The relationship between group A streptococcal infections and Tourette syndrome: a study on a large service-based cohort

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This article is commented on by Gilbert. To view this paper visit http://dx.doi.org/10.1111/1469-8749.2011.04029.x.

PUBLICATION DATA
Accepted for publication 11th April 2011.
Published online.

ABBREVIATIONS

ABGA	Anti-basal ganglia antibodies
ASO	Anti-streptolysin O
GAS	Group A *Streptococcus*
OCS	Obsessive–compulsive symptoms

AIM To evaluate the relationship between diagnosis and clinical course of Tourette syndrome and group A *Streptococcus* (GAS).

METHOD GAS infections, anti-streptococcal, and anti-basal ganglia antibodies (ABGA) were compared between 168 patients (136 males, 32 females) with Tourette syndrome; (median [range] age [25th–75th centile] 10y [8–11y]); median Tourette syndrome duration (25th–75th centile), 3y (1y 3mo–5y 9mo) and a comparison group of 177 patients (117 males, 60 females) with epileptic or sleep disorders median age [25th–75th centile], 10y [8y–1y 6mo]). One hundred and forty-four patients with Tourette syndrome were followed up at 3-month intervals; exacerbations of tics, obsessive–compulsive symptoms, and other psychiatric comorbidities were defined by a bootstrap procedure. The effect of new GAS infections and identification of new ABGA upon risk of exacerbation was assessed using logistic regression analysis.

RESULTS Cross-sectionally, patients with Tourette syndrome exhibited a higher frequency of GAS infection (8% vs 2%; p=0.009), higher anti-streptolysin O (ASO) titres (246 [108–432] vs 125 [53–269]; p<0.001), and higher ABGA frequency (25% vs 8%; p<0.001) than the comparison group. On prospective analysis, ASO titres were persistently elevated in 57% of patients with Tourette syndrome; however, new infections or newly identified ABGA did not predict clinical exacerbations (all p>0.05).

INTERPRETATION Patients with Tourette syndrome might be more prone to GAS infections and develop stronger antibody responses to GAS, probably as a result of underlying immune dysregulation. New GAS infections are unlikely to exert, years after their onset, a major effect upon the severity of neuropsychiatric symptoms.

Tourette syndrome is a chronic multiple-tic disorder affecting 0.8 to 1% of the paediatric population, associated with an impairment in daily functioning and quality of life.[1,2] The course of this illness is variable, and the severity of tics and comorbid psychiatric manifestations, such as obsessive–compulsive symptoms (OCS), fluctuates over time.[2]

Tourette syndrome is considered a multifactorial condition; however, the search for major aetiological factors has been largely inconclusive.[2] Among environmental factors, the possible involvement of group A *Streptococcus* (GAS) infection has been proposed, mainly following the description of a rare group of patients with Tourette syndrome in whom tics and behavioural features fluctuated in association with variations in the immunological response to GAS (the so-called Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal [PANDAS] infection).[3,4] Nevertheless, widely accepted consensus-based criteria for the definition of PANDAS are currently lacking.[5]

Irrespective of the controversy concerning the concept of PANDAS, preliminary but consistent data support the presence of abnormalities of immune regulation in children with Tourette syndrome.[6–9] These alterations suggest that environmental factors acting on the immune system, such as GAS infections, could modify the course of the severity of tics and associated symptoms in Tourette syndrome. Most previous prospective studies compared the exposure to GAS infections and/or the immunological response to GAS between patients with Tourette syndrome who fulfilled criteria for PANDAS and those who did not.[10–13] In the present study,

*A list of other members of the Italian Tourette Syndrome Study Group can be found in Appendix S1 (supporting information online).

we explored the link between GAS infection and Tourette syndrome in a multicentre, service-based Italian paediatric cohort, representative of the general population of patients with Tourette syndrome referred to tertiary outpatient clinics. We first aimed to show cross-sectionally the relationship between the diagnosis of Tourette syndrome and GAS pharyngeal infections, the specific antibody response to this pathogen, and the expression of anti-neuronal antibodies previously associated with post-streptococcal neuropsychiatric disorders (anti-basal ganglia antibodies [ABGA]);[14] then, we followed up prospectively a subgroup of patients with Tourette syndrome to evaluate the effect of GAS-related parameters on the temporal course of symptom severity in this condition.

