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Serum B cell activating factor and interleukin 10 levels in common variable immunodeficiency: relationship with clinical findings

Serumski nivoi B ćelijskog aktivacionog faktora i interleukina 10 u običnoj promenljivoj imunodeficijenciji: povezanost sa kliničkim nalazima

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Abstract

Background/Aim. Common variable immunodeficiency (CVID) is an immunologically and clinically heterogeneous disorder. Disturbed cytokine production is implicated in dysfunctional immune response. The aim of this study was to investigated B-cell activating factor (BAFF) and interleukin (IL)-10 levels in CVID patients. **Methods.** The study included 28 CVID patients diagnosed and followed during a 20-year period (mean follow-up 14.5 years). Control groups consisted of 4 patients with X-linked agammaglobulinemia (XLA) and 21 healthy subjects. According to clinical characteristics, the CVID patients were divided into four groups which partly overlap: chronic pulmonary diseases (n = 21), splenomegaly (n = 13), autoimmune diseases (n = 9) and patients with recurrent infections despite regular intravenous immunoglobulin (IVIg) substitution (n = 4). The serum levels of BAFF and IL-10 were measured by commercial ELISA. **Results.** The BAFF levels were found to be higher in all CVID patients compared to the healthy controls ($p < 0.01$). The most significant differences were observed in the patients with pulmonary diseases and splenomegaly ($p < 0.0001$). Also, concentrations of IL-10 were higher in all CVID patients in comparison with the XLA patients ($p < 0.05$) and healthy subjects ($p < 0.01$). A statistically significant positive correlation ($r = 0.86$; $p < 0.01$) was found between the levels of BAFF and IL-10 in the CVID patients with autoimmune diseases. We demonstrated that the CVID patients with chronic pulmonary diseases had higher levels of IL-10, while the CVID patients with recurrent infections had higher BAFF concentrations in comparison to the patients without these features ($p < 0.05$). **Conclusion.** In spite of the limited number of patients, this is the first report from Serbia, examining the serum levels of BAFF and IL-10 in the CVID patients. Our study showed significantly increased concentrations of serum BAFF and IL-10 in the patients with CVID compared to the healthy subjects. Further studies are needed to confirm our findings that the BAFF levels are more pronounced in patients with recurrent infections while IL-10 levels are higher in patients with chronic pulmonary diseases.

Key words:
common variable immunodeficiency; b-cell activating factor; cytokines; interleukins; lung diseases; splenomegaly; autoimmune diseases.

Apstrakt

Uvod/Cilj. Obična promenljiva imunodeficijencija (CVID) je imunološko i kliničko heterogeno oboljenje. Poremećena citokinska produkcija utiče na disfunkcionalan imunski odgovor. Cilj rada bio je da se ispitaju nivoi faktora aktivacije B-limfocita (BAFF) i interleukina (IL)-10 u serumu kod bolesnika sa CVID. **Metode.** Studijom je bilo obuhvaćeno 28 bolesnika sa CVID-om koji su dijagnostikovani i praćeni tokom 20 godina (srednje vreme praćenja iznosilo je 14,5 godina). Kontrolne grupe činila su: četiri bolesnika sa X vezanom agamaglobulinemijom (XLA) i 21. zdrava osoba. Prema kliničkim karakteristikama bolesnici sa CVID bili su podeljeni u četiri grupe koje su se delimično preklapale: hronična plućna bolest (n = 21), splenomegalija (n = 13), autoimunske bolesti (n = 9) i ponavljajuće infekcije koje su bolesnici imali uprkos redovnoj primeni intravenskih imunoglobulina (IVIg) (n = 4). Serumski nivoi BAFF i IL-10 mereni su standardnom ELISA metodom. **Rezultati.** Nivoi BAFF-a bili su povišeni kod svih bolesnika sa CVID u poređenju sa zdravim ispitanicima ($p < 0.01$). Najznačajnije razlike nađene su kod bolesnika sa plućnim bolestima i splenomegalijom ($p < 0.0001$). Takođe, koncentracije IL-10 u serumu bile su više kod svih bolesnika sa CVID-om u odnosu na bolesnike sa XLA ($p < 0.05$) i zdrave ispitanike ($p < 0.01$). Statistički značajna pozitivna korelacija između koncentracija BAFF i IL-10 nađena je kod bolesnika sa CVID sa autoimunskim bolestima ($r = 0.86$; $p < 0.01$).

