improve on infection resolution. Our review of chest CT scans also revealed an unexpectedly high incidence (28/31) of mosaic attenuation, which is indicative of reduced perfusion of poorly ventilated lung regions. This might reflect inflammatory small-airway disease or result from viral respiratory tract infections.

Patients with APDS had a high incidence (34%) and wide range of inflammatory/autoimmune manifestations. Enhanced PI3K*d* activity has been reported in patients with autoimmune diseases, such as systemic lupus erythematosus,[28] and PI3K*d* modulates regulatory T-cell function.[29] Our findings suggest a role for PI3K*d* in the genesis or perpetuation of autoimmunity and potentially for PI3K*d* inhibition in treating such conditions. Activating somatic *PIK3CD* mutations have been associated with lymphoid malignancy.[30] We identified 7 lymphomas in this series of 53 patients with a spectrum of pathologic subtypes but identified no solid malignancies, perhaps reflecting the young age of our cohort or the predominant expression of p110*d* in leukocytes. Although PI3K*d* is described as leukocyte restricted, expression is also found in cells of breast or melanocytic origin,[31] lung fibroblasts,[32] and TNF-*a*–stimulated endothelial and synovial cells.[33] p110*d* has recently been shown to regulate epithelial cell polarity,[34] which is of potential import for respiratory epithelial function. It is tempting to speculate that induction of p110*d* expression by locally produced TNF-*a* during inflammation might impair epithelial barrier functions and aggravate local inflammation. Thus the lung phenotype might be the result of interplay between immune functions of p110*d* and epithelium-intrinsic roles of p110*d*.

Almost one fifth of our cohort experienced neurodevelopmental morbidity, from speech delay to global developmental delay. PI3K*d* is expressed broadly in the developing CNS, as well as in specific adult brain regions (including the hippocampus, cerebral cortex, and thalamus) of reporter mice (Fig 3).[16] PI3K*d* has been implicated in schizophrenia; pharmacologic inhibition reversed prepulse inhibition deficits in a rat model of schizophrenia and blocked amphetamine-induced hyperlocomotion in a mouse model of psychosis-like behavior.[35] Interestingly, loss-off-function phosphatase and tensin homolog *(PTEN)* mutations (with consequent enhanced PI3K-dependent signaling) are associated macrocrania and autism spectrum disorders.[36] One patient with APDS had macrocrania, and in addition to the single patient with a formal diagnosis of autism in our cohort, before submission of this manuscript, we were informed of an additional patient with APDS with autism spectrum disorder (personal communication; Professor P. Martin von Hagen, Erasmus MC, The Netherlands). These findings suggest PI3K*d* might play an important but little-understood role in the CNS, and this aspect of APDS warrants further study.

HSCT has been seemingly curative in 3 patients with APDS described herein and an additional 5 patients described by Imai et al,[37] supporting its use in carefully selected cases; however, longer-term follow-up to determine the degree of donor chimerism needed to achieve cure is required. Lucas et al[2] reported a single patient in whom the mammalian/mechanistic target of rapamycin inhibitor rapamycin improved circulating T-cell profiles. Four patients within our cohort experienced a decrease in nonneoplastic lymphoproliferation while taking rapamycin, and this drug also led to regression of cutaneous T-cell lymphoma. Nevertheless, direct inhibition of activated PI3K*d* might be a more attractive approach in patients with APDS. Selective PI3K*d* inhibitors are currently in clinical trials for a range of cancers and inflammatory disorders, and one compound is already approved for treatment of B-cell malignancies.[38,39] Such disease-specific therapy could address both the infectious and noninfectious complications of APDS, but the reported side effect profile and significant immunoparesis in mice lacking PI3K*d* function[25] emphasize the need for careful dosing to restore normal rather than abolish PI3K*d* activity, particularly given that long-term treatment is contemplated.

In conclusion, APDS is a combined immune deficiency with a variable phenotype complicated by recurrent sinopulmonary bacterial and herpesvirus infections, bronchiectasis, lymphoid hyperplasia, autoimmunity, and, less frequently, neurodevelopmental delay and lymphoma. The rapidly increasing number of patients identified since the initial description of APDS in 2013 suggests this is a clinically significant cause of primary immunodeficiencies, which should be considered in patients presenting with atypical or inherited primary antibody deficiency, bronchiectasis, severe herpesvirus infections, and lymphoma. The severity of complications and significant mortality rate support

the consideration of HSCT in young patients, as well as clinical trials of selective PI3K*d* inhibitors for this condition.

We thank Erwan Dumontet, Marie-Céline Deau, and Rémy Rodriguez for technical support and Dr Hideki Sano for his contribution.

Clinical implications: The variable clinical phenotype with severe complications of bronchiectasis, bacterial and viral infections, and lymphoma suggests that patients who fit this clinical profile should be screened for APDS-causing mutations.

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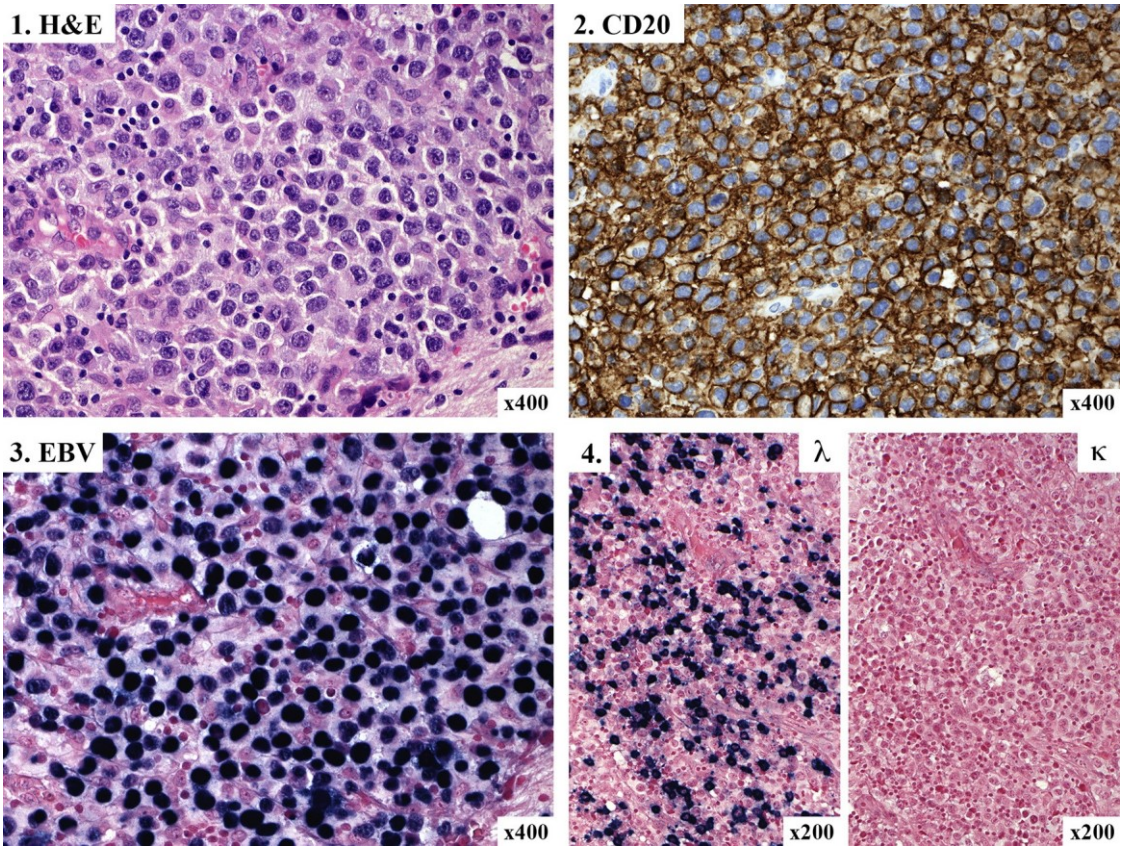

FIG E1. EBV-positive diffuse large B-cell lymphoma in patients with APDS. *1*, A diffuse infiltrate of large atypical lymphoid cells and some atypical plasmacytoid cells was present in the cerebellum. *2*, Immunohistochemical staining showed large B cells expressing CD20, CD79a, Pax5, and interferon regulatory factor 4 but not Bcl6 or CD10. *3*, Most neoplastic cells showed positive *in situ* hybridization for EBV EBER. *4*, Plasmacytoid cells expressed CD138 and showed **/** restricted immunoglobulin light chain *in situ* hybridization. *H&E*, Hematoxylin and eosin.