What this paper adds

- Cross-sectionally, children and adolescents with Tourette syndrome exhibit a higher frequency of group A streptococcal (GAS) throat infections, higher mean anti-streptococcal antibody titres, and anti-basal ganglia antibodies (ABGA) than children with illnesses unrelated to tic disorders.
- Anti-streptococcal antibody titres are persistently elevated in almost 60% of patients with Tourette syndrome.
- New infections or newly identified ABGA do not predict exacerbations of symptoms in Tourette syndrome.
- These findings support the view that patients with Tourette syndrome might be more prone to GAS infections and develop stronger immune responses to this pathogen.

METHOD

Participants

Children and adolescents fulfilling Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for Tourette syndrome[15] were consecutively recruited between June 2007 and June 2008 in four Italian tertiary referral centres for this condition. The comparison group comprised children and adolescents with epileptic or sleep disorders who had never suffered from tics or OCS. Patients with moderate to severe learning disability[†] fulfilling DSM-IV criteria[15] were excluded from either group. The study was approved by the ethics committees of each recruiting institution, and informed consent (plus informed assent from children >12y) was obtained from each participant.

Study design and clinical/laboratory assessment

Patients recruited in each centre were assessed by the same child neuropsychiatrist with expertise in Tourette syndrome, who also collected the throat specimens by a standardized procedure.

Cross-sectional study

For this part of the study, case and comparison groups of patients were assessed using the following: (1) a standardized clinical interview and examination; (2) psychometric assessment; and (3) collection of throat and peripheral blood specimens.

The standardized interview consisted of the Italian version of the interview developed by Robertson and Eapen,[16] and gathered past and current symptoms of Tourette syndrome (tics, OCS, echo-, pali-, and coprophenomena, self-injurious behaviour, etc.) in order to measure the Diagnostic Confidence Index;[17] the current treatment regime was also recorded. The psychometric assessment comprised the following scales: Wechsler Intelligence Scale for Children-Revised; Diagnostic Confidence Index; Yale Global Tic Severity Scale;[18] Children's Yale-Brown Obsessive Compulsive Scale;[19] Child Behaviour Checklist;[20] Children's Depression Inventory;[21] Multidimensional Anxiety Scale for Children.[22] Attention-deficit–hyperactivity disorder (ADHD) symptoms were measured using the sub-item N of Conners' Parent Rating Scale-Revised.[23]

The pharyngo-tonsillary specimen was collected in duplicate swabs with transport medium, and immediately sent to the local microbiology service, where receiving technicians had been trained in the specimen processing technique (pour-plate in agar Columbia with sheep blood). Bacterial identification and DNA isolation were performed as described elsewhere,[24] and determination of the *emm* type was performed by comparison with the sequences available in the Center for Disease Control and Prevention database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm/).

Peripheral venous blood specimens were centrifuged and analysed at the local chemistry laboratory for erythrosedimentation rate. An aliquot of 0.5 to 1 mL of serum was stored at −80°C and subsequently sent to the University of Bari for immunological analyses, which included (1) anti-streptolysin O (ASO) and anti-deoxyribonuclease B (anti-DNAse B) antibody titres by standardized immunonephelometry (Dade-Behring II), and (2) search for ABGA using a previously described western immunoblotting method.[14] An ASO titre of more than 200 international units (IU)/mL and an anti-DNAse B titre of more than 300 IU/mL were considered indicative of recent streptococcal infection. The presence of ABGA was screened using a protein homogenate of striatum (obtained at autopsy from two donors without evidence of neurological disease and stored at −80°C), and confirmed using the human recombinant forms of the three target antigens (aldolase C, γ-enolase, and pyruvate-kinase M1), prepared as previously described.[25] Samples were considered ABGA-positive only if the presence of ABGA was shown against both striatum and at least one of the recombinant antigens.