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Bolesnici sa CVID sa hroničnim plućnim bolestima imali su značajno više nivoe IL-10, dok su bolesnici sa CVID sa recidivirajućim infekcijama imali povišene koncentracije BAFF u serumu, u poređenju sa ispitanicima bez navedenih komplikacija ($p < 0.05$). **Zaključak.** Uprkos malom broju bolesnika, ovo je prva studija iz Srbije koja je ispitivala nivoe BAFF I IL-10 kod bolesnika sa CVID-om. Bolesnici sa CVID su u našoj studiji imali značajan porast nivoa serumskog BAFF i IL-10 u odnosu na zdrave ispitanike. Za potvrdu naših rezultata o značajno višim serumskim nivoima BAFF kod bolesnika sa recidivirajućim infekcijama, i značajno višim serumskim nivoima IL-10 kod bolesnika sa hroničnim plućnim bolestima, potrebna su dalja ispitivanja.

Ključne reči:
obična promenljiva imunodeficijencija; faktor aktivacije b-ćelija; citokini; interleukini; pluća, bolesti; splenomegalija; autoimunske bolesti.

Introduction

Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency (PID) with the prevalence of 1 : 25,000 to 1 : 50,000 in general population [1]. CVID is characterized by a normal or low number of B-cells, dysregulation of B-cell differentiation and maturation accompanied by low levels of immunoglobulins, impaired response to vaccines and susceptibility to infections, mainly respiratory ones [1, 2]. Moreover, patients with CVID are often affected with various inflammatory, autoimmune, lymphoproliferative diseases, malignancy and granulomas [1, 2]. X-linked agammaglobulinemia (XLA) is inherited PID, characterized by the absence of B cells, profound antibody deficiency and recurrent bacterial infections. However, XLA patients are not prone to a variety of immunoinflammatory conditions characteristic for CVID [3].

Although numerous B and T cell abnormalities have been described in CVID, dysfunctional immune responses might be, at least partially, explained by disturbed cytokine production and dysregulation of a complex cytokines network [4]. Many studies addressed the possibility that disturbed cytokine production of B-cell activating factor (BAFF) and interleukin-10 (IL-10), in conjunction with other factors, might contribute to the creation of certain CVID phenotypes [4]. The BAFF and IL-10 in chronic inflammation, autoimmunity and immune dysregulation had been extensively examined [5, 6]. Single nucleotide polymorphisms in promotor region of several cytokines genes [IL-10, tumor necrosis factor (TNF)-alpha and interferon gamma] are found to be associated with susceptibility to CVID [7, 8].

BAFF and proliferation-inducing ligand (APRIL) are involved in B-cell development, promoting the survival of mature B cell and class-switching [5]. Reduced expression of BAFF receptor (BAFF-R) was found in some CVID patients with severe defect in B-cell development [9]. Mutations affecting BAFF-R genes in a subset of CVID patients were also described [10]. Several studies revealed elevated levels of BAFF in the sera of CVID patients, but until now no obvious association between serum levels of BAFF and clinical complications of CVID has been demonstrated [9–13]. On the other hand, it was shown that mice carrying a BAFF transgene, leading to BAFF overexpression are prone to develop high titer of autoantibodies and a systemic lupus erythematosus (SLE)-like disease [14]. Serum levels of BAFF were found to be elevated in various autoimmune diseases, especially in SLE [14]. Moreover, anti-BAFF monoclonal antibody is now used for the treatment of SLE patients.

IL-10 is an anti-inflammatory cytokine with pleiotropic effects in the immune regulation. It is primarily produced by monocytes and, to a lesser extent, lymphocytes. IL-10 downregulates the expression of Th1 cytokines, costimulatory molecules on macrophages, but enhances B cell survival and proliferation [15, 16]. Similar to BAFF, serum levels of IL-10 were found to be markedly increased in patients with autoimmune diseases and correlate with disease activity [17, 18]. Besides that, numerous studies revealed the heterogeneous secretion of IL-10 profile in CVID patients, but its role in immune dysregulation in the CVID specific subgroups still remains unelucidated [4].

