The Significance of B-cell Subsets in Patients with Unclassified Hypogammaglobulinemia and Association with Intravenous Immunoglobulin Replacement Requirement

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ABSTRACT

Background: Patients with unclassified hypogammaglobulinemia (UCH) constitute a diagnostic and therapeutic dilemma, because information concerning the clinical and immunological characteristics of these patients is insufficient. **Objective:** To evaluate B-cell subsets in cases with UCH and common variable immunodeficiency (CVID) and their association with treatment requirement in UCH patients. **Methods:** The study included 41 UCH, 25 CVID, and 36 healthy individuals between the ages of 4-18 years. **Results:** The absolute count of total memory and switched memory B-cells were lower in the CVID cases in comparison to the control group. Additionally, the absolute count of marginal zone-like B cells in the 4-10 year age group, and the absolute count of switched plasmablasts in the 10-18 year age group were lower in CVID cases when compared to both the control and UCH groups. The UCH group was categorized based on IVIG replacement therapy. Therefore, the percentage of switched memory B cells was significantly lower in the IVIG-receiving group (10.6% ± 3.10%) compared to the control group (14.0% ± 5.60%). However, there was no significant difference between the IVIG-receiving group and the CVID group. Regarding the comparison of the non-IVIG replacement group and the CVID group, the absolute count of total memory B cells, marginal zone-like B cells, and switched memory B cells were significantly higher in the UCH group. **Conclusion:** B-lymphocyte subsets in UCH cases that did not require IVIG replacement were similar to the control group. On the other hand, the percentage of switched memory B-cells in the UCH cases that required IVIG replacement was not different from that of the CVID cases.

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INTRODUCTION

Common variable immunodeficiency (CVID) is the most prevalent symptomatic primary immune deficiency (1). The incidence of CVID has been reported between 1/50,000 and 1/200,000 (2). Both T- and B-cells have been shown to be dysfunctional in CVID. However, hypogammaglobulinemia has been documented as the prevailing manifestation due to B-cell dysfunction (3,4,5). Cases with CVID have increased mortality rates due to recurrent infections and non-infectious complications (autoimmune cytopenia, polyclonal lymphocytic proliferation, and persistent unexplained enteropathy) (6). Unclassified hypogammaglobulinemia (UCH) is a disease characterized by reduced gamma globulin levels, insufficient vaccine response, and inadequate antibody production. According to the primary immune deficiency classification of European Society of Immunodeficiencies (ESID), patients that do not fully meet the diagnostic criteria for CVID are categorized as UCH (7). Although UCH is frequently encountered in clinical practice, information regarding its prevalence, clinical and immunological features is lacking. Comparison of UCH and CVID with regard to immunological parameters and pathophysiological aspects is important to better understand: i) the frequency and severity of complications in UCH; ii) how to clarify whether UCH is a clinically relevant antibody deficiency; and iii) how to develop appropriate treatment strategies (8). Recent studies have reported that, while CVID and UCH are similar in terms of the frequency of infection, other complications including autoimmunity, granulomatous diseases, and hepatomegaly are less frequent in UCH (9).

The present study aims: i) to analyze B-cell subsets in CVID and UCH patients; ii) to identify similarities and differences between the two diseases regarding B cell subsets; and iii) to investigate their association with clinical findings and treatment requirements.

MATERIALS AND METHODS

Study Population. This study included patients between the ages of 4 and 18 years. These patients were admitted to the Pediatric Immunology Outpatient Clinic of Dr. Behçet Uz Pediatrics and Pediatric Surgery Training and Research Hospital, and were diagnosed with either CVID or UCH according to ESID 2016 diagnostic criteria (7). A total of 102 age-matched individuals (25 diagnosed with CVID, 41 diagnosed with UCH, and 36 healthy controls) were included in the study. Two patients diagnosed with CVID received immunosuppressive therapy: one of them due to granulomatous lung disease and the other due to inflammatory bowel-like disease. Therefore, these two patients were excluded. The study was approved by the ethics committee of Dr. Behçet Uz Pediatrics and Pediatric Surgery Training and Research Hospital and written informed consents were also obtained from all participants. For all participants, data regarding consanguinity, presence of any family member with immunodeficiency, age at the onset of symptoms, types of symptoms, frequency and type of infections, treatments received, presence of autoimmunity, age at diagnosis, follow-up time, immunoglobulin (Ig) levels, lymphocyte and leukocyte counts, and vaccination responses (anti-tetanus IgG and anti-HBs) were collected from medical records or a face-to-face interview with the family. For comparison of B-cell subsets, patient and control groups were divided into two subgroups: 4-10 years and 10-18 years age groups.

