Neopterin concentrations in cerebrospinal fluid and serum as an aid in differentiating central nervous system and peripheral infections in children

Michael M. Millner, Wolfgang Franthal, Gabriela H. Thalhammer, Andrea Berghold, Reingard M. Aigner, Gerhard F. Füger, and Gilbert Reibnegger

Neopterin is a sensitive indicator for cellular immune activation. Its concentrations were determined in cerebrospinal fluid (CSF) and serum specimens from 91 children with no evidence of central nervous system (CNS) or peripheral inflammations, 43 with definite neuroborreliosis, 51 with other CNS infections, and 33 with peripheral infections. The aim of our study was (a) to establish a range of normal CSF neopterin concentrations in control children, and (b) to inquire into the diagnostic potential of neopterin measurements in both body compartments for aiding in differential diagnosis of inflammatory vs noninflammatory diseases, and CNS vs peripheral inflammations. CSF neopterin concentrations in controls were invariably low (up to 9.3 nmol/L), but in children with neuroborreliosis and, even more so, with other CNS infections neopterin concentrations were significantly \( (P < 0.0001) \) increased. Children with peripheral infections, however, rarely showed raised CSF neopterin concentrations. Serum concentrations of neopterin, on the other hand, were not significantly different between controls and children with neuroborreliosis. Although serum concentrations were significantly different between controls and children with other CNS infections, diagnostic efficiency was poor for this comparison. Peripheral infections, in contrast, were associated with significantly higher \( (P < 0.0001) \) serum neopterin concentrations when compared with controls.

A classification tree was constructed on the basis of CSF and serum neopterin concentrations, allowing with high accuracy the discrimination between controls, children with CNS infections, and children with peripheral infections. Thus, on the basis of a comparatively large control group, our data underline the diagnostic validity of neopterin as an aid in differential diagnosis of inflammatory vs noninflammatory diseases, and confirm that CSF neopterin concentrations are not correlated with serum neopterin concentrations, and, therefore, CSF neopterin appears to be produced intrathecally.

The catchment area of the Pediatric Department of Graz, Austria, is endemic for Lyme borreliosis. If clinical symptoms conform with laboratory tests, diagnosis of a neuroborreliosis is easy. Because diagnostic methods for defining Lyme borreliosis are not standardized in cases of suspected neuroborreliosis, a certain discriminating limit would be very helpful.

Neopterin belongs to the class of pteridines that are pyrazino-[2,3-d]-pyrimidine compounds occurring ubiquitously in living cells. Neopterin has been shown to be a sensitive indicator for the activation of cell-mediated immune reactions \([1, 2]\) and thus, determination of neopterin concentrations in various body fluids is of diagnostic interest in a variety of diseases in which T lymphocytes and macrophages are involved \([3, 4]\).

Although many studies have dealt with neopterin measurements in peripheral blood and urine, few investigators have determined the concentration of this immune activation marker in cerebrospinal fluid (CSF)\(5\). The

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5 Nonstandard abbreviations: CSF, cerebrospinal fluid; CNS, central nervous system; CART, classification and regression trees; and CI, confidence interval.
latter studies were devoted to infections with HIV-I [5, 6], morbilli [7], and meningitis with afebrile convulsions [8], reviewed in a recent article [9].

Here, we present results of neopterin measurements in CSF specimens and in sera from children without or with infections either in the central nervous system (CNS) or in the periphery of the organism. Particular weight has been put on the problem of defining “normal ranges” for neopterin in children’s CSF specimens and on the question of whether CSF neopterin provides an improvement in diagnostic accuracy in comparison with CSF lymphocytic cell count.

**Patients and Methods**

**Patients**

Children from ages 4 to 18 years were included in this study while admitted to the Department of Pediatrics of the University Hospital of Graz during 1991–1995 because of suspicion of CNS disease. Not included into the study were children who showed a disturbed blood–brain barrier according to the method of Reiber [10], children with neurological disease other than aseptic meningitis and (or) neuroborreliosis, and children whose CSF specimens were contaminated by blood. A total of 218 children (124 boys, 94 girls) from ages 4 to 16.6 years (median age 8.4 years) were finally included.