Only a few studies investigated association between the serum levels of BAFF and IL-10 with clinical features of CVID patients up to now [4, 10]. The aim of this study was to evaluate aberrations in cytokine production in a cohort of Serbian patients with CVID divided into four clinical groups in order to examine relationship between BAFF, IL-10 and certain common complications of CVID.

Methods

This study included 28 CVID patients diagnosed and followed during a 20-year period (1995–2015, median follow-up was 14.5 years) at the Clinic of Allergy and Immunology, Clinical Center of Serbia, Belgrade, Serbia. All 28 patients fulfilled the criteria for CVID (decrease of serum IgG < 2 standard deviations below the mean for age and reduced serum IgA and/or IgM; absence of isohemagglutinins or poor response to vaccines; age greater than two years; exclusion of other causes of hypogammaglobulinemia) according to the European Society for Immunodeficiencies (ESID). Four patients with clinical characteristics corresponding with XLA with genetically confirmed mutations in the gene for Bruton's tyrosine kinase were used as a disease control. Twenty-one healthy control (HC) subjects were recruited as gender- and age-matched control group. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade (Protocol Number 29/XI-9) and all participants gave their written informed consent.

All subjects were free from current infections and were not on immunosuppressive therapy when blood samples were collected. Original medical records of the patients were used to obtain laboratory results, clinical signs and duration of symptoms before the diagnosis of CVID. Diagnostic delay was considered as the time between the onset of symptoms and the time when the diagnosis of PID was established. All CVID and XLA pa-

tients were on regular monthly intravenous immunoglobulin (IVIg) therapy. Blood was taken 30 minutes before the regular monthly IVIg substitution. We checked regularly the serum IgG levels to achieve a minimum concentration of 5 g/L.

Clinical groups of the CVID patients

The CVID patients were categorized into four main clinical groups: 21 of 28 patients had chronic pulmonary diseases with clinical characteristics as followed – 12 suffered from bronchiectasis [determined by the high resolution computed tomography (HRCT)], 4 had bronchial asthma, 4 had chronic obstructive bronchitis and 1 had pulmonary fibrosis. Thirteen of 28 patients displayed splenomegaly defined as spleen length more than 11 cm as determined by ultrasound or HRCT. Nine of 28 patients had autoimmune diseases: 4/9 had atrophic gastritis, 3/9 had autoimmune thyroiditis, 1/9 had systemic vitiligo and 1/9 had autoimmune thrombocytopenia (ITP). Four of 28 CVID patients, despite regular IVIg treatment, suffered from recurrent severe infections defined as more than three episodes of elevated numbers of leukocytes and increased level of C-reactive protein (CRP), body temperature higher than 38.5°C in the previous year.

Quantification of cytokines concentrations in serum

The cytokines BAFF and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA) (RnDSystems, Abingdon, UK). Immunoassays were calibrated against a highly purified recombinant human BAFF and IL-10, respectively. Minimum detectable dose (MDD) of BAFF ranged from 1.01-6.44 pg/mL (the mean value 2.68 pg/mL). MDD of IL-10 was less than 3.9 pg/mL. Cytokine concentrations were expressed in pg/mL.

Quantification of immunoglobulins in serum

The serum concentrations of IgM, IgG and IgA classes were measured by nephelometric method (Minineph, The Binding Site, Birmingham, UK) at the time of diagnosis and during follow-up.

Statistical analysis

Descriptive analysis used medians, percentage, range and interquartile ranges. Statistical comparisons were based on the nonparametric Mann–Whitney U test for two groups of continuous variables and the nonparametric one-way analysis of variance (ANOVA) and the Kruskal-Wallis test for more than two groups of continuous variables. Correlations between continuous variables were evaluated by the Spearman's correlation coefficient. The *p*-value less than 0.05 was considered statistically significant in all statistical analyses. Data were analyzed by using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA) and the Statistical Package for Social Science (SPSS) for Windows (version 20, SPSS Inc., Chicago, IL, USA).

