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Adults with common variable immunodeficiency: a single-center experience*

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Background/aim: In this study, we aimed to assess the clinical and immunological findings of our patients with common variable immunodeficiency (CVID).

Materials and methods: We analyzed the records of 31 adult patients with CVID (12 females, 19 males). The patients were classified into clinical and immunophenotypic subgroups for statistical comparisons.

Results: Our patients had some clinical signs in considerable frequencies, such as low body weight (45.2%), urinary tract infections (41.9%), various dermatoses (35.5%), and oral aphthae (32.3%). The histological findings in the biopsy specimens of the gastrointestinal tract (nodular lymphoid hyperplasia, villous atrophy, and lymphocytic infiltrates at mucosa) were significantly associated with splenomegaly, hepatomegaly, or low body weight (P = 0.005, 0.045, and 0.007, respectively). The patients with low CD4/CD8 ratios had lower IgG levels and a lower percentage of CD19+ B cells, but a higher percentage of activated T cells (P = 0.023, 0.011, and 0.028, respectively).

Conclusion: In adults with CVID, there existed some clinical signs at considerable frequencies, but these are not overemphasized in the literature. The CD4/CD8 ratio is an important factor in antibody production and the clinical presentation of CVID. It seems that the adaptive immune system is on alert and subclinical immune activation insidiously continues in patients with CVID.

Key words: Immunodeficiencies, immunophenotype, classification of common variable immunodeficiency

1. Introduction

Common variable immunodeficiency (CVID) is a mysterious primary immunodeficiency disorder (PID) characterized by impaired immunoglobulin production and an unpredictable clinical course (1). Two peaks in childhood (before the age of 10 years) and adulthood (between 30 and 40 years of age) are seen for this disorder. In spite of recent improvements in diagnostic tools, the diagnosis of mild or moderate CVID is often delayed, especially in adult patients, due to the lack of awareness of this illness among medical professionals. The most remarkable clinical feature of CVID is recurrent infections. Some autoimmune diseases, allergies, and malignant conditions are the other frequent clinical associations. The prevalence of CVID is the second most common among the PIDs, which is between 1/25,000 and 50,000. In spite of many clinical and laboratory investigations, the current knowledge of CVID is not enough to clarify its pathogenesis. Mutations in the genes *ICOS* (inducible T-cell co-stimulator on chromosome 2q), *TACI* (transmembrane activator and CAML-interactor on chromosome 17p), *BAFF* (B-cell activating factor on chromosome 22q), and *CD19* (on chromosome 16p) were identified in only a small proportion of patients with CVID

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(2). While a familial transmission pattern of the disease is seen in about 10% of patients, a sporadic pattern of disease occurs in most of the patients (3).

Studies focusing on the clinical and immunological phenotypes of CVID revealed some considerable relationships among certain findings (4). Accordingly, the Freiburg, Paris, and EUROClass research groups suggested some classification schemes for CVID. Briefly, these classification systems were developed on the basis of B-cell phenotypes and clinical properties. It is clearly predictable that these schemes will be applied to improve the management of this complex disorder.

We believe that all PID centers should analyze their experiences related to CVID and share their findings. Thus, the accumulated data would provide the most convenient and practical approach for the evaluation and management of patients with CVID. Therefore, we herein aimed to assess the clinical and immunological findings of our own patients with CVID and to reveal possible relationships by their analysis.

2. Materials and methods

2.1. Patients

We retrospectively analyzed the records of 31 adult patients with CVID (19 males, 12 females) who were referred to our clinic by their primary care physicians between January 2008 and May 2014. At their first visit, 7 patients were already diagnosed with CVID by another center and were receiving intravenous immunoglobulin (IVIG) treatment, and 7 other patients had CVID diagnosis without IVIG treatment. Seventeen patients with CVID were diagnosed by our clinic after their follow-up period. The European Society of Immune Deficiencies (ESID) and the Pan American Group for Immune Deficiency (PAGID) diagnostic criteria were used for the diagnosis of CVID (5). According to these criteria, patients who have markedly reduced serum immunoglobulin (Ig) G concentrations in combination with low levels of serum IgA and/or IgM should fulfill all of the following criteria: a) more than 2 years of age at the onset of immunodeficiency, b) absence of isohemagglutinins and/or poor response to vaccines, and c) exclusion of other causes leading to hypogammaglobulinemia. All patients completed the questionnaires specifically prepared for CVID. The study was conducted according to the Helsinki Declaration. Ethics committee approval and informed consent of the patients were received in accordance with the procedure.

2.2. Physical measurement

Ideal body weights (IBWs) of the patients were calculated via the IBW formula [50 kg + 0.9 kg/each cm over 152 cm (–4.5 kg if female)] (6). A weight of less than the IBW was considered a low body weight.

2.3. Immunological analyses

While immunoglobulin (IgG, IgA, IgM) and complement (C3c, C4) levels in sera were measured via a nephelometer (Siemens Healthcare Diagnostics, BN II system), lymphocyte subsets were determined in peripheral blood samples with EDTA using flow cytometry (Becton Dickinson, FACSCanto system). As per the manufacturers' instructions, kits compatible with the devices were used for these analyses. The absolute counts of each of the lymphocyte subsets (number of cells/μL) were calculated with the following formula: white blood cell (WBC) count × lymphocyte percentage × lymphocyte subset percentage (7).