All children were investigated by experienced neurologists, and were classified into one of four diagnostic categories: Group 1 was 91 children in whom, after initial suspicion of CNS inflammatory disease, an inflammation of CNS or periphery could be excluded by laboratory tests (Table 1), by clinical course, and partly by a normal magnetic resonance image. Notably, these children initially were definitely not healthy but had a wide spectrum of diseases: cranial nerve palsy of unknown etiology (n = 42), strong headache (n = 17), first generalized seizure (n = 12), acute strabismus (n = 8), transient gait disturbance (n = 5), paresthesia of unknown etiology (n = 2), acute confusional state due to migraine (n = 2), benign paroxysmal vertigo (n = 2), and progressive hypacusis of unknown etiology (n = 1). Because in these children CSF or peripheral infections were excluded by clinical and laboratory criteria, CNS specimens collected initially from them were used to define normal ranges of CSF neopterin concentration. Group 2 comprised 43 children with definitive neuroborreliosis presenting as clinically aseptic meningitis (18–1280 lymphocytic cells/mm³) and additional cranial nerve palsy in 51%. For defining criteria [11] see Table 1. Clinically heterogeneous group 3 consisted of 51 children with meningoencephalitis due to morbilli (n = 5), Central European encephalitis (n = 5), varicella (n = 1), subacute sclerosing panencephalitis (n = 1), or unknown etiology (n = 39). Neuroborreliosis was excluded. Group 4 contained 33 children with peripheral inflammatory disease in whom CNS inflammation could be excluded by laboratory tests. These children presented with stiff neck and confirmed diagnosis of tonsillitis (n = 15), sinustis (n = 8), salmonellosis (n = 4), mononucleosis (n = 2), or otitis media (n = 4).

**Laboratory Methods**

Neopterin concentrations in CSF and serum specimens were determined by a commercially available RIA (Immuo Biological Laboratories). For serum neopterin, a concentration of 10 nmol/L is generally accepted as upper limit of normal. As shown in a large population of 76 000 healthy voluntary blood donors [12], this value corresponds to the 98th percentile of the distribution of neopterin concentrations in sera from healthy subjects. An earlier study involving parametric evaluation techniques [13] identified the same neopterin concentration as suitable cutoff value.

For CSF neopterin concentrations, a well-defined upper limit of normal does not exist, mostly because of the ethical impossibility to recruit enough subjects for collecting CSF. A review of studies investigating CSF neopterin has been recently published [9]; the problems with normal values are discussed herein.

Lymphocytic cell count in CSF was determined by routine technique.

**Statistical Analysis**

The Mann–Whitney U-test was used to test for statistical significance of differences of laboratory variables between the different patient groups. Program BMDP3S (BMDP Statistical Software) was used for this purpose. The same program was also used for performing Spearman rank correlation analyses between the variables.

To evaluate the ability of both CSF and serum neopterin concentrations and of CSF lymphocytic cell count to differentiate between controls and neuroborreliosis, CNS infections, and peripheral infections, ROC curve analyses were performed with program CLABROC, kindly provided by Charles E. Metz, University of Chicago [14].

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**Table 1. Diagnostic laboratory variables in all children.**

<table>
<thead>
<tr>
<th>Specific analytes in CSF</th>
<th>CSF/serum index (Bb4-IgG) &gt;2.0</th>
<th>ELISA Bb4-IgG in CSF</th>
<th>CSF Bb&quot; culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonspecific analytes in CSF</td>
<td>CSF cells</td>
<td>CSF protein</td>
<td>CSF neopterin</td>
</tr>
<tr>
<td>Specific analyte in serum</td>
<td>Serum ELISA Bb4-IgG</td>
<td>White blood count</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>Nonspecific analytes in serum</td>
<td>C-reactive protein</td>
<td>Neurotropic viruses &quot;</td>
<td>Serum neopterin</td>
</tr>
</tbody>
</table>

"Borrelia burgdorferi"  
*Herpes simplex, varicella zoster, cytomegalo, Central European encephalitis, mumps, morbilli, rubeola, Epstein Barr viruses."

Defining criteria in suspected neuroborreliosis was at least one of the specific variables in CSF (ref. 11).
program not only calculates maximum likelihood estimates of the parameters (in particular, the area under the ROC curve) of binormal ROC curves for two different diagnostic tests performed on the same subjects, but also permits estimation of the statistical significance of the difference between the two resulting ROC curves with different statistical tests. We have chosen area under the two ROC curves for statistical comparisons (null hypothesis: The data sets arose from binormal ROC curves with equal areas beneath them). An area index of 0.50 would indicate a worthless diagnostic test. Levels of significance ($P$) are always for two-sided comparisons.

