Prospective Longitudinal Study of Children With Tic Disorders and/or Obsessive-Compulsive Disorder: Relationship of Symptom Exacerbations to Newly Acquired Streptococcal Infections

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ABSTRACT. Background. It has been proposed that infection by group A β-hemolytic streptococci (GABHS) can trigger acute symptom exacerbations among patients with Tourette’s syndrome (TS) or obsessive-compulsive disorder (OCD), via autoimmune mechanisms.

Objective. To examine the temporal relationship between newly acquired GABHS infections (and other immunologic indices) and acute exacerbations of tic and obsessive-compulsive symptoms.

Methods. Pediatric patients (7–17 years of age) with TS and/or OCD (N = 47) and healthy control subjects (N = 19) were prospectively monitored for newly acquired GABHS infections, nonspecific markers of acute inflammatory responses, and D8/17-reactive cells (a marker of rheumatic fever). Objective monthly ratings of tic and obsessive-compulsive symptom severity were used to determine the timing of symptom exacerbations.

Results. The overall rate of acute exacerbations of neuropsychiatric symptoms was 0.56 exacerbations per patient per year. The average rate of new GABHS infections, using a stringent definition, was 0.42 infections per subject per year among patients, compared with 0.28 infections per subject per year for control subjects. The association between symptom exacerbations and new GABHS infections among patients was no greater than that expected on the basis of chance. At baseline, patients demonstrated significantly higher levels of D8/17-reactive cells and neopterin, compared with control subjects, but there was no consistent pattern of change when exacerbation time points were compared with baseline or follow-up time points.

Conclusions. The results suggest no clear relationship between new GABHS infections and symptom exacerbations in an unselected group of patients with TS and/or OCD. Pediatrics 2004;113:578–585. URL: http://www.pediatrics.org/cgi/content/full/113/6/e578; Tourette’s syndrome, obsessive-compulsive disorder, PANDAS, group A β-hemolytic streptococci, D8/17, neopterin, C-reactive protein.

ABBREVIATIONS. ADHD, attention-deficit/hyperactivity disorder; ASO, anti-streptolysin O; BRR, B repeat region; CRP, C-reactive protein; CRR, C repeat region; Y-BOCS, Yale-Brown Obsessive-Compulsive Scale; GABHS, group A β-hemolytic streptococci; OCD, obsessive-compulsive disorder; OC, obsessive compulsive; PANDAS, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections; RF, rheumatic fever; TS, Tourette’s syndrome; YGTS; Yale Global Tic Severity Scale; IgG, immunoglobulin G.

Tic disorders, obsessive-compulsive disorder (OCD), and related conditions affect as many as 3% of children and adolescents.¹–⁶ The factors that contribute to the pathogenesis of these disorders are poorly defined. The hypothesis that infections can modulate the clinical appearance of tic disorders dates from the 1800s.⁷ The past decade has seen the reemergence of the hypothesis that postinfectious immune mechanisms account for at least some cases of Tourette’s syndrome (TS) and OCD.

It is well known that group A β-hemolytic streptococci (GABHS) can trigger immune-mediated diseases.⁸–¹⁰ Rheumatic fever (RF), one of the most well-recognized examples of a delayed nonsuppurative complication of GABHS infection, usually occurs a few weeks to several months after streptococcal infection among susceptible persons. RF typically involves the heart, joints, and central nervous system. The central nervous system manifestations usually take the form of chorea (Sydenham’s chorea). However, some patients with RF also display motor or phonic tics, obsessive-compulsive (OC) symptoms, or features suggesting attention-deficit/hyperactivity disorder (ADHD).¹¹–¹³ On the basis of these associations, Swedo et al¹⁴ proposed that pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) represents a distinct clinical entity that includes cases of TS and OCD.