Results

Table 1 describes the demographic characteristics of the patients and controls, including age at presentation and the delay in the diagnosis. All XLA patients were males, significantly younger at the time when a diagnose was established comparing to the CVID patients (median: 4 vs. 33 years; $p < 0.05$). In the group of the CVID patients, 43% were males. Delays in the diagnosis of the CVID and XLA patients were similar (median: 5.5 vs. 5 years). Concentrations of all immunoglobulin classes in serum at the time of diagnosis showed no significant differences between the XLA and CVID patients (Table 2).

Clinical characteristics of the CVID patients

The Table 1 reveals demographic characteristics of the total study population, the CVID patients and defined CVID groups. Out of 28 patients with CVID, 75% had pulmonary disease, 46% had splenomegaly, 32% had autoimmune disorders and 14% had severe recurrent infections. Figure 1 indicates the distribution and partially overlapping features in the main clinical groups of our CVID patients.

There were no differences in the age and gender between the main CVID groups (Table 1). The patients with autoimmune diseases were the oldest at the time of diagnosis (43 years) comparing to other groups. The diagnostic delay was longer for the CVID patients with severe recurrent infections and the patients with autoimmune diseases (13.5 and 11 years respectively) comparing to other groups, but without a statistical significance.

Table 1

Demographics and clinical data of the study groups

Variable	Patients (n)	Gender M/F	Age (years) median (range)	Age (years) at Dg median (range)	Delay in Dg (years) median (range)
HC	21	9/12	42 (18–59)	/	/
XLA	4	4/0	29.5 (28–42)	4 (1–16)	5 (0–9)
CVID total	28	12/16	47.5 (17–62)	33 (10–59)	5.5 (0–31)
pulmonary diseases	21	10/11	46.5 (31–61)	30 (10–59)	6.5 (0–31)
splenomegaly	13	6/7	46.5 (17–61)	33 (13–59)	6 (1–12)
autoimmune diseases	9	5/4	51 (29–62)	43 (25–56)	11 (0–24)
recurrent infections	4	1/3	45.5 (38–53)	20 (15–51)	13.5 (2–31)

Median and range are indicated for all groups.
CVID – common variable immunodeficiency; HC – healthy controls; XLA – X-linked agammaglobulinemia; Dg – diagnosis;M – male; F – female.

Table 2

The immunoglobulin levels at the time of the diagnosis of the CVID and XLA patients and the serum cytokine levels of the study groups

Variable	Patients (n)	IgG (g/L) median (range)	IgA (g/L) median (range)	IgM (g/L) median (range)	BAFF (pg/mL) median (range)	IL-10 (pg/mL) median (range)
HC	21	/	/	/	1070 (658–1628)	0 (0–8.56)
XLA	4	2.805 (2.20–3.90)	0.185 (0–0.25)	0.25 (0.08–0.32)	8,024 (6,393–11,400)	0 (0–9.24)
CVID total	28	1.80 (0.29–4.11)	0.20 (0–0.32)	0.18 (0.09–1.04)	3,306 (1,021–11,300)	7.88 (0–26.82)
Pulmonary diseases	21	1.80 (0.53–4.11)	0.20 (0.04–0.27)	0.17 (0.09–1.04)	4,355 (1,021–11300)	9.22 (0–26.82)
Splenomegaly	13	2.28 (0.33–4.11)	0.20 (0.09–0.32)	0.19 (0.09–1.04)	4,157 (1,021–1,1300)	9.24 (2.90–16.18)
Autoimmune diseases	9	1.98 (0.29–3.70)	0.20 (0–0.27)	0.20 (0.10–0.60)	4,355 (1,113–1,0500)	5.06 (0–26.82)
Recurrent infections	4	1.96 (1.80–3.60)	0.175 (0.11–0.25)	0.125 (0.10–0.20)	7,615 (5,074–1,0500)	12.5 (4.34–24.30)
p	/	ns	ns	ns	< 0.0001	< 0.0001

Median and range are indicated for all groups, as well as the results of Kruskal–Wallis test and the one-way analysis of variance (ANOVA).
CVID – common variable immunodeficiency; HC – healthy controls; XLA – X-linked agammaglobulinemia; ns – not significant.

