Clinical features and immunoglobulin replacement therapy outcomes of adults with common variable immunodeficiency: a single centre experience

Abstract

Background/aim: Common variable immunodeficiency (CVID) characterized by defective immunoglobulin production is the most prevalent form of symptomatic primary immunodeficiency (PID) in adults. We aimed to reveal the clinical features of adults with CVID and to evaluate the effects of immunoglobulin replacement treatment (IRT) on hemato-immunological findings.

Materials and methods: This study included 26 adult patients receiving IRT. Two measurements of complete blood counts and major immunoglobulin levels measured at the beginning-end of follow-up period were used for comparisons. Lymphocyte subsets and B-cell subgroups were measured only at the time of presentation**.**

Results: The most common complications were related to respiratory and digestive systems, and organomegaly. Chronic diarrhoea and low body weight were positively correlated with the percentage of $CD8^+$ T cells (P = 0.019 and P = 0.003 respectively) but negatively correlated with the CD4/CD8 ratio and the percentage of $CD19^+$ B cells (P = 0.019 and P = 0.005 for both parameters, respectively). At the end of period, the distribution of haematological parameters significantly improved, and immunoglobulin M (IgM) level increased to detectable levels (P = 0.035).

Conclusions: There are apparent relationships among chronic diarrhoea and low body weight, and deterioration of T and B cell immunity in adults with CVID. IRT improves the whole blood parameters and stimulates IgM production. The later effect supports the immunomodulatory feature of this therapy.

Key Words: Common variable immunodeficiency, intravenous immunoglobulins, diarrhoea

1. Introduction

Common variable immunodeficiency (CVID) characterized with antibody deficiencies is the most common symptomatic primary immune deficiency (PID) in adults [1]. Heterogeneous genetic and phenotypic features of CVID make it difficult to classify this disease. The European Society of Immune Deficiencies (ESID) diagnostic criteria (2014) were used for the diagnosis of CVID. Accordingly, in absence of any other secondary immunodeficiency state, a patient with markedly reduced serum concentrations of immunoglobulin G (IgG) in combination with low level of immunoglobulin A (IgA) and/or immunoglobulin M (IgM), and weak or absent response to immunizations is diagnosed as CVID [2]. This disorder is characterized by recurrent infections (primarily sinopulmonary, gastrointestinal, septic arthritis, meningitis, sepsis) and immune dysregulation leading to autoimmunity including autoimmune haemolytic anaemia (AIHA), immune thrombocytopenia (ITP), rheumatoid arthritis and rheumatoid-like arthritis, pernicious anaemia, autoimmune thyroiditis, and vitiligo, in addition to a variety of inflammatory disorders, granulomatous diseases, allergic diseases and malignancies (non-Hodgkin lymphoma, gastric cancer).

The mainstay of treatment is immunoglobulin replacement for those patients who have substantial impairments in its production and non-responsive to both protein and polysaccharide vaccines [3]. Despite its high cost, immunoglobulin replacement therapy can decrease the burden of recurrent infections and their complications. The commercial preparations used for this purpose consist of immunoglobulins, most commonly contain IgG, purified from pooled human plasma. Immunoglobulin preparations are referred to

by the route of administration; intravenous immunoglobulin (IVIG) or subcutaneous immunoglobulin (SCIG). These parenteral preparations predominantly contain purified polyvalent IgG (≥ 95%) with a physiological subclass distribution, and a very small amounts of IgA, trace amounts of IgM, immunoglobulin E (IgE), cytokines, some soluble molecules spilled from cell surface [human leukocyte antigens (HLA), cluster of differentiation molecules: CD4 and CD8), and adjuvants for stabilization of IgG molecules [3,4].

The IVIG dose is titrated according to treatment purposes, such as replacement or immunomodulation. The mechanism of action of IVIG is overly complex. The immunodeficient patients are treated with replacement level of IVIG (400 - 600 mg/kg), whereas the patients with autoimmune and inflammatory diseases are administered high doses of IVIG (1 - 2 g/kg) [3,4]. Intravenous immunoglobulin provides adequate diversity and concentrations of antibodies against a broad range of pathogens for clearance of infections in patients with hypogammaglobinaemia and other immunodeficiency states. Also, IVIG has many immunosuppressive and anti-inflammatory effects, including modulation of immunoglobulin production, lymphocyte and dendritic cell functions, Fc receptor expressions and functions, cytokine production, complement regulation, and clearance of pathogenic IgG at high doses [5,6]. In the literature, few studies have investigated the effects of these actions on clinical parameters in CVID patients. Therefore, the present study aimed to reveal the clinical features of adults with CVID and to evaluate the effects of immunoglobulin replacement treatment on some haemato-immunological findings.

2. Materials and methods

2.1. Patients

The medical records of 26 adult patients with CVID (19 females, 7 males) that presented to our outpatient clinic between October 2017 and June 2020 were retrospectively reviewed. At the time of presentation, 8 of the patients had been previously diagnosed with CVID at another institution and initiated IVIG treatment, whereas the other 18 patients were diagnosed at our outpatient clinic following their clinical evaluation and initiated IVIG treatment. The European Society of Immune Deficiencies diagnostic criteria (2014) were used for the diagnosis of CVID.

Demographic features, and all clinical and laboratory characteristics were obtained from the patients' files. Ideal body weight (IBW) in each patient was calculated using the IBW formula: 50 kg + 0.9 kg for every cm > 152 cm (- 4.5 kg if female) [7]. Weight below the IBW was considered low body weight. At the time this manuscript was prepared, all patients were still receiving IVIG treatment and were under regular follow-up. None of the patients received immunosuppressive therapy during follow up. However, various antibiotics had been used to treat or prevent the infections in all patients whenever they were needed.

The study protocol was approved by Institutional Review Board of our centre. Written informed consent was received form all the participants, and the study was conducted in accordance with the Declaration of Helsinki.

2.2. Standard haematological parameters

Standard haematological parameters [complete blood count (CBC)] were measured using an automated haematology analyser (Cell-Dyn Ruby, Abbot Diagnostics, Santa Clara, CA, USA). The reference ranges provided by the manufacturer were used for interpretation of each test result.

2.3. Immunological tests

While the levels of IgG, IgA, and IgM in sera were measured using Clinical Chemistry Analyzer (Architect C8000, Abbot Diagnostics, Santa Clara, CA, USA), the levels of total IgE were measured using IMMULITE® 2000 CLEIA system (chemiluminescent enzyme immunoassay) (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Reference ranges for immunoglobulin measurements are respectively: IgG: 7.1 - 16 g/L, IgA: 0.7 - 5.2 g/L, IgM: 0.3 - 2.9 g/L, and total IgE < 87 IU/mL.