2.4. Biochemical analyses

Complete blood counts and blood tests for measurements of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), vitamin B12, folate, and ferritin levels were performed with appropriate methods in the biochemistry department of our institute.

2.5. Statistical analysis

Commercial statistical software (SPSS 11.5, SPSS Inc., Chicago, IL, USA) was used for the statistical analyses of this study. For the tests of normality, we used the Kolmogorov–Smirnov test. The Mann–Whitney U or the chi-square test was used for the comparisons of two independent groups.

The patients were classified into clinical and immunophenotypic subgroups for the comparisons. Clinical subgroups were determined according to the existence of splenomegaly, hepatomegaly, lymphadenopathy, low body weight, and chronic complications of the lower respiratory tract and the gastrointestinal system. The immunophenotypic subgroups consisted of the patients who had a low percentage of CD19+ B cells (less than or equal to 2%) or a low CD4/CD8 ratio (less than or equal to the lower reference limit of 0.8). Accordingly, while SG-1 and SG-2 respectively include patients with or without a low percentage of CD19+ B cells, SG-3 and SG-4 include patients with or without a low CD4/CD8 ratio. The relationships among all parameters were shown by the Spearman rank test or the Pearson test as appropriate. A simple linear regression model was also used for the relationships between the variables. $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. General characteristics of the patients and analyses

The CVID patients referred to our clinic originated from different regions of Turkey. All of them were Caucasian and they were not asked about ethnic origin. The demographic, diagnostic, and treatment characteristics of the patients with CVID are summarized in Table 1.

Table 1. The demographic, diagnostic, and treatment characteristics of the patients with CVID.

Characteristics	
Sex (male/female)	19/12
Parental consanguinity [a]	4 (12.9)
Current age (years) [b]	28 (42)
Age at diagnosis (years) [b]	23 (48)
Delay in diagnosis (years) [b]	14 (42)
Graduation [a]	
Primary school	19 (61.3)
High school	12 (38.7)
Treatments at the first visit [a]	
IVIG	7 (22.6)
Antibiotic prophylaxis	4 (12.9)
Surgical operation	3 (9.6)

[a] n (%); [b] median (range).

An individualized dose of IVIG regimen at a certain interval was started for newly diagnosed patients and the patients who had only CVID diagnosis at their first visit (in total 24 patients). While 4 patients with CVID were receiving antibiotic prophylaxis at their first visit, we started antibiotic prophylaxis for the 9 newly diagnosed CVID patients. Thus, in total 13 patients with CVID (41.9%) were given both IVIG and antibiotic prophylaxis regimens after a certain follow-up period. Daily full-dose amoxicillin, trimethoprim/sulfamethoxazole, or azithromycin was given every other day for prophylaxis. Three different patients at their first visit had a surgery history due to chronic complications of recurrent infections. These were lung lobectomy, drainage of hepatic abscess, and tonsillectomy.

The clinical characteristics of patients with CVID are given in Table 2. The 10 warning signs of PID for adults suggested by the Jeffrey Modell Foundation were used for the clinical characteristics of the patients. It is noteworthy that apart from these 10 warning signs other clinical presentations of CVID, such as low body weight (45.2%), urinary tract infections (41.9%), various dermatosis (35.5%), oral aphthae (32.3%), and meningitis (6.5%), were also observed in our patients.

While 14 patients (45.1%) had no comorbid condition, 17 patients (54.9%) had one or a few different comorbid conditions. The frequency of gastrointestinal comorbidities such as celiac-like disease, gastroduodenitis, and gastritis was the highest (38.7%), followed by Hashimoto thyroiditis (9.6%) and malignant comorbidities in remission (9.6%) such as acute lymphoblastic leukemia (ALL), papillary thyroid carcinoma, and Hodgkin disease. Other comorbid conditions such as nephrotic syndrome with

Table 2. The clinical characteristics of patients with CVID.

Disease	Affected patients (n)	Frequency (%)
Sinusitis [a]	26	83.9
Otitis [a]	14	45.2
Pneumonia [a]	20	64.5
Chronic diarrhea with weight loss	6	19.3
Recurrent viral infections [b]	31	100
Recurrent use of parenteral antibiotics	27	87.1
Recurrent skin abscesses	2	6.5
Persistent fungal infections	0	0
Infection with tuberculosis like bacteria	0	0
Family history of PID [c]	3	9.6

[a] According to warning signs of the Jeffrey Modell Foundation, frequencies of sinusitis and otitis greater than or equal to 2 within 1 year and frequency of pneumonia once a year for more than 1 year were considered as significant for patients. [b] Colds, herpes, warts, condylomata. [c] Three first-degree relatives of the patients had humoral immune defects, two of whom had selective IgA deficiency (1 sister, 1 cousin) and 1 of whom had IgG_2 deficiency (cousin).

amyloidosis, type 2 diabetes, aplastic anemia, idiopathic thrombocytopenic purpura (ITP), Behçet disease, and reactive arthritis were separately observed in six different patients.

All of the patients had chronic structural changes in the lungs such as emphysema, atelectasis, or pleural thickening according to high-resolution computed tomography, and these findings were associated with bronchiectasis or not at the percentages of 61.2% and 32.2%, respectively. Other chronic complications of CVID in the patients were enlargements of the spleen, the lymph nodes, and/or the liver. Accordingly, the frequencies of splenomegaly, lymphadenopathy, and hepatomegaly were 61.3% (19 patients), 48.4% (15 patients), and 22.6% (7 patients), respectively, while 22.5% of the patients had none of these complications. Isolated enlargements of the spleen and lymph nodes but not of the liver were found in 19.3% and 16.1% of the patients, respectively. Involvements of two or more of these organs were found in 38.7% of the patients.