Additionally, the UCH group was divided into two subgroups according to whether patients were receiving intravenous immunoglobulin (IVIG) replacement therapy. These two subgroups were compared to control and CVID groups with regard to B-cell subsets without the consideration of age. IVIG replacement was initiated in all patients of the CVID group. In the UCH group, IVIG replacement was started in only 16 patients that had frequent infections and could not be regulated by antibiotic prophylaxis. Ig levels, blood samples, and vaccination responses (anti-tetanus IgG and anti-HBs) were evaluated before IVIG replacement.

Definition of Antibody Deficiency. Serum Ig levels were measured by nephelometry. Ig results were evaluated according to age-specific reference levels in Turkish children reported by Aksu *et al.* (10).

Study Exclusion Criteria. For the patient groups, study exclusion criteria were hypogammaglobulinemia secondary to any other cause (i.e. drug, infection), presence of syndromic findings, being on immunosuppresive therapy, and B-cell counts less than 1% of the total lymphocyte count. For healthy controls, the study exclusion criteria were presence of infection at the time of study, any known chronic disease, and timeline of drug use.

Flow cytometric Analysis and Assignment of B-cell Patterns. All participants provided 3 ml of venous blood drawn into tubes containing EDTA. The blood samples were immediately transferred to our Immunology laboratory and were stained with a fluorochrome using conjugated monoclonal antibodies [CD19 PC7 (Beckman Coulter IO test, Clone: J3-119, Isoype: IgG1 Mouse), CD21 FITC (Beckman Coulter IO test, Clone: BL13, Isoype: IgG1 Mouse), CD27 PC5 (Beckman Coulter IO test, Clone: IA4CD27, Isoype: IgG1 Mouse), CD38 PC5 (Beckman Coulter IO test, Clone: LS198-4-3, Isoype: IgG1 Mouse), IgD FITC (Beckman Coulter IO test, Clone: IA6-2, Isoype: IgG2a), and IgM PE (Beckman Coulter IO test, Clone: SA-DA4, Isoype: IgG1 Mouse)]. After completion of the incubation, the erythrocytes were lysed and leukocytes were stabilized and fixed by TQ-Prep (Coulter). The appropriate isotype controls were used. At least 10,000 cells from each sample were analyzed on the Cytomics FC500 (Beckman Coulter) flow cytometer, and the data were processed with CXP cytometer software. Additionally, a complete blood count was performed for each case to measure total lymphocyte count. Using monoclonal antibodies that are in the Euroclass classification, B-cell subsets were defined as follows (11): B cells (CD19+), Non-memory B cells (CD19+CD27–), Naive B cells (CD19+CD27–IgD+, IgM+), Transitional B cells (CD19+CD38 high, IgM high)**,** Memory B cells (CD19+CD27+)**,** Marginal zone like B cells (CD19+CD27+IgM+, IgD+), Switched memory B cells (CD19+CD27+IgD–, IgM–)**,** Activated B cells (CD19+CD21 low, CD38 low)**,** Switched plasmablasts (CD19+IgM–CD38 high).

Statistical Analysis. Statistical analyses were performed using SPSS version 22.0 (IBM Corporation, Armonk, New York, United States) program. Normality assessment was made with Shapiro-Wilk test, and variance homogeneity was evaluated with Levene test. Comparison of two independent groups for quantitative data was made with either independent-samples t-test together with Bootstrap results or Mann-Whitney U-test together with Monte Carlo results. Comparison of more than two groups for quantitative data was made with One-Way ANOVA and Kruskal-Wallis H tests. Post-hoc analyses were made with Dunn's Test and Fisher's Least Significant Difference (LSD) test. Categorical variables were compared with Fisher Exact test. Quantitative variables were expressed as mean ± standard deviation (SD) and median with range (Minimum-

Maximum). However, categorical variables were expressed as n (%). Variables were analyzed within 95% confidence interval, and a p-value of less than 0.05 was accepted as statistically significant.

RESULTS

The number of female patients was 17 (47.2%) in the control group, 16 (39%) in UCH group, and 13 (52%) in the CVID group. There was no statistically significant difference between the groups regarding distribution of sex (p>0.05). The average age was 7.2 (4-18) years in the control group, 6.6 (4-18) years in the UCH group, and 8.2 (4-18) years in the CVID group. The mean age among cases in 4-10 years age group was 6.1 ± 1.6 years in the control group, 6.6 ± 1.5 years in the UCH group, and 8.2 years in the CVID group.

Table 1. Demographic properties of cases with CVID and UHC.