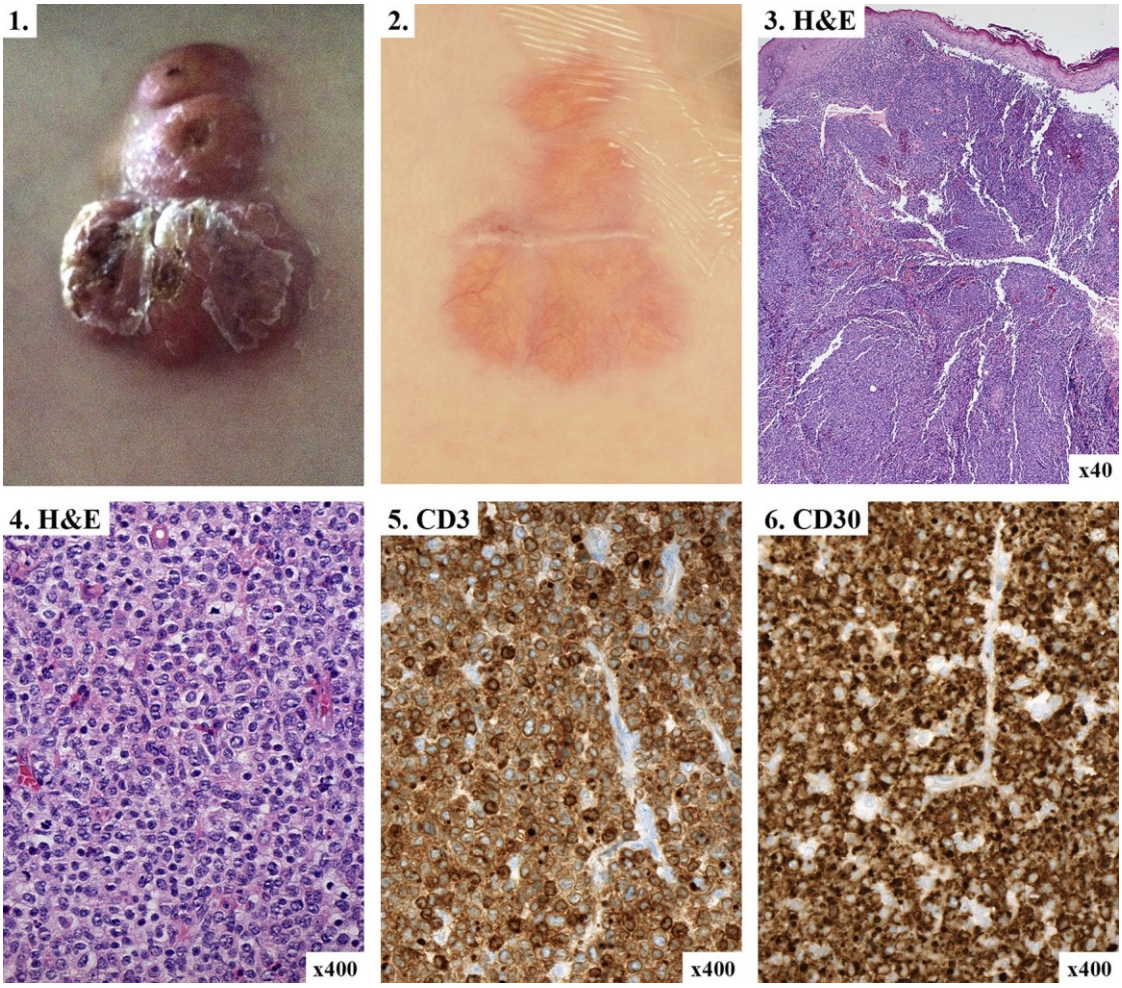

FIG E2. Primary cutaneous anaplastic large cell lymphoma in patients with APDS. *1* and *2*, A multinodular cutaneous tumor on the chest of an 11-year-old boy (Fig E2, *1*), which regressed to a flat plaque (Fig E2, *2*) on 6 weeks of treatment with rapamycin. *3* and *4*, The dermis and subcutis contained a diffuse infiltrate of large atypical lymphoid cells. *5* and *6*, Immunohistochemical staining showed large T cells expressing CD3 (Fig E2, *5*), CD30 (Fig E2, *6*), CD2, interferon regulatory factor 4, T-cell receptor *b*, and perforin but not CD4, CD8, or ALK. *H&E*, Hematoxylin and eosin.

TABLE E1. Comparison of the frequency of complications in patients with APDS and common variable immune deficiency

Clinical feature	Frequency (%) in APDS cohort	Frequency (%) in CVID cohort
Pneumonia	85	32-77[E1-E4]
Bronchiectasis	60	23-64[E1,E3,E5-E7]
Splenomegaly	58	15-30[E1,E3-E6]
Autoimmunity	42	22-29[E1-E3]
Enteropathy	25	9[E1,E4,E5]
Granuloma*	0	8-9[E1,E2,E5]
Meningitis/encephalitis	1.9	3-4[E1,E4]
Lymphoma	11	3-8[E1,E2,E5]
Living patients currently receiving immunoglobulin replacement therapy	77	80[E1]

CVID, Common variable immune deficiency.
*Two patients with cutaneous granulomatous inflammation after BCG vaccination were not included.

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Disease evolution and response to rapamycin in Activated Phosphoinositide 3-Kinase δ syndrome: the european society for immunodeficiencies-Activated Phosphoinositide 3-Kinase δ syndrome registry

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Activated phosphoinositide 3-kinase (PI3K) δ Syndrome (APDS), caused by autosomal dominant mutations in *PIK3CD* (APDS1) or *PIK3R1* (APDS2), is a heterogeneous primary immunodeficiency. While initial cohort-descriptions summarized the spectrum of clinical and immunological manifestations, questions about long-term disease evolution and response to therapy remain. The prospective European Society for Immunodeficiencies (ESID)-APDS registry aims to characterize the disease course, identify outcome predictors, and evaluate treatment responses. So far, 77 patients have been recruited (51 APDS1, 26 APDS2). Analysis of disease evolution in the first 68 patients pinpoints the early occurrence of recurrent respiratory infections followed by chronic lymphoproliferation, gastrointestinal manifestations, and cytopenias. Although most manifestations occur by age 15, adult-onset and asymptomatic courses were documented. Bronchiectasis was observed in 24/40 APDS1 patients who received a CT-scan compared with 4/15 APDS2 patients. By age 20, half of the patients had received at least one immunosuppressant, but 2–3 lines of immunosuppressive therapy were not unusual before age 10. Response to rapamycin was rated by physician visual analog scale as good in 10, moderate in 9, and poor in 7. Lymphoproliferation showed the best response (8 complete, 11 partial, 6 no remission), while bowel inflammation (3 complete, 3 partial, 9 no remission) and cytopenia (3 complete, 2 partial, 9 no remission) responded less well. Hence, non-lymphoproliferative manifestations should be a key target for novel therapies. This report from the ESID-APDS registry provides comprehensive baseline documentation for a growing cohort that will be followed prospectively to establish prognostic factors and identify patients for treatment studies.

Keywords: activated phosphoinositide 3-kinase δ syndrome, PiK3cD, PiK3r1, registry, natural history, rapamycin

iNtrODUctiON

Heterozygous gain-of-phosphoinositide 3-kinase (PI3K) δ-function mutations in *PIK3CD* or *PIK3R1* cause an autosomal-dominant primary immunodeficiency (PID) called activated phosphoinositide 3-kinase δ syndrome (APDS) or PASLI (p110-delta-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency) 1 and 2, respectively (1–4). The main clinical and immunological characteristics of APDS 1 and 2 have been recently described in two major retrospective cohort studies (5, 6). Recurrent respiratory infections and benign lymphoproliferation emerged as key clinical aspects of the disease in both cohorts. Bronchiectasis was noted as a frequent complication with 60% in the APDS1 cohort and less frequently (18%) in the APDS2 cohort study. Additional immune dysregulation including cytopenias, glomerulonephritis, arthritis, and colitis was reported in these studies. An increased risk for lymphoma was also highlighted with 13% among the APDS1 patients and 28% in the APDS2 cohort. Non-immunological characteristics included neurodevelopmental delay (19% of APDS1 and 31% of APDS2) and growth impairment, especially among APDS2 patients (45%). Immunologically, hypogammaglobulinemia with increased IgM levels was frequent. B-cell lymphopenia, worsening with age, and expansion of transitional B cells were the main B-cell alterations. A reduction in the frequency of naïve $CD4^+$ and $CD8^+$ T cells with an increased frequency of effector/effector

memory $CD8^+$ T cells was reported. These first two important retrospective analyses of the disease illustrated clinical and immunological characteristics but did not address the dynamics of the disease evolution over time. Furthermore, although both reports showed that the majority of APDS patients receive supportive therapies in terms of immunoglobulin-replacement treatment (IGRT) or antimicrobial prophylaxes, data regarding immunosuppressive treatments were only reported for a limited number of patients. Here, we use an initial report from the European Society for Immunodeficiencies (ESID)-APDS prospective registry to address some of these questions.