In this prospective longitudinal study, the temporal relationship between periods of neuropsychiatric symptom exacerbation and newly acquired GABHS infections was examined. On the basis of previous studies of RF, TS, and OCD patients,¹⁵–²² we also measured the levels of peripheral blood B lymphocytes expressing the D8/17 surface antigen at baseline, at exacerbation, and 2 months after neuropsychiatric symptom exacerbation. Nonspecific markers of acute inflammation, ie, neopterin and C-reactive protein (CRP), were also measured at those 3 time points.
METHODS

Prospective Study Design

This report combines data from an initial, longitudinal, pilot study with data from a larger, ongoing, longitudinal, case-control study. The primary distinction between the pilot and case-control studies is that the latter study includes flow cytometric measurements of T-lymphocyte subsets (to be presented in a separate report). Measurements for D8/17-reactive cells (see below) were obtained for the entire pilot study and continued through April 2001 of the ongoing case-control study; this report includes all surveillance periods for which D8/17 measurements were made, and no data are omitted.

Forty-seven children (7–17 years of age) who had been diagnosed as having a tic disorder, OCD, or both and 19 control children (with neither of those disorders was assessed at baseline before entry into the study). In both studies, each participating family was assigned to an experienced clinical assessor, who monitored the child and family monthly throughout the surveillance period, except for time gaps for 3 patients (as noted in the Results). After the baseline assessments, monthly clinical ratings were completed for the patients; for 8 patients, the surveillance period for clinical ratings did not extend beyond the baseline time point. In addition, for subjects in an ongoing longitudinal study, in-person visits occurred approximately every 4 months, for additional monitoring of clinical severity and collection of blood and microbiologic specimens. For all patients, if an exacerbation of symptoms occurred, then the patient was immediately reevaluated to confirm that a clinically significant exacerbation had occurred; at that time, additional throat cultures and blood specimens were collected. Approximately 2 months after the exacerbation, an identical follow-up assessment was completed. Blood was obtained from 17 patients at the time of entry only (baseline), and no additional serologic surveillance was performed; the remaining 30 patients were monitored for ≥3 months, as were 17 of the 19 control subjects.

Subjects

All subjects entered the study between October 1997 and April 2001. To be included in the study, subjects were required to have an IQ of >75 and to be free of serious medical illnesses, major sensory handicaps (blindness or deafness), major neurologic diseases (including seizure disorders), previous head trauma that resulted in loss of consciousness, and any current (past 6 months) psychiatric disorder that could interfere with participation, such as major depression, psychosis, autism, or another pervasive developmental disorder. All parents provided written informed consent, and the study was described to them in detail. A separate assent form was used to ensure the informed participation of the child and adolescent subjects. All patients were monitored at the Yale Child Study Center Tic Disorder–Obsessive-Compulsive Disorder Specialty Clinic. Patients were not selected for the diagnosis of PANDAS. Control subjects were ascertained from regional telemarketing lists, for identification of individuals (matched for age, gender, socioeconomic status, and season of the year at entry into the study) without symptoms of TS or OCD. Control subjects were selected only if there was no lifetime personal history, for the patient or any first-degree relative, of a Diagnostic and Statistical Manual of Mental Disorders (4th ed) diagnosis of a chronic tic disorder, TS, OCD, or ADHD. No ethnic or racial groups were excluded from the study. Information on past GABHS infections was not used in making decisions regarding participation in the study.

Initial Clinical Assessments

When a family entered the study, diagnostic information concerning the patient was collected in a 2-stage process. The first stage consisted of the collection of information concerning symptoms associated with TS and OCD, using a self-and-family report based on the tic inventory, ordinal severity scales of the Yale Global Tic Severity Scale (YGTSS), and the symptom checklist and ordinal scales of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS). The PANDAS data were prospectively collected, with the National Institute of Mental Health PANDAS Diagnostic Interview. From June 2000 onward, in the second stage of assessment, an experienced clinician reviewed these symptom ratings with each family member to ensure their accuracy and validity.

