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NIVOI SERUMSKOG B ĆELIJSKOG AKTIVACIONOG FAKTORA I INTERLEUKINKA 10 U OBIĆNOJ PROMENLJIVOJ IMUNODEFICIJENCIJI: POVEZANOST SA KLINIČKIM NALAZIMA

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

Nivoi serumskog B čelijskog aktivacionog faktora i interleukina 10 u običnoj promenljivoj imunodeficijenciji: povezanost sa kliničkim nalazima

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e) Running Title: BAFF and IL-10 in CVID
Abstract

**Background/Aim.** Common variable immunodeficiency (CVID) is an immunologically and clinically heterogeneous disorder. Disturbed cytokine production has been implicated in dysfunctional immune response. This study 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: a) 4 patients with X-linked agammaglobulinemia (XLA) and b) 21 healthy subjects. According to clinical characteristics, CVID patients were divided into four groups which partly overlap each other: 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). Serum levels of BAFF and IL-10 were measured by commercial ELISA. **Results.** BAFF levels were found to be higher in all CVID patients, compared to healthy controls (p<0.01). The most significant differences were observed in patients with pulmonary diseases and splenomegaly (p<0.0001). Also, concentrations of IL-10 were higher in all CVID patients in comparison with XLA patients (p<0.05) and healthy subjects (p<0.01). Statistically significant positive correlation (r=0.86; p<0.01) was found between levels of BAFF and IL-10 in CVID patients with autoimmune diseases. We demonstrated that CVID patients with chronic pulmonary diseases had higher levels of IL-10, while CVID patients with recurrent infections had higher BAFF concentrations in comparison to 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 CVID patients. Our study showed significantly increased concentrations of serum BAFF and IL-10 in patients with CVID compared to healthy subjects. Further studies are needed to confirm our findings that BAFF levels are more pronounced in patients with recurrent infections while IL-10 levels are higher in patients with chronic pulmonary diseases.

**Keywords:** common variable immunodeficiency (CVID); cytokines; B-cell activating factor (BAFF); interleukin-10; pulmonary diseases; splenomegaly; autoimmunity; recurrent infections
Apstrakt

Uvod/Cilj. Obična promenljiva imunodeficijencija (CVID) je imunološko i kliničko heterogeno oboljenje. Poremećena citokinska produkcija utiče na disfunkcionalan imunski odgovor. Ova studija je ispitivala nivoa faktora aktivacije B-limfocita (BAFF) i interleukina (IL)-10 u serumu kod pacijenata sa CVID. Metode. Studija je uključila 28 pacijenata sa CVID-om koji su dijagnostikovani i praćeni tokom 20 godina (srednje vreme praćenja je bilo 14,5 godina). Kontrolne grupe su činile: a) 4 pacijenta sa X vezanom agamoglobulinemijom (XLA) i b) 21 zdrava osoba. Prema kliničkim karakteristikama pacijenti sa CVID su podeljeni u 4 grupe koje su se delimično preklapale: hronična plućna bolest (n=21), splenomegalija (n=13), autoimunske bolesti (n=9) i ponavljajuće infekcije uprkos redovnoj primeni intravenskih imunoglobulina (IVIg) (n=4). Serumski nivoi BAFF i IL-10 su mereni standardnom ELISA metodom. Rezultati. Nivoi BAFF-a su bili povišeni kod svih CVID pacijenata u poređenju sa zdravim kontrolama (p<0.01). Najznačajnije razlike su nađene kod pacijenata sa plućnim bolestima i splenomegalijom (p<0.0001). Takođe, koncentracije IL-10 su bile više kod svih CVID bolesnika u odnosu na pacijente sa XLA (p<0.05) i zdravih kontrola (p<0.01). Statistički značajna pozitivna korelacija između koncentracija BAFF i IL-10 je nađena kod CVID pacijenata sa autoimunskim bolestima (r=0.86; p<0.01). CVID pacijenti sa hroničnim plućnim bolestima imali su značajno više nivoa IL-10, dok su CVID pacijenti sa recidivirajućim infekcijama imali povišene koncentracije BAFF u serumu, u poređenju sa pacijentima bez navedenih komplikacija (p<0.05). Zaključak: Uprkos malom broju pacijenata, ovo je prva studija iz Srbije koja je ispitivala nivo BAFF I IL-10 kod pacijenata sa CVID u Srbiji. Pacijenti sa CVID su u našoj studiji imali značajan porast nivoa serumskog BAFF i IL-10 u odnosu na zdrave kontrole. Potrebna su dalja ispitivanja za potvrdu naših rezultata da je BAFF značajno viši kod pacijenata sa recidivirajućim infekcijama, dok je IL-10 značajno viši kod pacijenata sa hroničnim plućnim bolestima.