Lymphocyte subsets and B cell subgroups were analysed in peripheral blood samples with EDTA using Flow Cytometry (Becton, Dickinson and Company, BD Biosciences, FACS Canto II system). As per the manufacturers' instructions, kits compatible with the devices were used for these analyses. BD Multitest^{TM} 6 - Color TBNK reagent (Becton, Dickinson and Company, BD Biosciences, San Jose, CA, USA) was used for detection of the percentages of T, B, and natural killer (NK) cells as well as the CD4 and CD8 subpopulations of T cells in peripheral blood. The reagents used for the analysis of B cell subgroups were supplied from the same manufacturer. For this purpose, a panel including fluorochrome labelled monoclonal antibodies against CD45 (APC - Cy7), CD19 (PerCP - Cy5-5), CD21 (PE), CD24 (PE - Cy7), CD27 (PE - Cy7), CD38 (FITC), IgM (APC), and IgD (Alexa Flour 700) markers were used.

In accordance with the panels, blood samples were prepared with a multicolour reagent in only one tube for lymphocyte subset analysis, while 2 tubes were used for the same procedure in B cell subgroup analysis. Before the analyses, 50 µl of reagents and 100 µl of blood samples drawn previously into tubes with EDTA were collected into a single tube, and the cells were labelled via incubation for 20 minutes in the dark and at room temperature (20°C – 25°C). At the end of this period, erythrocytes were lysed using

FACS lysing solution (Becton Dickinson Co.) and removed by washing with phosphate-buffered saline (PBS). Then, the tubes were prepared for analysis by the addition of PBS. Finally, analyses were made using the software (BD FACSCanto™ clinical software) pre-loaded on the flow cytometry device.

The complete blood counts and major immunoglobulin levels measured in the patients that had not any infection or active inflammation at the beginning and the end of follow up period were statistically compared with each other in order to determine the effect of IVIG treatment on haemato-immunological parameters. Lymphocyte subset analysis and B cell subgroups were only performed at presentation.

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v.21.0 (IBM Corp., Armonk, NY). The Kolmogorov-Smirnov test was used to determine the normality of the distribution of data. Wilcoxon Signed Ranks Test was used for comparison of 2 dependent variables, whereas Mann-Whitney U Test was used to compare 2 independent groups. In addition, the relationships between all variables were investigated using Spearman's rank test. A simple linear regression model was also used for the relationships between the variables. All directional P values were 2-tailed, and the level of statistical significance was set as $P < 0.05$. Statistical comparisons of clinical and laboratory parameters were made according to before and after follow up period of IVIG treatment.

3. Results

The baseline demographic features, and clinical characteristics of the patients are shown in Table 1. Mean age at onset of symptoms was 17.1 ± 11.8 years, mean age at diagnosis was 37.1 ± 15.7 years, and mean duration of delay to diagnosis was 19.9 ± 9.3 years. The

mean IVIG dose was 38.2 ± 5.1 gr for every 3 weeks. The frequency of consanguineous marriages in the patients' parents was 15.4% and the family history of cancer rate was 53.8%. In addition, the rate of smoking in the patients was 5%.

Most common complication was upper respiratory tract infections (100%), followed by gastritis/duodenitis (73.1%), pneumonia (65.4%), organomegaly [lymphadenopathy (57.7%), splenomegaly (46.2%), hepatomegaly (46.2%)], and diffuse nodular lymphoid hyperplasia (30.8%), respectively. In patients with a history of respiratory symptoms or pneumonia, lung screening with computed tomography was performed, and bronchiectasis was noted in 3 (11.5%), and 19 (73.1%) had pathological lung findings. Other complications are shown in Table 1. Some significant associations were naturally observed among enlargements of the lymphoid organs or lymphoid hyperplasia (data not shown). Low body weight was observed in 6 (23.1%) of the patients, of which all had a history of chronic diarrhoea; low body weight and diarrhoea were positively correlated ($R = 0.891$, $P < 0.001$). Low body weight was also positively correlated with splenomegaly ($R = 0.408$, $P = 0.038$), and diffuse nodular lymphoid hyperplasia ($R = 0.624$, $P = 0.001$). In compatible with these correlations, the gastrointestinal tract complications (splenomegaly, chronic diarrhoea, and nodular hyperplasia) were significantly more common in the patients with low body weight than in those with IBW (83.3% vs 35%, $P = 0.037$; 83.3% vs 0%, $P < 0.001$; 83.3% vs 15%, $P = 0.001$, respectively).

Haemato-immunological parameters measured before and after follow up period of IVIG treatment were given in Table 2. In terms of the correlation between low body weight and chronic diarrhoea, and standard haematological measurements (CBC), a negative correlation was noted between the erythrocyte count and these complications at

baseline (R = -0.444, P = 0.023; R = -0.423, P = 0.031; respectively). Low body weight and chronic diarrhoea were also correlated with some immunological measurements; both were positively correlated with an elevated percentage of $CD8^+$ T cells (R = 0.566, P = 0.003; R = 0.456, P = 0.019, respectively), but both were negatively correlated with the CD4/CD8 ratio and the percentage of $CD19^+$ B cells (R = -0.530, P = 0.005, R = -0.455, P = 0.019, respectively). Low body weight and chronic diarrhoea was not correlated with other CBC parameters. As shown in Figure 1, accordingly, the $CD8^+$ T cell rate was higher in the patients with low body weight (P = 0.005), but erythrocyte count, the CD4/CD8 ratio and $CD19^+$ B cell rate were lower, as compared with those with IBW, and these differences were significant (P = 0.026, P = 0.008, and P = 0.008 respectively).

There were also some significant relationships among the laboratory parameters. The haemoglobin and haematocrit levels were negatively correlated with the percentage of $CD8^+$ T cells (R = -0.499, P = 0.009; R = -0.488, P = 0.011, respectively), whereas both parameters were positively correlated with the CD4/CD8 ratio (R = 0.394, P = 0.046; R = 0.402, P = 0.042, respectively) and the percentage of $CD19^+$ B cells (R = 0.440, P = 0.024; R = 0.466, P = 0.022, respectively). As shown in Figure 2a - f, the effects of $CD8^+$ T cell rate, CD4/CD8 ratio and $CD19^+$ B cell rate on haemoglobin and haematocrit levels were described with the simple linear regression model and were found to be statistically significant. Additionally, $CD19^+$ B cell rate was negatively correlated with $CD8^+$ T cell rate (R = -0.782, P < 0.001), but positively correlated with CD4/CD8 ratio (R = 0.595, P = 0.001). As shown in Figure 2g and 2h, the effects of $CD8^+$ T cell rate and CD4/CD8 ratio on $CD19^+$ B cell rate were described with the simple linear regression model and were found to be statistically significant.