Comorbid psychiatric diagnoses (including ADHD) were made on the basis of all available information, including data collected with the Kiddie Schedule for Affective Disorders and Schizophrenia. As previously described in detail, clinical data were reviewed for those PANDAS cases. PANDAS diagnoses were made according to both minimal and stringent criteria. The minimal criteria required two-tenths of a log10 increase in either the ASO or anti-DNase B titer between 2 consecutive blood samples. This value corresponds to a 1.6-fold increase in titer. Titer of <50 units were treated as 50 (log10 1.70).

Stringent criteria for a new GABHS infection required satisfaction of the minimal criteria plus a GABHS-positive throat culture and/or an absolute titer for either ASO or anti-DNase B exceeding the upper limit of normal values. Three slightly different definitions of the upper limits of normal values were considered, ie, 1) ≥800 units for either ASO or anti-DNase B titer between 2 consecutive blood samples. This value corresponds to a 1.6-fold increase in titer. Titer of <50 units were treated as 50 (log10 1.70).

The percentages of D8/17-positive cells were measured for 141 and 56 of the blood samples collected from the patients and...
control subjects, respectively. The percentages of D8/17-positive cells were measured with immunofluorescence microscopy, as described. A minimum of 200 anti-HLA-DR-positive peripheral blood mononuclear cells, representing B lymphocytes and monocytes, were counted after mounting with an antiquenching agent. Individual anti-HLA-DR-positive cells were scored for fluorescein labeling attributable to bound D8/17 monoclonal antibody plus anti-mouse immunoglobulin M conjugate; the percentages of anti-HLA-DR-positive cells that were also stained with the D8/17 monoclonal antibody were calculated. At the beginning of the study, experiments were performed to ensure that there was minimal variation between investigators in terms of scoring positive cells; in addition, by using samples from healthy adult control subjects, day-to-day variations were shown to be minimal. Little batch-to-batch variation in D8/17 immunoreactivity was observed.

Nonspecific Markers of Acute Inflammation

Measures of CRP and neopterin were made in microtiter plates with standard kits, in accordance with the instructions provided by the manufacturer (high-sensitivity CRP enzyme immunoassay test kit and neopterin enzyme immunoassay kit; both from ICN Diagnostics, Costa Mesa, CA). Samples were tested in duplicate, values were averaged, and the measurements of optical density at 450 nm were compared with standard curves generated with known quantities of CRP or neopterin.

Data Analyses

Continuous variables were expressed as mean ± SD for normally distributed data or as median and range for nonparametric data. Categorical data were expressed as frequencies and percentages. Differences between groups were determined with either Student’s t test or the Mann-Whitney U test (depending on data distribution) for continuous variables or with Fisher’s exact test for discrete variables.

With the use of either the minimal or stringent criteria (see above), the average number of new GABHS infections per subject per year was calculated as (number of new infections ± number of subjects under surveillance) / (average number of years of surveillance per patient). This rate calculation assumes that the acquisition of multiple GABHS infections by an individual is unaffected by previous infections. Exacerbation rates for patients were calculated as (number of exacerbations ± number of patients under surveillance) / (average number of years of surveillance per patient).

Survival curves and survival probabilities were generated with the Kaplan-Meier method. Survival time was defined as the time from baseline to either symptom exacerbation or a new GABHS infection. A multivariate, Cox proportional-hazards model that included age as a covariate was also applied.

RESULTS AND DISCUSSION

Subjects

The demographic and clinical characteristics of the patients and control subjects are summarized in Table 1. Of the 47 patients, 37 (79%) met the diagnostic criteria for TS and 27 (57%) had OCD; 17 patients (36%) were diagnosed as having both disorders. Of the 47 patients, 34 were assessed for PANDAS at baseline. Of those patients, 8 (25%) met the case definition for probable PANDAS. Sixteen patients experienced a total of 23 acute exacerbations of TS and/or OCD symptoms. The overall rate of acute exacerbations was 0.56 exacerbations/patient per year, which is equivalent to 1 exacerbation/patient every 1.8 years (mean surveillance time: 12.7 months).