Among the patients who underwent gastrointestinal endoscopy and biopsy (11 patients), 6 (54.5%) had nodular lymphoid hyperplasia, 3 (27.2%) had villous atrophy, and 2 (18.1%) had lymphocytic infiltrates of the mucosa. In addition, reactive hyperplasia was recorded in histopathologic investigations of excisional biopsy samples of the lymph nodes obtained from 3 of the 15 patients who had lymphadenopathy. Neither incisional nor excisional biopsy was performed in the patients with an enlarged spleen or liver due to its invasiveness. Of the 11 skin biopsy specimens of the patients with dermatitis, 7 (63.6%) were histopathologically diagnosed as neutrophilic dermatosis. Granuloma formation was not detected in any biopsy specimens.

In investigation of stool specimens of the patients with diarrhea, 3 (23.1%) cases of *Giardia intestinalis*, 3 (23.1%) of *Candida*, 2 (15.3%) of both *Giardia intestinalis* and amoebae, and 2 (15.3%) of *Escherichia coli* were determined using either light microscopy or cultures of the specimens.

Various laboratory test results and immunological measurements are given in Tables 3 and 4, respectively. Leukocyte differential counts and biochemical tests of the patients, except for one, were found to be normal according to the reference ranges. The median levels of ALP were higher than the upper reference limit. Increased ALP levels were found in 17 patients (54.8%). All of the patients had decreased levels of serum IgG and also low levels of serum IgA and/or IgM at diagnosis. Isolated low immunoglobulin levels in sera were found in 9.6% of the patients, but low levels of 2 or 3 major immunoglobulins in sera were respectively found in 29.0% and 61.2% of the patients. Isolated low immunoglobulin levels were detected only during the first admission of the patients to our clinic who had already been diagnosed and treated with IVIG. While the median percentages of CD3+ T cells and CD8+ T cells were within the reference ranges, the median percentage of CD4+ T cells was low in the patients. Accordingly, the patients had a low median ratio of CD4+ T cells to CD8+ T cells compared to the lower limit of the reference range. Although the median percentage of B cells of the patients was lower than the lower limit of the reference range, the median absolute count of B cells of the patients was normal. In addition, the patients had normal median percentages and absolute counts of natural killer (NK) cells and activated T cells (CD3+HLADR+).

The existence of chronic complications of the gastrointestinal tract (nodular lymphoid hyperplasia, villous atrophy, and lymphocytic infiltrates of the mucosa) was positively correlated with splenomegaly, hepatomegaly, and low body weight ($r = 0.496$, $P = 0.005$; $r = 0.363$, $P = 0.045$; $r = 0.477$, $P = 0.007$, respectively). Another significant association was found between daily stool frequency and low body weight ($r = 0.455$, $P = 0.010$). As expected, there was a positive relationship between daily stool frequency and patients' hematocrit levels.

Some typical correlations were separately observed among immunoglobulin levels and lymphocyte subsets (data not shown). Neither the percentage nor the absolute count of CD19+ B cells was correlated with immunoglobulin levels. However, IgG levels were negatively correlated with the percentages of CD3+ and CD8+ T cells ($r = 0.508$, $P = 0.004$; $r = 0.530$, $P = 0.002$; respectively). Accordingly, there was a positive correlation between IgG levels and CD4/CD8 T cell ratio ($r = 0.380$, $P = 0.035$). The effect of CD4/CD8 ratio on IgG production was described with the simple linear regression model and was found to be statistically significant (Figure 1a). On the other hand, serum B12 levels were positively correlated with the percentage of CD3+ T cells ($r = 0.639$, $P = 0.006$), but negatively correlated with serum IgM levels and the percentage of CD19+ B cells ($r = 0.513$, $P = 0.035$; $r = 0.566$, $P = 0.018$, respectively). However, B12 levels were found to have an effect only on the percentage of CD3+ T cells according to the simple linear regression model (Figure 1b).

3.2. Comparisons of patient subgroups

There were no statistically significant differences between male and female patients regarding the clinical characteristics except for one. Urinary tract infection was significantly more frequent in female patients than in male patients (75% vs. 21.1%: $P = 0.008$). Diagnostic delay was predominant in the patients who were educated at a primary school level compared to the patients who were educated at a high school level. On the other hand, neutrophilic dermatoses in better educated patients were more frequent than in less educated patients. However, these differences were not statistically significant (data not shown).

Table 3. The laboratory measurements of the patients with CVID.