There were some alterations in standard haematological parameters and immunoglobulin levels at the end of the follow up period. As shown in Table 2 and Figure 3, increase in haemoglobin, haematocrit, erythrocyte count and lymphocyte percentage, and decrease in neutrophil count and percentage were statistically significant. Similarly, it was observed that IgM production increased significantly to measurable levels (P = 0.035) at the end of the follow-up period. However, IgA and IgE production was not changed after this period.

3.1. The comparisons of B cell subsets between the patient subgroups

B cell subset analysis was conducted in 13 of the CVID patients, of which 6 had gastrointestinal system (GIS) involvement, and 7 had prominent respiratory system symptoms and signs. All patients with GIS involvement had diffuse nodular lymphoid hyperplasia, especially in duodenum. As shown in Figure 4, the median percentage of $CD19^{+}$ B cells was significantly lower (3.1% [range: 6.0%] vs. 10.8% [range: 22.1%]) and the percentage of $CD21^{low}CD38^{-}$ B cells was significantly higher (12.8% [range: 10.2%) vs. 5% [range: 12%]) in the 6 patients with GIS involvement than in those the 7 with prominent respiratory system symptoms and signs. The other B cell subsets did not differ between these 2 patient subgroups (data not shown).

4. Discussion

CVID is the most common form of PID in Caucasian populations [8]. All our patients from different regions of country were Caucasian. The rate of parental consanguinity varies for CVID in different studies. For instance, this rate was reported to be 5.4%, 7%, and 8% by Oksenhendler et al., Malphettes et al., Aghamohammadi et al., respectively [9-11]. In addition, in 2 cohorts from Turkey the reported consanguinity rate was 30%

and 12.6% [12,13]. In the present study, the rate was 15.4% which is close to our previously reported rate [13].

In the present study, mean delay in diagnosis was 19.9 ± 9.3 years, which is comparable to our previous report of a median 14 years (range: 42) [13]. Data on diagnostic delay in CVID patients are similar across studies. The mean age at diagnosis and onset of symptoms in patients with CVID was, respectively, 22 years and 12 years in Carvalho et al.'s study [14]. Ardeniz et al. reported that the median age at diagnosis and median delay in diagnosis was 33 years (range: 17 - 73 years) in females and 28 years (range: 13 - 49 years) in males, and 15 years (range: 1 - 32 years) in females and 8 years (range: 1 - 31 years) in males, respectively [12].

In the present cohort, non-Hodgkin lymphoma (NHL) developed in only 1 patient during follow-up, but the family history of cancer was 53.8% in the cohort. It is well known that there is an increased risk of malignancy in CVID patients, particularly lymphoma (NHL is the most frequent malignancy), followed by epithelial tumors of the stomach, breast, bladder, and cervix; however, Mellemkjaer et al. reported that no increase in the overall risk of malignancy was observed in relatives of CVID patients [15,16]. The pathological mechanisms for increased risk of malignancy are not fully known; however, immune dysregulation, impaired clearance of oncogenic viruses, genetic predisposition, impaired genetic stability, and iatrogenic causes might contribute to the development of malignancy in CVID patients [17].

The frequencies of the clinical findings at presentation in the present study were slightly different than those reported in earlier Turkish studies [12,13]. Ardeniz et al. reported that the frequency of recurrent sinusitis and pneumonia was 91.3% and 61%,

respectively, versus 83.9% and 64.5%, respectively, according to our previous report [12,13]. In the present study, chronic sinusitis and pneumonia occurred in 38.5% and 65.4% of the patients, respectively. The differences in the frequencies of recurrent sinusitis and pneumonia between our previous and current studies may depend on different demographic characteristics of both cohorts. Ardeniz et al. observed that 52.1% of CVID patients had chronic diarrhoea (without weight loss and/or malabsorption), versus 23% and 29%, according to Oksenhendler et al. and Carvalho et al., respectively [9,12,14]. In contrast, the present study's 19.2% of patients with chronic diarrhoea had weight loss.

The chronic complications of CVID involving the lungs, spleen, lymph nodes, and/or liver were observed in some of the present study's patients. In all, 46.2%, of the present study's patients had splenomegaly, versus 82.6%, 61.3%, 38% according to Ardeniz et al., Musabak et al., and Oksenhendler et al., respectively [9,12,13]. The frequency of lymphadenopathy in the present study's patients was 57.7%, which is much higher than reported by Chapel et al. (30%) and Wehr et al. (26.2%) [18,19]. The higher rate of lymphadenopathy in the present study might have been due to routine use of ultrasonographic screening for organomegaly.

As the associations among the enlargement of lymphoid organs found in the present study, splenomegaly and hepatomegaly coexisted or existed separately in our previous study. In addition, enlargement of these organs was associated with each other, low body weight, and chronic GIS complications [13]. Among the most important findings of the present study is the association between low body weight and chronic GIS complications such as chronic diarrhoea, splenomegaly, and diffuse nodular lymphoid

hyperplasia. To the best of our knowledge only 1 earlier study reported similar findings as those in the present study all CVID patients with hypogammaglobinaemia had diarrhoea and weight loss, but there was not a significant correlation between daily stool frequency and low body weight [20].

Low body weight and chronic diarrhoea in the present study's CVID patients were inversely correlated with the erythrocyte count before the follow up period, which were lost at the end of this period. All the patients with low body weight had history of chronic diarrhoea. Malabsorption due to diarrhoea and chronic inflammation might have caused anaemia in these patients. In addition, the patients in the present study with low body weight had a high percentage of $CD8^+$ T cells and, accordingly, a low CD4/CD8 ratio. This subgroup of patients also had a low percentage of CD19+ B cells. These findings are compatible with our earlier study in which a low CD4/CD8 ratio and low percentage of $CD19^+$ B cells were considered to be risk factors for poor outcome in CVID patients due to suppression of humoral immunity [13]. To the best our knowledge the present study and our earlier study are the first to report that low body weight in CVID patients is associated with T cell and B cell immunity.

In addition, the levels of haemoglobin and haematocrit were inversely correlated with the percentage of $CD8^+$ T cells in the present cohort; accordingly, there were positive correlations between the CD4/CD8 ratio and these parameters. A positive association was also found between the percentage of $CD19^+$ B cells and the levels of haemoglobin and haematocrit. Additionally, $CD19^+$ B cell rate was negatively related to $CD8^+$ T cell rate, but positively related to CD4/CD8 ratio. Simple linear regression model has shown that the haemoglobin and haematocrit levels of the patients depend on the percentages of major T cell subsets and B cells in the peripheral blood, and the rates of major T cell

subsets influence on B cell rate. Briefly, these findings support the notion that cellular and humoral immune dysfunction is associated with antibody deficiency in CVID patients and may be a risk factor for anaemia due to malabsorption.