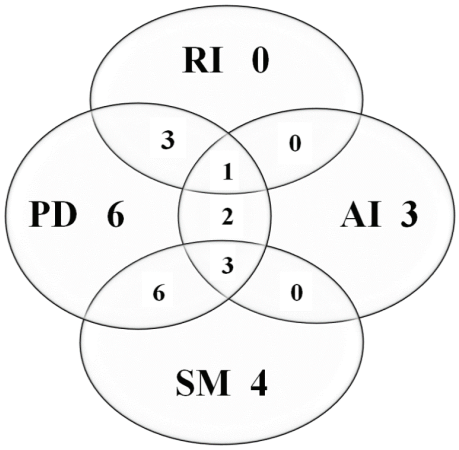

Fig. 1 – Venn diagram illustrating a distribution of the common variable immunodeficiency (CVID) patients into four main clinical groups. Numbers represent the patients in each group. One patient may belong to more than one group, as indicated.
RI: recurrent infections; PD – pulmonary diseases; SM – splenomegaly; AI – autoimmune diseases.

Serum levels of BAFF and IL-10

Median and range of the BAFF and IL-10 levels in sera of the CVID patients and controls are shown in Table 2. The age at diagnosis, the actual age of the patients and the diagnostic delay did not correlate significantly with the concentrations of BAFF and IL-10 in total CVID patients, CVID groups and XLA patients. Also, there were no correlations found between concentrations of immunoglobulins and concentrations of BAFF and IL-10.

The BAFF levels were higher in all CVID patients and CVID groups compared to HC ($p < 0.01$). The most significant differences were found between the patients with pulmonary diseases and splenomegaly ($p < 0.0001$; Figure 2A) and HC. Figure 2A shows that the patients with XLA had higher levels of BAFF than the patients with CVID ($p < 0.05$).

The IL-10 levels were also higher in all CVID patients and all CVID groups compared to HC ($p < 0.01$). The most pronounced differences appeared between the groups of patients with pulmonary diseases, recurrent infections and splenomegaly [$p < 0.0001$; (Figure 2B)]. The patients with CVID were found to have higher levels of IL-10 than the patients with XLA ($p < 0.05$). There was no difference in the IL-10 levels between the XLA patients and HC (Figure 2B).

There were no significant relationships between the BAFF and IL-10 levels in all CVID patients. Further analysis for defined clinical groups of CVID revealed positive correlation between the BAFF and IL-10 levels only for the group of patients with autoimmune diseases [$r = 0.86$; $p = 0.003$; (Figure 3)].

Figures 4A and 4B reveal the differences in concentrations of BAFF and IL-10 among all CVID patients with and without defined clinical complications. The patients with severe recurrent infections, despite regular IVIg therapy, had significantly higher BAFF concentrations than the patients without this complication [$p < 0.05$; (Figure 4A)]. The IL-10 levels were significantly higher in the patients with chronic pulmonary diseases, compared to the patients without these complications [$p < 0.05$; (Figure 4B)]. Also, the patients with bronchiectasis had higher level of IL-10 than the patients without bronchiectasis and other chronic pulmonary diseases ($p < 0.05$).