MetHODs

the esiD-APDs registry: Goals and Design

The ESID is a not-for-profit association whose aim is to improve knowledge in the field of PIDs (www.esid.org). The ESID Registry is an international Internet-based database for basic epidemiological (level 1), and more extensive disease-specific (level 3) data on patients with PID. The APDS Registry is the first prospective level 3 project that was initiated to better define the natural history of patients with APDS. The study is carried out in accordance with the recommendations of Section 15 of the Code of Conduct of the General Medical Council of Baden-Württemberg, Germany. The protocol was approved by the Ethics committee of the University of Freiburg (IRB approval No. ESID registry: 493/14; IRB approval No. APDS registry: 458/15). All subjects gave written informed consent in accordance with the Declaration of Helsinki. The goals of the project are to characterize disease evolution over time, to establish prognostic factors and biomarkers, to assess the impact of various treatment strategies, and to identify patients who could be eligible for novel treatments and interventions. Entry into the database requires an initial retrospective documentation, followed by yearly prospective follow-ups. Because of required patient consent, deceased patients cannot be registered. Each patient is evaluated at entry for eligibility by one of the three chief investigators to ensure that only patients with functionally validated APDS-associated mutations are registered. The APDS registry is supported by the pharmaceutical companies Novartis, GlaxoSmithKline, and UCB UK, who financed development and maintenance of the online level 3-documentation-section for APDS as well as project management including ethics submission in all participating countries, data management, and quality controls.

resULts

Disease Manifestations and their evolution Over time

By December 2017, 77 patients had been enrolled in the APDS Registry, 51 with APDS1, and 26 with APDS2. Detailed clinical and immunological information of 68 patients [39 of them not published in the cohort papers (5, 6)] from 59 unrelated families was available for this initial analysis. Forty-five of these 68 patients were diagnosed with APDS1 (43 with the E1021K and 2 with the C416R mutation) and 23 with APDS2 (all with mutations leading to skipping of exon 11). At the time of evaluation, living patients (65) had a mean age of 17.9 years (range 3–47 years). The main clinical features reported in APDS1 and APDS2 are summarized in **Figures 1A,B**. As in the previously reported cohorts, recurrent respiratory infections were by far the most frequent manifestation, occurring in 96% of the patients. Upper respiratory tract infections, otitis media, and sinusitis were the leading diagnoses, and, importantly, 59% of the patients had experienced at least one episode of pneumonia. Cumulative retrospective data highlight that the respiratory infections begin very early in life, with almost all patients being affected by the age of 15 (**Figure 1C**). The registry data confirmed the previously described (5, 6) high incidence of bronchiectasis (28 patients out of the 55 who underwent a CT-scan), which was documented early in life (age range: 2–39 years; mean: 11.2 years). As already suggested by a previous retrospective review of the literature (7), the majority of patients with bronchiectasis had APDS1 (24 patients out of the 40 who had a CT-scan). Abnormal lung function was noted in 17 out of 35 patients who performed these tests. Acute viral infections (with varicella and herpes simplex) as well as chronic viral infections/reactivations were frequently documented in APDS1 and APDS2 patients (**Figure 1A**). The most frequently reported chronic infection in both cohorts was Epstein–Barr virus infection (16/68). Among the non-respiratory bacterial infections, the most frequent was infectious lymphadenitis (14/68). Five patients suffered from chronic mucocutaneous candidiasis and three developed local infection following vaccination with bacillus Calmette–Guérin. Consistent with the two published cohorts, chronic non-neoplastic lymphoproliferation was reported in the majority of patients (87%). Persistent peripheral lymphoproliferation, splenomegaly, and lymphoid hyperplasia were frequent and they were often concomitantly reported in the same patients (**Figure 1D**). Across the cohort, lymphoproliferation occurred with later onset than respiratory infections (**Figure 1C**) but preceded gastrointestinal manifestations and the development of autoimmunity.

Benign lymphoproliferation may be difficult to distinguish from malignant disease, the risk of which is increased in APDS patients. Eight of the registry-documented patients (5 APDS1, 3 APDS2) developed lymphoma between the age of 11 and 25 years, including two patients with Hodgkin lymphoma, one of whom subsequently developed an intestinal diffuse large B-cell lymphoma. Six patients were diagnosed with non-Hodgkin lymphomas (two diffuse large B-cell lymphomas, one anaplastic lymphoma, one marginal zone lymphoma, two without detailed histologic information). Five patients achieved a complete remission on treatment, one patient achieved only a partial remission, one patient was still under treatment at the time of registration, while in the remaining case, the lymphoma was sadly fatal. One of these eight patients also had a benign ovarian serous cystadenoma. One patient developed a B-cell chronic lymphocytic leukemia at the age of 40 years. In addition to the established high incidence of hematological malignancy, 2 cases of solid organ malignancy or pre-malignancy were noted: one case of ductal breast carcinoma-*in situ* (diagnosed in an APDS2 patient at the