Finally, by using CSF and serum neopterin concentrations and liquor lymphocytic cell count as candidate variables, we constructed a classification tree for the discrimination between the four diagnostic categories (controls, neuroborreliosis, other CNS infections, and peripheral infections) by using the classification and regression tree (CART) technique [15]. Briefly, the CART method starts with the whole measurement space (which is by definition the matrix containing all measurement vectors) and proceeds by repeated splits of subsets of the measurement space into two descendant subsets. The basic idea is to select such splits that the data in the descendant subsets are “purer” with respect to the classification problem at hand, i.e., each subset should contain the greatest possible number of members (measurement vectors) belonging to one certain category, and at the same time, the fewest possible members belonging to all remaining categories. Each split is evaluated by one of various possible statistical criteria; we have used the well-known $\chi^2$ test for evaluation. The CART method belongs to computer-intensive procedures because a systematic search for the optimum splits in the above-mentioned sense is required. The reward for the computational effort is an effective and easy-to-perform algorithm to use laboratory (or other diagnostic) information for clinical decisions.

**Results**

**NEOPTERIN CONCENTRATIONS AMONG ALL DIAGNOSTIC GROUPS**

Table 2 shows median neopterin concentrations in CSF and serum samples, and median lymphocytic cell counts in CSF samples, together with their ranges, grouped according to diagnostic classification of the patients.

<table>
<thead>
<tr>
<th>Table 2. Neopterin concentrations in CSF and serum samples, and lymphocytic cell count in CSF samples.</th>
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<tbody>
<tr>
<td><strong>CSF neopterin, nmol/L</strong></td>
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<tr>
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<tr>
<td>Group 1</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>4</td>
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</tbody>
</table>

To specify the differences between the four diagnostic categories in more detail, Mann–Whitney $U$-tests were performed. Although the difference of CSF neopterin values between controls and patients with borreliosis was highly significant ($P <0.0001$), serum concentrations between both groups were not significantly different ($P = 0.061$). In children with other CNS infections, both serum and CSF neopterin concentrations differed significantly from those in controls ($P <0.0001$). Both in CSF and serum, neopterin concentrations differed significantly between controls and children with peripheral infections ($P <0.0001$). However, as Table 2 shows, the neopterin concentrations in CSF of these patients were only slightly higher than in controls; the statistical significance is a result of the small variance in both groups. Notably, when we compared CSF or serum concentrations in a pairwise fashion among children with borreliosis, with CNS infections, and with peripheral infections, invariably highly significant differences were detected ($P <0.0001$).

Neopterin concentrations in CSF and serum were poorly correlated with each other. Overall, Spearman’s rank correlation coefficient was 0.30 (218 data pairs); when computed separately in each diagnostic category, Spearman’s rank correlation coefficients were 0.12 (controls), 0.13 (borreliosis), 0.36 (CNS infections), and 0.39 (peripheral infections).

There was a significant but not nearly perfect correlation between CNS neopterin and liquor lymphocytic cell count in children with neuroborreliosis [linear correlation coefficient $r = 0.53$; 95% confidence interval (CI) 0.29–0.70; $P = 0.0001$] and with other peripheral infections ($r = 0.34$; CI 0.07–0.57; $P = 0.015$).

**NEOPTERIN CONCENTRATIONS IN CONTROLS**

From an ethical viewpoint, CSF specimens cannot be collected from clinically healthy children; thus, group 1 children appeared to us to be best suited for the purpose of comparison with the three remaining diagnostic categories.

The distribution of neopterin concentrations found in serum specimens from 91 control children was in good agreement with literature data: In 88 specimens, neopterin concentration was ≤10.0 nmol/L; in three children, slightly increased concentrations between 10.1 and 11.0 nmol/L were found. Notably, the distribution of CSF neopterin data in the same children was not too different: The mean value was shifted slightly towards smaller values (Table 2); 88 specimens had neopterin concentrations ≤9.0 nmol/L; and in three CSF samples, neopterin concentrations were between 9.1 and 9.3 nmol/L.

**ROC CURVE ANALYSES**

For both CSF and serum neopterin concentrations and for CSF lymphocytic cell count, ROC curves were computed to evaluate their power to discriminate between controls and either of the three other diagnostic categories.

Figure 1 shows the results of ROC analyses for the
comparison between group 1 (controls) and group 2 (neuroborreliosis). The best discrimination is provided by CSF neopterin (area index = 0.9927), but CSF lymphocytic cell count discriminates essentially equally well between both groups (area index = 0.9864); the difference between both tests is not statistically significant \((P = 0.47)\). Serum neopterin is nearly worthless for this comparison (area index = 0.6153); both CSF neopterin and CSF cell count are significantly better discriminators \((P < 0.0001)\).