Quantitative tests	n	Median (range)	Reference ranges
Leukocyte differential count			
WBCs (cells/μL)	31	7700 (23,500)	(4100–11,200)
Lymphocytes (%)	31	28.1 (42.6)	(18.8–50.8)
Lymphocytes (cells/μL)	31	1780 (4830)	(1200–3600)
Monocytes (%)	31	6.2 (7.5)	(2.0–12.2)
Monocytes (cells/μL)	31	372 (1126)	(300–900)
Granulocytes (%)	31	62.3 (44.2)	(40.9–79.3)
Granulocytes (cells/μL)	31	4203 (21,730)	(1900–7100)
Biochemical tests			
AST (U/L)	31	25 (50)	(10–35)
ALT (U/L)	31	19 (73)	(10–35)
ALP (U/L)	31	124 (293)	(30–120)
GGT (U/L)	31	28 (75)	(7–38)
B12 (pg/mL)	31	349 (529)	(197–866)
Qualitative tests	n [a]	%	References
Anti-TPO [b]	4	19	Negative
Viral markers			
HBsAg	0	0	Negative
Anti-HCV	0	0	Negative
Anti-HIV	0	0	Negative
Anti-HBs [c]	4	12.9	Negative
Blood groups			
A	11	35.5	NA
B	7	22.6	NA
AB	3	9.7	NA
O	10	32.3	NA
Isohemagglutinin titer [d]	21[e]	75	1/32–1/512

Anti-TPO: Thyroid peroxidase antibody; HBsAg: hepatitis B surface antigen; Anti-HCV: hepatitis C antibody; Anti-HIV: human immunodeficiency virus antibody; Anti-HBs: hepatitis B antibody; NA: not applicable.

[a] The number of patients who had positive test results. [b] Anti-TPO was investigated in 21 patients. [c] The patients with anti-HBs positivity had no history of vaccination. While 4 anti-HBs-positive patients were diagnosed in other centers and 3 of them were under IVIG treatment, the remaining 3 patients had positive serology for this marker before IVIG treatment. [d] Isohemagglutinin titer was measured in 28 patients who had O, A, or B blood groups. [e] The number of patients who had a low isohemagglutinin titer (<1/32).

Table 4. The immunological measurements of the patients with CVID.

Immunological tests	n	Median (range)	n (%) [a]	Reference ranges
IgM (g/L)	31	0.2 (3.7)	22 (70.9%)	(0.46–3.04)
IgA (g/L)	31	0.2 (2.2)	29 (93.5 %)	(0.82–4.53)
IgG (g/L)	31	4.6 (6.4)	31 (100 %)	(7.51–15.6)
IgE (IU/mL)	31	6 (115)	-	(0–85)
C3c (g/L)	31	1.2 (1.3)	3 (9.6 %)	(0.79–1.52)
C4 (g/L)	31	0.2 (0.5)	4 (12.9 %)	(0.16–0.38)
CD3 (%)	31	78.1 (36.3)	3 (9.6)	(63–85)
CD3 (cells/μL)	23	1101 (1848)	3 (13.0)	(700–2100)
CD4 (%)	31	30.8 (48.9)	19 (61.2)	(4–64)
CD4 (cells/μL)	23	370 (1543)	9 (39.1)	(300–1400)
CD8 (%)	31	38.7 (58.1)	1 (3.2)	(19–48)
CD8 (cells/μL)	23	677 (1851)	2 (8.6)	(200–900)
CD4/CD8 (%)	31	0.7 (2.3)	16 (51.6)	(0.8–3.3)
CD19 (%)	31	6.3 (23.8)	15 (48.3)	(7–23)
CD19 (cells/μL)	23	112 (2870)	10 (43.4)	(100–500)
CD3-CD16+56+ (%)	31	9.1 (26.9)	9 (29.0)	(6–29)
CD3-CD16+56+ (cells/μL)	23	139 (288)	3 (13.0)	(78–470)
CD3+HLADR+ (%)	31	6.3 (36.7)	7 (22.5)	(3.6–25.9)
CD3+HLADR+ (cells/μL)	23	145 (686)	-	(30–500)
CD45 (%)	31	95.6 (9.3)	-	(88–100)

[a] : Patients had decreased levels according to reference ranges.

No statistically significant difference was found between male and female patients with CVID regarding the measurements of leukocyte differential counts, biochemical tests, and immunological parameters. The comparisons of parametric tests were also not significantly different from one another among the clinical subgroups of the patients according to clinical characteristics, except for two. The first subgroup, including the patients with low body weight, had a lower CD4/CD8 T cell ratio than the subgroup with normal body weight [median (range): 0.4 (2.2) vs. 1.2 (2.3)] (Figure 2a). The second one, the patients with hepatomegaly, had higher AST and GGT levels than those without hepatomegaly [median (range): 39 (44) vs. 23 (34); 57 (65) vs. 25 (63); respectively] (Figures 2b and 2c).

The patients with a low percentage of CD19+ B cells (SG-1) had lower IgM levels but a higher percentage of CD3+HLADR+ T cells than the remaining patients (Table 5; Figures 2d and 2e). In addition, a lower level of IgG and lower percentage of CD19+ B cells but a higher percentage of CD3+HLADR+ T cells were found in the patients who had a low CD4/CD8 ratio (SG-3) than the remaining patients (Table 5; Figures 2f–2h). The differences in the comparisons of other nonparametric and parametric test results did not reach statistical significance between these patient subgroups.

4. Discussion

CVID is a prevalent form of PID in Caucasian populations (8). All of our patients were Caucasian and originated from different regions of Turkey. While parental consanguinity in our patients was found to be 12.9%, this rate was found to be 5.4%, 7%, and 8% in the studies reported by Oksenhendler et al., Malphettes et al., and Aghamohammadi et al., respectively (9–11). The highest percentage for consanguineous marriage was given as 30% in Ardeniz et al.'s study (12).