Prospective study

The second part of the study comprised a prospective evaluation limited to patients with Tourette syndrome only. The visit in which cross-sectional data were collected represented visit 1; patients entering the prospective study underwent follow-up visits at intervals of 11 to 13 weeks. Each follow-up visit (visits 2–4) included psychometric assessment (the same as in visit 1, except for the Diagnostic Confidence Index), and collection of throat and blood specimens. Parents of children entering the prospective study kept a semi-structured diary of the patients' health status and exposure to infections/inflammatory states between consecutive visits, with special attention to episodes of 'sore throat'. Medications for tics, OCS, and

[†]North American usage: mental retardation.

other neuropsychiatric conditions were allowed throughout the study, as well as antibiotics for concurrent infections, but all treatment changes were recorded. No patient received immune-modifying or antibiotic treatments specifically aimed at the resolution of neuropsychiatric symptoms. Visiting physicians were blinded to the result of throat cultures and serum analyses throughout the course of the study.

Exacerbations of tics, OCS, ADHD symptoms, and symptoms related to depression and anxiety were based on score changes between two consecutive visits on the Yale Global Tic Severity Scale, Children's Yale-Brown Obsessive Compulsive Scale, Conners' Parent Rating Scale-Revised, Children's Depression Inventory, and Multidimensional Anxiety Scale for Children scores respectively. According to the method proposed by Lin et al.,[26] for each of the five severity scores a bootstrap procedure (5000 replications) was applied to any pair of consecutive observations to extract one-tailed 95% lower confidence limits for the 90th and the 75th centiles of the symptom severity visit-to-visit variation ('90 lower change' and '75 lower change' respectively); these centile levels were chosen to favour specificity (90th centile) or sensitivity (75th centile) in pointing out exacerbations. The same bootstrap procedure was applied to extract one-tailed 95% upper confidence limits for the 50th centile (median) of the second observation of the pair ('50 upper final level'). For each of the five severity scores, two different definitions of exacerbation were used in the analysis: 'more stringently defined clinical exacerbations' occurred if the change was higher than '90 lower change' and the final level was higher than '50 upper final level' (the latter condition also aiming at increasing specificity); 'less stringently defined clinical exacerbations' occurred if the change was higher than '75 lower change' and the final level was higher than '50 upper final level'.

Changes in throat culture and anti-streptococcal antibody titres between consecutive visits were used to define new GAS infections. For a GAS-positive throat culture to be useful for the diagnosis of new infection, the previous culture had to be either negative or positive for a different GAS strain. Similarly, for an antibody titre rise to be diagnostically useful, the titre at the second of two consecutive visits had to be more than 200IU for ASO and more than 300IU for anti-DNAseB antibodies. In addition to the conditions above, a 'definite' new GAS infection was defined if either of the following conditions were fulfilled: (1) isolation of a GAS strain with a corresponding $\log_{10}$ rise in either ASO or anti-DNAseB titre of at least 0.2; (2) a $\log_{10}$ rise of at least 0.2 in both ASO and anti-DNAseB titres. A 'possible' new GAS infection was defined if either of the following conditions were fulfilled: (1) isolation of a GAS strain without a corresponding rise in antibody titres; (2) a $\log_{10}$ rise of at least 0.2 in either ASO or anti-DNAseB titre. Finally, for an ABGA-positive serum sample to be indicative of a mounting autoantibody response, the specimen from the previous visit had to be ABGA-negative.

Statistical analysis

For the cross-sectional study, we hypothesized that, compared with the comparison group, the Tourette syndrome group would manifest a higher frequency of GAS-positive throat specimens, higher ASO and anti-DNAse B titres, and higher frequency of ABGA-positive serum specimens. Differences between groups were analysed by *t*-test or Mann–Whitney *U* test (as appropriate) for continuous variables, and chi-squred or Fisher's exact test for categorical variables. Homogeneity of variance across groups was measured with Levene's test. The cross-sectional study had a 1-β power value of 0.90 to detect a twofold higher proportion of GAS-positive throat specimens in the Tourette syndrome group compared with the comparison group, assuming a prevalence of GAS carriage in the general population of 15%, and a significance level α of 0.05 in a chi-squared test; under these conditions at least 161 participants were needed in each group. A p value <0.05 was considered significant.