	UHC (n = 41)	CVID (n = 25)	P-value
Age at diagnosis*	4.5 ± 2.2	4.6 ± 2.7	0.973
Age at the onset of symptoms*	2.0 ± 1.9	2.1 ± 1.9	0.912
Diagnostic delay*	2.4 ± 2.1	2.4 ± 1.9	0.996
Follow-up time	2.4 ± 1.9	4.2 ± 2.7	**0.000**
Consanguinity, n (%)	7 (17)	6 (24)	
Family history of immunodeficiency, n (%)	3 (7.3)	5 (20)	
History of infection at presentation			
Frequent URTI, n (%)	25 (60.9)	14 (56)	
Recurrent otitis, n (%)	3 (7.3)	2 (8)	
Pneumonia, n (%)	10 (24.3)	7 (28)	
Autoimmune disease, n (%)			
Psoriasis		1 (4)	
Hypothyroidism		1 (4)	
Celiac disease	1 (2.4)		
Vitiligo	1 (2.4)		
ITP	1 (2.4)		
Chronic lung disease, n (%)		3 (12)	
Splenomegaly, n (%)		1 (4)	
Hepatitis B vaccine response, n (%)	38 (92.6)	20 (80)	**0.046**
Tetanus vaccine response, n (%)	36 (87.8)	19 (76)	0.131
	UCH -receiving IVIG replacement therapy (n=16)	CVID (n=25)	
Initial immunoglobulin levels			
IgG (mg/dL)	526.6 ± 91.5	506.0 ± 105.8	0528
IgA (mg/dL)	76.5 ± 52.8	34.2 ± 24.2	**0.001**
IgM (mg/dL)	102.2 ± 47.5	48.6 ± 33.3	**0.000**
IgE (IU/ml)	66.0 ± 59.1	30.8 ± 37.9	0.088
	UCH-not receiving IVIG replacement therapy (n=25)	CVID (n=25)	
Initial immunoglobulin levels			
IgG (mg/dL)	555.2 ± 80.8	506.0 ± 1058	0.810
IgA (mg/dL)	77.2 ± 36.3	34.2 ± 24.2	**0.000**
IgM (mg/dL)	105.4 ± 89.2	48.6 ± 33.3	**0.049**
IgE (IU/ml)	74.2 ± 85.1	30.8 ± 37.9	0.098

URTI: upper respiratory tract infection, ITP: idiopathic thrombocytopenic purpura, * Mean ±SD years.

The mean age among cases in the 10-18 years age group was 12.8 ± 1.7 years in the control group, 12.6 ± 3 years in the UHC group, and 13.9 ± 3 years in the CVID group, respectively. There was no statistically significant difference between groups regarding age ($p>0.05$). The mean age at diagnosis, mean age at onset of symptoms, and mean diagnostic delay were 4.5 ± 2.2, 2.0 ± 1.9, and 2.4 ± 2.1 years, respectively, in the UHC group, and 4.6 ± 2.7, 2.1 ± 1.9, and 2.4 ± 2.4 years, respectively, in the CVID group. There was no statistically significant difference between the groups regarding these variables ($p>0.05$). The mean follow-up time was 2.4 ± 1.9 years in the UHC group, and 4.2 ± 2.7 years in the CVID group. There was consanguinity in 7 (17%) cases in the UHC group, and 6 (24%) in the CVID group. Familial history of immunodeficiency was present in 3 (7.3%) cases in the UHC group, and 2 (8%) cases in the CVID group. With regard to history of infections in the UCH group, 25 (60.9%) cases had frequent upper respiratory tract infections, 3 (7.3%) had recurrent otitis, and 10 (24.3%) had a history of pneumonia. However in the CVID group, 14 (56%) had frequent upper respiratory tract infections, 2 (8%) had recurrent otitis, and 7 (28%) had a history of pneumonia. With regard to autoimmunity findings in the UCH group, 1 case had celiac disease, 1 case had vitiligo, and 1 case had idiopathic thrombocytopenic purpura. In the CVID group, 1 case had psoriasis and 1 case had autoimmune hypothyroidism. In the CVID group, 3 (12%) had chronic lung disease and 1 case had splenomegaly. In the UCH group, a positive vaccine response was observed in 38 (92.6%) cases for hepatitis B vaccine and 36 (87.8%) cases for tetanus vaccine. While in the CVID group, positive vaccine response was observed in 20 (80%) cases for hepatitis B vaccine and 19 (76%) cases for tetanus vaccine. In the UCH group, 24 (58.5%) cases received antibiotic prophylaxis and 16 (39%) cases received IVIG replacement. In the UCH group, those cases that received IVIG replacement had mean IgG, IgA, IgM and IgE levels at first presentation of 526.6 ± 91.5, 76.5 ± 52.8, 102.2 ± 47.5, and 66.0 ± 59.1 mg/dL, respectively. In the group that did not receive IVIG, the levels were 555.2 ± 80.8, 77.2 ± 36.3, 105.4 ± 89.2, and 74.2 ± 85.1 mg/dL, respectively. In the CVID group, the mean IgG, IgA, IgM and IgE levels were 506.0 ± 105.8, 34.2 ± 24.2, 48.6 ± 33.3, and 30.8 ± 37.9 mg/dL, respectively. When initial Ig levels in IVIG and non-IVIG subgroups in the UCH group was compared to the initial Ig levels of the CVID group, there was no statistically significant difference regarding IgG and IgE levels ($p>0.05$). However, initial IgA and IgM levels were significantly lower in the CVID group compared to both the IVIG-receiving and non-IVIG-receiving cases with UHC ($p<0.05$) (Table 1).