At entry into the study (baseline), there was no significant difference in the proportions of individuals with elevated antistreptococcal titers (ASO and/or anti-DNase B), in a comparison of patients (14 of 45 patients, 31.1%) and control subjects (7 of 17 subjects, 41.2%) for whom material was available for serologic analysis. In contrast to several previous reports and in agreement with others, we did not find that our patients had higher titers of antistreptococcal antibodies. The reason for these discrepancies is unclear but is possibly related to regional and seasonal differences and the composition of the patient sample. In the northeastern United States, GABHS infection usually peaks during the winter months (November through April). In the present study, there was no seasonal bias in the time of entry into the study for the patient versus control groups (P = .18, Fisher’s exact test).

Incidence of Newly Acquired GABHS Infections

During the course of the longitudinal surveillance study, patient and control subjects (N = 30 and N = 17, respectively) (Table 1) were monitored for evidence of newly acquired GABHS infections, as indicated by serologic findings. GABHS infections result in a host antibody response, which leads to clearance of the infecting organism by ~2 weeks after infection. Antistreptococcal antibody titers can continue to increase for several weeks. In clinically inapparent GABHS infections, the levels of antistreptococcal antibodies are elevated in the absence of clinical in-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Subjects</th>
<th>Patients</th>
<th>Patients Monitored Longitudinally</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. per cohort</td>
<td>19</td>
<td>47</td>
<td>30</td>
</tr>
<tr>
<td>Gender, % female</td>
<td>32</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>Ethnicity, % white</td>
<td>95</td>
<td>94</td>
<td>90</td>
</tr>
<tr>
<td>Age at baseline, y (SD)</td>
<td>12.7 (2.7)</td>
<td>11.8 (3.0)</td>
<td>11.7 (2.9)</td>
</tr>
<tr>
<td>No. with TS (including chronic tics)</td>
<td>NA</td>
<td>37 (79%)</td>
<td>23 (77%)</td>
</tr>
<tr>
<td>No. with OCD</td>
<td>NA</td>
<td>27 (54%)</td>
<td>20 (67%)</td>
</tr>
<tr>
<td>No. with TS and OCD</td>
<td>NA</td>
<td>17 (36%)</td>
<td>13 (43%)</td>
</tr>
<tr>
<td>No. with PANDAS</td>
<td>NA</td>
<td>8/34 (24%)*</td>
<td>7/26 (27%)*</td>
</tr>
</tbody>
</table>

* Thirteen of the 47 patients were not assessed.
† Four of the 30 patients in this subset were not assessed.
dication of pharyngitis. GABHS can also produce a
carrier state in the upper respiratory tract with no
increase in antibody titer, presumably because the
organism is not undergoing active growth.

Eighteen time points among 16 patients met the
minimal criteria for a newly acquired GABHS infec-
tion, on the basis of serologic findings. However, 1
patient exhibited increasing antistreptococcal anti-
body titers at 3 consecutive time points, approxi-
mately 4 months apart. According to our criteria,
each of these time points would constitute a newly
acquired GABHS infection. However, because the
GABHS isolates recovered from throat cultures ob-
tained at the 2 latter time points were of the same
emm type (emm1), we counted this as a single infec-
tion.

For several of the newly acquired GABHS infec-
tions, the absolute values for both ASO and anti-
DNase B titers were low. Our stringent definition of
a newly acquired GABHS infection was then applied.
With the stringent criteria, 12 new infections among
12 patients were identified. This yielded a conserva-
tive estimate of 0.42 new infections/year in the pa-
tient sample. For the control subjects, 6 new GABHS
infections among 6 different subjects were identified
with the minimal criteria; 4 of these events also met
the stringent criteria. This yielded a conservative
estimate of 0.28 new infections per year among the
control subjects.