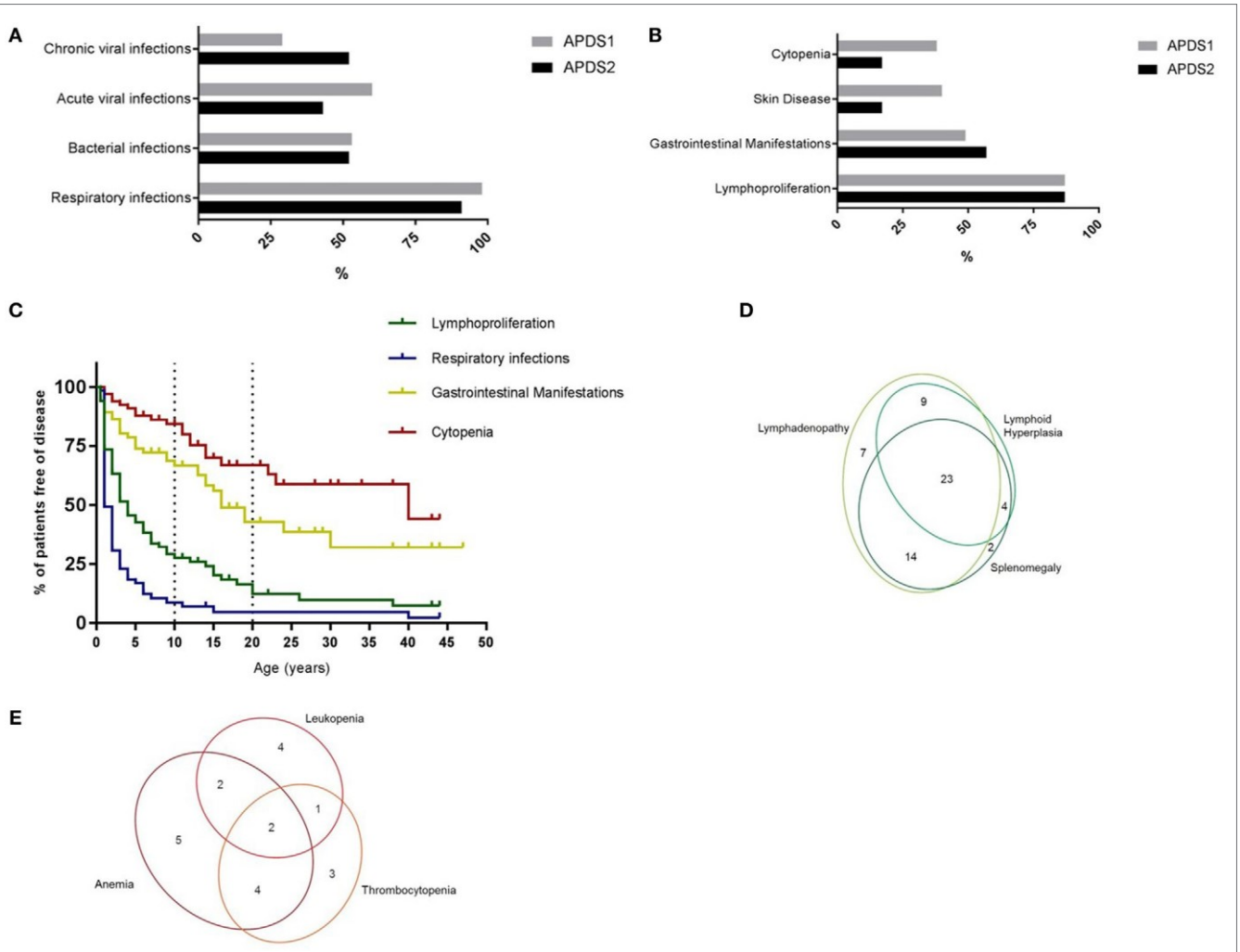

FiGUre 1 | **(A)** Incidence of infections in APDS1 and APDS2 patients. **(B)** Incidence of manifestations of immune dysregulation in APDS1 and APDS2 patients. **(c)** Evolution of disease manifestations over time. Information regarding age at onset available for: respiratory infections $n = 62/65$, lymphoproliferation $n = 59/59$, gastrointestinal manifestations $n = 33/35$, cytopenia $n = 20/21$ patients. **(D)** Diagram showing the different types of benign lymphoproliferative manifestations. **(e)** Diagram showing the different blood lineages affected in patients with cytopenias.

age of 33) and one case of rhabdomyosarcoma (diagnosed in an APDS1 patient at the age of 13).

Gastrointestinal manifestations were the third most frequent disease manifestation (51%) and across the cohort occurred before the other features of immune dysregulation, such as cytopenias or arthritis, but typically much later than the respiratory infections and the benign lymphoproliferation (**Figures 1B,C**). Small or large bowel inflammation was histologically confirmed in 17 patients, in 11 of them by the age of 10 years. Granulomas were reported in only one patient. Protracted diarrhea with no identified underlying cause was the second commonest reported gastrointestinal problem and was often severe enough to require hospitalization. Two patients were diagnosed with autoimmune hepatitis but no cases of sclerosing cholangitis were reported, in contrast with the two patients reported by Coulter et al. (5) and the two reported by Hartman et al. (8). Of note, 14/68 patients of the APDS-Registry cohort had eczema. Elkaim et al. (6) noted only three APDS2 patients with chronic eczema and no inflammatory skin disease was mentioned in the published APDS1 cohort (5). Cytopenias were the fourth major disease manifestation affecting around 30% of patients, usually later in life (**Figures 1B,C**) than the other main features and frequently affecting multiple blood lines (**Figure 1E**). The autoimmune origin of the cytopenias could be documented in the majority of the patients. Other autoimmune diseases were also reported, all occurring after the age of 10 years: two patients had autoimmune thyroiditis, three had arthritis, and three glomerulonephritis.

Concerning non-immunological manifestations, short stature (>2 SD) was reported in 11 patients, with a predominance of APDS2 individuals (8/13), consistent with previous reports (6, 7). Neurodevelopmental delay was diagnosed in three patients. Specific neuropsychiatric disorders were also reported: one patient had Asperger Syndrome, one had autism, one suffered from a mixed anxiety and depression disorder, and two other

patients had mild disorders of speech and language development. It is unclear if these findings reflect the impact of a severe physical illness or the impact of enhanced PI3Kδ signaling in the central nervous system.

immunological Abnormalities

One of the objectives of the ESID-APDS registry is to collect immunological data prospectively. An initial analysis of the immunological profile in the registry cohort confirmed the already published T- and B-cell alterations. No clear difference between APDS1 and 2 was detected in the current cross-sectional data set. In the future, the longitudinal collection and analysis of these data will offer the possibility to explore associations between specific disease manifestations and immunological alterations, to evaluate the response of immunological alterations to the different types of treatment, and to establish the predictive value of immunological parameters for disease prognosis.

current therapies

Supportive therapy is a key component of the management of APDS patients. In the APDS-registry, 54 patients received antibiotic prophylaxis, whereas only eight received antifungal prophylaxis, which appears justified given the absence of reported invasive fungal infections. IGRT was administered in 44 patients (28/45 APDS1, 16/23 APDS2), was in general very well tolerated, and was started early in life (**Figure 2A**), mirroring the early presentation with respiratory infections. The majority of patients also received immunosuppressive treatments. Thirty-one patients received corticosteroids and 27 of them showed at least a partial clinical benefit. More than half

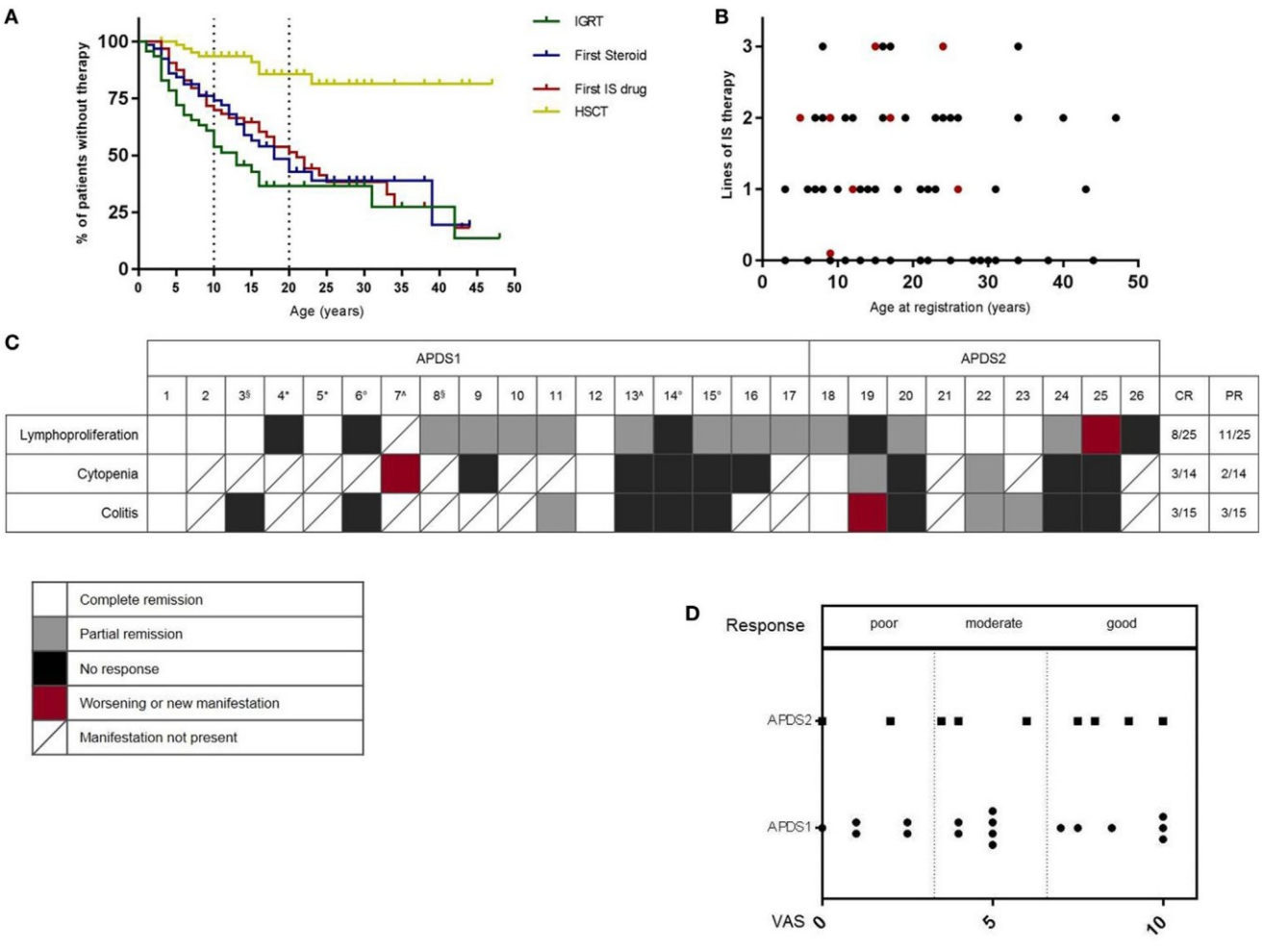

FiGUre 2 | (A) Use of treatment modalities over time. IGRT, immunoglobulin-replacement-treatment; IS, immunosuppressive drug; HSCT, hematopoietic stem cell transplantation. Information regarding age at first therapy available for: IGRT $n = 28/44$, steroid therapy $n = 31/31$, IS therapy $n = 35/36$, HSCT = 8/8. **(B)** Number of lines of immunosuppressive treatments (steroids, immunosuppressive drugs, rituximab) by the time of registration; red: patients who had undergone HSCT by the time of registration. **(c)** Response to rapamycin treatment. *White*: complete response; *gray*: partial response; *black*: no response; *red*: worsened or new manifestation; *boxes with a diagonal*: manifestation not present in this patient. CR, complete remission; PR, partial remission. Rapamycin stopped because of: *non-compliance, °inefficiency, ^side effects, §clinical trial. **(D)** Overall clinical benefit (Visual Analog Scale) according to physician's evaluation.