Individuals in the same age groups were compared across UHC, CVID, and control groups with regard to B-cell subsets. Therefore, the percentage of non-memory B-cells was significantly higher in the CVID group compared to the control group, and the percentage of naive B cells was significantly higher in the CVID group compared to both the control and UCH groups for those individuals in the 4-10 year age group. In the CVID group, the number of memory B-cells and switched memory B-cells were significantly lower compared to the control group ($p<0.05$). Additionally in CVID group, the number of marginal zone like B-cells was significantly lower compared to both the control and the UCH groups ($p<0.05$). There was no significant difference between UCH and control groups regarding B-cell subsets (Table 2).

Table 2. Comparison of B cell subsets between patient and control groups between the age of 4-10 years.

	Control = I	UCH = II	CVID = III	P-value		
	(n=26)	(n=35)	(n=15)	I-II	I-III	II-III
ALC / mm^3 **	3476.5 ± 1268.3	2792.5 ± 1034.4	3086 ± 922.8	#	#	#
B cells / mm^3*	290.5 (72-928)	266 (66.90-717)	244 (102-785)	#	#	#
B cells %**	10.4 ± 3.7***	10.9 ± 4.0***	10.3 ± 3.5***	#	#	#
Non- memory B cells , %**	83.4 ± 5.9	85.0 ± 5.4	88.0 ± 5.5	0.268	**0.014**	0.090
Non- memory B cells / mm^3 *	251 (86.6-795)	215.6 (61.2-642)	209 (80-687)	#	#	#
Naive B cells , %**	69.24± 9.0	70.1 ± 7.6	76.7 ± 7.3	0.661	**0.006**	**0.010**
Naive B cells /mm^3**	256.7± 148.4	217.6 ± 129.1	246.47± 145.3	#	#	#
Transitional B cells , %*	0.40 (0.0-3.2)	0.3 (0.1-4.4)	0.3 (0.1-3.6)	#	#	#
Transitional B cells / mm^3 *	1.5 (0.0-12.6)	1 (0.1-6.9)	0.8 (0.2-6.7)	#	#	#
Memory B cells , %**	16.58± 5.9	14.49± 5.9	11.9 ± 5.5	#	#	#
Memory B cells /mm^3 *	47.4 (22-148.8)	36 (5.6-126.3)	29 (12.3-98)	0.203	**0.007**	0.279
Activated B cells , %*	13.15 (5.3-35.5)	14.7 (3.3-28.6)	13 (4.4-29.9)	#	#	#
Activated B cells /mm^3 *	41.0 (15.7-185)	4.90 (9-158.4)	30 (12-112.1)	#	#	#
Switched memory B cells , %**	13.62± 5.3	11.9 ± 4.5	9.8 ± 4.4	#	#	#
Switched memory B cells /mm^3 *	38.50 (16.50-134)	29.90 (4.63-114.40)	24 (9.67-86.90)	0.188	**0.011**	0.403
Marginal zone like B cells, %*	1.9 (0.3-7.7)	2.4 (0.2-7)	1.2 (0.5-6.2)	#	#	#
Marginal zone like B cells /mm^3*	7.2 (1.9-23.5)	6.4 (0.4-19.6)	2.6 (1.7-14.8)	1	**0.012**	**0.030**
Switched plasmablasts, %*	0.8 (0.1-20)	0.4 (0.1-7.4)	0.7 (0.1-5.4)	#	#	#
Switched plasmablasts/mm^3*	1.6 (0.2-88.4)	1.4 (0.1-38.4)	1.6 (0.1-42.)	#	#	#

* Median (Min-Max.), **Mean ± SD, ***Percentage of the B cells was assessed in the lymphocytes gate and the percentages of B cell subsets were assessed in B cells gate, #: p>0.05, ALC: Absolute lymphocyte count

In comparison of the B-cell subsets of individuals aged 10-18 years across the groups, the number of memory B-cells and switched memory B-cells were significantly lower in the CVID group compared to the control group. Additionally in the CVID group, plasmablast count was significantly lower compared to both control and UCH groups, whereas the percentage of plasmablast was lower compared to UCH group (p>0.05). There was no statistically significant difference between the UCH and the control groups regarding B cell subsets in this age group (Table 3).