Ključne reči: obična promenljiva imunodeficijencija; citokini; faktor aktivacije B-limfocita (BAFF); interleukin 10; plućne bolesti; splenomegalija; autoimunost; rekurentne infekcije
INTRODUCTION

Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency (PID) with the prevalence of 1:25000 to 1:50000 in general population.\(^1\) CVID is characterized by 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.\(^1,2\) 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 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.\(^7\)

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 decribed.\(^10\) 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. 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 down-regulates the expression of Th1 cytokines, costimulatory molecules on macrophages, but enhances B cell survival and proliferation. Similar to BAFF, serum levels of IL-10 were found to be markedly increased in patients with autoimmune diseases and correlate with disease activity. Besides that, numerous studies revealed the heterogeneous secretion IL-10 profile in CVID patients but its role in immune dysregulation in the CVID specific subgroups still remains unelucidated.

Only a few studies investigated association between the serum levels of BAFF and IL-10 with clinical features of CVID patients up to now. This study was performed to evaluate aberrations in cytokine production in a cohort of Serbian patients with CVID. Patients were 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 who were 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 twenty-eight patients fulfilled 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 European Society for Immunodeficiencies (ESID). Four patients had 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 Medical faculty, 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 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 patients were on regular monthly intravenous immunoglobulin (IVIg) therapy. Blood was taken 30 minutes before the regular monthly IVIg substitution. We checked regularly serum IgG levels to achieve minimum concentration of 5 g/l.

**Clinical groups of CVID patients**

CVID patients were categorized into four main clinical groups: 21/28 patients had chronic pulmonary diseases with clinical characteristics as followed: 12/21 suffered from bronchiectasis (determined by the high resolution computed tomography – HRCT), 4/21 had bronchial asthma, 4/21 had chronic obstructive bronchitis and 1/21 had pulmonary fibrosis. 13/28 patients displayed splenomegaly defined as spleen length more than 11 cm as determined by ultrasound or HRCT. 9/28 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). 4/28 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, 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 analyses

Descriptive analysis used medians, percentage, interquartile ranges and range. Statistical comparisons were based on nonparametric Mann–Whitney U test for two groups of continuous variables and nonparametric one-way analysis of variance (ANOVA) Kruskal-Wallis for more than two groups of continuous variables. Correlations between continuous variables were evaluated using Spearman’s correlation coefficient. P-value less than 0.05 was considered statistically significant in all statistical analyses. Data were analyzed 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

Demographics and immunoglobulins levels in CVID and XLA patients

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 diagnose were established comparing to CVID patients (median: 4 vs 33 years; p<0.05). In the group of CVID patients 43% were males. Delays in the diagnosis of 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 XLA and CVID patients (Table 2).

Clinical characteristics of CVID patients

The Table 1 reveals demographic characteristics of the total study population 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 distribution and the 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). Patients with autoimmune diseases were the oldest at the time of diagnosis (43 years) compared to other groups. The diagnostic delay was longer for CVID patients with severe recurrent infections and patients with autoimmune diseases (13.5 and 11 years, respectively) comparing to other groups, but without statistical significance.

**Serum levels of BAFF and IL-10**

Median and range of BAFF and IL-10 levels in sera of 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 (data not shown).

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

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 groups of patients with pulmonary diseases, recurrent infections and splenomegaly (p<0.0001; see Fig. 2B). Patients with CVID were found to have higher levels of IL-10 comparing to patient with XLA (p<0.05). There was no difference of IL-10 levels between XLA patients and HC (Fig. 2B).