In Carvalho et al.'s study, the ages at diagnosis and the first symptoms were respectively found to be 22 and 12 years in patients with CVID (13), similar to those of our patients. In Ardeniz et al.'s study, the median values of age at diagnosis and delay in diagnosis were respectively 33 (range: 17–73) years for females and 28 (range: 13–49)

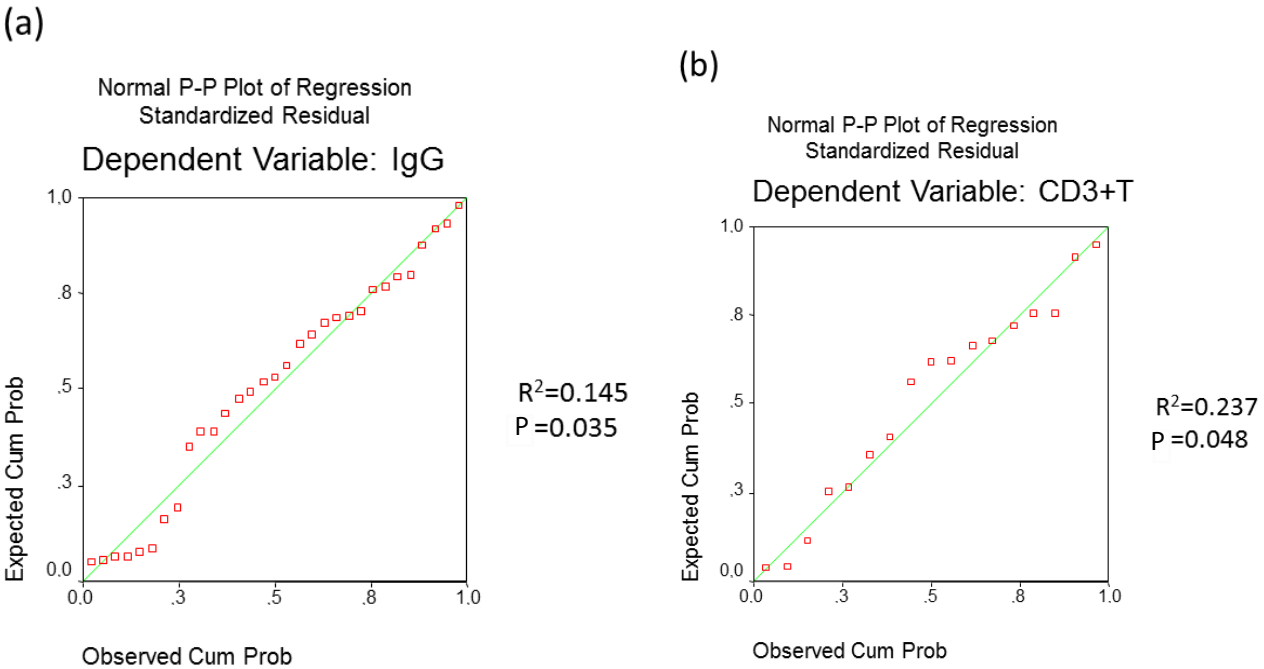

Figure 1. P-P plots of the relationships between IgG levels and the ratio of CD4/CD8 (a) and B12 levels and the percentage of CD3+ T cells (b).

years for males, and 15 (range: 1–32) years for females and 8 (range: 1–31) years for males (12). Similarly, these parameters were respectively 47 (range: 13–72) years vs. 30 (range: 5–56) years in females and males, and 7.0 (range: 1–45) vs. 3.0 (range: 0.8–30) years in females and males in Thickett et al.'s study (14). The findings of the last two studies including a small number of patients are comparable to those of the EUROClass trial and the DEFI study including large series (4,9).

Since the clinical spectrum of CVID varies from mild to severe, some cases are easily missed by physicians. However, awareness of this disorder is also at undesired levels among people in general regardless of the level of development of their countries (15). Therefore, we categorized our patients into 2 educational levels and compared them with each other with regard to diagnostic delay. However, we could not find any significant difference between the patient subgroups.

The dose of IVIG used for the treatment of our patients was changed between 400 and 600 mg/kg (median: 600 mg/kg) every 3 to 4 weeks. The dose was adjusted based on recurrence of infection and the IgG levels of our patients. In some reports from a single-center case series, IVIG dosage and the range of frequencies were similar to those of our study (11,12,14). However, the data of multicenter cohorts (EUROClass and DEFI) showed that various doses of IVIG (median: 129–750 mg/kg monthly) have been used for CVID treatment (4,9).

Prophylactic antibiotic therapy was required in 41.9% of our patients who had persistent infections despite IVIG therapy. As in our study, 43% of the patients in Thickett et al.'s study were given antibiotic therapy in continuously or monthly rotating manners (14). However, the rate of prophylactic antibiotic usage of the patients with CVID in Ardeniz et al.'s study was found to be 21.7% (12). No additional major surgery was needed in our patients after the optimal dose of IVIG therapy and/or antibiotic prophylaxis.

The frequencies of clinical characteristics of our patients on admission were either slightly or considerably different from those of other reports (9,12,13). Briefly, in these reports and the current study, the ranges of frequencies of sinusitis, otitis, and pneumonia are between 63% and 91.3%, 35.2% and 91.3%, and 58% and 64.5%, respectively. Patients with chronic diarrhea (without weight loss and/or malabsorption) were more frequent in our study and in that of Ardeniz et al. (38.7%, 52.1%) than in those of Oksenhendler et al. and Carvalho et al. (23%, 29%). As in the other reports, *Giardia* was found to be the main causative agent in our patients with chronic diarrhea (8). Since these clues are acquired from the patients by investigating standard signs, these data mostly depend on subjective information and the physician's experience. We therefore think that this may be a reason for the differences in the frequencies of the clinical signs noted in the literature.