The UCH group was divided into two subgroups according to IVIG replacement requirement, and the groups were compared to the control and CVID groups with regard to B-cell subsets. Accordingly in the IVIG-receiving group, the percentage of switched memory cells was 10.63% ± 3.10%, and was significantly lower compared to the control group (14% ± 5.59%) (p=0.041). There was no statistically significant difference between the IVIG-receiving UCH group and the CVID group regarding count or percentage of B-cell subsets (p>0.05). In the non-IVIG-receiving UCH group, the percentage of switched memory B-cells was (12.59% ± 5.14%), which was significantly higher compared to the CVID group (9.38% ± 6.49%). There was also a significant difference regarding the number of switched memory B-cells. Additionally in the non-IVIG-requirement UCH group, the numbers of total memory B-cells and marginal zone-like B-cells were significantly higher compared to the CVID group. There was no statistically significant difference between the non-IVIG-requirement UCH group and the control group in terms of B cell subsets (Table 4).

Table 3. Comparison of B cell subsets between patient and control groups between the age of 10-18 years.

	Control =I	UCH=II	CVID=III	P-value		
	(n=10)	(n=6)	(n=10)	I-II	I-III	II-III
ALC / mm^3 **	2648 ± 464.2	2716.6 ± 884.4	2256 ± 688.3	#	#	#
B cells/ mm^3 *	229 (175-486)	243.2 (137-440)	159 (79-414)	#	#	#
B cells %**	10.9 ± 4.3***	9.5 ± 2.1***	9.1 ± 5.3***	#	#	#
Non- memory B cells, %**	82.3 ± 7.0	85.4 ± 6.3	86.5 ± 12.7	#	#	#
Non- memory B cells /mm^3 *	198.6 (129.8-401.5)	201.3 (110-402)	146.1 (53.4-390)	#	#	#
Naive B cells, %**	67.8 ± 11.8	71.2 ± 6.2	69.8 ± 15.0	#	#	#
Naive B cells / mm^3*	170.8 (86.7-350)	161.9 (99.1-349)	107.6(37.6-357.2)	#	#	#
Transitional B cells, %*	0.3 (0.1-2.2)	0.6 (0.2-4.3)	0.5 (0.2-6.2)	#	#	#
Transitional B cells / mm^3 *	0.8 (0.2-9.3)	2.0 (0.3-6.7)	0.8 (0.3-5.3)	#	#	#
Memory B cells, %**	17.6 ± 7.0	14.5 ± 6.3	13.6 ± 13.3	#	#	#
Memory B cells /mm^3*	47.6 (12.7-87)	32.4 (17-71.4)	19.6 (2.8-41)	1	**0.015**	0.233
Activated B cells, %*	12.4 (2.3-34.2)	7.3 (3.8-38.2)	14 (2.8-55.4)	#	#	#
Activated B cells /mm^3*	22.8 (5.7-146)	23.9 (4.9-70.6)	13.1 (3.5-131.2)	#	#	#
Switched memory B cells, %**	15 ± 6.2	11.0 ± 4.3	8.6 ± 9.0	#	#	#
Switched memory B cells /mm^3 *	34.3 (10.4-78.5)	24.5 (13.6-50.5)	15.1 (1.2-24)	0.663	**0.003**	0.289
Marginal zone like B cells, %*	1.2 (0.6-5.5)	2.5 (1.2-5.2)	2.1 (0.4-13.3)	#	#	#
Marginal zone like B cells / mm^3*	5.9 (1.8-15.6)	5.7 (2.8-16.1)	3.1 (0.7-18.9)	#	#	#
Switched plasmablasts, %*	0.8 (0.1-2.4)	1.3 (0.2-5.4)	0.2 (0.1-1.1)	0.580	0.126	**0.008**
Switched plasmablasts /mm^3*	1.6 (0.1-10.2)	3.5 (0.2-21.5)	0.4 (0.1-0.9)	1	**0.035**	**0.012**

* Median (Min-Max.), **Mean ± SD, *** Percentage of the B cells was assessed in the lymphocytes gate and the percentages of B cell subsets were assessed in B cells gate, #: p>0.05, ALC: Absolute lymphocyte count

DISCUSSION

While antibody deficiency is the prevailing finding in CVID and UCH, their etiology is still not clear. Several recent studies have demonstrated abnormalities in B-cell subsets in peripheral blood in CVID patients (12-14). The majority of these studies have evaluated adult patients with very limited data from the childhood period. Furthermore, very few studies analyzed B-cells subsets in UCH (13).

Çipe FE *et al.* analyzed B cell subsets in their study, and reported a mean age of 23 patients with UCH as 6.7 (3-17) years (15). In the study by Berron-Ruiz L *et al.*, disease onset age of patients with CVID was 12.2 years, the mean age at diagnosis was 14.6 years, and the diagnostic delay was 5.4 years (16). In our study, the median age in UCH was 6.6 (4-18) years, and the median age in CVID was 8.2 (4-18) years. Interestingly, diagnostic delay was 2.4 ± 1.9 years in CVID and 2.4 ± 2.1 years in UHC. In their study, Çeliksoy MH *et al.* reported 55% of patients had UCH, and 54% of patients with CVID were male (17). Çipe FE *et al.* reported 52% of patients with UCH were male (15), while Yazdani R *et al.* reported 66% of patients with CVID were male (18). Consistent with the literature, 61% of our patients had UCH and 48% of the patients with CVID were male in our study. Piątosa B *et al.* reported the initial presenting sign of 69.4% of patients with CVID was recurrent respiratory tract infection (19).