There were no significant relationships between BAFF and IL-10 levels in all CVID patients (data not shown). Further analysis for defined clinical groups of CVID revealed positive correlation between BAFF and IL-10 levels only for the group of patients with autoimmunity (r=0.86; p=0.003; see Fig. 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. Patients with severe recurrent infections despite regular IVIg therapy had significantly higher BAFF concentrations than patients without this complication (p<0.05; Fig. 4A). IL-10 levels were significantly higher in patients with chronic pulmonary diseases, compared to patients
without these complications (p<0.05; Fig. 4B). Also, patients with bronchiectasis had higher level of IL-10 than patients without bronchiectasis and other chronic pulmonary diseases (p<0.05) (data not shown).

DISCUSSION

Our study confirmed the heterogeneity of CVID with wide range of clinical manifestations often with overlapping features (Tables 1,2; Fig. 1). Symptoms of CVID may appear during the childhood, adolescence or adult life, but the diagnosis is usually established, as in our study group (Table 1), in the their thirties. The median diagnostic delay in our center was higher (5.5 years) than the average delay in the greatest cohort of CVID patients (4.1 years). Patients with autoimmune diseases had the longest delay in diagnosis in our CVID group in a accordance with earlier findings (Table 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. According to a dominant clinical manifestation, we divided our CVID patients into four main groups (Fig 1). Splenomegaly as a one of the most common features in patients with CVID and its relationship with a variety of immunological and cytokine disturbances has been investigated in previous cohort studies. Kutukculer et al. used splenomegaly as criterion for severe forms of CVID. They found higher prevalence of splenomegaly and lymphadenopathy in a group of CVID patients lacking switched memory B cells. Also, splenomegaly was more frequent in the group of CVID patients characterized by the absence of memory B cells. Giovannetti et al. 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 typical finding in patients with CVID, and it has been showed that up to 90% of patients have abnormalities on chest CT scan. Mortality in CVID is found to be linked to both structural and functional lung impairment. It is very important that some patients despite the regular IVIg supplementation and antibiotic treatment have recurrent infections. Moreover, complexity of cellular and cytokine dysregulation in CVID was thought to produce various autoimmune phenomena. Refer 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 (Fig.1) were analogous to previously published investigations in CVID.\textsuperscript{1,3,20}

Identification of the factors governing BAFF-R and TACI is crucial to understanding B-cell biology and CVID pathogenesis. BAFF-induced signals are essential for the development of functional B cell compartment. BAFF levels inversely correlated with the numbers and the percentage of circulating B cells and the availability of BAFF receptors.\textsuperscript{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.\textsuperscript{9} BAFF expression is upregulated by proinflammatory responses, during viral infections and in various autoimmune conditions.\textsuperscript{9,14} We found highly elevated BAFF levels in both 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 of CVID patients, we found significantly higher levels of BAFF only in patients with severe recurrent infections despite the regular IVIg treatment (Figure 4A). Quinti et al. recorded that 13.3% patients continued to have episodes of recurrent pneumonia and otitis media despite regular IVIg treatment, which is similar to our result of 14.3%.\textsuperscript{24} Giovannetti et al. described the strong positive correlation between the number of naive CD4\textsuperscript{+} lymphocytes and disease severity, including history of severe respiratory tract infections.\textsuperscript{21} Our research showed that patients with severe recurrent infections had significantly higher levels of BAFF, comparing to patients without them (Fig. 4A). The limitation of our study was the small number of patients in this group (Fig.2A; Table 2). This finding could be explained by the fact that BAFF is essential co-stimulatory factor for humoral immune response to capsular polysaccharides of encapsulated bacteria (\textit{S. pneumoniae} and \textit{H. influenzae}), which are the commonest cause of recurrent infections (sinus, lungs, ears) in CVID patients.\textsuperscript{26} Also, Kreuzaler concluded that long-term increase in BAFF levels in response to chronic infections and inflammation cannot be excluded.\textsuperscript{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 CVID patients (Fig.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.\textsuperscript{27-29}

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

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

CONCLUSION

This is the first report from Serbia, examining the serum levels of BAFF and IL-10 in CVID patients. To the best of our knowledge, this is the first report that analyzed BAFF and IL-10 in CVID patients suffering from severe recurrent infections despite the regular IVIg substitution. We demonstrated that 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 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.