had received steroid treatment by the age of 20 (**Figure 2A**). Thirty-six patients received other immunosuppressive drugs, including azathioprine (n = 1), mycophenolate (n = 3), cyclosporine (n = 5), or rapamycin (n = 27); clinical benefit was reported in 28 of these patients. Rituximab was given to eight patients, with clinical benefit in all. **Figure 2B** illustrates the multiple lines of immunosuppressive treatments (steroids, immunosuppressive drugs, or rituximab), which had already been received by patients by the time of enrollment into the registry. Five patients underwent splenectomy (4 APDS1 and 1 APDS2) because of cytopenias or splenomegaly and 25 patients (12 APDS1 and 13 APDS2) underwent tonsillectomy (age range: 1–12 years), with clear benefit in only seven of them. The only available curative option is hematopoietic stem cell transplantation (HSCT) and the first experiences in this field have been published (9). Among the patients in the registry, 8/68 patients had undergone HSCT (7 APDS1 and 1 APDS2) by the time of registration (**Figure 2A**), with fatal outcome in one.

rapamycin therapy in APDs

Consistent with activation of mTOR signaling downstream of the activated PI3Kδ, patients with APDS may benefit from rapamycin (2). In the APDS2 cohort-paper (6), six patients had been treated with rapamycin, but the time of follow-up was too short to evaluate the response to treatment in four of them. Six of the patients in the reported APDS1 cohort (5) were treated with rapamycin for benign lymphoproliferation; five of them had a treatment response, but in one case, the therapy was stopped due to side effects. Additional case reports of rapamycin therapy have also been published (7, 10). In the ESID-APDS-registry cohort, rapamycin was the most frequently used immunosuppressive drug. We, therefore, decided to evaluate the experience with rapamycin (Sirolimus) in 26 patients (1 patient was not included because treatment was started and terminated before the diagnosis of APDS and the response to therapy was not well documented), 17 with APDS1, and 9 with APDS2. The main indications for treatment were lymphoproliferation, colitis, and/or cytopenia. Physicians were asked to judge the degree of severity of each manifestation as mild, moderate, or severe at the start of therapy, following 3–6 months of treatment and at the latest follow-up (average time of therapy monitoring: 1.6 years). Overall response judged by the physician visual analog scale was good in 10, moderate in 9, and poor in 7 (**Figure 2D**). Lymphoproliferation showed the best response (8 complete, 11 partial, 6 no remission), while bowel inflammation (3 complete, 3 partial, and 9 no remission) and cytopenia (3 complete, 2 partial, 9 no remission) responded less well, as shown in **Figure 2C**. Notably, of the eight patients who were on steroids at initiation of treatment with rapamycin (No. 1, 7, 9, 13, 19, 22, 23, 25), seven were able to stop steroids and one (No. 25) was able to reduce the dose. Two patients (No. 4, 5) stopped therapy because of poor compliance, in three cases (No. 6, 14, 15), the reason for cessation was lack of efficacy. Two patients (No. 7, 13) suffered from side effects (severe headaches, anorexia, renal toxicity) that led to the complete interruption of the treatment, whereas in three cases, the therapy was paused because of side effects (aphthous ulcers, liver toxicity, renal toxicity) but could be started again. Two patients (No. 3, 8) stopped despite efficacy because of enrollment in a clinical trial with PI3Kδ inhibitors. In two other individuals (No. 11, 12), treatment was interrupted after prolonged usage; in one patient (No. 20), this was due to the patient planning for pregnancy and, in another (No. 19), it followed the development of a lymphoma. Of note, three patients (No. 14, 18, 25) received also Rituximab during and one (No. 10) shortly before the treatment with rapamycin. One patient (No. 20) concomitantly received Adalimumab because of arthritis. Interestingly, some patients did not show any relevant alterations in the disease manifestations after 3–6 months of therapy but did show either improvement (No. 1, 8, 10, 18, 22, 23) or worsening (No. 6, 14, 19) after a longer period of observation on treatment (about 2 years).

DiscUssiON

We present an initial analysis of the prospective ESID-APDS registry, a longitudinal cohort study of patients with APDS1 and APDS2. This overview expands the known information regarding the clinical manifestations of the disease by adding the aspect of the evolution of the features over time. The emerging picture is the one of a PID characterized by the early occurrence of respiratory infections (mostly upper respiratory infections), followed by the development of chronic benign lymphoproliferation and subsequently other features of immune dysregulation, in particular, gastrointestinal manifestations and autoimmune cytopenias. We again noted the higher incidence of bronchiectasis in APDS1 compared with APDS2 patients; however, the numbers remain small and differences in CT uptake cannot be excluded as a confounder. However, this observation may stimulate future studies of the roles of the *PIK3CD* and *PIK3R1* genes and their proteins in the respiratory system. In the future, further analysis of the clinical evolution in this prospective cohort will allow better definition of long-term prognosis for this disease. In addition, the correlation of clinical features with the immunological abnormalities and their relationship with outcome parameters will help defining clinical and biological biomarkers of outcome.

The choice of treatment is a key issue in these patients who often present with severe concomitant manifestations not only of immunodeficiency but also of immune dysregulation. According to the registry, the combination of supportive therapy to prevent recurrent infections and the immunosuppressive treatment of immune dysregulation is often initiated early in life, with many patients undergoing multiple treatments. Rapamycin inhibits the biologically relevant downstream PI3K effector mTOR pathway, and it has been widely used with good efficacy in other PIDs, in particular, autoimmune lymphoproliferative syndrome (11, 12). Our interrogation of the ESID-APDS registry aligns with previous reports (7, 10) in suggesting that rapamycin reduces the severity of benign lymphoproliferative disease also in APDS. However, a less satisfactory response was documented regarding the non-lymphoproliferative manifestations, in particular, intestinal disease and cytopenias, which can be highly detrimental for the patients' quality of life. It is important to relate these registry results to the first results of targeted therapy with the PI3Kδ inhibitor leniolisib that have recently been published (13). In the first six patients, the drug showed an excellent control of the lymphoproliferation (6/6 patients) and in part also improved the cytopenias at the end of treatment (day 84). Three of the

six patients normalized their thrombocytopenia, one patient resolved his anemia, and three of four patients improved their lymphopenia, while there was no correction of the neutropenia observed in two patients; however, respiratory and gastrointestinal symptoms and outcomes were not reported in this study. Furthermore, our registry analysis highlighted that also colitis and skin disease can cause significant symptoms in these patients and should, therefore, be carefully evaluated in future clinical studies on novel therapies, particularly given previous reports of colitis associated with PI3K inhibitors (14). Longitudinal data capture on APDS patients in the ESID-APDS registry will be critical to observe the long-term benefits and/or side effects of these therapies, in particular, their effect on the incidence of lymphomas. It is noteworthy that one patient developed lymphoma while taking rapamycin. Another key question, where the registry will be helpful, is the question if and when to perform HSCT. The analysis of this question will profit from the principles established in the P-CID study, a prospective natural history study on profound combined immunodeficiency in which matched pairs of transplanted and non-transplanted patients with similar disease burden and immunological alterations are followed (15).