For the prospective study, we hypothesized that clinically relevant changes in markers of infection and autoimmunity and new GAS infections would predict temporally related exacerbations of tics, OCS, ADHD symptoms, and symptoms related to depression and anxiety. We performed logistic regression to measure the effect of new, definite, and possible GAS infections and new detection of ABGA in serum upon the risk of occurrence of both more and less stringently defined exacerbations of tics, OCS, ADHD symptoms, and symptoms related to depression and anxiety, as defined above. The odds ratio (OR; a measure of effect size which is an approximation of the relative risk[27]) was computed for definite or possible GAS infections on each type of exacerbation in separate analyses, adjusting for referral centre, age, time interval between visits, increased dosage or change of medication in the treatment regime for neuropsychiatric symptoms, and occurrence of additional non-GAS infections. Confidence intervals at the 95% level (95% CI) were computed using robust standard errors to take into account repeated observations collected from the same individuals. All statistical analyses were performed using a commercial statistical software package (Stata 11; Stata Corp, College Station, TX, USA).

RESULTS

Cross-sectional study

One hundred and sixty-eight patients with Tourette syndrome (136 males, 32 females; median [range] age [25th–75th centile] 10y [8–11y]; median Tourette syndrome duration [25th–75th centile] 3y [1y 3mo–5y 9mo]) and 177 comparison patients (117 males, 60 females; median age [25th–75th centile] 10y [8y–11y 6mo]) entered the cross-sectional study. There was no significant difference between case and comparison patients in the number of parents' years of education (median 13y vs 12.5y, $p>0.05$). Groups did not significantly differ for period of the year of study entry (Tourette syndrome: 91 out of 168 between October and March, 77 out of 168 between April and September; controls: 105 out of 177 between October and March, 62 out of 177 between April and September; $\chi^2=2.62$, $p=0.11$).

Patients with Tourette syndrome scored significantly higher on the Child Behaviour Checklist (on internalizing, externaliz-

Table I: Cross-sectional comparison of demographic and clinical characteristics between patients with Tourette syndrome and those in the comparison group

	Patients with Tourette syndrome (*n*=168)	Comparison patients (*n*=177)	Test statistics (df for *t* or *z*=343; df for χ^2=1); *p*
Age	10 (8–11)	10 (8–11.5)	*t*=1.72; *p*=0.04
Sex (M/F)	136/32	117/60	χ^2=9.72; *p*=0.002
WISCR–TIQ	97 (86–104)	90 (83.2–101.5)	*t*=1.51; *p*=0.07
CBCL intern.	10 (4–15)	6 (3.2–13.2)	*t*=3.4; *p*=0.0004
CBCL extern.	10 (5–19)	3 (0–7.5)	*z*=−5.95; *p*<0.001
CBCL total	34.5 (16–49)	14 (7.2–31.5)	*t*=6.74; *p*<0.001
CDI	9 (4–12)	7 (4–11)	*t*=2.98; *p*=0.002
MASC	44 (34–53)	27 (10.5–45.5)	*z*=−6.34; *p*<0.001
CPRS-R sub-item N	24 (13.5–41)	7 (2.5–15)	*z*=−8.38; *p*<0.001
YGTSS severity subscale	25 (16.5–31)	0	NA
CY-BOCS	8 (0–18.2)	0	NA
Tourette syndrome DCI	40 (26.5–55)	0	NA
Erythrocyte sedimentation rate, (SD)	7 (2–12.5)	6.5 (2.5–12.5)	*t*=0.09; *p*=0.46
GAS-positive throat specimen *n* (% of positivity)	13[a] (7.7)	3[a] (1.7)	Fisher's exact test *p*=0.009
Anti-streptolysin O titre (SD)	246 (108.5–432)	125 (53–269)	*t*=3.41; *p*<0.001
% of pathological titre (>200IU)	57.7 (*n*=97)	33.9% (*n*=60)	χ^2=19.75; *p*<0.001
Anti–DNAse B titre (SD)	351 (153–635)	275 (137–630)	*t*=0.51; *p*=0.3
ABGA *n* (% of positivity)	40/158 (25.3)	14/171 (8.2)	χ^2=17.56; *p*<0.001
Aldolase C (40kDa)	14/158 (8.9)	3/171 (1.7)	Fisher's exact test *p*=0.005
γ-Enolase (45kDa)	14/158 (8.9)	6/171 (3.5)	χ^2=4.12; *p*=0.04
Pyruvate kinase (60kDa)	18/158 (11.4)	6/171 (3.5)	χ^2=7.55; *p*=0.006