With the minimal criteria definition, a Cox regres-
sion analysis showed no significant difference in the
rates of new GABHS infections for patients versus
control subjects (adjusted hazard ratio: 1.49, not sig-
nificant). However, the effect of age approached sig-
nificance (adjusted hazard ratio: 0.84, P = .07), indi-
cating a trend that younger individuals were at
slightly greater risk. Similar results were obtained
when the stringent criteria were applied (adjusted
hazard ratio: 1.60, not significant; age-adjusted haz-
ard ratio: 0.84, P = .12).

Among patients, only 5 of 133 throat swabs (3.8%) tested positive for GABHS; among control subjects, 2
of 58 throat swabs (3.4%) yielded GABHS. The rate of
GABHS recovery was lower in this study, compared
with several recent reports on GABHS carriage rates.46–49 GABHS were recovered in winter, au-

tumn, and summer, and emm types included emm1, emm2, emm12, and emm44/61. Therefore, serologic
findings provided evidence for many more newly
acquired GABHS infections among these subjects
than did throat cultures.

Tempor al Relationships Between Infections and
Exacerbations

Of the 30 patients who underwent longitudinal
surveillance (Table 1), 28 had surveillance data for
both neuropsychiatric symptoms and serologic anal-
yses, with all blood samples being collected within
time periods of <5 months (mean surveillance time
per patient in the subset: 10.3 months). Considering
only the surveillance time periods in which both
monthly neuropsychiatric ratings and frequent (ie,
<5 months) serologic analyses were performed, the
rate of acute exacerbations of neuropsychiatric
symptoms was 0.71 exacerbations per patient per
year (17 exacerbations). Within this subset of pa-
tients, the rates for new GABHS infections were 0.67
and 0.50 infections per patient per year, according to
the minimal and stringent criteria, respectively.
Therefore, both events were fairly common in the
patient cohort. The null hypothesis assumes that
acute exacerbations and GABHS infections are inde-
pendent events. With multiplication of the 2 calcu-
lated rates, a new GABHS infection (stringently de-
fined) is expected to be temporally linked, with an
acute exacerbation at an average rate of 0.36 times
per patient per year, simply by chance. To correct for
the order of events (ie, infection preceding exacerba-
tion), the expected rate was divided in half, yielding
a corrected rate of 0.18 times per patient per year.

The observed rate of temporally linked GABHS infec-
tions and acute neuropsychiatric episodes could
also be calculated. Four new infections, all of which
met the stringent definition, coincided with or were
followed within 5 months by an acute exacerbation
(mean time interval: 1.2 months) among the 28 pa-
tients under continuous joint surveillance. This
yielded an observed rate of 0.17 temporally linked
infections and exacerbations per patient per year.
The observed rate was nearly equal to the rate ex-
pected on the basis of chance alone (0.18 temporally
linked infections and exacerbations per patient per
year). Therefore, with this unselected group of pa-
tients, there is little evidence to support the hypoth-
esis that GABHS infections trigger acute episodes of
neuropsychiatric disease.

Host Immune Responses to GABHS Surface Antigens

In addition to type-specific antigens, M proteins
contain highly conserved epitopes that are exposed
on the bacterial cell surface. In a previous cross-
sectional analysis, patients with acute RF displayed
significantly elevated serum IgG immunoreactivity to
peptides corresponding to the CRR of M protein,
compared with patients with pharyngitis and healthy
control subjects.36 Furthermore, the CRR of M
protein shares an antigenic epitope with myosin,56
which may underlie immunologic cross-reactivity with
the heart. Increased serum levels of IgG directed to M
protein can serve as an additional marker for newly
acquired GABHS infections. Consecutive blood
samples drawn from each subject were analyzed for
increases in serum IgG immunoreactivity to one or
both of the CRR peptides tested with kinetic enzyme-linked immunosorbent assays.