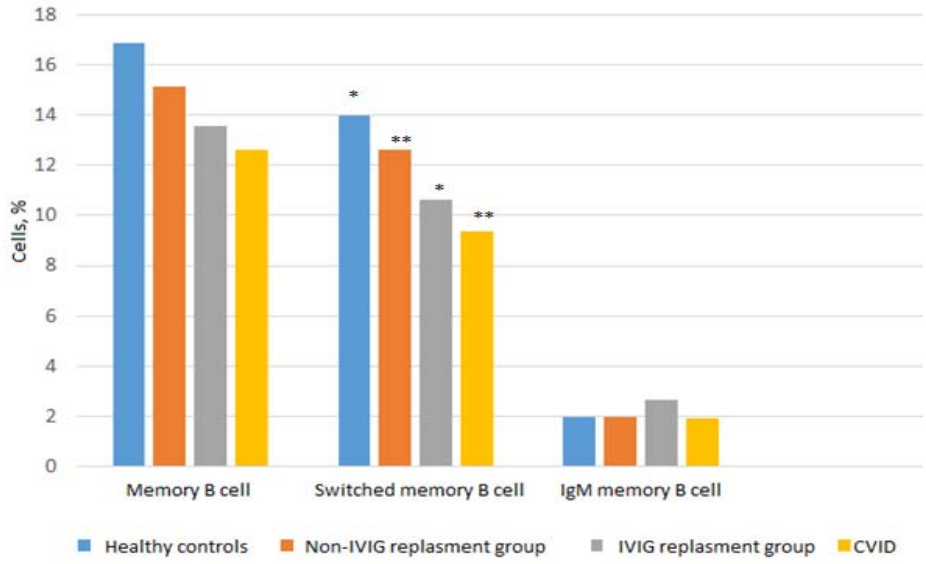

Figure 1. Comparison of IVIG-receiving UCH group, non-IVIG-receiving UCH group, CVID group and control group for B cell subsets in the CD19+ gate. Patients with UCH who received IVIG replacement had lower percentage of switched memory B cells compared to control group (p=0.041)*, however, it was similar in comparison to CVID group (p>0.05). In the UCH group that did not receive IVIG replacement, while percentage of switched memory B cells did not show significant difference in comparison to the control group (p>0.05), it was significantly higher compared to CVID group (p=0.039)**.

In our study, 60.9% of patients with UCH had recurrent upper respiratory tract infection, 7.3% had recurrent otitis, and 24.3% had history of pneumonia. Conversely, 56% of patients with CVID had recurrent upper respiratory tract infection, 8.3% had recurrent otitis, and 28% had history of pneumonia. Driessen GJ *et al.* reported that non-infectious clinical complications were observed in CVID but not in UCH (8). Wehr *et al.* found an association between reduced switched memory B-cells among B-cell subsets, and splenomegaly and granulomatous disease in CVID. They reported that this was caused by a defect in the germinal center (11). In their study, including 49 cases with CVID, Piątosa B *et al.* found splenomegaly, hepatomegaly, and lymphadenopathy in 51.1%, 36.7%, and 71.7% percent of cases, respectively. They observed bronchiectasis in eight cases and pulmonary fibrosis in five cases (19). Berron-Ruiz L *et al.* reported bronchiectasis in 56% of patients and lymphadenopathy in 37% of patients with CVID (16). Consistent with the literature, we did not observe chronic lung disease in patients with UCH. However, 3 patients (12%) in the CVID group had chronic lung disease and 1 patient had splenomegaly. Additionally, one had granulomatous disease in both lungs and liver and the other had inflammatory bowel disease among two patients that were not included in the study due to administration of immunosuppressive treatment. Marginal zone-like B-cells are thought to have important roles in protection against encapsulated microorganisms (20). It has been reported that patients with reduced number of marginal zone-like B-cells had pneumonia more frequently; and therefore, marginal zone-like B-cells could be used to predict the risk of infection with encapsulated bacteria in patients with CVID (21). Studies have shown that 44-62% of

childhood-age group patients with CVID were able to develop adequate antibody responses against protein antigens (22).

Table 4. Comparison of UCH groups that does and does not receive IVIG replacement with CVID, and control groups and between themselves regarding B cell subsets.