In the present study, haemoglobin, haematocrit, and erythrocyte count improved with IVIG treatment at the end of follow up period. In addition, there was an increase in lymphocyte ratio, versus a decrease in the neutrophil count and neutrophil ratio. These findings indicate that IVIG treatment declines the systemic inflammation by immunomodulation even at replacement doses. There was not an any patient that haemolysis developed due to IVIG treatment. It is well known that while haemolysis occurs with high dose IVIG especially used in autoimmune diseases, this complication rarely occurs in the replacement doses [21].

Usually, all 3 immunoglobulin classes were reduced and/or undetectable in our patients. In the EURO Class trial, the IgG level significantly decreased in CVID patients, and the percentage of patients with low/undetectable IgA and IgM levels was similar to that observed in the present study [19]. All the CVID patients in the present study had a low serum IgG level before the follow up period. Although the serum IgG level in these patients naturally increased by regular IVIG therapy, the change in the IgA level was not significant; however, the serum IgM level significantly increased to detectable levels at the end of the follow up period. Salehzadeh et al. observed that the IgM and IgA levels were stable over time after IVIG treatment [22].

The rate of a detectable IgE level increased in the present study after the follow up period, but not significantly. Although earlier studies have suggested that the IgE level can be low in CVID patients, work up for patients with recurrent infections and suspected

hypogammaglobinaemia does not include routine measurement of serum IgE [23]. Lawrence et al. reported that the frequency of an IgE level below the lower limit of detection (2 IU/mL) was 74.0%, and the frequency of an IgE level below the lower limit of normal was 93.4%. They also noted that a serum IgE level < 2 IU/mL was observed in only 3.3% (95% CI: 1.9 - 5.7) of the general population, but the calculated pooled estimate of an undetectable IgE level in CVID patients based on a random effect meta-analysis was 75.6% (95% CI: 76.6 - 85.7). Furthermore, they posited that in immunoglobulin replacement treatment there is an insufficient quantity of IgE to change the total serum IgE level.

The percentage of $CD19^{+}$ B cells were lower in present study's CVID patients that had GIS involvement with nodular lymphoid hyperplasia than in those with respiratory system involvement. In addition, the percentage of $CD21^{low}CD38^{-}$ B cells were higher in the first subgroup of patients than in the later subgroup of patients. The $CD21^{-/low}$ B cell subgroup constitutes more than 20% of total B cells in peripheral blood and its frequency is often increased in CVID patients [24]. Moreover, this subgroup of B cells is implicated in autoimmunity. As CVID patients are prone to chronic inflammatory disorders, it is reasonable to think that the profile of B cell subsets might play a role in the development of inflammation in gastrointestinal tract [25].

The main limitation of our study is the small number of total patients included in the study. In addition, the fact that some patients referred to our outpatient clinic from external centers had already started IVIG treatment and had not performed flow cytometric tests at the time of diagnosis were important handicaps. On the other hand, some patients who applied to our hospital for the first time did not have health insurance. Therefore, flow cytometric tests, which had high costs, could only be paid once by these

patients. Thus, the number of patients who had 2 measurements for lymphocyte subsets and B cell subgroups at the beginning and end of the follow-up period was not sufficient to make a strong statistical comparison and to obtain a reliable p-value. Similarly, the effect of gender difference on the measured parameters could not be evaluated statistically due to the small number of male patients in our cohort.

In conclusion, the systemic inflammation is declined by IVIG treatment even at replacement doses. The most important finding of the present study is the evidence of clear associations between chronic diarrhoea and low body weight, and deterioration of T and B cell immunity. In addition, cellular and/or humoral immune dysfunction before IVIG treatment might result in anaemia due to malabsorption. Because of these, the CVID patients with low body weight and chronic diarrhoea should be carefully evaluated and managed as a separate subgroup of CVID. The course of disease in this patient subgroup is not as good as in those with the other form of the disorder. Another important finding of the present study is that IVIG therapy had stimulated IgM production at the end of the follow up period in the CVID patients. This finding supports that there is an immunomodulatory effect of immunoglobulin replacement therapy. However, more comprehensive, and multicentre studies conducting in phenotypically different CVID subgroups are needed to obtain more accurate and valid data.

Acknowledgement

The authors thank the technicians working in the flow cytometry laboratory: Şale Şirvan, Dilek Ünalan, Hanife Haberal Bal, and Sedef Işık.

Conflict of interest

The authors of this article declare no conflict of interest that may have influenced either the conduct or the presentation of the research. No financial support for the research was received.

References

1. Abbott JK, Gelfand EW. Common variable immunodeficiency: diagnosis, management, and treatment. Immunology and Allergy Clinics of North America 2015; 35 (4): 637–658. doi: 10.1016/j.iac.2015.07.009.
2. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clinical immunology 1999; 93 (3): 190–197. doi: 10.1006/clim.1999.4799.
3. Hooper AJ. The history and evolution of immunoglobulin products and their clinical indications. The Journal of Inherited Immune Disorders (LmyphoSign Journal) 2015; 2 (4): 181–194. doi.org/10.14785/lpsn-2014-0025.
4. Kazatchkine MD, Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immunoglobulin. The New England Journal of Medicine 2001; 345 (10): 747–755. doi: 10.1056/NEJMra993360.
5. Chaigne B, Mouthon L. Mechanisms of action of intravenous immunoglobulin. Transfusion and Apheresis Science 2016; 56 (1): 45–49. doi: 10.1016/j.transci.2016.12.017.
6. Perez EE, Orange JS, Bonilla F, Chinen J, Chinn IK, et al. Update on the use of immunoglobulin in human disease: A review of evidence. The Journal of Allergy and Clinical Immunology 2017; 139 (3S): 1–46. doi: 10.1016/j.jaci.2016.09.023.