Acknowledgement

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Figure 1: Venn diagram illustrating the distribution of common variable immunodeficiency (CVID) patients into four main clinical groups. Numbers show 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
Figure 2: Serum levels of BAFF (A) and IL-10 (B) (pg/ml). Common variable immunodeficiency (CVID) patients may appear in more than one group, as indicated in Fig. 1. HC: healthy controls; XLA: X-linked agammaglobulinemia; AI: autoimmune diseases; PD: pulmonary diseases; RI: recurrent infections; SM: splenomegaly. Statistics were performed using the one-way analysis of variance (ANOVA) Kruskal–Wallis, with post-hoc test. *p < 0.05; **p<0.01; ***p < 0.001
Figure 3. Positive correlation between serum levels of B-cell activating factor (BAFF) with interleukin-10 in group of patients with autoimmune diseases. Statistics were performed using Spearman’s correlation coefficient.
Figure 4: Differences in BAFF (A) and IL-10 (B) concentrations between 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 using the Mann–Whitney U test. *p < 0.05
Table 1: Demographics and clinical data of the study group

<table>
<thead>
<tr>
<th></th>
<th>Pts</th>
<th>Gender M/F</th>
<th>Age years (range)</th>
<th>Age at Dg years (range)</th>
<th>Delay of Dg years (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>21</td>
<td>9/12</td>
<td>42 (18-59)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>XLA</td>
<td>4</td>
<td>4/0</td>
<td>29.5 (28-42)</td>
<td>4 (1-16)</td>
<td>5 (0-9)</td>
</tr>
<tr>
<td>CVID total</td>
<td>28</td>
<td>12/16</td>
<td>47.5 (17-62)</td>
<td>33 (10-59)</td>
<td>5.5 (0-31)</td>
</tr>
<tr>
<td>Pulmonary diseases</td>
<td>21</td>
<td>10/11</td>
<td>46.5 (31-61)</td>
<td>30 (10-59)</td>
<td>6.5 (0-31)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>13</td>
<td>6/7</td>
<td>46.5 (17-61)</td>
<td>33 (13-59)</td>
<td>6 (1-12)</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>9</td>
<td>5/4</td>
<td>51 (29-62)</td>
<td>43 (25-56)</td>
<td>11 (0-24)</td>
</tr>
<tr>
<td>Recurrent infections</td>
<td>4</td>
<td>1/3</td>
<td>45.5 (38-53)</td>
<td>20 (15-51)</td>
<td>13.5 (2-31)</td>
</tr>
</tbody>
</table>

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

Table 2: Immunoglobulin levels at the time of the diagnosis of CVID and XLA patients and serum cytokine levels of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Pts</th>
<th>n</th>
<th>IgG g/l (range)</th>
<th>IgA g/l (range)</th>
<th>IgM g/l (range)</th>
<th>BAFF pg/ml (range)</th>
<th>IL-10 pg/ml (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>21</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>1070 (658-1628)</td>
<td>0 (0-8.56)</td>
</tr>
<tr>
<td>XLA</td>
<td>4</td>
<td>2.805</td>
<td>0.185 (2.20-3.90)</td>
<td>0.25 (0.08-0.32)</td>
<td>3306 (1021-11300)</td>
<td>9.22 (0-26.82)</td>
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<tr>
<td>CVID total</td>
<td>28</td>
<td>1.80</td>
<td>0.20 (0.29-4.11)</td>
<td>0.18 (0.09-1.04)</td>
<td>3306 (1021-11300)</td>
<td>16.18 (0-26.82)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary diseases</td>
<td>21</td>
<td>1.80</td>
<td>0.20 (0.53-4.11)</td>
<td>0.17 (0.04-1.04)</td>
<td>4355 (1021-11300)</td>
<td>2.90 (0-26.82)</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>13</td>
<td>2.28</td>
<td>0.20 (0.33-4.11)</td>
<td>0.19 (0.09-1.04)</td>
<td>4157 (1021-11300)</td>
<td>12.5 (0-26.82)</td>
<td></td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>9</td>
<td>1.98</td>
<td>0.20 (0.29-3.70)</td>
<td>0.20 (0.10-0.60)</td>
<td>4355 (1113-10500)</td>
<td>5.06 (0-26.82)</td>
<td></td>
</tr>
<tr>
<td>Recurrent infections</td>
<td>4</td>
<td>1.96</td>
<td>0.175 (1.80-3.60)</td>
<td>0.125 (0.10-0.20)</td>
<td>7615 (5074-10500)</td>
<td>12.5 (4.34-24.30)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>/</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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