Clinical usefulness of serum neopterin in children with juvenile idiopathic arthritis

Abstract: The aim of this study was to analyze the usefulness of the serum concentration of neopterin (NPT) as a marker of juvenile idiopathic arthritis (JIA). The study included 67 children with JIA (36 girls and 31 boys), aged between 3.8 and 17.9 years (mean 13.7 ± 3.4 years), and 105 healthy controls (47 girls and 58 boys) of similar age, with no evidence of acute or chronic inflammation. Serum NPT was determined immunoenzymatically. The median serum concentration of NPT and prevalence of elevated serum NPT (>11 nmol/L) were significantly higher in children with JIA than in the controls: 6.044 vs. 4.734 nmol/L (p < 0.001) and 30% vs. 5% (p < 0.001), respectively. The serum concentration of NPT did not correlate with body temperature (R = 0.00, p = 0.97), erythrocyte sedimentation rate (R = 0.09, p = 0.47), leukocyte count (R = −0.05, p = 0.70), C-reactive protein (R = −0.14, p = 0.25), and procalcitonin levels (R = 0.07, p = 0.56). Furthermore, serum NPT was not associated with the type of JIA. However, children with exacerbation of JIA presented with significantly higher median serum level of NPT (10.912 vs. 4.471 nmol/L, p < 0.001) and higher prevalence of serum NPT >11 nmol/L (50% vs. 0%, p < 0.001) than did patients with remission. These data suggest that elevated serum concentration of NPT is an accurate marker of JIA exacerbation.

Keywords: autoimmune disorders; disease activity; exacerbation; markers; remission.

Introduction

Juvenile idiopathic arthritis (JIA) is the most common autoimmune disorder diagnosed in pediatric patients. JIA is underlied by inappropriate immune response that is triggered by incompletely understood extrinsic factors, mostly infectious ones, and concomitant genetic predisposition. Excessive inflammation leads to disruption of immunological tolerance to autoantigens and injury of tissues. The inappropriate immune response manifests as a predominance of pro-inflammatory cytokines (TNF, INF-γ, IL-6, IL-12) over the anti-inflammatory ones (e.g., IL-10), which is typical for most of chronic inflammatory conditions [1, 2]. The clinical course of JIA during the initial 6 months (i.e., during its early phase) frequently determines the further clinical outcome of the condition. JIA can begin as a systemic, oligoarticular, or polyarticular disease, and is highly heterogeneous in terms of manifestation and outcome [3].

A disrupted balance between various immune components, namely innate and acquired immunity and cellular and humoral response, may be responsible for the occurrence of exacerbations and remissions of JIA [2]. Therefore, the aim of ongoing studies is to identify novel, more specific serological markers of the above-mentioned condition that would enable accurate monitoring of its outcome and treatment efficacy.

Neopterin (NPT), detected in early 1980s, is considered a biochemical marker of cellular immune response [4]. Activated lymphocytes T were proved to synthesize interferon-γ, which stimulates the release of NPT from monocytes/macrophages [5–7]. Previous studies confirmed the usefulness of NPT as a clinical marker of many autoimmune conditions [5, 8, 9], e.g., in gastroenterology [10–13], endocrinology [14], and rheumatology [15, 16]. As components of both cellular and humoral response are involved in the pathogenesis of JIA, one may expect...
elevated concentrations of NPT in blood and other body fluids of patients diagnosed with this condition. Moreover, the concentration of NPT may prove to be a useful marker of JIA activity. Therefore, the aim of this study was to analyze the usefulness of serum concentration of NPT as a clinical marker in children with JIA.