Among the 17 control subjects with longitudinal
data, 8 subjects (47%) demonstrated ≥2-fold in-
creases in anti-CRR activity; among the 30 patients
with longitudinal data, 16 individuals (53%) demon-
strated ≥2-fold increases in anti-CRR activity (P = .77, Fisher’s exact test). The data failed to show an
obvious difference between patients and control sub-
jects with respect to increased titers of antibody to
the conserved region of M protein. However, 1 of the
patients (patient 5) (Table 2) experienced an exacer-
bation of neuropsychiatric symptoms after an in-
cidence that GABHS infections trigger acute episodes of
neuropsychiatric disease.
The BRR that is present in some M proteins was previously shown to elicit an antibody that is cross-reactive with brain tissue. Therefore, it was of interest to establish the relationship between anti-BRR reactivity and neuropsychiatric disease. However, the BRR was poorly immunogenic in the enzyme-linked immunosorbent assay; none of the 213 blood samples drawn from patients or control subjects exhibited anti-BRR serum IgG reactivity exceeding the low threshold.

Putative PANDAS Cases, Based on Serologic Findings

Although we found no greater than a chance association between newly acquired GABHS infections and symptom exacerbations in an unselected group of patients with TS or OCD, these data do not exclude the possibility that infections and exacerbations are temporally linked, dependent events for a small subset of patients. As indicated by antistreptococcal antibody (ASO and anti-DNase B) titers, 4 patients experienced acute exacerbations of neuropsychiatric symptoms that were temporally linked to newly acquired GABHS infections. Therefore, each is a putative PANDAS case (Table 2, patients 1 through 4). Patient 5 demonstrated a sharp increase in anti-CRR reactivity preceding an exacerbation. Patient 6 experienced an exacerbation 4 months after an infection but beyond the surveillance period and is another PANDAS candidate. For the 6 patients combined (all male), the mean time between infection and exacerbation was 1.9 ± 1.8 months (Table 2). Of note, 3 of the 5 serologic assay-defined PANDAS cases had been identified previously as being probable PANDAS cases, on the basis of the diagnostic interviews before longitudinal surveillance. Another patient (a female patient with OCD, representing a probable PANDAS case) experienced an exacerbation 11 months after a newly acquired GABHS infection.

D8/17 Cell Marker Expression

The D8/17 surface antigen is expressed by B lymphocytes. Patients with RF and their siblings have significantly greater proportions of D8/17-reactive cells, compared with control populations. The percentages of anti-HLA-DR-staining cells that were immunoreactive with the D8/17 monoclonal antibody were compared for patients and control subjects at the baseline time point (Fig 1). The mean percentages of D8/17-positive cells were 19.3% and 13.1% for patients and control subjects, respectively; this difference was highly significant (2-sided t test, t = 2.73, df = 64, P = .0029). With a cutoff value of less than or equal to 12% D8/17-positive cells, 9 of 19 control subjects (47.4%) and 9 of 47 patients (19.1%) could be considered to have nonelevated D8/17-positive cell pools. With a statistical test for independence (Fisher’s exact test), the difference between patients and control subjects was significant (P = .03). However, it should be noted that the percentage of D8/17-positive cells among the control subjects in this study was higher than the average ranges (5%–7%) reported for several other studies. Otherwise, the observation of significantly greater percentages of D8/17-positive cells among pediatric patients with TS and/or OCD, compared with control subjects, confirms the findings reported by other investigators.

The fluctuations in serial measurements of percentages of D8/17-positive cells were compared for the patient and control groups, with time intervals of 6 months between measures. The magnitude of fluctuations (ie, net changes in the percentages of D8/17-positive cells) in the patient population was significantly greater than that in the control group (2-sided t test, t = 2.03, df = 118, P = .03). This finding is consistent with the idea that the D8/17-positive cell population is more active in patients