7. Maple-Brown LJ, Lawton PD, Hughes JT, Sharma SK, Jones GR, et al. Study Protocol-accurate assessment of kidney function in Indigenous Australians: aims and methods of the eGFR study. BMC Public Health 2010; 10: 80. doi: 10.1186/1471-2458-10-80.
8. Cunningham-Rundles C. How I treat common variable immune deficiency. Blood 2010; 116 (1): 7–15. doi: 10.1182/blood-2010-01-254417.
9. Oksenhendler E, Gerard L, Fieschi C, Malphettes M, Mouillot G, et al. Infections in 252 patients with common variable immunodeficiency. Clinical Infectious Diseases 2008; 46 (10): 1547–1554. doi: 10.1086/587669.
10. Malphettes M, Gérard L, Carmagnat M, Mouillot G, Vince N, et al. Late-onset combined immune deficiency: a subset of common variable immunodeficiency with severe T cell defect. Clinical Infectious Diseases 2009; 49 (9): 1329–1338. doi: 10.1086/606059.
11. Aghamohammadi A, Abolhassani H, Moazzami K, Parvaneh N, Rezaei N. Correlation between common variable immunodeficiency clinical phenotypes and parental consanguinity in children and adults. Journal of Investigational Allergology and Clinical Immunology 2010; 20 (5): 372–379. PMID: 20945602.
12. Ardeniz O, Başoğlu OK, Gunşar F, Unsel M, Bayraktaroğlu S, et al. Clinical and immunological analysis of 23 adult patients with common variable immunodeficiency. Journal of Investigational Allergology and Clinical Immunology 2010; 20 (3): 222–236. PMID: 20635788.
13. Muşabak UH, Demirel F, Yeşillik S, Baysan A, Selçuk A, et al. Adults with common variable immunodeficiency: a single-centre experience. Turkish Journal of Medical Sciences 2017; 47 (1): 1–12. doi: 10.3906/sag-1503-22.

14. Carvalho KI, Melo KM, Bruno FR, Snyder-Cappione JE, Nixon DF, et al. Skewed distribution of circulating activated natural killer T (NKT) cells in patients with common variable immunodeficiency disorders (CVID). PLoS One 2010; 5 (9): e12652. doi: 10.1371/journal.pone.0012652.

15. Kiaee F, Azizi G, Rafiemanesh H, Zainaldain H, Rizvi FS, et al. Malignancy in common variable immunodeficiency: a systematic review and meta-analysis. Expert Review of Clinical Immunology 2019; 15 (10): 1105–1113. doi: 10.1080/1744666X.2019.1658523.

16. Mellemkjaer L, Hammarstrom L, Andersen V, Yuen J, Heilmann C, et al. Cancer risk among patients with IgA deficiency or common variable immunodeficiency and their relatives: a combined Danish and Swedish study. Clinical and Experimental Immunology 2002; 130 (3): 495–500. doi: 10.1046/j.1365-2249.2002.02004.x.

17. Manesh AT, Azizi G, Heydari A, Kiaee F, Shaghaghi M, et al. Epidemiology and pathophysiology of malignancy in common variable immunodeficiency? Allergologia et Immunopathologia 2017; 45 (6): 602–615. doi: 10.1016/j.aller.2017.01.006.

18. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood 2008; 112 (2): 277–286. doi: 10.1182/blood-2007-11-124545.

19. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood 2008; 111 (1): 77–85. doi: 10.1182/blood-2007-06-091744.

20. Ghoshal UC, Goel A, Ghoshal U, Jain M, Misra A, et al. Chronic diarrhea and malabsorption due to hypogammaglobulinemia: a report on twelve patients. Indian Journal of Gastroenterology 2011; 30 (4): 170–174. doi: 10.1007/s12664-011-0111-y.

21. Quinti I, Pulvirenti F, Milito C, Granata G, Giovannetti G, et al. Haemolysis in patients with antibody deficiencies on immunoglobulin replacement treatment. Transfusion 2015; 55 (5): 1067–1074. doi: 10.1111/trf.12939.

22. Salehzadeh M, Aghamohammadi A, Rezaei N. Evaluation of immunoglobulin levels and infection rate in patients with common variable immunodeficiency after immunoglobulin replacement therapy. Journal of Microbiology, Immunology and Infection 2010; 43 (1): 11–17. doi: 10.1016/S1684-1182(10)60002-3.

23. Lawrence MG, Palacios-Kibler TV, Workman LJ, Schuyler AJ, Steinke JW, et al. Low serum IgE is a sensitive and specific marker for common variable immunodeficiency (CVID). Journal of Clinical Immunology 2018; 38 (3): 225–233. doi: 10.1007/s10875-018-0476-0.

24. Thorarinsdottir K, Camponeschi A, Gjertsson I, Martensson IL. $CD21^{-/low}$ B cells: A snapshot of a unique B cell subset in health and disease. Scandinavian Journal of Immunology 2015; 82 (3): 254–261. doi: 10.1111/sji.12339.

25. Agarwal S, Cunningham-Rundles C. Autoimmunity in common variable immunodeficiency. Current Allergy and Asthma Reports 2009; 9 (5): 347–352. doi: 10.1007/s11882-009-0051-0.

446 **Table 1. Demographic and clinical characteristics of patients with CVID (n = 26).**

Demographics	
Sex (F/M)	19 / 7
Age (years) [a]	41.5 ± 15.2
Age at onset of symptoms [a]	17.1 ± 11.8
Age at diagnosis [a]	37.1 ± 15.7
Duration of delay to diagnosis [a]	19.9 ± 9.3
Duration of treatment [a]	4.4 ± 4.5
Dose of IVIG	38.2 ± 5.1 [c]
Consanguineous marriage	4 (15.4)
Family history of cancer	14 (53.8)
History of smoking [b]	4 (15.4)
Complications	
Allergic rhinitis [b]	5 (19.2)
Chronic sinusitis [b]	10 (38.5)
Asthma [b]	8 (30.8)
Upper respiratory tract infections [b]	26 (100)
Pneumonia [b]	17 (65.4)
Bronchiectasis [b]	3 (11.5)
Lymphadenopathy [b]	15 (57.7)
Splenomegaly [b]	12 (46.2)
Hepatomegaly [b]	12 (46.2)
Gastritis/duodenitis [b]	19 (73.1)

Nodular lymphoid hyperplasia [b]	8 (30.8)
Chronic diarrhoea [b]	5 (19.2)
Low body of weight [b]	6 (23.1)
Oral aphthous ulcer [b]	11 (42.3)
Genitourinary tract infection [b]	7 (26.9)
Dermatitis [b]	6 (23.1)

Explanations: "a" value was given as "mean ± standard deviation" notation, "b" value was given as n (%) notation, "c" gram for every 3 weeks. Ideal body weight (IBW) in each patient was calculated using the IBW formula explained in text.

