PROLIFERATION OF B-LYMPHOCYTES IN INFLAMMATORY AND HEMATOLOGICAL DISEASES

Tamara TEŠIĆ1, Dajana LENDAK2,3, Ivana UROŠEVIC2,4, Igor MITIĆ2,4 and Vanja ANDRIĆ2

Summary
Introduction. A proliferation-inducing ligand is a membrane binding protein that represents one of the main survival factors for immature, naive and activated B-cells, and is involved in the global immune response. The objective of this study was to determine whether plasma levels of a proliferation-inducing ligand may be used to assess the proliferation of B-lymphocytes in patients with bacterial infections, B-cell malignancies and autoimmune inflammatory disorders.

Material and Methods. The study included 91 patients divided into three groups and 30 blood donors assigned to the control group. Group 1 included 34 patients with bacterial infections confirmed by microbiology and/or radiology diagnostic tests; group 2 included 32 patients with B-cell malignancies; and group 3 included 25 patients with autoimmune inflammatory diseases. All plasma samples were assayed for a proliferation-inducing ligand using enzyme-linked immunosorbent assay. The differences between groups were examined by one-way analysis of variance test and post hoc analysis.

Results. One way analysis of variance test showed a statistically significant difference in concentrations of a proliferation-inducing ligand in the examined groups. The highest mean value of a proliferation-inducing ligand was found in patients with established bacterial infections (x̄ = 8,294 ng/ml). Post hoc analysis showed that a proliferation-inducing ligand levels in the plasma samples of patients with bacterial infections were significantly higher than in healthy controls, and patients with hematological and autoimmune diseases, respectively. Conclusion. B-cell proliferation was increased in patients with bacterial infections in regard to patients with other disorders. Therefore, a proliferation-inducing ligand can be used to differentiate bacterial infections from other inflammatory disorders and may be helpful in decision making whether to start antibiotic treatment or not.

Key words: B-Lymphocytes; Bacterial Infections; Autoimmune Diseases; Hematologic Diseases; Tumor Necrosis Factor Ligand Superfamily Member 13; Biomarkers; Diagnosis, Differential

Sažetak

Ključne reči: B-limfociti; bakterijske infekcije; autoimune bolesti; hematološke bolesti; transplantansenski protein APRIL; biomarkeri; diferencijalna dijagnoza

Introduction
Recent studies have shown that determination of serum concentrations of a proliferation-inducing ligand (APRIL) may be useful in differentiation of bacterial infections and nonbacterial inflamma-
dictions. APRIL induces proliferation of B-cells in autoimmune and malignant diseases, as well as a response to bacterial infections [1]. The objective of this study was to determine whether plasma levels of APRIL can be used to assess the proliferation of B-lymphocytes in patients with bacterial infections, B-cell malignancies and autoimmune inflammatory disorders.

B-lymphocytes produce immunoglobulins as a response to infection, cytokines as immunoregulatory cells, and as antigens they participate in T-B cooperation [2, 3]. Elevated concentrations of cytokines and markers of the B-lymphocyte function are present in various autoimmune diseases (rheumatoid arthritis, systemic lupus, Sjogren's Syndrome) [4, 5], as well as in B-cell malignancies [6–8], while the role of B-lymphocytes as regulatory immune cells in response to bacterial infections remains the object of many studies. Newer researches both in animal models [6] and in human population [2, 3] highlighted individual forms of B-lymphocytes as one of the main regulators of the immune response to infectious agents. The main regulators of B-lymphocytes’ function and activity are tumor necrosis factor alpha (TNF-α) superfamily members [9–11]. APRIL, as one of the superfamily members, is a type II transmembrane protein which releases as a trimer in a soluble form, and it is produced by monocytes, tissue macrophages and dendritic cells. APRIL exhibits physiological effects by binding to two B-lymphocyte receptors, transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA) which promote B-cell proliferation, stimulate production of immunoglobulin M (IgM) antibodies and survival of plasma cells, stimulate lymphocyte maturation and antibody production [12–14]. Considering that APRIL plays an important role in the survival, proliferation and maturation of B-lymphocytes, it is assumed that determination of this biomarker’s concentration may serve as a predictor of bacterial infections severity [1].

Material and Methods

The study included 91 patients who were hospitalized at the Clinic of Infectious Diseases, Clinic of Hematology and the Clinic of Nephrology and Clinical Immunology of the Clinical Center of Vojvodina (CCV). The control group included 30 healthy blood donors. Determination of serum concentration of APRIL is currently available for research purposes only, so there are no defined reference values for healthy persons. Blood samples from the control group were collected at the Blood Transfusion Institute of Vojvodina. Patients were divided into three groups. The first group included 34 patients with bacterial infections confirmed by microbiology and/or radiology diagnostic tests. The bacterial infection origin was identified by physical examination, radiological procedures and microbiological analysis of various samples obtained on admission that were sent to the Microbiology Center of the Institute of Public Health of Vojvodina. The second group included 32 patients with B-cell malignancies in remission (multiple myeloma, Hodgkin’s and non-Hodgkin’s lymphoma) diagnosed at the Clinic of Hematology of the CCV. The third group included 25 patients with inactive autoimmune inflammatory disorders.