### TABLE 2. Putative PANDAS Cases, Based on Serologic Findings

<table>
<thead>
<tr>
<th>Data Points*</th>
<th>Patient No.</th>
<th>Average (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>OCD/ADHD</td>
<td>OCD</td>
</tr>
<tr>
<td>Age, y†</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>PANDAS (based on interview)</td>
<td>ND</td>
<td>Male</td>
</tr>
<tr>
<td>Time intervals, mo</td>
<td>A to B</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>B to C</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>C to D</td>
<td>ND</td>
</tr>
<tr>
<td>D8/17-positive cells, %</td>
<td>A</td>
<td>13.5</td>
</tr>
<tr>
<td>Neopterin, nmol/L</td>
<td>A</td>
<td>3.0</td>
</tr>
<tr>
<td>CRP, μg/mL</td>
<td>A</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Data points are represented as follows: A, preinfection; B, postinfection (same as preexacerbation); C, exacerbation; D, postexacerbation. All data points shown are sequential. ND indicates not determined.
† Age at the time of the GABHS infection that was later linked to a neuropsychiatric exacerbation.
demonstrated increases in the percentages of D8/17-acerbation time points. Of the 21 exacerbations, 57% D8/17-positive cells at the preexacerbation and exacerbation time points (data not shown). Taken together, the data indicate that there were no significant changes in the D8/17-positive cell population with either GABHS infections or acute neuropsychiatric symptoms.

Nonspecific Markers of Immune Activation

Neopterin is a pteridine that is produced by human monocytes/macrophages during Th1-type immune responses, after stimulation with interferon-γ, and serves as a biomarker of cell-mediated immunity. Elevated levels of neopterin have been observed in association with several autoimmune diseases.53–56 CRP is an acute-phase protein that is secreted by hepatocytes and is thought to modulate inflammatory responses, although the exact mechanism remains unclear; serum levels of CRP are often elevated among patients with acute RF.8,57 Therefore, it was of interest to determine the relationships between neuropsychiatric diseases and neopterin and CRP levels in serum derived from patients and control subjects.

Comparison of the levels of neopterin for patients (N = 45) versus control subjects (N = 18) at a single time point (baseline) revealed significant differences (2-sided t test, t = 1.50, df = 61, P = .03) between the 2 groups. The mean levels of neopterin at baseline were 8.1 ± 10.2 and 4.5 ± 2.0 nmol/L for patients and control subjects, respectively. Serum levels of neopterin of >10 nmol/L, as determined with a standard test kit, are considered elevated. Ten of 45 patients (22%) displayed elevated neopterin levels, compared with no control subjects (Fisher’s exact test, 2-tailed, P = .03).

To address more thoroughly the possibility that acute exacerbations of neuropsychiatric symptoms are mediated by a cellular immune response, neopterin levels were measured at preexacerbation and exacerbation time points (N = 23); no significant differences were noted (mean time interval: 3.2 months). For 17 exacerbations, neopterin levels at follow-up time points were also known. No significant differences were observed when the exacerbation or preexacerbation time points were compared with the follow-up time points. Of the 3 time points, the mean neopterin level was lowest at exacerbation. The data failed to provide evidence that changes in neopterin levels corresponded to worsening of clinical symptoms among unselected patients with TS and/or OCD. Although we did observe increased neopterin levels at baseline when patients were compared with control subjects, in contrast to an earlier cross-sectional study of patients with TS,58 the significance of our results is cast in doubt by the fact that there was no clear relationship between changes...
in neopterin levels during the course of the study and changes in clinical state. Unlike neopterin levels, a comparison of CRP levels for patients versus control subjects at baseline indicated no significant difference between the 2 groups. The mean CRP levels at baseline were 2.0 and 1.5 g/mL for patients and control subjects, respectively. Serum levels of CRP of >5 g/mL are regarded as elevated; only 6 of 47 patients (13%) and 1 of 18 control subjects (6%) exhibited elevated CRP levels at baseline (Fisher’s exact test, not significant). No differences in CRP levels were observed in comparisons of preexacerbation, exacerbation, and follow-up time points. As observed for neopterin, CRP levels were lowest at the exacerbation time point. The data failed to reveal an association between serum CRP levels and neuropsychiatric disease.