463 **Table 2.** Haemato-immunological parameters before and after follow up period of IVIG

464 treatment (n = 26)

	Before the follow up	**After the follow up**	**P value**
Complete blood count			
Haemoglobin (g/dL) [a]	12.6 (8.9 - 15.9)	13 (9.7 - 17.4)	**0.001**
Haematocrit (%) [a]	38.7 (27.8 - 48.8)	39.7 (28.7 - 52.4)	**0.007**
Erythrocyte count ($\cdot 10^6$/µL)	4.7 (2.9 - 5.6)	4.9 (2.9 - 6.3)	**0.027**
Leucocyte (/µL) [a]	7005 (3600 - 12700)	6175 (581 - 9320)	0.112
Thrombocyte (/µL) [a]	240 (55 - 447)	201 (65 - 334)	0.073
Neutrophil (/µL) [a]	4245 (1134 - 10200)	3720 (1700 - 6360)	**0.04**
Neutrophil (%) [a]	63.7 (31.8 - 88.7)	59.1 (32.7 - 74.2)	**0.03**
Lymphocyte (/µL) [a]	1660 (483 - 2990)	1675 (890 - 2980)	0.946
Lymphocyte (%) [a]	25.3 (9.4 - 57.6)	29.2 (16.1 - 48.7)	**0.022**
Monocyte (%) [a]	7.1 (1.3 - 19.4)	7.5 (5.1 - 17.3)	0.527
Eosinophil (%) [a]	1.7 (0.1 - 7.7)	1.9 (0.3 - 4.5)	0.875
Basophil (%) [a]	0.74 (0.1 - 1.5)	0.67 (0.3 - 1.52)	0.898
Immunoglobulin levels			
IgG (g/L) [a]	4.1 (0.6 - 5.8)	10.1 (5.1 - 18.1)	**<0.001**
IgM (g/L) [b]	18 (69.2)	24 (92.3)	**0.035**
IgA (g/L) [b]	14 (53.8)	16 (61.5)	0.575
IgE (IU/mL) [b]	8 (30.8)	11 (42.3)	0.388

Lymphocyte subgroups			
$CD3^+$ (%) [a]	78.1 (56.9 - 94.4)	-	-
$CD4^+$ (%) [a]	34.6 (22.2 - 50.9)	-	-
$CD8^+$ (%) [a]	35.6 (17.6 - 59.7)	-	-
$CD4^+/CD8^+$ [a]	1.1 (0.3 - 2.4)	-	-
$CD19^+$ (%) [a]	6.7 (0 - 40.6)	-	-
$CD3^-CD16^+CD56^+$ (NK) [a]	11.1 (2.1 - 25.1)	-	-
$CD3^+CD4^-CD8^-$ (DNT) [a]	4.9 (0.8 - 12.5)	-	-

Abbreviations: CD: cluster of differentiation, NK: natural killer, DNT: double negative T cell. **Explanations:** "a" value was given as "median (min-max)" notation, "b" n (%) of patients who had detectable levels of immunoglobulin. Values are given in bold when the level of significance is less than 0.05 in comparisons.

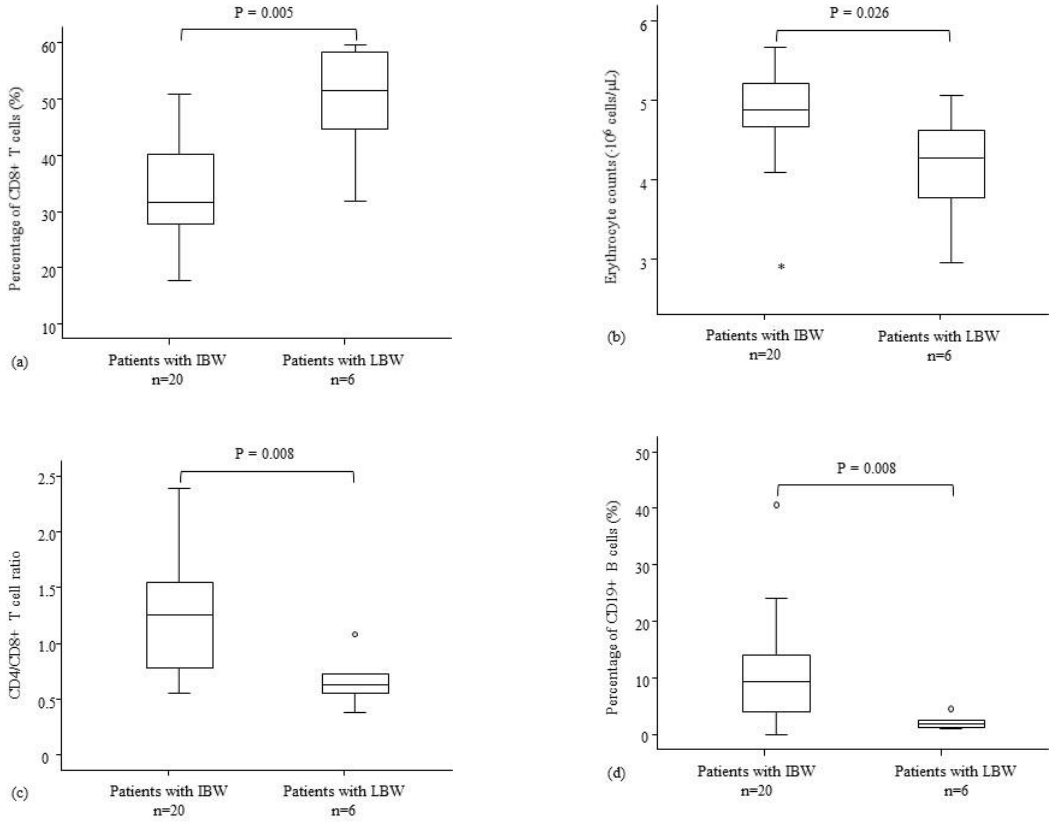
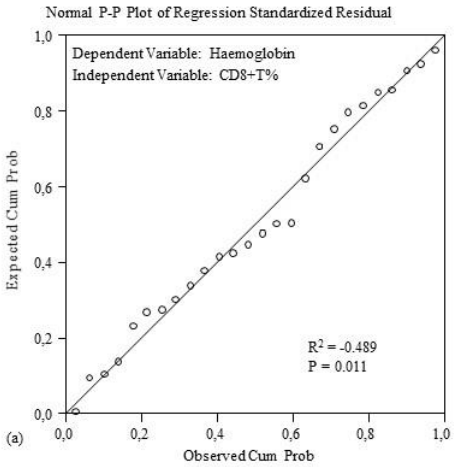
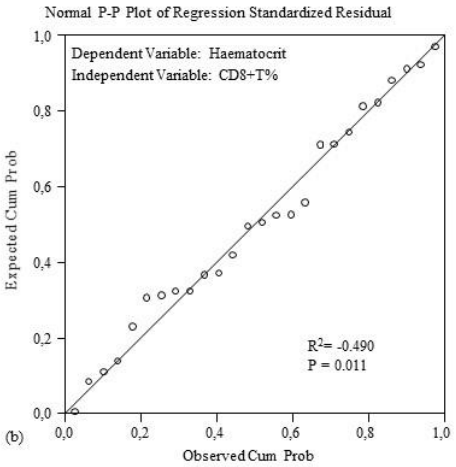
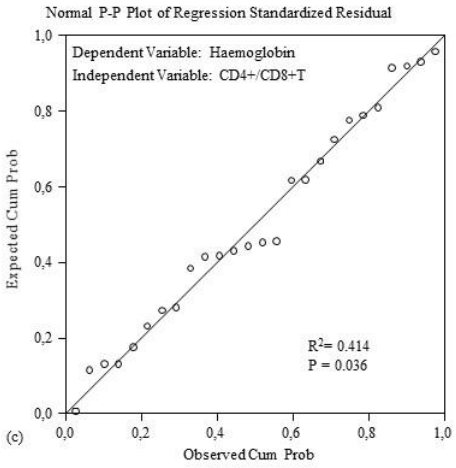
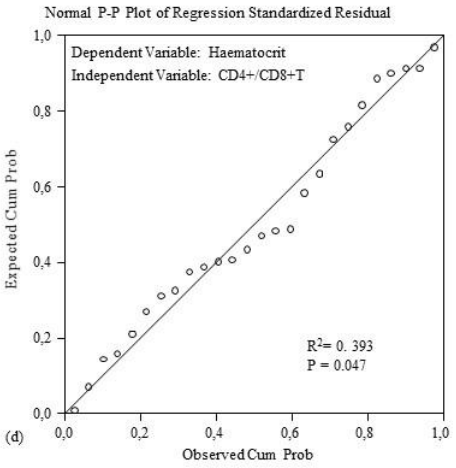