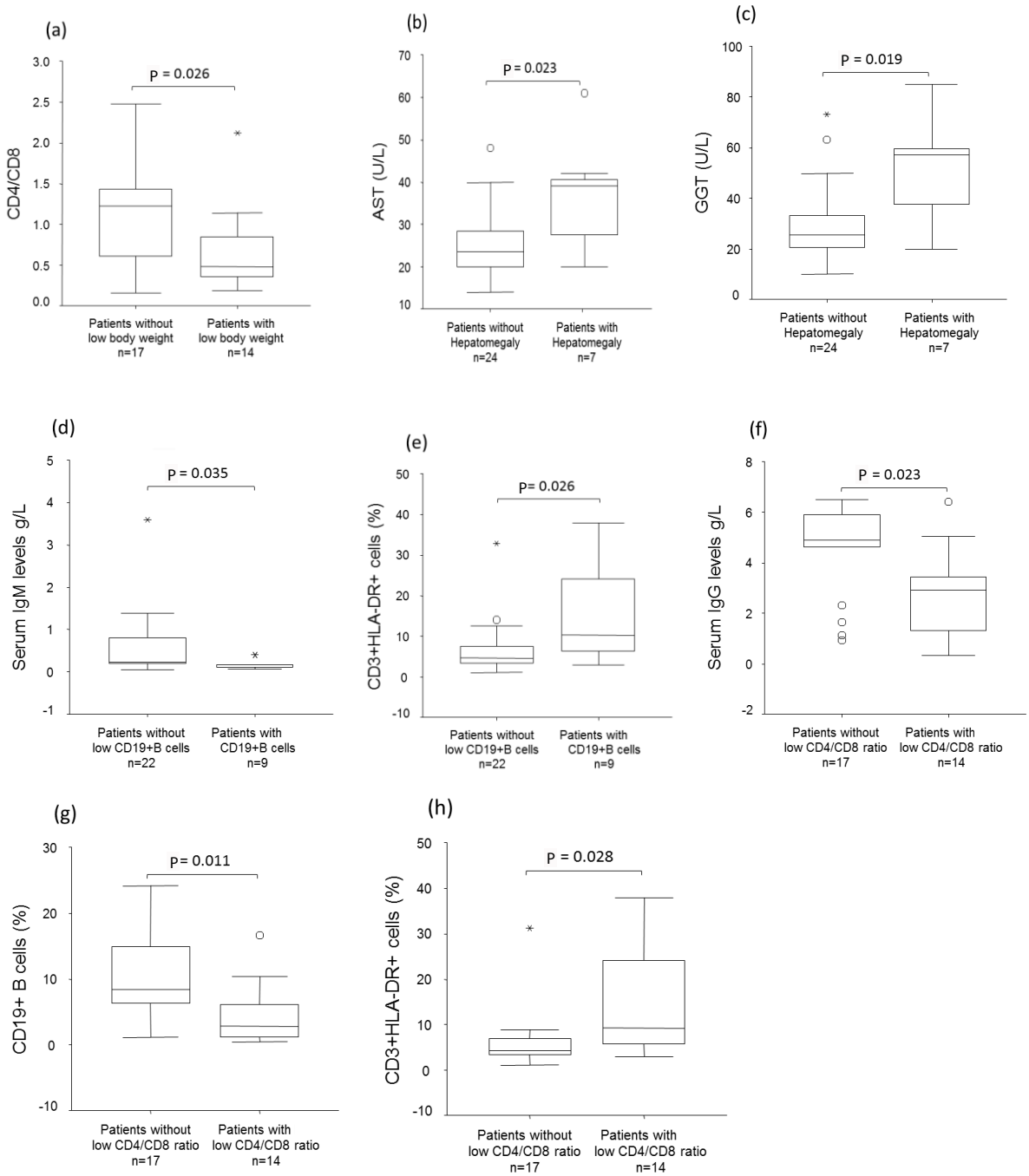

Figure 2. The differences in laboratory measurements between subgroups of patient with CVID. Patients were subdivided according to clinical or immunophenotypic properties. Accordingly, (a) shows the comparisons of patients with or without low body weight; (b) and (c) show comparisons of patients with or without hepatomegaly; (d) and (e) show comparisons of the patients with or without low percentage of CD19+ B cells; and (f), (g), and (h) show the comparisons of the patients with or without low CD4/CD8 ratio. Boxes show the ranges of 1st and 3rd quartiles and extreme values. Horizontal bars represent median values. The differences between two groups were evaluated by the Mann–Whitney U test. P-values are indicated above the boxes when a level of significance of $P < 0.05$ was reached in the comparisons of study groups. SG: Subgroup.

We indeed found some additional clinical signs, frequencies of which were nonnegligible in the course of CVID. The main ones were urinary tract infections, various dermatosis, oral aphthae, and meningitis. In the current study, the female patients had a higher frequency of urinary tract infection than male patients,

Table 5. Comparisons between the subgroups of patients with CVID.