Continuous data are summarized using median value (25th–75th centile). [a]*emm* types in patients with Tourette syndrome and the comparison group: 6 (four Tourette syndrome), 89 (three Tourette syndrome, one comparison), 12 (two Tourette syndrome, one comparison), 1 (one Tourette syndrome, one comparison), 3 (one Tourette syndrome), 87 (one Tourette syndrome), 12 (one Tourette syndrome). WISCR-TIQ, Wechsler Intelligence Scale for Children Revised–total intellectual quotient; CBCL, Child Behaviour Checklist; intern, internalizing score (possible range, 0–26), extern, externalizing score (possible range, 0–34); total score (possible range, 0–130); CDI, Child Depression Inventory (possible range, 0–54); MASC, Multidimensional Anxiety Scale for Children (possible range, 0–90); CPRS-R, Conners' Parent Rating Scale-Revised (sub-item N; possible range, 0–54); YGTS, Yale Global Tic Severity Scale (severity score; possible range, 0–50); CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale (possible range, 0–40); DCI, Diagnostic Confidence Index (possible range, 0–100); GAS, Group A *Streptococcus*; DNAse B, deoxyribonuclease B; ABGA, anti-basal ganglia antibodies; NA, not applicable.

ing, and total scores), Children's Depression Inventory, Multidimensional Anxiety Scale for Children, and Conners' Parent Rating Scale-Revised score for ADHD (Table I). Mean ASO titre was significantly higher in the group with Tourette syndrome ($p<0.001$), whereas the group mean anti-DNAseB titres did not differ between patients with Tourette syndrome and the comparison group ($p=0.3$). There was a significantly higher frequency of positive throat culture in patients with Tourette syndrome (13 out of 168, 8%) than the comparison group (three out of 177, 2%; $p=0.009$; Table I). ABGA testing was available for 158 of the 168 Tourette syndrome (94%) patients, and for 171 of the 177 comparison participants (97%): the frequency of serum ABGA was significantly higher among patients with Tourette syndrome (40 out of 158, 23%) than among those in the comparison group (14 out of 171, 8%; $p<0.001$). There was no difference in ASO and anti-DNAse B titres between ABGA-positive and ABGA-negative patients with Tourette syndrome, comparing both group mean values and percentage of pathological ASO titre or anti-DNAse B titre (all $p>0.05$). Mean erythrosedimentation rates did not differ between the two groups. Given that male sex was overrepresented in the case group, between-group comparisons were repeated in the male population only, yielding similar results to those obtained from the general population (data not shown).

Prospective study

One hundred and forty-four patients with Tourette syndrome entered the prospective study. Of these, 76 underwent four visits at 3-monthly intervals, 33 underwent three visits, and 35 underwent two visits only; the whole prospective study yielded a total number of 473 visits, with an average number per patient equal to 3.3, and a total number of 329 pairs of consecutive visits.