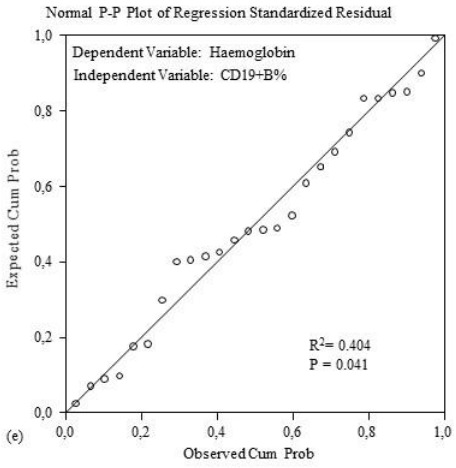
Figure 1. Comparisons of data obtained from peripheral blood samples of patients with CVID who had ideal body weight (IBW) or low body weight (LBW). Boxes show the ranges of 1st and 3rd quartiles and extreme values with the thick horizontal bars representing median values. The differences between two independent groups were evaluated by the Mann–Whitney U-test. P-values are indicated above the boxes when a level of significance < 0.05 was reached in comparisons of the study groups.

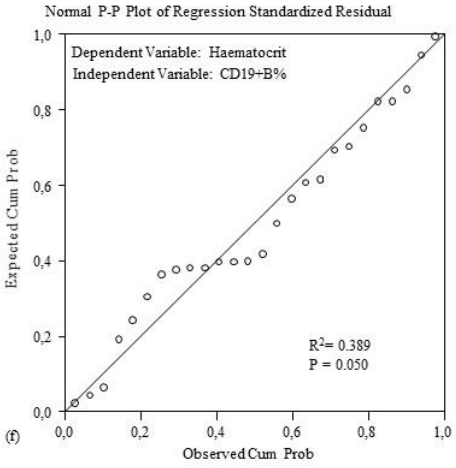

Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Haemoglobin
Independent Variable: CD8+T%
R^2 = -0.489
P = 0.011
Expected Cum Prob
Observed Cum Prob
(a)


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Haematocrit
Independent Variable: CD8+T%
R^2= -0.490
P = 0.011
Expected Cum Prob
Observed Cum Prob
(b)


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Haemoglobin
Independent Variable: CD4+/CD8+T
R^2= 0.414
P = 0.036
Expected Cum Prob
Observed Cum Prob
(c)


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Haematocrit
Independent Variable: CD4+/CD8+T
R^2= 0. 393
P = 0.047
Expected Cum Prob
Observed Cum Prob
(d)


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Haemoglobin
Independent Variable: CD19+B%
R^2= 0.404
P = 0.041
Expected Cum Prob
Observed Cum Prob
(e)


Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Haematocrit
Independent Variable: CD19+B%
R^2= 0.389
P = 0.050
Expected Cum Prob
Observed Cum Prob
(f)

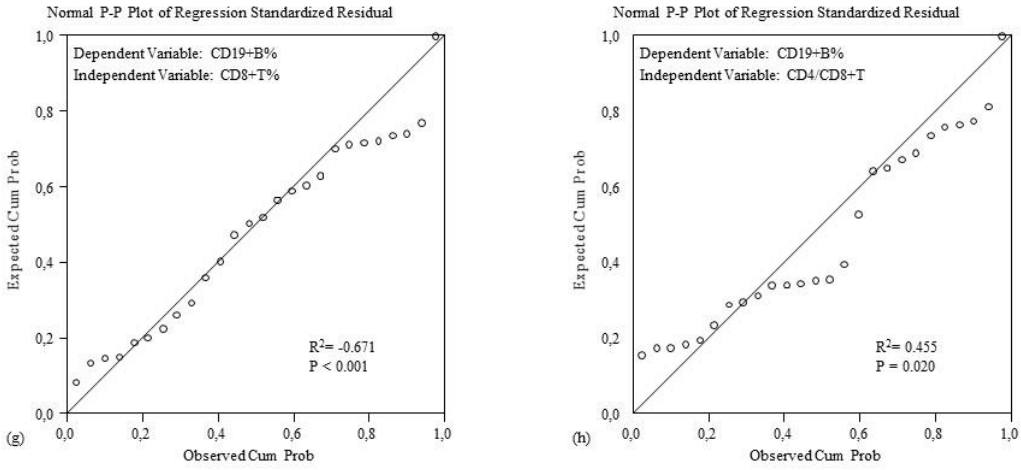
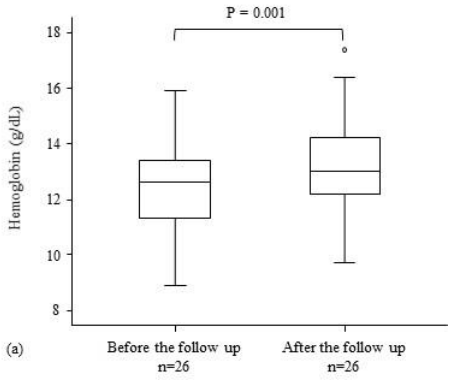
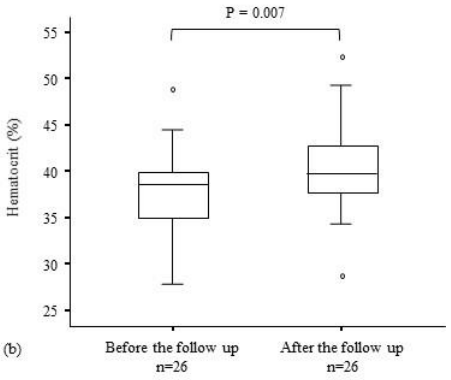
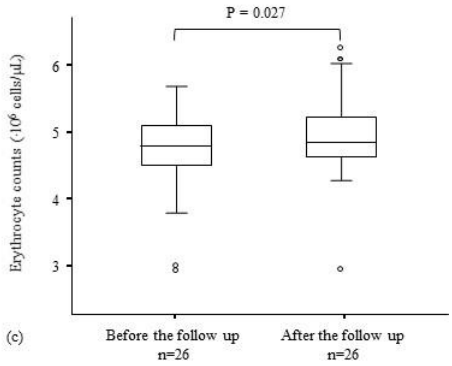
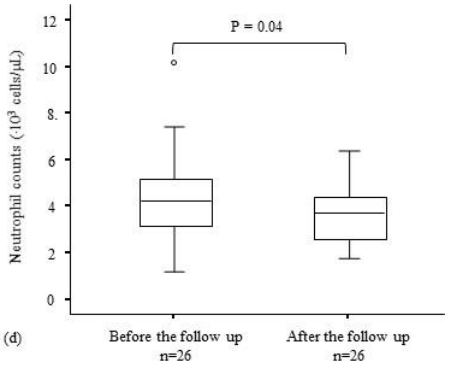