Finally, an attractive goal for the registry is to involve patients and their families directly in data acquisition. This could in the future allow collecting information about the quality-of-life of APDS patients, thus ameliorating the evaluation of the disease burden in all its complexity. In summary, thanks to the collaborative work of the participating centers, the ESID-APDS registry will comprise a valuable resource for physicians dealing with this disease and for shaping future research questions.

etHics stAteMeNt

The study is carried out in accordance with the recommendations of Section 15 of the Code of Conduct of the General Medical Council of Baden-Württemberg, Germany. The protocol was approved by the Ethics committee of the University of Freiburg (IRB approval No. ESID registry: 493/14; IRB approval No. APDS registry: 458/15). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUtHOr cONtriBUtiONs

MM collected analyzed and interpreted data and wrote the manuscript. HA, AA, ALA, OA, CB, SAB, FB, HB, MB, SOB, CC, ANDC, PC, MC, ANIC, FC, TC, LD, JE, SF, AF, MG, LH, MH, SJ, EK, AK, DK, BG, HL, NM, TM, FM, DM, AM, ON, BN, PO, AO, JP, CP, SP, JR, SS, ALS, ANS, SS, ASH, MS, PS, AUS, FS, WR, FT, JM, KW, AW, and PW repeatedly referred and registered patients. AN, GK and AU coordinated the registry. SR and RS provided the export data from the online-registry and gave informatic support. SK, ALC, and SE interpreted the data and wrote the manuscript. All the authors edited the manuscript.

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Conflict of Interest Statement: The APDS registry is supported by the pharmaceutical companies Novartis, GlaxoSmithKline, and UCB UK, who have financed development and maintenance of the online level 3 documentation section for APDS as well as project management including ethics submission in all participating countries, data management, and quality controls. The financial support also allows some reimbursement of documentation activities for the participating centers. For those patients who have specifically agreed to this in the registry consent, anonymized data from the APDS Registry are available to industry partners for their purposes (e.g., designing a drug trial or data submission for regulatory approvals).

Czech Hizentra Noninterventional Study With Rapid Push: Efficacy, Safety, Tolerability, and Convenience of Therapy With 20% Subcutaneous Immunoglobulin

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ABSTRACT

Purpose: Immunoglobulin substitution therapy is an essential therapeutic approach for patients with primary antibody deficiencies. Different methods of administration, including intravenous immunoglobulin (IVIG) or subcutaneous immunoglobulin (SCIG) preparations, provide effective and tolerable treatment and enable the adjustment of therapy to patients' needs. A new 20% SCIG represents a new therapeutic option and a new route of administration using rapid-push application. The aim of the Czech Hizentra Noninterventional Study With Rapid Push (CHHINSTRAP) is to evaluate patient satisfaction with as well as the tolerability and efficacy of nonmedical switch to 20% SCIG from previous treatment with IVIG or SCIG and rapid push as a new way to administer SCIG. CHHINSTRAP is the first Phase IV, noninterventional, open-label, prospective, multicentric study of this type conducted in Central and Eastern Europe.

Methods: Primary end points, including efficacy, adverse effects, convenience of use, and overall satisfaction, were evaluated by Treatment Satisfaction Questionnaire for Medication version II. Secondary end points, such as serum IgG trough levels, infusion duration, number of application sites, frequency of infections, related hospital admissions, and antibiotic consumption, were obtained from patients at each follow-up visit.

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Findings: Together, 50 eligible patients with primary antibody deficiency were switched from SCIG or IVIG to an equivalent dose of 20% SCIG and were followed up for 12 months during 5 consecutive visits. The results indicate that patients switched from previous IVIG or SCIG preparations had significantly higher serum trough IgG levels and a lower incidence of infections and related events, such as hospital admissions or consumption of antibiotics. These findings were also reflected in gradually increasing convenience of use and overall satisfaction reported by patients. Apart from duration of application, no differences were found between patients previously receiving SCIG or IVIG. Moreover, our study found a high level of safety of 20% SCIG rapid push, which was comparable to other preparations and application methods.

Implications: On the basis of the results of CHHINSTRAP study, we conclude that 20% SCIG is a tolerable and effective immunoglobulin preparation, representing a new therapeutic approach in patients with primary antibody deficiencies. Its efficacy and tolerability have been found in patients on nonmedical switch from previous treatment with IVIG or SCIG. (*Clin Ther*. 2019;XX:XXX**e**XXX) © 2019 Elsevier HS Journals, Inc. (*Clin Ther*. xxxx;xxx:xxx) © 2019 Elsevier Inc. All rights reserved.

Keywords: Nonmedical switch, Primary antibody deficiency, Rapid push, Subcutaneous immunoglobulin replacement therapy, Treatment satisfaction.

INTRODUCTION

Primary antibody deficiencies (PADs) are the most prevalent inborn errors of immune system, representing more than half of all patients with primary immunodeficiencies (PIDs).[1,2] PADs are characterized by impaired immunoglobulin production with broad spectrum of manifestations, ranging from selective deficiencies, which are often asymptomatic, such as selective IgA deficiency or IgG1 to IgG4 subclass deficiencies, to complex disorders with disturbed production of specific antibodies and immune system dysregulation, such as common variable immunodeficiency. The patients with PADs are particularly endangered by recurrent bacterial respiratory infections, including rhinosinusitis, bronchitis, or pneumonia, which are the hallmark of PADs and manifest in most patients.[3] The therapeutic approach is based on regular immunoglobulin replacement, which leads to significant reduction of infections.[4] Crucial progress in the treatment of PADs occurred in 1952, when Ogden Carr Bruton described an inborn X-linked agammoglobulinemia (later called Bruton syndrome) and provided a specific treatment in the form of IgG administration.[5] Since then, many application forms of immunoglobulin replacement therapy have been developed in attempts to cover patients' needs. Currently, 2 application routes are available in current clinical practice: intravenous immunoglobulin (IVIG) and subcutaneous immunoglobulin (SCIG). IVIG was established in the late 1970s, and SCIG was implemented into clinical practice in 1990s.[6,7] Both approaches are recommended as substitution therapy in primary and secondary antibody immunodeficiencies[8]; however, they differ in many aspects, such as pharmacokinetic properties, adverse reactions, or modes of administration.[9]

The new manufacturing technologies and processes enabled the development of 20% SCIG* stabilized in proline, which offers the possibility of achieving higher concentrations and administering higher amounts of immunoglobulins to a limited subcutaneous space. Moreover, a new application method, rapid push, was introduced with 20% SCIG, which does not require the use of infusion pump for its administration. Its efficacy and tolerability in adult and pediatric patients have now been proved in multiple Phase III studies.[10e12] On the basis of these results, 20% SCIG was successfully registered by the State Institute for Drug Control of the Czech Republic in 2013.

*Trademark: Hizentra® (CSL Behring, King of Prussia, Pennsylvania).

On the other hand, various aspects of the nonmedical switch from different IVIG or SCIG to 20% SCIG remained unresolved because only a limited number of studies were previously performed in Europe on this subject.[13,14] We present the results of the Phase IV, noninterventional, open-label, prospective, multicentric study Czech Hizentra Noninterventional Study With Rapid Push (CHHINSTRAP), which focused on patient satisfaction with and the tolerability and efficacy of nonmedical switch from previous treatment with IVIG or SCIG preparations.

METHODS

The study was reviewed and approved by the Ethical Committee of Motol University Hospital, Prague, Czech Republic, and further approved by the State Institute for Drug Control of the Czech Republic. Patients were recruited from 2014 to 2016 and followed up for 12 months. Before enrollment, informed consent was obtained from all patients. The inclusion criteria were as follows: (1) diagnosis of PID (based on diagnostic criteria defined by European Society for Immunodeficiency[15]), (2) indication for immunoglobulin therapy,[8] (3) previous immunoglobulin replacement therapy, and (4) eligibility for the rapid-push method of application. Patients with hypersensitivity to any IVIG or SCIG preparation and/or patients with hyperprolinemia were excluded.

All enrolled patients were treated with equivalent dose of 20% SCIG, which was administered before study initiation. In patients previously treated with IVIG, the application dose was calculated according to following formula: total monthly dose of IVIG (in grams) divided by 4 (planned number of 20% SCIG applications per month). The dose of 20% SCIG in patients previously treated with SCIG in weekly intervals remained unchanged. Apart from the single administration at the baseline visit (V1), all remaining applications were performed at the patients' residence by themselves without the presence of a health care professional.