Figure 2 presents ROC curves for the comparison between group 1 (controls) and group 3 (other CSF infections). CSF neopterin perfectly discriminates between both groups, i.e., there was no overlap between CSF neopterin concentrations of both groups. This situation is termed “degenerated;” the program CLABROC terminates without performing the ROC computations. To circumvent this problem, the CSF neopterin concentration of one control child was incremented by 10 nmol/L, yielding an “elevated” result. Notably, the area index thus computed for CSF neopterin (area index = 0.9992) is slightly underestimated. CSF lymphocytic cell count also discriminates nicely between both groups (area index = 0.9592); the differences between CSF and serum neopterin \((P < 0.0001)\) and between serum neopterin and CSF cell count \((P = 0.0083)\) are significant. When comparing group 1 with pooled groups 2 and 3, the difference between the area indices of CSF neopterin (0.9981) and CSF cell count (0.9707) remain statistically significant \((P = 0.019)\). This indicates superior discrimination between controls and patients with CNS infections (including neuroborreliosis) by CSF neopterin concentrations.

Figure 3 compares the ROC curves for the three variables obtained for the discrimination between group 1 (controls) and group 4 (peripheral infections). As expected, the best discriminator for this diagnostic dilemma is serum neopterin concentration (area index = 0.9744). The discrimination was perfect: To circumvent the degeneracy of the data set, we applied the same procedure as above. CSF neopterin (area index = 0.8371) and particularly CSF cell count (area index = 0.5071) discriminate significantly \((P < 0.0001)\) worse.

An interesting comparison, group 2 (neuroborreliosis) and group 3 (other CNS infections), is demonstrated in Fig. 4: CSF neopterin (area index = 0.8004) is a weak discriminator but still significantly \((P < 0.0001)\) superior to CSF cell count (area index = 0.5236) in this situation.

**CART ANALYSIS**

Figure 5 shows the decision tree resulting from the CART procedure: Starting with all individuals investigated, the optimum initial split is based on CSF neopterin \(<13.4 \text{ vs } >13.4 \text{ nmol/L})\). Of 91 children with CNS infections, 89 fell into one subset; the remaining two children together with all controls and all children with peripheral infections comprised the second subset. This split produced an extremely high \(\chi^2\) value (206.2); the lymphocytic
cell count was slightly inferior ($\chi^2 = 185.6$): The “impurity” of the descendant subsets would have been higher. In the group with CSF neopterin $<13.4$ nmol/L, a second split based on the question “serum neopterin below or above $10.7$ nmol/L?” yields a nearly perfect classification of controls and children with peripheral infections; only one subset remained “contaminated” with two falsely classified children with neuroborreliosis. The discrimination between children with neuroborreliosis and with other CNS infections could not be made perfectly on the basis of the laboratory variables investigated; the best split obtainable was based on the fact that CNS neopterin concentrations were considerably higher in patients with CNS infections other than neuroborreliosis.

**Discussion**

Neopterin originates from GTP, which is transformed by a specific enzyme, GTP-cyclohydrolase I, into the first detectable intermediate of pteridine biosynthesis, 7,8-dihydronopterin triphosphate. From this key intermediate of pteridine biosynthesis, either neopterin is formed by oxidation and cleavage of the triphosphate moiety by phosphatases, or 5,6,7,8-tetrahydrobiopterin is synthesized by several enzymatic steps. This latter pterin derivative has long been known to be an essential cofactor for the enzymes involved in hydroxylation of aromatic amino acids (i.e., Phe, Tyr, and Trp [16]) in ether–lipid cleavage [17], and, more recently, to play a role in the inducible nitric oxide synthase reaction [18]. Nearly all human and animal cells capable of constitutive or inducible pteridine biosynthesis produce 5,6,7,8-tetrahydrobiopterin. The only known exceptions are monocytes/macrophages from humans or primates, and cultured cell lines derived from such cells [19]. Neopterin production in these cells is inducible by cytokines; interferon-$\gamma$ is the most potent stimulator of neopterin biosynthesis.