	Control=I	UCH-receiving IVIG =II	UCH- not receiving IVIG =III	CVID=IV	p				
	(n=36)	(n=16)	(n=25)	(n=25)	I-II	I-III	II-IV	III-IV	II-III
ALC / mm^3 **	3246.3 ± 1160.2	2978.1 ± 1049.8	2655.6 ± 974.2	2754 ± 920.1	#	#	#	#	#
B cells/mm^3*	276.5 (72-928)	242.4 (132-643)	289 (66.9-717)	237.6 (79-785)	#	#	#	#	#
B cells %**	10.6±3.8***	9.6±3.1***	11.4±4.2***	9.8±4.3***	#	#	#	#	#
Non- memory B cells, %**	83.1 ± 6.1	86.4 ± 3.9	84.2 ± 6.2	87.4 ± 8.9	#	#	#	#	#
Non- memory B cells /mm^3*	235.9 (86.6-795)	211.8 (106.6-595)	215.6 (61.8-642)	204 (53.4-687)	#	#	#	#	#
Naive B cells , %**	68.8 ± 9.7	71.8 ± 6.7	69.3 ± 7.7	74 ± 11.3	#	#	#	#	#
Naive B cells /mm^3*	196.4 (72.5-657)	180 (89.6-526.9)	193.8 (41.7-515)	166 (37.6-625)	#	#	#	#	#
Transitional B cells , %*	0.4 (0.1-3.20)	0.50 (0.1-2.6)	0.3 (0.1-4.4)	0.3 (0.1-6.2)	#	#	#	#	#
Transitional B cells /mm^3*	1.1 (0.1-12.6)	1.3 (0.2-6.94)	1 (0.1-6.7)	0.8 (0.2-6.7)	#	#	#	#	#
Memory B cells , %**	16.8 ± 6.1	13.5 ± 3.9	15.1 ± 6.8	12.6 ± 9.2	#	#	#	#	#
Memory B cells /mm^3 *	47.4 (12.7-148.8)	34.8 (16-77.2)	36 (5.6-126.3)	26 (2.8-98)	0.322	1	0.564	**0.023**	1
Activated B cells , %*	13.1 (2.3-35.5)	14.1 (4.4-38.2)	9.9 (3.3-28)	13.2 (2.8-55.4)	#	#	#	#	#
Activated B cells /mm^{3*}	34.1 (5.7-185)	39.1 (12.7-86.5)	32.1 (4.9-158.40)	29 (3.5-131.2)	#	#	#	#	#
Switched memory B cells , %**	14 ± 5.5	10.6 ± 3.1	12.5 ± 5.1	9.3 ± 6.4	**0.041**	0.319	0.476	**0.039**	0.262
Switched memory B cells /mm^3*	37.9 (10.4-134)	27.6 (13.3-57.6)	30.3 (4.6-114.4)	21.6 (1.2-86.9)	0.167	1	0.652	**0.016**	1
Marginal zone like B cells, %*	1.9 (0.3-7.7)	2.6 (0.2-5.9)	1.9 (0.3-7)	1.9 (0.4-13.3)	#	#	#	#	#
Marginal zone like B cells /mm^3*	6.6 (1.8-23.5)	6.3 (0.4-19.6)	6.7 (0.5-16.1)	3.0 (0.7-18.9)	0.925	0.998	0.054	**0.021**	0.804
Switched plasmablasts, %*	0.8 (0.1-20)	0.3 (0.1-6.5)	0.9 (0.1-7.4)	0.5 (0.1-5.4)	#	#	#	#	#
Switched plasmablasts/mm^3*	1.6 (88.4-0.1)	0.8 (21.5-0.1)	1.7 (38.4-0.1)	0.6 (42.3-0.1)	#	#	#	#	#

* Median (Min-Max.), **Mean ± SD, *** Percentage of the B cells was assessed in the lymphocytes gate and the percentages of B cell subsets were assessed in B cells gate, #: p>0.05, ALC: Absolute lymphocyte count