The primary end points were evaluated by a standardized Treatment Satisfaction Questionnaire for Medication version II (TSQM). The questionnaire provides 4 dimensions, which were evaluated as primary points: efficacy, adverse events (AEs), convenience of use, and overall satisfaction data. The score of each component ranges from 0 to 100 points, with a higher score representing a higher satisfaction and fewer AEs.[16] Furthermore, the number of infections and related hospital admissions, antibiotic consumption, volume per administration, number of injection sites, duration of infusions, IgG dose, and serum trough levels were evaluated as secondary end points.

The entire study period was divided into 5 follow-up visits (V1–V5). The evaluation of eligibility for enrollment was performed during a screening visit (V0) based on inclusion and exclusion criteria, IgG trough level, patient history (including number of infections and hospital admissions), and antibiotic consumption in the past 12 months. Patient education, including the demonstration of application method and various aspects of the switch, was conducted at V1. Further visits (V2–V5) were performed in 3-month intervals. TSQM, serum IgG trough level, and patient diary recording duration of infusions and number of application sites, number of infections, related hospital admissions, and antibiotic consumption were evaluated on each visit.

All obtained data were statistically analyzed by repeated-measures ANOVA test (for independent variables) and Fisher exact test (for nominal variables) using GraphPad software, version 6.04 (GraphPad Software Inc, San Diego, California). The differences were considered significant at $P < 0.05$.

RESULTS

Patient Characteristics

A total of 50 white patients (21 males and 29 females; mean age, 43.3 years; age range, 12–78 years) from 9 Czech national centers for diagnosis and treatment of PIDs were enrolled during the recruitment period; 48 of them completed all scheduled visits. Two patients withdrew their consents. The largest subgroup consisted of 11 patients (22.9%) 30–39 years of age followed by 9 patients (18.7%) 40–49 years of age. Common

Table I. List of diagnoses of the patients in the Czech Hizentra Noninterventional Study With Rapid Push.

Diagnosis	No. (%) of Patients (N ¼ 48)
Common variable immunodeficiency	34 (71)
IgG subclass deficiency	9 (18.5)
Hyper-IgM syndrome	1 (2)
Activated PI3 kinase d syndrome	1 (2)
Hyper-IgE syndrome	1 (2)
X-linked agammaglobulinemia	1 (2)
Severe combined immunodeficiency	1 (2)

variable immunodeficiency (34/48 [71%]) and IgG subclass deficiency (9/48 [18.5%]) were the most common diagnosis (Table I). Hyper-IgM syndrome, activated PI3 kinase d syndrome, hyper-IgE syndrome with antibody deficiency, X-linked agammaglobulinemia, and severe combined immunodeficiency were all represented by 1 patient per group. All patients had been previously treated with IVIG (35/48 [73%]) or SCIG (13/48 [27%]). In total, 2896 20% SCIG administrations were monitored for 48 patient-years. No significant differences were found between the SCIG and IVIG groups in sex, age, trough serum IgG levels, and dose of administration at baseline. Patient characteristics are summarized in Table II.

Efficacy

All included patients were switched to the treatment scheme with an equivalent mean (SD) dose of 2.20 (1.22) g per administration and a cumulative dose of 9 (4.88) g per 4-week interval (corresponding to a monthly mean [SD] dose of 170 [0.123] mg/kg) at V1, which remained unaltered for the duration of the study (mean [SD] dose of 2.53 [1.11] g per administration and 10.2 [4.44] g per 4-week cumulative dose [240 (0.217) mg/kg per month]) compared with V5. These differences corresponded to the minimal changes of the dosage from V1 to V5, which were statistically insignificant. Despite the unchanged application dose, the serum IgG trough levels were constantly increasing. The mean (SD) serum IgG trough concentration at screening (baseline) before the switch was 4.9 (2.39) g/L compared with 6.5 (2.5) g/L at the end of the study (V5). The differences were statistically significant ($P < 0.001$). No differences were observed between patients receiving previous treatment with IVIG and SCIG (Figure 1).

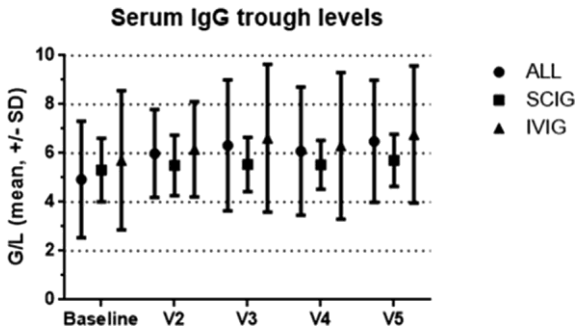

Figure 1. Serum IgG trough levels from Baseline to End of study (V5) showed gradual statistically significant increase (***p <0,001), no significant differences were observed between IVIG and SCIG group.

The increase of IgG serum concentration was paralleled with the decrease in number of infections and related hospital admissions for infection. Both parameters were the most important clinical markers assessed in this study. One year before study initiation, 33 patients (69%) experienced infectious episodes and were treated accordingly with antibiotics and/or antimycotic drugs. Seven of these patients (15% of all included patients) received antibiotics twice, and 3 patients (6% of all included) received antibiotics 3 times during the study period. In total, 46 courses of antibiotics and/or antimycotics were reported. As for severe bacterial infection, 11 episodes of pneumonia

Table II. Characteristics of patients in the Czech Hizentra Noninterventional Study With Rapid Push.

Characteristic	Total (N ¼ 48)	IVIG (n ¼ 35)	SCIG (n ¼ 13)
Males, No. (%)	19 (39.6)	15 (31.3)	4 (8.3)
Females, No.(%)	29 (60.4)	20 (41.7)	9 (18.7)
Age, mean (SD), y	43.3 (16.09)	40.6 (15.58)	52.5 (17.46)
Baseline serum IgG, mean (SD), g/L	4.93 (2.39)	5.06 (2.52)	4.57 (2.04)
Monthly baseline dose, mean (SD), mg/kg	170 (123)	140 (112)	180 (127)

IVIG ¼ intravenous immunoglobulin; SCIG ¼ subcutaneous immunoglobulin.

occurred before the study initiation. A total of 13 patients (27%) had to be hospitalized because of infection. Together, 21 patients (44%) experienced infections during the study duration, and a total of 34 courses of antibiotics were prescribed. Only 4 patients (8%) were hospitalized during the study period, and only 1 episode of pneumonia was recorded.

Tolerability

The mean (SD) volume of the first application was 11.2 (6.08) mL, with only a slight increase of 1.6 (0.53) mL during the 12-month study period. The mean single application volume at V5 (the end of study) was 12.8 (5.55) mL, which was administered in 4-week intervals. Overall, this volume was very well tolerated, and the drug was administered into a single application site in most patients. No significant differences were noted in the number of injection sites between patients receiving previous IVIG or SCIG treatment. However, we found significant differences in duration of infusions. The mean (SD) time of single administration in a group of patients previously treated with SCIG was significantly shorter than in those treated with IVIG (21.6 [10.08] minutes at V1 and 21 [10.1] minutes at V5 in the SCIG group compared with 30.6 [15.55] minutes and 32.2 [16.87] minutes in the IVIG group). The mean (SD) application time for both groups (all patients) was 28.2 (14.74) minutes and 29.1 (16.03) minutes, respectively (Figure 2).

Only a minimal number of AEs were observed during the study period. Together, 3 AEs were reported during the study, which was equivalent to a prevalence of 6.25% patient-years and 0.1% of all applications compared with 4 AEs reported in the 12-month prestudy period (equivalent to 8.33% patient-years, 0.36% of all applications). None of the AEs were regarded as severe; in fact, all reactions were graded as mild according to the Common Terminology Criteria for Adverse Events[17] (grade 1 or 2) and mostly presented as pain and/or skin reaction at the site of administration. None of these events led to termination of the study. Statistical analysis was not performed because of the low occurrence of AEs in both periods of data collection.

Satisfaction

The patients' satisfaction with the treatment was the most important parameter and was appointed as the primary end point of the study. This parameter was composed of 4 attributes: efficacy, AEs, convenience of use, and overall satisfaction. These attributes were all evaluated by patients using TSQM. At V1, the mean (SD) efficacy, convenience, and overall satisfaction was 66 (9.74) points, 63.6 (13.77) points, and 67.1 (13.81) points, respectively, reflecting the level of satisfaction with the previous treatment

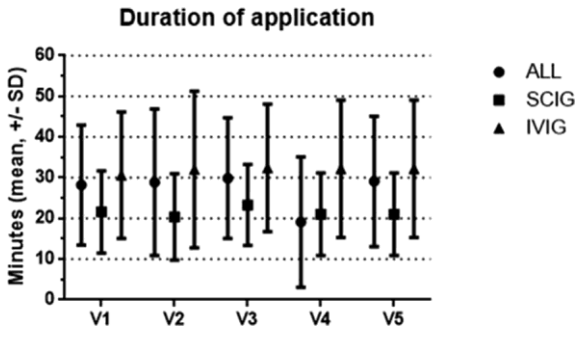

Figure 2. Duration of applications was stable during entire study (V1 e V5) without any significant changes, however the time of duration in SCIG group was significantly shorter (*p <0,05).