The numbers of more stringently and less stringently defined exacerbations of tics, OCS, ADHD symptoms, and symptoms related to depression and anxiety identified from the 329 pairs of consecutive visits are shown in Table II. The 329 pairs of consecutive visits yielded seven definite and 32 possible new GAS infections, with a frequency of 2% and 10% respectively. Newly positive throat cultures were identified in 10 out of 144 patients (7%), and rises in ASO and anti-DNAseB titres in 26 (18%) and 11 (8%) patients respectively. In contrast, 82 out of 144 patients (57%) had ASO titres that were persistently elevated (i.e. >200IU/mL) over at least two consecutive visits, whereas only three out of 144 (2%) had a persistently positive throat culture. All 13 patients with a positive throat culture at visit 1 and all 10 patients in whom a newly positive throat culture was identified during the prospective observation (seven definite and three possible GAS infections) were treated with a standardized cycle of amoxicil-

Table II: Frequency of more and less stringently defined exacerbations of tics, obsessive–compulsive symptoms, and symptoms related to attention-deficit–hyperactivity disorder, depression, and anxiety identified in the prospective study

Severity rating scale	*N* of more stringently defined exacerbations (% on the total *n* of pairs of consecutive visits)	*N* of less stringently defined exacerbations (% on the total *n* of pairs of consecutive visits)
YGTSS	22 (6.7)	47 (14.2)
CY-BOCS	29 (8.8)	57 (17.3)
Conners' Parent Rating Scale-Revised	32 (9.7)	56 (17)
CDI	23 (7)	31 (9.4)
MASC	23 (7)	51 (15.5)

YGTSS, Yale Global Tic Severity Scale; CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale; CDI, Child Depression Inventory; MASC, Multidimensional Anxiety Scale for Children.

lin and clavulanic acid. Additional non-GAS-related (presumably viral) episodes of 'sore throat' were recorded in 30 visits (9%). A new identification of ABGA in patients who were ABGA-negative at the previous visit was observed in 29 visits (9%) from 29 out of 144 patients (20%), whereas 20 out of 144 (14%) tested persistently positive for ABGA over at least two consecutive visits.

Logistic regression analyses were adjusted for referral centre, age, time lag between visits, change of treatment regimen for neuropsychiatric symptoms, and occurrence of non-GAS 'sore throat' episodes. None of the seven definite new GAS infections was temporally linked to any type of more stringently defined exacerbation. Only one and two definite infections were linked to less stringently defined exacerbations of, respectively, depressive (OR 1.74, 95% CI 0.22–14.05; p=0.60) and anxiety (OR 2.87; 95% CI 0.47–17.63; p=0.26) symptoms. Combined definite/possible GAS infections occurring during the 3-monthly interval between two consecutive visits did not significantly predict the development of more stringently defined (Table III) or less stringently defined (Table IV) exacerbations of tics, OCS, ADHD, or depressive or anxiety symptoms. Likewise, a new identification of ABGA did not predict the occurrence of an exacerbation of any of the five different categories of symptoms explored (Tables III and IV).

DISCUSSION

When analysed cross-sectionally, children and adolescents with Tourette syndrome exhibited a higher frequency of GAS throat infection, higher mean ASO antibody titres, and higher frequency of ABGA than those with illnesses unrelated to tic disorders. Although ASO titres were persistently elevated (i.e.

Table III: Logistic regression analysis assessing the effect of group A *Streptococcus* (GAS) infections and of new identification of anti-basal ganglia antibodies (ABGA) on the risk of developing more stringently defined exacerbations of tics, obsessive–compulsive symptoms, and symptoms related to attention-deficit–hyperactivity disorder (ADHD), depression, and anxiety identified in the prospective study

	Tics (YGTSS)	Obsessive–compulsive symptoms (CY-BOCS)	ADHD symptoms (CPRS-R)	Depressive symptoms (CDI)	Anxiety symptoms (MASC)
Score (OR [95% CI]; *p*)					
Definite or possible GAS infection (*n*=39)	0.37 (0.05–2.98); 0.35	1.57 (0.60–4.11); 0.36	1.34 (0.44–4.04); 0.61	1.62 (0.51–5.21); 0.42	1.90 (0.59–6.11); 0.28
Newly positive ABGA (*n*=29)	NE	1.04 (0.23–4.64); 0.96	NE	0.49 (0.08–3.19); 0.46	NE

Odds ratio (OR) could not be estimated (NE) because no participant with newly positive ABGA developed more stringently defined exacerbations of tics, ADHD symptoms, or anxiety symptoms. YGTSS, Yale Global Tic Severity Scale; CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale; CPRS-R, Conners' Parent Rating Scale-Revised, sub-item N; CDI, Child Depression Inventory; MASC, Multidimensional Anxiety Scale for Children; CI, confidence interval.