In our study, the vaccine response rate was 80% to hepatitis B vaccine and 76% to tetanus vaccine in the CVID group, and 92.6% to hepatitis B vaccine and 87.8% to tetanus vaccine in the UCH group. Al Kindi M *et al.* reported that absolute counts of all B-cell subsets were reduced in patients with CVID (23). Yazdani R *et al.* found both the number and percentage of total B-cells were lower in CVID compared to the controls (18). In our study, we did not find any statistically significant difference between CVID, UCH, and the control groups in regard to both the number and percentage of total B lymphocytes. There have been several classification systems used for B-cell subsets (12,24). In our study, B-cell subsets were analyzed using the monoclonal antibodies described in the EURO class classification (11). Since the levels of B-cell subsets vary with age, their levels in patients should be analyzed and compared to an age-matched control group. For this reason, we included a control group and analyses were performed after selecting groups based on age. While not present in the cord blood, memory B-cells show progressive increases in the first year of life. They constitute 10-20% of B-cells by the end of the second year and 30-60% of B-cells in adulthood (20). Marginal zone-like B-cells predominantly synthesize IgM, whereas switched memory B-cells synthesize IgG, IgM, IgA and IgE (25). Huck K *et al.* reported that 86% of patients with CVID had switched memory B-cell levels below 10th percentile in comparison to age-matched healthy controls (26). Driessen GJ *et al.* found both switched memory B-cells and marginal zone-like B-cells were reduced in patients with CVID (8). Wehr C *et al.* found reduced switched memory B-cells in patients with CVID (11). Çeliksoy MH *et al.* found a reduced percentage of switched memory B-cells and a reduced count and percentage of marginal zone-like B-cells in patients with CVID compared to controls (17). Yazdani R *et al.* reported reduced numbers and percentages of total memory cells, marginal zone-like B-cells, IgM-only memory B cells, switched memory B cells, and switched plasmablasts compared to the control group (18). In their study, Bukowska-strakova K *et al.* reported that, while total memory cells showed increase with age in the control group of individuals aged between 5 and 18 years, switched memory B-cell levels did not increase with age in patients with CVID. Although some of these cases had normal initial total B-cell counts, the levels decreased over time (26). Al Kindi M *et al.* studied children and adults along with a control group older than 18 years of age and found a reduced count and percentage of plasmablast cells in patients with CVID compared to the control group (23). Consistent with the literature, we found that patients with CVID in the 4-10 years age group had reduced numbers of total memory B-cells, switched memory B-cells, and marginal zone-like B-cells. Conversely, CVID patients in the 10-18 years age group had lower numbers of total memory B-cells, switched memory B-cells, and switched plasmablasts compared to the control group. Çeliksoy MH *et al.* compared patients with CVID to a control group and found a lower percentage of naive B-cells in patients with CVID (16). Al Kindi M *et al.* reported a lower count and percentage of transitional B-cells and a lower count of activated B-cells in patients with CVID compared to controls (23). Yazdani R *et al.* found a lower count and percentage of activated B-cells and a lower count of naive B-cells in patients with CVID compared to the control group (17). Driessen GJ *et al.* reported reduced transitional B-cells in patients with CVID (8). Bukowska-strakova *et al.* did not find a difference between CVID and control groups regarding activated B-cell count (27). In our study, patients with CVID in the 4-10 years age group had lower

percentages of non-memory B-cells and naive B-cells compared to the controls even though there was no significant difference regarding their counts. There was also no significant difference between CVID patients and controls in the 10-18 years age group regarding both the number and percentage of non-memory B-cells. Several studies found similar B-cell subsets between patients with UCH and an age-matched healthy control group (15,9,17). Similarly, we found that B-cell subsets were similar between the UCH and control groups in the same age group. In relation to IVIG replacement requirement, patients with UCH that required IVIG replacement had a lower percentage of switched memory B-cells compared to the control group. On the other hand, there was no difference between patients with CVID that required IVIG replacement, and patients with CVID in terms of both the number and percentage of switched memory B-cells. Driessen GJ *et al.* compared B-cell subsets of patients with CVID and UCH to that of the healthy controls and reported that total memory B-cell count reached adult levels in the healthy control group by approximately 2 years of age. However, they found the majority of patients with CVID had reduced memory B-cells while memory B-cells were far less affected in UCH. They also found that the distribution of B-cell subsets in patients with UCH did not overlap with that of patients with CVID (8). In our study, in 4-10 years age group, the percentage of naive B-cells was higher and the number of marginal zone-like B-cells was lower in patients with CVID compared to patients with UCH. In the 10-18 years age group, both the number and percentage of switched plasmablasts were lower in the CVID group compared to the UCH group. On the other hand, the numbers of total memory cells and marginal zone-like B-cells and both the number and percentage of switched memory B-cells were lower in CVID group when compared to patients with CVID and patients with UCH that did not require IVIG replacement. Patients with UHC that required IVIG replacement, however, were not different than CVID group in terms of B-cell subsets. Bukowska-Strakova K *et al.* reported that memory B-cell levels could be used as a predictive marker for the development of CVID in patients that are followed up due to hypogammaglobulinemia (27). In our study, we found that B-cell subsets were similar between the control group and patients with UCH that did not require IVIG replacement. Interestingly, those that required IVIG replacement had a lower percentage of switched memory B-cells compared to control group but were no different than the CVID group in terms of B-cell subsets. In our opinion, B-cell subset analysis may be of benefit for treatment strategies in UCH cases, as well as prognostic for future CVID development.

In conclusion and consistent with the literature, our study results showed a reduced number and percentage of memory B-cell subsets and an increased percentage of naive B-cells in cases with CVID. B-cell subsets in patients with UCH that did not require IVIG replacement overlapped with that of the control group. However, patients with UCH that required IVIG replacement had a reduced percentage of memory B-cells. In particular, the number and percentage of switched memory B-cells did not differ from the CVID group, which suggests that these cases are likely to develop CVID in the future. In cases with UCH, the B-cell subset analysis should be considered for predicting IVIG replacement requirement.

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