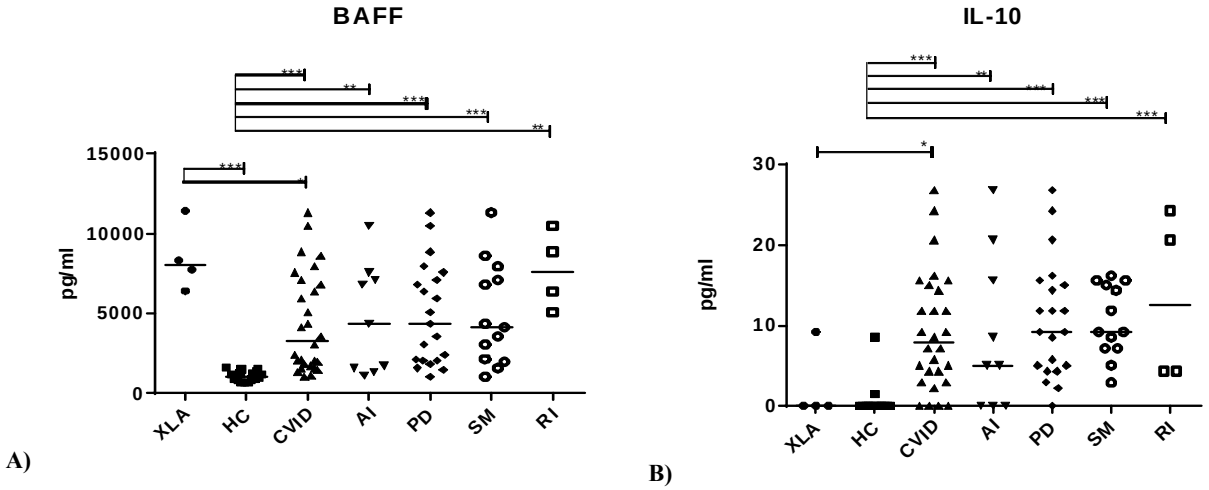

Fig. 2 – Serum levels of A) B-cell activating factor (BAFF) (pg/mL) and B) IL-10 (pg/mL). The common variable immunodeficiency (CVID) patients may appear in more than one group, as indicated in Figure 1 HC: healthy controls; XLA: X-linked agammaglobulinemia; AI: autoimmune diseases; PD: pulmonary diseases; RI: recurrent infections; SM: splenomegaly. Statistics were performed by using the one-way analysis of variance (ANOVA) and Kruskal–Wallis test, with post-hoc test. *$p < 0.05$; **$p<0.01$; ***$p < 0.0001$.

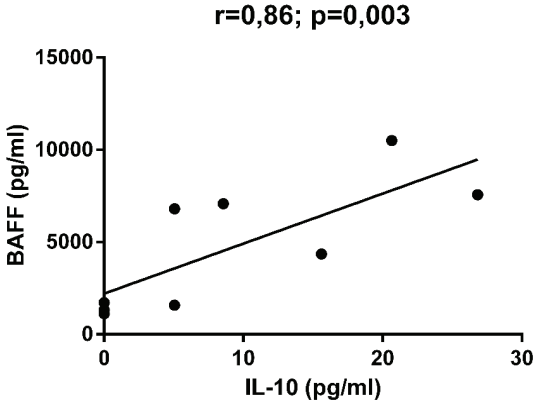

Fig. 3 – A positive correlation between serum levels of B-cell activating factor (BAFF) and interleukin-10 (IL-10) in the group of patients with autoimmune diseases. Statistics were performed by using the Spearman's correlation coefficient.

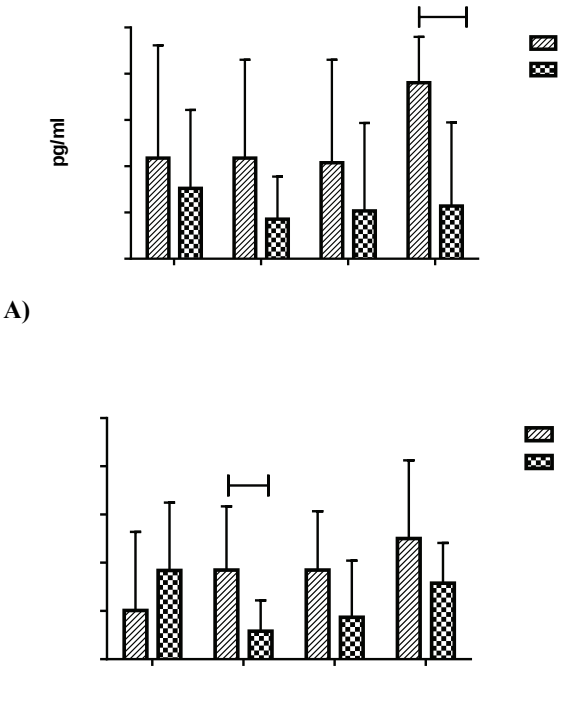

Fig. 4 –Differences in A) B-cell activating factor (BAFF) and B) IL-10 concentrations between the patients with and without particular clinical findings (medians and interquartile ranges). AI – autoimmune diseases; PD – pulmonary diseases; RI – recurrent infections; SM – splenomegaly. Statistics were performed by using the Mann–Whitney U test (*$p < 0.05$).