Table IV: Logistic regression analysis assessing the effect of group A *Streptococcus* (GAS) infections and of new identification of anti-basal ganglia antibodies (ABGA) on the risk of developing less stringently defined exacerbations of tics, obsessive–compulsive symptoms, and symptoms related to attention-deficit–hyperactivity disorder (ADHD), depression, and anxiety identified in the prospective study

	Tics (YGTSS)	Obsessive–compulsive symptoms (CY-BOCS)	ADHD symptoms (CPRS-R)	Depressive symptoms (CDI)	Anxiety symptoms (MASC)
Score (OR [95% CI]; *p*)					
Definite or possible GAS infection (*n*=39)	0.30 (0.07–1.39); 0.13	1.05 (0.47–2.37); 0.91	0.81 (0.31–2.10); 0.67	1.77 (0.60–5.22); 0.30	1.36 (0.59–3.13); 0.47
Newly positive ABGA (*n*=29)	1.13 (0.30–4.28); 0.86	0.78 (0.25–2.44); 0.67	0.49 (0.11–2.29); 0.37	0.79 (0.18–3.54); 0.76	0.28 (0.04–2.12); 0.22

YGTSS, Yale Global Tic Severity Scale; CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale; CPRS-R, Conners' Parent Rating Scale-Revised, sub-item N; CDI, Child Depression Inventory; MASC, Multidimensional Anxiety Scale for Children; OR, odds ratio; CI, confidence interval.

>200IU/mL) in most patients with Tourette syndrome, our prospective evaluation showed that neither GAS infections nor newly identified ABGA predict exacerbations of neuropsychiatric symptoms over a period of 3 months. These findings do not support the inclusion of microbiological and serological screening for GAS infections and of ABGA testing in the routine follow-up assessment of children with Tourette syndrome. This seems consistent with the study by Singer et al.,[12] which showed that immune markers and GAS infections do not correlate with clinical exacerbations in a group of children with tic disorders.

Two retrospective community-based studies showed that children with tics/OCS are more exposed to GAS infections before disease onset.[28,29] Although a third study did not confirm this,[30] several cross-sectional, service-based reports documented an enhanced immune response to GAS in patients with Tourette syndrome (summarized in Murphy et al.[4]). Strong evidence supporting the use of antibiotics to prevent the onset or exacerbation of tics/OCS is lacking, and it is unclear whether the increased exposure to GAS truly contributes to the occurrence of Tourette syndrome or is epiphenomenal. A recent study reported a decreased total immunoglobulin-A titre in children with Tourette syndrome,[8] which, when also involving mucose-secreted immunoglobulin-A, might explain the increased susceptibility to infections of the upper airways, including GAS infections. Consistent with this, our patients with Tourette syndrome exhibited a higher frequency of GAS-positive throat specimens years after disease onset (mean disease duration at visit 1: 3.2y).

An interesting finding of our study is that almost 60% of patients with Tourette syndrome had persistently elevated ASO titres, whereas only 2% manifested persistent throat GAS colonization. This suggests an enhanced immune response against GAS. Bombaci et al.[31] provided evidence supporting this, analysing cross-sectionally the antibody response to more than 100 recombinant GAS proteins: these authors found that patients with tics recognized as many antigens as children with pharyngitis, but their antibody titres were higher. Several reports documented a dysregulated cell-mediated and humoral immunity in Tourette syndrome.[9] The reduced percentages of T-regulatory lymphocytes and the immunoglobulin-A dysgammaglobulinemia suggest that these patients may be prone to autoimmunity,[7,8] and the higher frequency of ABGA in our patients with Tourette syndrome, consistent with previous studies,[9] is in line with this view. On the other hand, caution is necessary because these autoantibodies were identified in 8% of our comparison group of patients, and may, therefore, not be specific for Tourette syndrome; moreover, their pathogenic role still needs to be demonstrated.[9] Overall, the role of these antibodies targeting neuronal glycolytic enzymes in Tourette syndrome remains unclear.