Materials and methods

The study, conducted in 2012, included 67 children with JIA (36 girls and 31 boys), aged between 3.8 and 17.9 years (mean 13.7±3.4 years, median 14.4 years). The children were hospitalized at the Department of Pediatric Gastroenterology, Hepatology and Nutrition, Medical University of Gdansk, and at the Pediatric Ward, Rheumatologic Hospital in Sopot (Poland). The patients presented with three forms of JIA: systemic (n=9), oligoarticular (n=34), and polyarticular (n=24). On the basis of medical history, physical examination, and examination with the Child Health Assessment Questionnaire (C-HAQ) and Juvenile Arthritis Functional Assessment Scale (JAFAS) (developed specifically for children with JIA) [17], the children were qualified to one of the two groups: with remission (n=27) or exacerbation (n=40).

The control group consisted of 105 healthy children (47 girls and 58 boys), aged between 1 month and 17.99 years (mean 7.6±5.7 years; median 7.2 years), with no clinical and laboratory evidence of either acute or chronic inflammation. All the controls were hospitalized at the Clinic of Surgery and Urology for Children and Adolescents, Medical University of Gdansk (Poland) due to surgical correction of various congenital malformations.

The list of exclusion criteria included a history of vaccination or antibiotic therapy within 4 weeks preceding the study, administration of immunosuppressive or immunomodulatory agents within 3 months before enrollment, and signs of protein-calorie malnutrition.

The protocol of the study was approved by the local bioethical committee at the Medical University of Gdansk (decision no. NKEBN/942/2006).

Medical history was obtained from all the participants, and they were subjected to physical examination. Venous blood samples were obtained on the day of admission. The serum concentration of NPT was measured by means of immunoenzymatic assay (ELISA; ELItest Neopterin catalogue no. 99.1 and 95.4; BRAHMS, Hennigsdorf, Germany). Apart from NPT, also erythrocyte sedimentation rate, complete blood count with smear, and serum concentrations of C-reactive protein (CRP; latex turbidimetric test; COBAS INTEGRA, Roche Diagnostics GmbH, IN, USA) and procalcitonin (PCT; immunoluminometric assay, LIA PCT, catalogue no. 54.1; BRAHMS, Hennigsdorf, Germany) were determined for all children.

The normal distribution of continuous variables was verified with the Kolmogorov-Smirnov test, and their statistical characteristics were presented as arithmetic means and their standard deviations (SDs), or medians and interquartile ranges (IQRs). Depending on the type of distribution, Student t-test or Mann-Whitney U-test, as well as ANOVA or Kruskal-Wallis test, with relevant post hoc tests (Tukey test or Dunn test), were used for intergroup comparisons. The distributions of qualitative and discrete variables were compared with either Pearson’s χ²-test or Fisher’s exact test. Associations between continuous variables were tested with Spearman’s coefficient of rank correlation (R). All calculations were performed using Statistica 10 (StatSoft, Tulsa, OK, USA) software, with statistical significance defined as p<0.05.

Results

The serum concentration of NPT in the controls ranged between 2.872 and 14.779 nmol/L (median, 4.734 nmol/L). We used these values to determine the reference level of serum NPT. Irrespective of the patient’s age and sex, the cutoff value for serum concentration of NPT was set at 11 nmol/L, as previously proposed [18]. The serum concentration of NPT in children with JIA ranged between 2.811 and 22.765 nmol/L (median 6.044 nmol/L) and turned out to be significantly higher than in the controls (p<0.001; Table 1).

As many as 20 of 67 (30%) children with JIA presented with elevated levels of serum NPT (>11 nmol/L). The prevalence of elevated serum NPT in patients with JIA turned out to be significantly higher than in the controls (30% vs. 5%, p<0.001).

We did not observe significant correlations between the serum concentration of NPT and body temperature (R=0.00, p=0.97), erythrocyte sedimentation rate (R=0.09, p=0.47), leukocyte count (R=−0.05, p=0.70), CRP (R=−0.14, p=0.25), and PCT levels (R=0.07, p=0.56).