Serum neopterin and CRP levels were compared for blood samples obtained immediately before and after acquisition of a new GABHS infection. With a paired t test (for patients and control subjects combined), slight decreases in mean neopterin and CRP levels were observed between preinfection and postinfection time points. However, the changes were not statistically significant.

**Biomarkers for PANDAS Cases**

In an attempt to identify a biomarker that distinguishes the subset of serologically defined PANDAS candidates from other patients with TS and/or OCD, data on nonspecific markers were examined in greater detail. No obvious distinctions were observed for serologically defined PANDAS cases, in terms of the levels of D8/17-reactive cells or the serum levels of neopterin or CRP (Table 2). However, this study was underpowered for assessment of such relationships.

**Limitations**

There are several limitations to this investigation that are worthy of note. First, all of the patients were identified through a specialty clinic, which limits our ability to generalize these results to the general population of individuals with chronic tic disorders and OCD. Second, as noted above, the number of putative PANDAS cases was too small to allow any firm conclusions regarding the role of GABHS infections in triggering symptom exacerbations. Furthermore, some of these putative PANDAS cases are likely to be attributable to chance alone, just as some individuals experience newly acquired GABHS infections that are followed in time, by chance, by exacerbations of their neuropsychiatric symptoms. Third, because it has been postulated that GABHS infection must be the initial autoimmune response-eliciting event but subsequent symptom exacerbations can be triggered by viruses, other bacteria, or noninfectious immunologic responses, future studies will need to monitor patients systematically for other potential triggers. Much remains to be investigated in this area, including the potential roles of antineuronal antibodies, bacterial superantigens, host factors, and other regulators of immune responses, such as cytokines, chemokines, and specific subsets of T cells that have been implicated in autoimmune diseases. Additional prospective longitudinal studies are needed to clarify these mechanisms.

**CONCLUSIONS**

It has been suggested that GABHS infection is an important factor in the acute onset and “sawtooth” exacerbation pattern of tic and OC symptoms. In this study, we found no convincing association between the exacerbation of tic and OC symptoms and the occurrence of GABHS infections among an unselected group of patients with TS and/or OCD. Although a small number of exacerbations were preceded by GABHS infections, many others were not. Similarly, other patients experienced GABHS infections without any clear effect on their tic and OC symptom presentation. Although no overall association between infection and exacerbation was found for the unselected group of patients with TS and/or OCD, these data do not exclude the possibility that infection and exacerbation are temporally linked, dependent events for a small subset of patients (ie, PANDAS cases).

This is the first large-scale, prospective, longitudinal study of patients with tics and OCD that has systematically evaluated the role of newly acquired GABHS infections as potential triggers for symptom exacerbations. The results suggest no clear consistent relationship between new GABHS infections and symptom exacerbations among an unselected group of patients with TS and/or OCD.

**ACKNOWLEDGMENTS**

This research was funded in part by the Tourette Syndrome Association, the Donahue Medical Research Foundation, and National Institutes of Health grants MH493515, MH61940, RR00125, and RR06022.

We thank Romeo Caringal, Tracy Collingridge, and Christy Schilling for technical assistance. We also thank John Zabriskie and Floresta Chapman (Rockefeller University) for provision of the D8/17 monoclonal antibody used in this study, Edward Kaplan (University of Minnesota) for consultation regarding the criteria used for identification of newly acquired GABHS infections, and the anonymous reviewers for helpful insights. All ASO and anti-DNase values were determined in the laboratory of Elia Ayoub (University of Florida, Gainesville).

**REFERENCES**

Prospective Longitudinal Study of Children With Tic Disorders and/or Obsessive-Compulsive Disorder: Relationship of Symptom Exacerbations to Newly Acquired Streptococcal Infections

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_Pediatrics_ 2004;113;e578

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