Discussion

Our study confirmed the heterogeneity of CVID with a wide range of clinical manifestations often with overlapping features (Tables 1 and 2; Figure 1). Symptoms of CVID may appear during the childhood, adolescence or adult life, but the diagnosis is usually established in their thirties, as in our study group (Table 1) [1]. The median diagnostic delay in our center was higher (5.5 years) than the average delay in the greatest cohort of the CVID patients (4.1 years) [1]. The patients with autoimmune diseases had the longest delay in diagnosis in our CVID group (Table 1) in accordance with earlier findings [1].

The large multicenter studies, which primarily analyzed mortality, divided patients with CVID into four main phenotypes: isolated infection, polyclonal lymphoproliferation, autoimmune cytopenias and enteropathy [2].

According to a dominant clinical manifestation, we divided our CVID patients into four main groups (Figure 1). Splenomegaly as a one of the most common features in patients with CVID and its relationship with a variety of im-

munological and cytokine disturbances has been investigated in previous cohort studies [1, 8, 9, 11, 19]. Kutukculer et al. [19] using splenomegaly as the criterion for severe forms of CVID found higher prevalence of splenomegaly and lymphadenopathy in a group of CVID patients lacking switched memory B cells. Also, splenomegaly was more frequent in a group of CVID patients characterized by the absence of memory B cells [20]. Giovannetti et al. [21] showed that the lower numbers of naive CD4+T cells were significantly associated with an increased likelihood of splenomegaly (OR 4,78). Pulmonary involvement is typically found in patients with CVID, and it was showed that up to 90% of patients had abnormalities on chest CT scan [22]. Mortality in CVID was found to be linked to both structural and functional lung impairment [23]. It is very important that some patients, despite the regular IVIg supplementation and antibiotic treatment, had recurrent infections [21, 24]. Moreover, complexity of cellular and cytokine dysregulation in CVID was thought to produce various autoimmune phenomena [1, 25]. Referring to heterogeneity of CVID, different manifestations are often presented either at the same time or during the evolution of the disease in the same patient. Overlapping features, as described in our study (Figure 1), were analogous to previously published investigations in CVID [1, 3, 20].

Identification of the factors governing BAFF-R and transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI) is crucial for understanding B-cell biology and CVID pathogenesis. BAFF-induced signals are essential for the development of functional B cell compartment. BAFF levels inversely correlate with the numbers and the percentage of circulating B cells and the availability of BAFF receptors [9]. Therefore, the size of the B cell pool and the availability of BAFF receptors seem to be primary factors regulating a steady-state concentrations of soluble BAFF, although a long-term increase in BAFF levels in response to chronic infections and inflammation cannot be excluded [9]. BAFF expression is upregulated by proinflammatory responses, during viral infections and in various autoimmune conditions [9, 14]. We found the highly elevated BAFF levels both in CVID and XLA (Table 2, Figure 2A), diseases that have low numbers of circulating B cells that are blocked in differentiation into switched memory B cells or plasma cells.