<table>
<thead>
<tr>
<th>Table 1. Average age among the groups</th>
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<tr>
<td><strong>Tabela 1. Prosečna starost unutar grupa</strong></td>
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<tr>
<td>N</td>
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<tr>
<td>Control group/Kontrolna grupa</td>
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<td>Bacterial infections/Bakterijske infekcije</td>
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<td>B-cell malignancies/B-ćelijski maligniteti</td>
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<td>Autoimmune inflammatory disorders</td>
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<tr>
<th>Table 2. Gender distribution in the groups</th>
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<tr>
<td><strong>Tabela 2. Distribucija po polovima unutar grupa</strong></td>
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<td>N</td>
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<tr>
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<td>B-cell malignancies/B-ćelijski maligniteti</td>
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<td>Autoimmune inflammatory disorders</td>
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Abbreviations

APRIL – a proliferation-inducing ligand
ELISA – enzyme-linked immunosorbent assay
R & D – Research and Development
CCV – Clinical Center of Vojvodina
BCMA – B cell maturation antigen
IgM – immunoglobulin M
flammmatory diseases (systemic lupus and autoimmune vasculitis) diagnosed at the Clinic of Nephrology and Clinical Immunology of the CCV.

Demographic data, age and sex, were collected from medical records of all patients included in the study, while the concentration of APRIL was done after sampling additional 5 ml of venous blood and prepared plasma samples according to manufacturer’s instructions. The concentration of APRIL was determined using the enzyme-linked immunosorbent assay (ELISA) technique of commercial reagents manufactured by Research and Development (R&D) Systems Europe. Reading was performed automatically on a spectrophotometer - Chemwell, USA. Reagents for the determination of APRIL concentrations were provided through the Project of the Provincial Secretariat for Science and Technological Development: “Noninvasive Markers in Diagnostics and Prognosis of Critical Conditions”. Statistical data processing was done using the software program Statistical Package for Social Sciences (SPSS) 21.0.

Results

The average age of patients in the first group was 59.50 ± 12.13 years, in the second 52.48 ± 14.30 years, and in the third group 55.32 ± 14.56 years. One-way ANOVA showed that there was no statistically significant difference in patient age among the groups (F = 1.514, p = 0.226), while the mean age of voluntary blood donors in the control group was 34.43 ± 11.67 years. There was no statistically significant difference in age among the examined groups of patients. Healthy blood donors were significantly younger than the remaining examined patients. There was no correlation between the age and plasma concentration of APRIL in patients with identified bacterial infections (t = 1.183, p = 0.240).

The gender distribution is shown in Table 2. A statistically significant difference between genders was found in the group of autoimmune inflammatory diseases, by using Hi-square test (p = 0.000). By comparing mean values of APRIL in men (4.27 ± 5.59 ng/ml) and women (3.27 ± 3.36 ng/ml), Student’s t-test showed that gender did not affect APRIL concentrations (t = 1.83, p = 0.240).

The origin of the bacterial infection was identified by microbiology and/or radiology tests in all patients in the first group. The most frequent origin was the urinary tract in 11 (32.3%) patients and soft tissues in 9 (26.5%), while respiratory infections were the least present. The results are shown in Graph 1.

Persons in the control group were significantly younger, so in order to determine the possible correlation between the APRIL concentrations and the age of patients, a correlation analysis was conducted. There was no statistically significant association between APRIL concentrations and age (p = 0.032, p = 0.727), indicating that age differences did not affect differences in APRIL concentrations.

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Plasma levels of APRIL in groups are given in Table 3. One-way ANOVA showed a statistically significant difference in APRIL concentrations among groups. Post-hoc analysis showed that plasma concentrations of APRIL in patients with diagnosed bacterial infections were significantly higher than in healthy subjects, patients with hematological disorders and patients with autoimmune disorders (p = 0.000, p = 0.000, p = 0.000). Although the mean concentrations of APRIL in patients with autoimmune and hematological diseases were slightly lower than in the healthy population, post-hoc analyses showed that the difference was not statistically significant (p > 0.05).

Discussion

The results of our study showed that there was no statistically significant difference in age among the examined groups of patients. Healthy blood donors were significantly younger than the remaining examined patients. There was no correlation between the age and plasma concentration of APRIL between examined subjects. There are no available data on the effects of patients’ age on APRIL concentrations in the literature. According to previous studies, men have been shown to be more prone to infection [14] and that was also confirmed in our

### Table 3. Plasma level of APRIL in plasma samples (ng/ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>x</th>
<th>SD</th>
<th>Median</th>
<th>Interval/Raspon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group/Kontrolna grupa</td>
<td>30</td>
<td>2.232</td>
<td>0.51970</td>
<td>2.00</td>
<td>1.50-3.75</td>
</tr>
<tr>
<td>Bacterial infection/Bakterijske infekcije</td>
<td>34</td>
<td>8.2941</td>
<td>6.85606</td>
<td>5.75</td>
<td>1.25-39.50</td>
</tr>
<tr>
<td>B-cell malignancies/B-ćelijski maligniteti</td>
<td>32</td>
<td>1.6875</td>
<td>0.47519</td>
<td>1.75</td>
<td>1.00-3.25</td>
</tr>
<tr>
<td>Autoimmune inflammatory disorders/Autoimun inflamatorni poremećaji</td>
<td>25</td>
<td>2.0900</td>
<td>0.65701</td>
<td>2.00</td>
<td>1.00-4.00</td>
</tr>
</tbody>
</table>