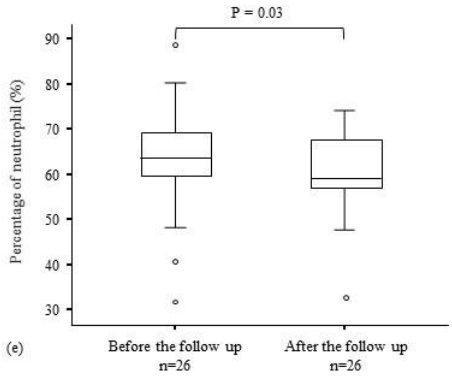
Figure 2. P-P plots of the relationships between haematological parameters (haemoglobin, haematocrit) and lymphocyte subsets (CD8+%, CD4/CD8, CD19+%) (a-f) and B cells (CD19+%) and T cell subsets (CD8+%, CD4/CD8) (g, h).

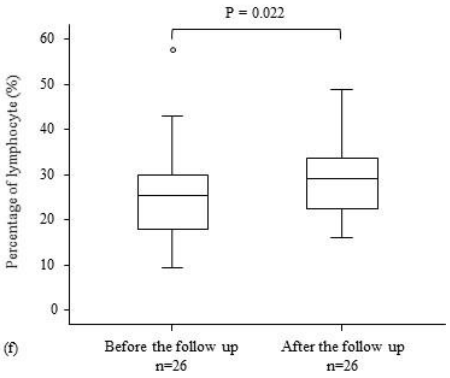

P = 0.001
Hemoglobin (g/dL)
18
16
14
12
10
8
(a)
Before the follow up
n=26
After the follow up
n=26


P = 0.007
Hematocrit (%)
55
50
45
40
35
30
25
(b)
Before the follow up
n=26
After the follow up
n=26


P = 0.027
Erythrocyte counts (10⁶ cells/μL)
6
5
4
3
8
(c)
Before the follow up
n=26
After the follow up
n=26


P = 0.04
Neutrophil counts (10³ cells/μL)
12
10
8.
6
4
2
0
(d)
Before the follow up
n=26
After the follow up
n=26


P = 0.03
Percentage of neutrophil (%)
90
80
70
60
50
40
30
(e)
Before the follow up
n=26
After the follow up
n=26


P = 0.022
Percentage of lymphocyte (%)
60
50
40
30
20
10
0
(f)
Before the follow up
n=26
After the follow up
n=26

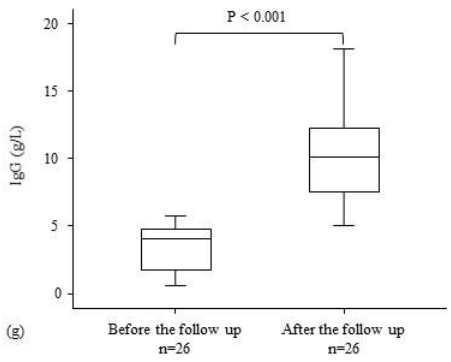
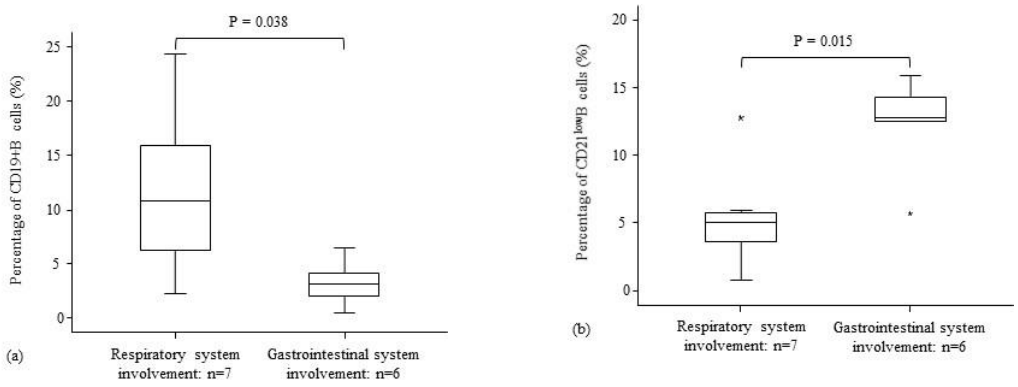

P < 0.001
IgG (g/L)
20
15
10
5
0
(g)
Before the follow up
n=26
After the follow up
n=26

Figure 3. Comparisons of data obtained from peripheral blood samples of patients with CVID before starting IVIG treatment (before the follow up period) and at the end of follow up period (after the follow up period). Boxes show the ranges of 1st and 3rd quartiles and extreme values. with the thick horizontal bars representing median values. The differences between two dependent groups were evaluated by the Wilcoxon Signed Ranks Test. P values are indicated above the boxes when a level of significance < 0.05 was reached in comparisons of the study groups.



505 **Figure 4. Comparisons of data obtained from peripheral blood B cells of patients**
506 **with CVID whose respiratory system or gastrointestinal system had been**
507 **prominently affected.** Boxes show the ranges of 1st and 3rd quartiles and extreme
508 values. with the thick horizontal bars representing median values. The differences
509 between two independent groups were evaluated by the Mann-Whitney U-test. P-values
510 are indicated above the boxes when a level of significance < 0.05 was reached in
511 comparisons of the study groups.

512