Serum concentrations of NPT in patients with various forms of JIA are presented in Table 2. The subsets of patients did not differ significantly in terms of mean NPT levels (p=0.40) and prevalence of results >11 nmol/L (p=0.20). The elevated serum concentration of NPT was documented in 5 (55%) children with systemic JIA, and in 8 (24%) and 7 (29%) patients with the oligoarticular and polyarticular form of this condition, respectively.

The relationships between serum concentration of NPT and activity of JIA are presented in Table 3. Children with exacerbation presented with significantly higher serum level of NPT than did patients with remission (p<0.001). Furthermore, serum concentration of NPT >11 nmol/L was documented in 20 of 40 (50%) patients with exacerbation and in none of the subjects with remission (p<0.001).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Lower quartile</th>
<th>Upper quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>105</td>
<td>4.734</td>
<td>2.872</td>
<td>14.779</td>
<td>4.050</td>
<td>5.292</td>
</tr>
<tr>
<td>JIA</td>
<td>67</td>
<td>6.044</td>
<td>2.811</td>
<td>22.765</td>
<td>4.471</td>
<td>12.578</td>
</tr>
</tbody>
</table>
Table 2: Serum concentration of neopterin, nmol/L, in children with various types of juvenile idiopathic arthritis.

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Lower quartile</th>
<th>Upper quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic</td>
<td>9</td>
<td>13.784</td>
<td>2.811</td>
<td>18.015</td>
<td>4.883</td>
<td>16.177</td>
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<tr>
<td>Oligoarticular</td>
<td>34</td>
<td>5.255</td>
<td>3.281</td>
<td>15.056</td>
<td>4.169</td>
<td>10.086</td>
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<tr>
<td>Polyarticular</td>
<td>24</td>
<td>6.347</td>
<td>3.976</td>
<td>22.765</td>
<td>5.149</td>
<td>12.378</td>
</tr>
</tbody>
</table>

Table 3: Serum concentration of neopterin, nmol/L, in children with remission and exacerbation of juvenile idiopathic arthritis.

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Lower quartile</th>
<th>Upper quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission</td>
<td>27</td>
<td>4.471</td>
<td>2.811</td>
<td>10.658</td>
<td>3.696</td>
<td>5.149</td>
</tr>
<tr>
<td>Exacerbation</td>
<td>40</td>
<td>10.912</td>
<td>3.914</td>
<td>22.765</td>
<td>5.953</td>
<td>14.196</td>
</tr>
</tbody>
</table>

Discussion

Our study showed that serum concentration of NPT in patients with JIA was significantly higher than in the controls. The lack of a significant association between the type of the disease (systemic vs. oligoarticular vs. polyarticular) and the serum concentration of NPT might reflect a low number of patients in particular subgroups. However, we revealed that the serum concentration of NPT differed significantly depending on activity of the disease, i.e., different during exacerbation and remission. While the peak serum concentration of NPT in patients with remission amounted to 10.66 nmol/L, the peak values documented during exacerbation of JIA reached up to 22.76 nmol/L. None of the patients with remission presented with a serum concentration of NPT above the predefined cutoff value (11 nmol/L). In contrast, this cutoff threshold of serum concentration of NPT was exceeded in as many as 50% of children examined at the time of exacerbation. We did not observe a significant correlation between the serum concentration of NPT and any of the analyzed inflammatory markers (erythrocyte sedimentation rate, leukocyte count, CRP, and PCT). This may be associated with the fact that NPT is an early marker of immune response and as such does not necessarily follow the expression patterns of other inflammatory indices [4].

To the best of our knowledge, none of previous studies analyzed the serum concentration of NPT as a clinical marker of JIA. However, many authors claimed on the usefulness of this parameter in patients with rheumatoid arthritis (RA) [16, 19–22]. Importantly, our findings are consistent with the above-mentioned data; this probably reflects similarities in the pathogenesis of JIA and RA, and the involvement of Th1 response in these two conditions.

