Research Article

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Serum Neopterin in Differential Diagnosis of Bacterial Diarrhea in Pediatric Patients

<https://doi.org/10.1515/pteridines-2019-0011>

received February 26, 2019; accepted April 9, 2019.

Abstract: Background: Neopterin, regarded as a marker of cellular immune activation, has been used in diagnosis of infection caused by intracellular pathogens. We have aimed to evaluate the clinical usefulness of serum neopterin (NPT) in acute bacterial diarrhea caused by group C enteropathogenic *Escherichia coli* (EPECs) and group D *Salmonella* spp.

Methods: Serum concentration of NPT was determined by ELISA. The study group included 47 children with diagnosis of bacterial diarrhea: 32 caused by group C enteropathogenic *Escherichia coli* (EPECs) and 15 by group D *Salmonella* spp. 105 healthy children constituted the control group.

Results: Serum concentration of NPT in children infected with group D *Salmonella* spp. turned out to be higher than in the other groups. The fraction of *Salmonella*-infected patients with serum neopterin above 11 nmol/l proved higher as compared to children with diarrhea caused by group C EPECs or to the healthy controls. The prevalence of a C-reactive protein (CRP) to NPT ratio of greater than 1 did not differ significantly between children with diarrhea of various etiology.

Conclusions: Neopterin can be used as a non-specific marker differentiating between bacterial diarrhea of various etiology.

Keywords: Neopterin; Bacterial gastroenterocolitis; Children; *Escherichia coli*; *Salmonella*.

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Introduction

Neopterin (NPT) belongs to the pteridine group, i.e. compounds composed of fused pyrimidine and pyrazine rings. Previous studies revealed that increased concentrations of NPT are associated with the cellular type of immune response, in the course of which interferon-gamma released from activated Th1 lymphocytes stimulates secretion of NPT from monocytes/macrophages [1]. Therefore, the concentration of NPT was proposed as a marker of immune response, and as such found applications in many medical disciplines (infectious diseases, pediatrics, oncology, rheumatology, transplantology and transfusiology), mostly in differential diagnosis, prognosis, and monitoring [2-8].

As a marker of cellular immune response, the role of NPT was studied extensively in patients with viral infections [9]. In contrast, little is known about the diagnostic value of NPT in bacterial infections of various etiology. Therefore, the aim of this study was to verify the clinical usefulness of serum NPT in children diagnosed with bacterial diarrhea.

Methods

Participants

The study, conducted between 2008 and 2012, included 47 children (20 girls and 27 boys) aged between 3 months and 15 years (mean 6.4 ± 4.4 years, median 6.9 years). All children were hospitalized at the Department of Pediatric Gastroenterology, Allergology and Nutrition, Medical University of Gdansk (Poland) due to bacterial diarrhea, confirmed based on stool culture. Microbiological examination identified the etiological factors as group C enteropathogenic *Escherichia coli* (EPECs, n=32) or group D *Salmonella* spp. (n=15).

The control group consisted of 105 healthy children (47 girls and 58 boys) aged between 1 month and 17 years (mean 7.6 ± 5.7 years; median 7.2 years). All 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 comprised of the presence of any chronic infection, autoimmune or neoplastic disease, history of vaccination or antibiotic therapy within four weeks preceding the study, administration of immunosuppressive or immunomodulatory agents within three months preceding the study, and body weight below third percentile for height.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Local Bioethical Committee at the Medical University of Gdansk and was conducted according to the principles of the Declaration of Helsinki.

Informed consent: Informed consent was obtained from legal guardians of all the participants included in the study, and from the participants older than 15 years.

Laboratory parameters

Venous blood samples were obtained on the day of admission, during the acute phase of infection. Serum concentration of NPT was measured by means of immunoenzymatic assay (ELISA; ELitest Neopterin catalogue no. 99.1 and 95.4, BRAHMS). Irrespective of the patient's age and gender, the cut-off value for serum concentration of NPT was set at 11 nmol/l, as previously proposed [10]. Apart from NPT, complete blood count with smear, and serum concentrations of C-reactive protein (CRP; latex turbidimetric test, COBAS INTEGRA, Roche Diagnostics GmbH) and procalcitonin (PCT; immunoluminometric assay, LIA PCT, catalogue no. 54.1, BRAHMS) were also determined for all children. Furthermore, serum level of creatinine was measured with a standard method, based on Jaffe's reaction, in order to exclude potential cases of renal failure.

Statistical analysis

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 (SD) or medians and interquartile ranges (IQR). Depending on the type of distribution, analysis of variance (ANOVA) or the 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 chi-squared 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, United States) software, with statistical significance defined as $p \leq 0.05$.