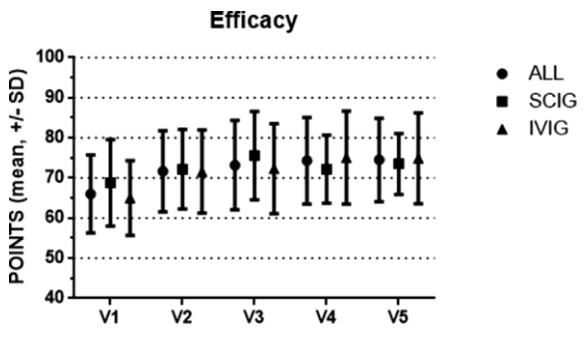

Figure 3. The level of efficacy evaluated by patients in TSQM was gradually increasing from V1 e V5, the results were statistical significant (***p <0,001), no significant differences were observed between IVIG and SCIG group.

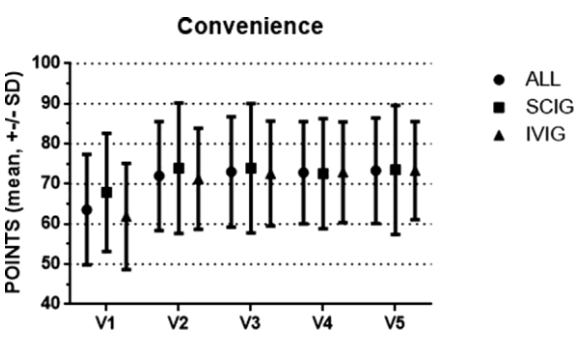

Figure 4. The level of convenience evaluated by patients in TSQM was gradually increasing from V1 **e** V5, the results were statistical significant (***p <0,001), no significant differences were observed between IVIG and SCIG group.

regimen. All 3 parameters were gradually increasing throughout the study, although the highest increases of 5.7 (0.37) points, 8.34 (0.19) points, and 5.2 (2.79) points, respectively, occurred in the first 3 months of the study between V1 and V2. At V5, the mean (SD) efficacy was 74.5 (10.38) points, the mean (SD) convenience was 73.3 (13.18) points, and the mean (SD) overall satisfaction was 76.3 (11.17)

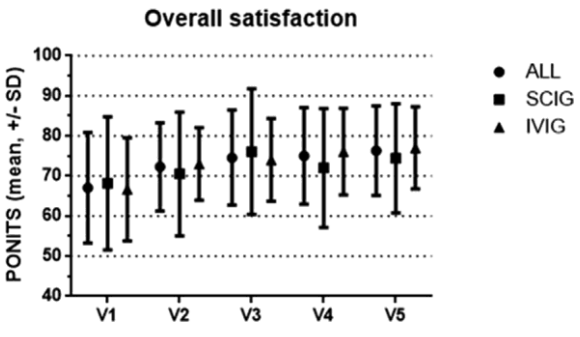

Figure 5. The level of overall satisfaction evaluated by patients in TSQM was gradually increasing from V1 **e** V5, the results were statistical significant (***p <0,001), no significant differences were observed between IVIG and SCIG.

points. The differences among individual visits were statistically significant (Figures 3**e**5).

DISCUSSION

We present the results of the first Phase IV, noninterventional, open-label, prospective, multicentric study regarding the nonmedical switch from IVIG and SCIG administered by infusion pump using the rapid push method conducted in Central and Eastern Europe. A total of 50 patients from 9 Czech national centers for diagnosis and treatment of PIDs were enrolled and followed up for 12 months in 5 consecutive visits in 3-month intervals. The primary end points of efficacy, AEs, convenience of use, and overall satisfaction and the secondary end points of IgG trough levels, duration of infusions, number of infections and hospitalizations, antibiotic consumption, and incidence of AEs were assessed.

All enrolled patients were treated with a dose of 20% SCIG equivalent to their prestudy IVIG or SGIG dose. Despite the insignificant change of dosage (170 mg/kg per month at V1 vs 240 mg/kg per month at V5), the serum IgG trough levels were rapidly and significantly increasing (from 4.9 g/L at baseline to 6.5 g/L at the end of the study). The increase was comparable in both group of patients previously treated with IVIG or SCIG. These findings support the European recommendations regarding an IVIG vs SCIG conversion ratio of 1:1 despite distinct pharmacokinetic properties. This is in the contrast with the US conversion method recommending the ratio 1:1.5.[18]

As expected, the higher serum IgG trough levels was accompanied with a decrease in incidence of infections (33 [69%] vs 21 [44%]), including pneumonias (11 vs 1 episode). Moreover, it led to the reduction of hospitalizations for infections (13 vs 4 events) and decreased use of antibiotics (46 vs 34 courses) compared with the prestudy period.[4] The application volume of 12**e**13 mL per 1 application site and 1 week was sufficient for most patients. The maximal recommended single-site application volume of up to 50 mL allows for the possibility of increasing the volume and thus extending the application intervals from 1 to 2 weeks, an approach whose efficacy was recently confirmed in clinical practice.[19] Not surprisingly, the duration of individual infusions were significantly lower in patients with previous experience with SCIG (21 min in the SCIG group vs

32.2 min in the IVIG group at V5). Lastly, administration of 20% SCIG by rapid push had a low incidence and low severity of AEs, which were comparable with previous treatment.

Essentially, the components of primary end points, including efficacy, convenience, and overall satisfaction, were gradually increasing as evaluated by patients throughout the study period. Interestingly, no differences were found between patients receiving previous treatment with IVIG and SCIG. The most prominent increase was observed in the first 3 months after the switch. These results are consistent with previously published studies.[13,14,20] However, administration with infusion pump was assessed in all these studies. CHHINSTRAP indicates that the rapid-push method, performed at patients' residences without the presence of a health care professional, was the preferred means of administration.

CONCLUSION

The new immunoglobulin preparation is subcutaneous use of a 20% concentration of IgG solution. The higher concentration allows reduction of application volume and provides a new rapid-push application method that enables the administration of IgG solution by hand without the need for an infusion pump, thus benefiting a heterogeneous group of patients with various PADs, ranging from common variable immunodeficiency, IgG subclass deficiency, X-linked agammaglobulinemia, activated PI3 kinase d syndrome, and hyper-IgM syndrome to other PIDs associated with hypogammaglobulinemia. The switch from SCIG or IVIG to an equivalent dose of 20% SCIG led to increased serum trough levels of IgG and a decrease in related infections rate. Apart from efficacy, our study also found a high level of tolerability comparable with other preparations and application methods. Moreover, the level of efficacy, convenience, and overall satisfaction, as evaluated by the patients, was significantly higher compared with their previous treatment. Thus, 20% SCIG represents a new and tolerable therapeutic option that facilitates individualized care for patients with PADs and significantly improves treatment adherence.

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CONFLICTS OF INTEREST

This clinical study was supported and sponsored by CSL Behring Biotherapies for Life, Switzerland and CSL Behring, Czech Republic. T. Milota, A. Sediva, P. Kralickova, D. Jilek, V. Novak, J. Litzman, H. Posova, L. Mrazova, J. Poloniova, M. Prucha, and P. Rozsiva were investigators on the studies described in the manuscript and received payment from the sponsor CSL Behring for their work. V. Rauschova and G. Philipp are full-time employees of the sponsor. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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T. Milota and A. Sediva substantially contributed to the conception and design of the work and its critical revising for important intellectual content and had final approval of the version to be published. M. Bloomfield, P. Kralickova, D. Jilek, V. Novak, J. Litzman, H. Posova, L. Mrazova, J. Poloniova, M. Prucha, and P. Rozsival substantially contributed to the conception and design of the work and to the acquisition, analysis, or interpretation of data. V. Rauschova and G. Philipp substantially contributed to the conception and design of the work.

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