Thus, neopterin determination is a valuable indicator of the activation of the cell-mediated immune system, although the biological reason for neopterin production by interferon-$\gamma$-stimulated human and primate monocytes/macrophages is not known. Most recent experiments have indicated that neopterin acts as an enhancer of several reactions involving oxygen and chlorine free radicals [20]; because these highly reactive substances participate in the effector functions of monocytes/macrophages, such a role would be compatible with the large amount of data accumulated on the use of neopterin as an immune activation marker.

All of our 51 children with CNS infections other than neuroborreliosis (group 3) had CSF concentrations well above the range found in the 91 control children. Two of 43 children with neuroborreliosis (group 2) had CSF neopterin concentrations overlapping with values found in controls (see also Fig. 4). But these children had their spinal tap not within the first three days but on day 16 and 14 of neurological disease, respectively.

The increase in neopterin is known to precede the appearance of specific antibodies in serum on average by 1 week. Neopterin release starts 3 days before the maximum of proliferation of T cells [21]. From a clinician’s
point of view, availability of an early marker of inflammation would be of particular interest in the very early phase of a disease before specific antibodies are produced. ROC analyses profoundly underscore the diagnostic potential of CSF neopterin, showing perfect specificity (none of the control children had CSF neopterin exceeding 9.3 nmol/L) and excellent sensitivities (95% for neuroborreliosis and 100% for other CNS infections).

As expected, CSF neopterin had only weak diagnostic potential in identifying children with systemic infections: At a cutoff value of 9.3 nmol/L, sensitivity was only 18%. Considering that the blood–brain barrier normally prevents neopterin transport from the peripheral circulation to CNS, and vice versa, this result was not surprising.

With serum neopterin, the situation was quite different: Both neuroborreliosis and other CNS infections were associated with poor sensitivities at the usual cutoff value of 10 nmol/L (12% and 51%, respectively; specificity was 97%). In contrast, for systemic infections serum neopterin had perfect sensitivity of 100% in our cases.

As was shown in previous studies by several authors (see ref. 9 for review), neopterin concentrations in CNS and peripheral circulation are practically not correlated with each other. Thus, our results confirm those of others that CSF neopterin evidently arises from intrathecal production. Only about 2–3% of CSF neopterin has been estimated to stem from sources outside the CNS. The source of neopterin in CSF is not definitively known; monocytic cells invading the CNS from peripheral blood or, more likely, brain cells such as astrocytes or microglia could be responsible for neopterin production. This speculation is underlined by the fact that human microglia cells are able to produce measurable amounts of neopterin [22].

Our study demonstrates the utility of neopterin measurements in CSF and serum in children with suspected CNS or peripheral infections as an aid in differentiating inflammatory vs noninflammatory disorders, and CNS vs peripheral disease. The comparatively large number of children who were eligible as controls because no CNS or peripheral inflammation was detectable by specific methods seems to yield a reliable distribution of control CSF neopterin concentrations that might aid others in judging results of CSF neopterin concentrations.

To correlate children's symptoms either to a CNS inflammation or to a peripheral one may sometimes be difficult. In these cases, neopterin as a nonspecific but highly sensitive marker of inflammation could add helpful information.

One might question the importance of CSF neopterin determination in view of the significant correlations between CSF neopterin and liquor cell count. However, the correlation between both variables was by no means perfect but significantly different from zero in both groups with CNS infections: For example, a linear correlation coefficient of 0.53 (neuroborreliosis group) means that only 28% (0.53^2 = 0.28) of the variation of one variable can be explained by the second variable. The most convincing argument for the utility of CSF neopterin, however, is provided by ROC analysis: Whereas CSF neopterin and CSF cell count are essentially equal in their discriminative potentials regarding controls vs neuroborreliosis cases, CSF neopterin is significantly superior in discriminating controls and patients with other CNS infections. Additionally, CSF neopterin allows at least tentative discrimination between neuroborreliosis and other CNS infections; cell count seems to be perfectly worthless for this discrimination. Finally, although both markers are excellent for the discrimination between controls and CNS infections including neuroborreliosis, area index of CSF neopterin is significantly higher than area index of cell count. A further indication for the superiority of CSF neopterin over cell count comes from CART analysis: All three variables, CSF and serum neopterin and CSF cell count, were candidate variables for this analysis. However, cell count was not entered by the algorithm for construction of the classification tree because it was slightly inferior compared with CSF neopterin. Our study suggests, therefore, that measurement of CSF neopterin contributes unique and statistically independent information for diagnostic and therapeutic decision making. Particularly in combination with serum neopterin concentrations, it might provide a significant aid for differential diagnosis.
References