APRIL - a proliferation inducing ligand/induktor profliseracije

**Graph 1. The origin of infection**

**Grafikon 1. Ishodište infekcije**
sample. Even though our study included more male subjects with B-cell malignancy, it was not found that men statistically more often suffer from this disease, which is consistent with previously conducted studies [8, 15]. Women are statistically more susceptible to developing autoimmune inflammatory diseases [16], as in our research. The explanation for this phenomenon was obtained through many studies [16, 17] that highlight the effect of sex hormones on humoral immunity, i.e. that androgens have immunosuppressive and estrogens immunostimulatory effects. The results of our research show that there is no statistically significant difference regarding concentration of APRIL in relation to sex. There are no available data on this matter in current literature.

The results of our study show that the most common source of bacterial infection is the urinary tract in contrast to literature data reporting that the respiratory tract is the leading source. This result is expected, since Vojvodina has a specialized institution for the treatment of respiratory tract infections, the Institute of Lung Diseases in Sremska Kamenica and those patients are rarely hospitalized at the Clinic of Infectious Diseases, mainly based on unclear evaluation of febrile conditions.

A proliferation-inducing ligand is an indicator of the function of our immune system and its concentrations in body fluids are not routinely determined, but only for research purposes. There are no clearly defined reference values of APRIL in healthy population, therefore plasma concentrations of this parameter were measured in 30 healthy individuals. The mean value of APRIL concentration in the healthy population of our sample was 2.22 ng/ml, ranging from 1.50 ng/ml to 3.75 ng/ml. Similar results were obtained by other researchers using the R&D Systems [18], and researchers who used tests of other manufacturers Plantinun ELISA Ebioscience, Austria and Bender Med Syst Vienna [7, 8].

A proliferation-inducing ligand concentrations were significantly elevated in patients with confirmed bacterial infections compared to healthy individuals, similar to the research of Roderburg et al. [1]. Elevated values of this biomarker in subjects with bacterial infection are expected, given the role of APRIL in B-lymphocytes homeostasis, as well as their role in pathogenesis of bacterial infections.

In the group of patients with B-cell malignancy, the mean APRIL value was 1.69 ng/ml, ranging from 1.00 ng/ml to 3.25 ng/ml. These values, although somewhat lower, did not show a statistically significant difference in relation to the average APRIL values in healthy subjects. Bolkun et al. pointed out that APRIL values at all stages of multiple myeloma were elevated in contrast to the control group [7]. Similar results were also obtained by Chiu et al. in their study in which they noted elevated APRIL values in subjects with Hodgkin’s lymphoma compared to healthy population [14]. On the other hand, Haiat et al. found that the APRIL concentrations were lower in their subjects with chronic lymphocytic leukemia and follicular lymphoma [6]. The fact that in our group the majority of patients had follicular lymphoma explains the correlation between our results and the results provided by Haiat et al., as opposed to studies that predominantly included patients with multiple myeloma and Hodgkin’s lymphoma.

In our study, the mean value of APRIL in the group suffering from autoimmune disease was 2.09 ng/ml. These values were slightly lower than median APRIL in healthy subjects, without a statistically significant difference. These findings are inconsistent with the majority of previous studies showing that APRIL values in autoimmune disorders are elevated in contrast to those in healthy population [5, 14]. In contrast, Vincent et al. revealed that APRIL values were lower than those in healthy population in some forms of systemic lupus, which may indicate certain differences in the serum level of APRIL within the autoimmune group [4]. Different studies show variations in APRIL concentration in healthy population depending on their selection. Vincent et al. included only patients with renal and neurological manifestations of systemic lupus [4], while Chiu et al. included only patients in the active phase of the disease [14]. It is assumed that research on a large number of subjects, which would include patients both in acute and remission phases of the disease, as well as patients with various manifestations, might provide more precise data.

The results of our study show a statistically significant difference in serum APRIL values in patients with confirmed bacterial infection in relation to healthy population, patients with hematological disorders and patients with autoimmune disorders. Currently, there are not many papers that compared APRIL as a biomarker of immune system function in such groups, but Roderburg et al. suggested that APRIL concentrations were significantly higher in septic patients compared to those with other causes of systemic inflammatory response [1, 19, 20]. Our new data show that APRIL serves as a sensitive and specific marker for septic patients [21].

**Conclusion**

The plasma concentrations of a proliferation-inducing ligand are significantly higher in patients with confirmed bacterial infections than in healthy subjects, patients with B-cell malignancies and those with autoimmune inflammatory disorders. Therefore, a proliferation-inducing ligand can be used to differentiate bacterial infections from other inflammatory diseases and hence help in decision making on the initiation of antibiotic therapy.
References