Our study is the first, to our knowledge, to investigate the association between newly incident GAS infections and the severity course of Tourette syndrome symptoms in children and adolescents from a European country. Several North American prospective studies failed to show different rates of temporally related exacerbations and antecedent GAS infections between patients with PANDAS and patients with Tourette syndrome not fulfilling PANDAS criteria.[10–13] Only two studies explored the link between GAS infections and exacerbations in patients with Tourette syndrome unselected for PANDAS criteria, both failing, in line with our study, to prove a clear temporal association.[32,33] However, Lin et al.[33] suggested that new GAS infections greatly increased the power of psychosocial stress in predicting future symptom severity, suggesting that these GAS infections do not exert a relevant effect upon the course of Tourette syndrome symptoms if not interacting in multiplicative manner with major determinants of psychosocial stress. This notwithstanding, the existence of a small minority of patients with Tourette syndrome who are particularly susceptible to developing clinical exacerbations triggered by GAS infections cannot be totally excluded on the basis of available evidence.

Our study has some limitations. The GAS carriage rates observed in the two groups compared in the cross-sectional study were lower than those expected from a recent meta-analysis assessing prevalence of GAS-positive throat cultures (12%).[34] However, the results of this meta-analysis documented a huge variation among studies, with estimates ranging between less than 3% and 26%, probably also because of geographical and inter-ethnic differences. The interval between consecutive visits (3mo) might have been too long to detect all the intervening infections and exacerbations. Recent reports have emphasized the need for frequent sequential collection of throat and serum specimens to define GAS infections more accurately.[4] We tried to reduce this limitation, at least for potential confounders like other infections and medication changes, by asking parents to keep a home diary. We tried to optimize homogeneity of multicentre data collection standardizing the throat culture procedure across centres, and using universally validated rating instruments to define exacerbations. Exposure to psychotropic and antibiotic drugs might have reduced the chance of detecting a temporal link between infections and exacerbations and/or altered the rate of clinical exacerbations in our patients. This is a limit of all prospective studies exploring this topic, and is difficult to overcome for ethical reasons; however, we adjusted our logistic regression analyses for changes in medications for tics and behavioural symptoms. Finally, as stated above, the course-modifying effect of GAS infections might be due to an interaction with stress,[33] which we did not investigate.

In conclusion, we documented an increased exposure to, and immune response against, GAS, and an increased expression of anti-neuronal antibodies previously associated with post-streptococcal neuropsychiatric illnesses in children and adolescents with Tourette syndrome. This supports the view that patients with Tourette syndrome might be more prone to GAS infections and to develop stronger humoral responses to this pathogen, probably as a result of underlying immune dysregulation. At the same time, new GAS infections are unlikely to exert, years after the onset, a major effect upon the severity of neuropsychiatric symptoms. Longitudinal studies on unaf-

fected children at risk of developing tics (e.g. first-degree relatives of patients with Tourette syndrome) might help to address the issue of whether GAS infections are a true risk factor for chronic tic disorders.

ACKNOWLEDGEMENTS

This study was sponsored by a Progetti di Rilevante Interesse Nazionale grant of the Italian Ministry for University and Research. The funder was not involved in study design, data collection, data analysis, manuscript preparation, and/or publication decisions. We are grateful to the Parkinson's UK Brain Bank, Institute of Neurology, London, for providing specimens of human striatal tissue, and to Dr P Candler and Professor G Giovannoni for providing preparations of human recombinant aldolase C, pyruvate kinase M1, and neuron-specific enolase for anti-basal ganglia antibody testing.

ONLINE MATERIAL/SUPPORTING INFORMATION SECTION

Additional material and supporting information for this paper may be found online.

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