	SG-1	SG-2	P	SG-3	SG-4	P
	n = 9	n = 22		n = 14	n = 17	
IgM (g/L)	0.17 (0.35)	0.23 (3.76)	**0.035**	0.18 (3.76)	0.21 (1.09)	0.164
IgA (g/L)	0.22 (0.46)	0.24 (2.17)	0.930	0.21 (0.59)	0.25 (2.17)	0.196
IgG (g/L)	2.7 (5.48)	4.7 (6.17)	0.407	2.9 (6.47)	4.9 (5.18)	**0.023**
CD3 (%)	84.4 (21.4)	75.8 (36.3)	0.016 [a]	83.6 (28.4)	75.2 (34.3)	0.059
CD4 (%)	22.1 (38.0)	32.3 (48.9)	0.408	21.5 (23.7)	37.1 (40.0)	<0.001 [a]
CD8 (%)	52.0 (31.2)	35.0 (58.1)	0.052	54.3 (40.2)	32.6 (28.6)	<0.001 [a]
CD4/CD8 (%)	0.42 (1.18)	0.94 (2.31)	0.107	0.40 (0.46)	1.26 (1.93)	<0.001 [a]
CD19 (%)	1.2 (1.7)	8.6 (20.6)	<0.001 [a]	2.8 (16.2)	8.4 (23)	**0.011**
CD3+HLADR+ (%)	10.3 (34.8)	4.6 (31.7)	**0.026**	9.3 (34.8)	4.3 (31.7)	**0.028**

Values are given as median (range). The patients were divided into the subgroups (SGs) according to their percentage of CD19+ B cells or CD4/CD8 ratio. While SG-1 and SG-2 included patients with or without a low percentage of CD19+ B cells, respectively, SG-3 and SG-4 included patients with or without a low CD4/CD8 ratio. [a] : Natural results for immunophenotyping due to using a gating strategy in flow cytometry. Values are given in bold when the level of significance is less than 0.05 in comparisons of study groups.

as in a healthy population (16). In Patuzzo et al.'s study, the frequencies were found to be 30.4% for urinary tract infection and 4.3% for both lichen planus and meningitis (17). While 2 patients (8.6%) had measles encephalitis and pneumococcal meningitis history in Ardeniz et al.'s study, the frequency of meningitis was 8% in Oksenhendler et al.'s study (9,12). One patient (4.3%) also had perivascular dermatitis in the former study. While we predominantly identified neutrophilic dermatosis in our patients, autoimmune disorders such as vitiligo and alopecia were reported as the most common forms of skin involvement in CVID in other reports (5,8). Notably, there are only 3 reports in the literature showing the relationship between CVID and aphthous-like ulceration (18–20). The latest one was published by Meighani et al., who reported that 5 patients with CVID (23.8%) in their study had oral aphthae (20).-

We found that the most common comorbidity associated with CVID was chronic inflammatory diseases of the gastrointestinal tract, followed by autoimmune thyroiditis and various malignant disorders in remission. These conditions developing in the course of CVID were already shown in many previous studies (21). The frequency of autoimmune thyroid disease shown by anti-TPO was 19% in our patients. The frequencies of thyroid autoantibodies were detected in a wide range in other studies on CVID (5,9,17,21). Although autoimmune cytopenia is one of the most common comorbidities associated with CVID, we diagnosed ITP only in 1 patient. It was reported in a multicenter study by Chapel et al. that the prevalence of autoimmune cytopenias in their study group was 7% for ITP, 4% for autoimmune hemolytic anemia (AIHA), and 1% for neutropenia (22). The EUROClass study group showed that the prevalence of autoimmune cytopenia was 20.2% in their patients (4).

As in other reports, chronic complications of CVID involving the lungs, spleen, lymph nodes, and/or liver were observed in some of our patients. Bronchiectasis frequency in our patients was close to that in the patients of Thickett et al. (61.2% vs. 68.1%) (14), but significantly higher than those in the patients of Oksenhendler et al. (37%), Patuzzo et al. (26.1%), and Chapel et al. (25.1%) (9,17,22). Similarly, the rate of patients with splenomegaly in our study was 61.3%, but rates were 38%, 30.4%, and 30% in the former 3 studies, respectively (9,17,22), and 40.5% in Wehr et al.'s study (4). We found the highest lymphadenopathy frequency in our patients (48.4%) as compared to those of Wehr et al., Chapel et al., and Patuzzo et al. (26.2%, 30%, and 43.4%, respectively) (4,17,22). The frequency of hepatomegaly that we found in our patients was also the highest compared to those that other studies found, which were between 0% and 15% (22).

In our study, splenomegaly and hepatomegaly either coexisted in the same patient or existed separately. In addition, enlargements of these organs were associated with each other, low body weight, and chronic complications of the gastrointestinal tract. There were also positive relationships between the last 2 features

and between daily stool frequency and low body weight. Ward et al. found similar results in their study suggesting that splenomegaly and hepatomegaly developed either jointly or separately in patients with CVID (23). They also showed a strong association between nodular regenerative hyperplasia of the liver and lymphoproliferation in their patients. Contrary to our findings, in Ghoshal et al.'s report, although all patients with hypogammaglobulinemia had diarrhea and weight loss, no significant association was determined between daily stool frequency and low body weight (24).

Histopathologic findings in biopsy specimens taken from the gastrointestinal tract of our patients were similar to those in other reports showing lymphoid hyperplasia, villous atrophy, and lymphocytic infiltration in the lamina propria (9,22).-

Neither viral hepatitis nor HIV infection was found in our patients. Viral hepatitis induced by hepatitis C virus, but not hepatitis B virus, was reported in patients with CVID in the studies by Oksenhendler et al. and Ardeniz et al. (9,12).