In previous studies of RA patients, the concentrations of NPT were determined in serum, urine, and synovial fluid. Several authors analyzed urinary concentration of NPT as a potential non-invasive marker of RA. Altindag et al. [19] compared urinary concentrations of NPT during exacerbation and remission of RA, and showed a significant relationship between this parameter and the activity of the disease. Furthermore, they observed a significant correlation between the urinary concentration of NPT and stage of RA. In turn, both erythrocyte sedimentation rate and CRP proved to be less useful clinical markers of the condition, and none of these parameters correlated significantly with urinary concentration of NPT [19]. Similar findings were reported by Reibnegger et al. [21], who showed that patients with RA are characterized by significantly higher urinary concentrations of NPT, and the latter parameter correlates significantly with both clinical status and radiological evidence of the disease. Longitudinal analysis showed that urinary concentration of NPT changes relatively quickly in response to exacerbation or remission of RA and treatment of this condition. Thus, the authors suggested that urinary NPT can serve as a marker of treatment efficacy. Apart from NPT, Reibnegger et al. [21] analyzed the usefulness of many other laboratory parameters (erythrocyte sedimentation rate, hemoglobin, leukocyte count, CRP, and rheumatoid factor) as potential markers of RA. However, they showed that the urinary concentration of NPT reflected the clinical outcome of the disease better that did the other parameters. Furthermore, they did not observe significant associations between the urinary concentration of NPT and the levels of the remaining parameters, apart from weak correlations with erythrocyte sedimentation rate and hemoglobin concentration. Similar to our study, urinary concentration of NPT did not correlate with leukocyte count. Therefore, the authors concluded that urinary concentration of NPT alone is as useful a marker of clinical activity of RA as a composite measure comprising NPT, CRP, and erythrocyte sedimentation rate. Furthermore, they observed that the urinary concentration of NPT in individuals with osteoarthritis did not differ significantly from values of this parameter in healthy controls, and was significantly lower than in patients with stage I RA [21].

Schroecksnadel et al. [22] analyzed the serum concentrations of NPT in RA patients, and showed that this parameter correlates significantly with the stage of the disease. Although both CRP and erythrocyte sedimentation rate turned out to be relatively accurate markers of the disease activity, it was the serum concentration of NPT that showed a stronger association with the stage of RA (I-IV)
according to the Steinbrocker staging system. Serum NPT proved to be as useful a marker of RA as the levels of antinuclear (ANA), anti-keratin (AKA), and anti-cyclic citrullinated peptide autoantibodies. However, contrary to the autoantibody titers, NPT was a useful marker irrespective of patient age [22]. Fagerer et al. [16] studied the serum concentrations of NPT and various chemokines (CCL2, CXCL13, CX3CL1) in 113 patients with RA. The concentrations of all these markers were high, especially in individuals with cardiovascular comorbidities. Furthermore, the authors documented a significant correlation between the concentration of NPT and the levels of studied chemokines [16].

The concentration of NPT in synovial fluid of RA patients deserves special attention. Krause et al. [20] analyzed the concentration of this marker in synovial fluid of adult patients with various conditions associated with joint involvement (RA, osteoarthritis, gout). The authors showed that compared to individuals with the other conditions, patients with RA present with significantly higher concentrations of NPT in synovial fluid. The latter parameter accurately reflected systemic rather than local activity of the disease. The concentration of NPT in synovial fluid reached its peak values during exacerbation of RA, markedly exceeding the serum level of this marker [20].

As the determination of NPT in serum and synovial fluid is an invasive procedure, future studies should center around the potential usefulness of urinary NPT as a potential clinical marker of JIA.

In conclusion, our study showed that elevated serum concentration of NPT is an accurate marker of JIA exacerbation. Nevertheless, determination of NPT in serum is an invasive procedure. Thus, taking into account the above-mentioned literature data on the clinical usefulness of urinary NPT in patients with RA, future studies should center around the potential application of this parameter as a potential marker of JIA.

References