Considering different complications in the CVID patients, we found significantly higher levels of BAFF only in the patients with severe recurrent infections despite the regular IVIg treatment (Figure 4A). Quinti et al. [24] recorded that 13.3% of patients continued to have episodes of recurrent pneumonia and *otitis media* despite regular IVIg treatment which is similar to our result of 14.3% [24]. Giovannetti et al. [21] described the strong positive correlation between the number of naive $CD4^+$ lymphocytes and disease severity, including history of severe respiratory tract infections. Our research showed that the patients with severe recurrent infections had significantly higher levels of BAFF, comparing to patients without them (Figure 4A). The limitation of our study was the small number of patients in this group. This finding could be explained by the fact that BAFF is the essential costimulatory factor for humoral immune response to capsular polysaccharides of encapsulated bacteria (*Streptococcus pneumoniae* and *Haemophilus influenzae*), which are the commonest cause of recurrent infections (sinus, lungs, ears) in the CVID patients [26]. Also, Kreuzaler et al. [9] concluded that long-term increase in the BAFF levels in response to chronic infections and inflammation could not be excluded [9]. Contrary to some systemic autoimmune diseases, we did not find elevated concentrations of BAFF in a subset of our CVID patients with autoimmune manifestations. In addition, a significant positive correlation between BAFF and IL-10 was found only for this subset of the CVID patients (Figure 3). Similar data were previously reported for immunoinflammatory and lymphoproliferative diseases such as active sarcoidosis, multiple myeloma and chronic lymphocytic leukemia, possibly through the induction of IL-10 production by transitional B cells [27–29].

We found high levels of IL-10 in all CVID patients and in all CVID groups (Figure 2B). Other authors also showed that CVID was associated with elevated serum levels of IL-10 [15, 30, 31]. Barssoti et al. [32] recently published that IL-10-producing regulatory B cells were decreased in CVID. Since IL-10 in conjunction with anti-CD 40 supports secretion of IgG, IgA, and IgM by B cells, many studies were performed to examine IL-10 production in CVID [4]. Zhou et al. [33] demonstrated that T cell secretion of IL-10 was deficient, but that monocyte-derived high levels of IL-10, plus a relative lack of IL-2 production, contributed to the defects of antigen induced cell proliferation in CVID. Holm et al. [34] found that impaired secretion of IL-10 by T cells from patients with CVID involved preserved function of cAMP/protein kinase A type I. In our investigation the XLA patients had significantly lower levels of serum IL-10 in comparison to the CVID patients (Figure 2B). Schmidt et al. [35] demonstrated that Bruton's tyrosine kinase was required for Toll-like receptor-induced IL-10 production. Barbosa et al. [36] examined monocyte activation in patients with CVID, XLA and healthy controls. They reported elevated markers of monocyte activation in CVID patients, but in contrast to CVID, the patients with XLA and healthy controls did not show increased markers associated with monocyte activation [36]. In this study authors showed that increased monocyte activation with the expansion of activated T cells, irrespective of the lipopolysaccharide levels, might have important role in the inflammation and lymphoproliferation.

In our study, the levels of IL-10 were significantly higher in the patients with chronic pulmonary diseases (Figure 4B) and in the patients with bronchiectasis comparing with HC. It was shown that the IL-10 levels could be affected by a single nucleotide polymorphisms of promoter region of the IL-10 gene [8]. A high production of IL-10 could be explained by a low frequency of low IL-10 producing haplotype in the CVID patients [7, 37]. It is well known that IL-10 is essential for maintaining the integrity of tissue epithelial layers [38]. It down-regulates production of several proinflammatory cytokines in macrophages, monocytes and T-cells [15]. In the CVID patients, IL-10 can limit the damage caused by infection, repress proinflammatory responses and decrease unnecessary tissue damage [37]. Moreover, it was

found that cytokine abnormalities, including IL-10 among other cytokines, were significantly higher in the patients with bronchiectasis [39]. Furthermore, high serum levels of IL-10 can induce some form of B cell "anergy" which is reversible and can be improved by maintenance of B cell in culture [40].

Conclusion

This is the first report from Serbia examining the serum levels of BAFF and IL-10 in the CVID patients. To the best of our knowledge, this is the first report that analyzed BAFF and IL-10 in the CVID patients suffering from severe recurrent infections despite the regular IVIg substitution. We demonstrated that the patients with CVID, in comparison with healthy controls, had higher serum concentrations of BAFF and IL-10. Severe respiratory infections, despite regular IVIG, were associated with higher levels of BAFF, while chronic pulmonary diseases were associated with higher levels of IL-10, compared to the patients without these manifestations. We emphasize that the dysregulation of cytokine production needs to be investigated separately in different subgroups of CVID patients during a long follow-up period.

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