In our study, the levels of 3 major immunoglobulin isotypes in the sera of patients with CVID were significantly lower than the lower limits of their ranges. In the EUROClass trial, IgG, IgA, and IgM levels were found to be significantly decreased in CVID patients, but the percentages of the patients with low/undetectable IgA and IgM levels were higher than those of our patients (50% vs. 29%, 79% vs. 22%, respectively). The results of the DEFI trial are compatible with these results, stating that respectively 41%, 40%, and 15% of patients with CVID at diagnosis had decreased IgG, IgA, and IgM levels according to certain reference ranges (9).

No relation was shown between immunoglobulin levels and clinical findings in our study. A similar finding was reported by Chapel et al., stating that very low serum IgG levels are not associated with increased numbers of severe infections prior to diagnosis (22).

We mainly found decreased percentages of CD4+ T cells in our patients. Accordingly, the CD4/CD8 ratio was lower than its lower reference limit. T-cell subset alteration in CVID was previously shown in Ardeniz et al.'s study, stating that 20 cases had decreased CD4+ T cell count but 6 had increased CD4+ T cell count (12). In another study, Batman et al. reported that a significant reduction in the number of naive CD4 T cells existed in CVID patients due to the lack of the replenishment of the CD4 T-cell pool by new thymic-derived cells (25). In an older study, Giovannetti et al. mentioned the contraction of CD4 naive T-cell pools in CVID by reduced thymic output and increased T-cell turnover and apoptosis. The results of these studies seem to be comparable to ours and strongly explain the reason for the inversion of the CD4/CD8 ratio (26).

In addition, serum IgG levels of our patients were positively correlated with the CD4/CD8 ratio but negatively correlated with CD8+ T cells. This relationship may be contributed to impaired IgG production besides defective molecular interaction between T cells and B cells as shown in the investigations by de Witt et al. and Bouaziz et al. (27,28). Therefore, we can speculate that the CD4/CD8 ratio is a risk factor for poor outcome of CVID via the suppression of humoral immunity. The ratio of CD4+ T cells to CD8+ T cells was low in our patients with a low body weight. These data are new knowledge for CVID and similar findings were reported in studies in which patients with HIV infection were the subject of research (29).

We observed that the patients with CVID had a decreased percentage of B cells and about half of them had a decreased B-cell percentage and/or absolute count. This is an important point because patients with CVID in large cohorts were classified into subgroups based on clinical course and B-cell percentage and then B-cell subsets (4).

In the current study, although the percentages and absolute counts of NK cells and activated T cells were within the normal limits, these cell subsets were found to be low in some patients with CVID. A similar result was earlier reported by Aspalter et al., suggesting that the patients had reduced absolute CD3-CD16+ NK cell numbers compared with normal subjects (30).

Decreased B cells associated with decreased IgM production but increased T-cell activity were found in our CVID patients (SG-1). Additionally, the patients with a diminished CD4/C8 ratio (SG-3) had low IgG production and B cells but increased T cell activity. In other words, on the one hand, both humoral and cellular immune responses were weakened in the patients with CVID. On the other hand, their T cells were on alert. This means that immune activation is maintained in CVID patients at a certain level and insidiously leads to chronic complications of the disease. Baumert et al. earlier categorized patients with CVID based on the CD4/CD8 ratio and found that the patients with a low ratio had increased CD4+ T cells bearing the activation marker HLADR (31). The study by Savasan et al. supports this hypothesis that Fas-mediated elimination of activated T lymphocytes was shown in children with Evans syndrome who had hypogammaglobulinemia (32). It is well known that Evans syndrome is characterized by ITP and AIHA, and it is one of the long-term complications resulting from immune dysregulation in CVID (33).

We found that serum B12 levels were related to an increased T-cell percentage, but decreased serum IgM levels and B-cell percentages. Tamura et al. reported decreased T cell subsets in B12 deficiency and emphasized the regulatory role of B12 on cellular immunity (34). On the other hand, it is well known that increased

serum B12 levels are reliable biomarkers of autoimmune lymphoproliferative syndrome (ALPS) and can be used to differentiate between ALPS and CVID (35,36). However, Roberts et al. also found increased serum B12 levels in CVID patients compared to healthy controls (36). We investigated the possible relationship between lymphoproliferation and serum B12 levels but failed to show one.

In our study, the liver function test results for AST and GGT were higher in the patients with hepatomegaly than in those without hepatomegaly. In Ward et al.'s study, abnormal liver function tests (especially for ALP) and hepatomegaly were present together in some patients with CVID and were associated with elevated GGT (23).

In conclusion, CVID has many clinical and laboratory signs having logical relationships with one another. The clinical presentations of CVID are more complex in adults than in children. Sometimes the disease insidiously progresses and threatens patients' life. The chronic complications of CVID and associated comorbid conditions determine the severity and prognosis of the disease. Therefore, all of these associations should be clarified for solving the pathogenesis of CVID and its effective treatment. For this purpose, authors should share and discuss their own experiences with CVID by comparing them with the data obtained from all other studies. On the other hand, every country should establish its own database and bring it into use for clinical immunologists as soon as possible. It should not be forgotten that this insidious disease is more common and dangerous than estimated, especially in adulthood. An early and accurate diagnosis of CVID can improve quality of life, reduce the morbidity and mortality of patients, and prevent economic losses due to wrong treatments.

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