Results

The serum concentration of NPT in children infected with group D *Salmonella* spp. was significantly higher than in patients with diarrhea caused by group C EPECs ($p = 0.05$) or the controls ($p < 0.001$). In contrast, the latter two groups did not differ significantly in terms of serum NPT levels ($p = 0.486$; Table 1). Moreover, the fraction of *Salmonella*-infected patients with serum NPT above 11 nmol/l proved significantly higher, as compared to children with diarrhea caused by group C EPECs ($p < 0.001$) or to healthy controls ($p < 0.001$); similarly as in the case of the absolute values, these two groups did not differ significantly in the prevalence of serum NPT above 11 nmol/l ($p = 0.352$; Table 2).

The prevalence of a CRP to NPT ratio greater than 1 did not differ significantly between children whose diarrhea resulted from group C EPEC infection and those infected with group D *Salmonella* spp. (26/32 vs. 10/15, $p = 0.271$). Moreover, we did not document any significant correlation between the serum concentration of NPT in diarrhea patients and their body temperature ($R = 0.30$, $p = 0.10$), concentration of CRP ($R = 0.16$, $p = 0.38$), leukocyte count ($R = -0.05$, $p = 0.78$), and serum procalcitonin level ($R = 0.12$, $p = 0.53$).

Discussion

Although the involvement of NPT in the processes associated with cellular immune response has been documented in many studies, the evidence is limited mostly to viral infections. An increase in NPT concentration proportional to the severity of the disease has been observed in many viral diseases including acute viral hepatitis [11], rubella [12], measles [13], mumps,

chickenpox, influenza [14], cytomegalovirus infection [15], mononucleosis [1, 2], and HIV infection [1, 16-19].

Only a few previously published reports documented an increase in NPT concentration during infection with intracellular bacteria, most commonly *Mycobacterium tuberculosis* [20, 21, 22]. Shaw [20] analyzed serum concentrations of NPT in untreated patients with tuberculosis; this author observed that serum NPT correlated with the severity of illness, and concentrations of NPT above 40 nmol/l showed 95.7% specificity for *M. tuberculosis* infection. Another study revealed that baseline urinary concentrations of NPT in HIV-positive patients with concomitant tuberculosis are significantly higher than in subjects infected with CMV or *Pneumocystis carinii* [22]. Horak et al. [21] analyzed urinary concentration of NPT in 7 children with lung tuberculosis, aged between 10 months and 6 years. Four of them showed normal or only slightly elevated baseline levels of NPT at diagnosis, while another three patients presented with very high NPT concentrations. Two patients from this latter group experienced a far more complicated clinical course of tuberculosis [21]. Akbulut et al. [23] monitored the outcomes of 30 patients with confirmed infection of another intracellular bacteria, *Brucella* spp. All of them showed high serum concentrations of NPT at diagnosis, but those who eventually did not respond to conventional therapy were characterized by extremely high serum NPT levels (42-109 nmol/l). Therefore, serum concentration of NPT was postulated to be an accurate prognostic marker of *Brucella* spp. infection [23].

To the best of our knowledge, there is only one published study of serum NPT levels in patients infected with *Salmonella* spp. Kozłowska-Murawska and Obuchowicz [24] determined serum levels of NPT in children with acute diarrhea caused by *Salmonella* spp. or rotavirus. They observed elevated concentrations of NPT in both rotavirus and *Salmonella* spp. infections, and thus concluded that this parameter is not helpful in the differential diagnosis of bacterial and viral diarrhea [24]. However, none of the previously published studies compared serum concentrations of NPT in patients with salmonellosis and other common etiology of bacterial diarrhea (e.g. diarrhea caused by enteropathogenic *E. coli*). Noticeably, our study revealed elevated serum concentration of NPT (above 11 nmol/l) in up to 93% of children infected with group D *Salmonella* spp. In contrast, the concentration of NPT was normal in more than 90% of our pediatric patients in whom group C EPECs were isolated from stool samples. Probably these differences were associated with different biology of bacterial replication: while enteropathogenic *E. coli*

represents extracellular pathogen, *Salmonella* is a typical intracellular bacterium and induces cellular immune response, similar to that observed during viral infection.

We realize that a small sample size is a limitation of the presented study and further research on serum NPT in bacterial diarrhea should be continued.

In conclusion we confirmed that increased serum levels of NPT can be observed during acute infections caused by intracellular bacteria, but are unlikely during invasion of extracellular pathogens. Therefore, serum NPT can be used as a non-specific marker differentiating between bacterial diarrhea of various etiology.

Conflict of interest: Authors declare no conflict of interest

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