Mycoplasma pneumoniae in chronic tic disorders

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ABSTRACT

Infectious pathogens may represent an environmental risk factor for chronic tic disorders (CTD). This study aimed to determine whether *M. pneumoniae* IgG positivity is associated with the presence or severity of tics. This cross-sectional study used baseline serum samples from the European Multicentre Tics in Children Studies (EMTICS) cohorts. We compared *M. pneumoniae* IgG positivity across three groups: children and adolescents (3-16 years) with CTD (n=302); first-degree relatives of people with CTD (3-10 years) who developed tics within a seven-year follow-up period (n=51); and first-degree relatives who remained unaffected and were ≥10-years-old at their last assessment (n=88). The relationship between *M. pneumoniae* IgG positivity and the presence and severity of tics was analysed using multilevel models controlling for site, family relatedness, sex, age, presence of comorbid obsessive-compulsive disorder and/or attention-deficit/hyperactivity disorder and use of psychotropic medication. *M. pneumoniae* IgG positivity was not associated with the presence of CTD, or the first development of tics as compared to first-degree relatives who remained unaffected. *M. pneumoniae* IgG positivity was associated with a moderately higher tic severity score among the CTD cohort (β = 2.64, s.e. = 1.15, p = 0.03). It may be that *M. pneumoniae* infection influences tic severity in CTD or, that having a more severe phenotype, increases the risk of infection. However, it is also possible that the two are not causally linked and that both infection and greater tic severity are epiphenomena of shared underlying mechanisms, with neither influencing the other directly.

INTRODUCTION

Chronic tic disorders (CTD) are common neurodevelopmental conditions characterised by the presence of motor tics, vocal tics or the combined presence of both motor and vocal tics known as Tourette syndrome (TS) (American Psychiatric Association, 2013). TS has an estimated worldwide prevalence of 0.3-0.9% (Knight et al., 2012). The aetiology of CTD is not fully understood. While there is a strong genetic component, with estimates of heritability between 0.25 and 0.77 (Mataix-Cols et al., 2015, Zilhão et al., 2017), environmental factors also play a substantial role (Hoekstra et al., 2013, Robertson et al., 2017).

Prior studies have suggested infectious agents may be involved in the pathogenesis of CTD (Krause and Müller, 2012, Martino et al., 2015, Martino et al., 2020a). The focus of research in this area has been on Group A β-haemolytic streptococcal infections given their apparent role in related movement disorders such as Sydenham's chorea and the post-streptococcal syndrome of tics and/or obsessive-compulsive disorders termed PANDAS (paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) (Swedo et al., 1998). However, a recent prospective cohort study of 715 children and adolescents with TS did not detect any association between recent exposure to Group A streptococcus and clinically relevant exacerbations of tics (Martino et al., 2021). Associations between tics and other infectious pathogens including *M. pneumoniae* (Müller et al., 2000, Ercan et al., 2008) and *Borrelia burgdorferi* (Riedel et al., 1998) have been described in case reports. There have also been a few small-scale case-control studies that have found elevated levels of antibodies in individuals with TS compared to controls including anti-*Toxoplasma gondii* immunoglobulin G (IgG) positivity (OR 5.827) (Akaltun et al., 2018), anti-*M. Pneumoniae* IgA positivity (31% vs. 3%) (Müller et al., 2004), and anti-

Chlamydia trachomatis IgG positivity (19.2% vs 0%) (Krause et al., 2012). However, no infectious agent has been consistently linked to CTD and evidence from larger sample sizes is limited.

M. pneumoniae is a ubiquitous organism that generally causes a mild acute respiratory infection (Atkinson et al., 2008). The prevalence of *M. pneumoniae* IgG positivity, which is most likely due to past infection, is around 12% in early childhood and increases to 50-60% in adolescence with no significant difference between sexes (Tuuminen et al., 2000). *M. pneumoniae* infection tends to be either asymptomatic or a self-limiting illness. However, it is also a common cause of community-acquired pneumoniae and extrapulmonary manifestations can affect any organ, including the brain (Korppi et al., 2004). There are two reports of children who developed movement disorders and/or basal ganglia lesions following *M. pneumoniae* encephalitis (Beskind and Keim, 1994, El Hafidi et al., 2012) and, as mentioned above, one study (n = 29) and two case reports have suggested that infection with *M. pneumoniae* could be associated with tics (Müller et al., 2004, Ercan et al., 2008, Müller et al., 2000).

This cross-sectional study investigated whether *M. pneumoniae* IgG positivity is associated with the presence of CTD, the first development of tics or tic severity. We used a sub-sample of the European Multicentre Tics in Children Studies (EMTICS) cohorts based on available baseline serum samples for analysis of *M. pneumoniae* IgG positivity. EMTICS is a large prospective European multicentre study of children and adolescents with CTD and first-degree relatives (mostly siblings) of individuals with CTD who themselves did not have tics at study entry and were observed for up to seven years (Schrag et al., 2019). This study investigated whether: (A) children with CTD have higher rates of *M. pneumoniae* IgG positivity compared to unaffected first-degree relatives; (B) the rate of baseline *M. pneumoniae* IgG positivity is higher in first-

degree relatives who developed tics compared to those who did not develop tics; (C) *M. pneumoniae* IgG positivity is associated with higher tic severity in participants with CTD. To our knowledge, this is the largest study to investigate *M. pneumoniae* IgG in CTD and is unique in its assessment of serum IgG positivity prior to the development of tics in an at-risk cohort.

PARTICIPANTS AND METHODS

Participants

Participants were from the EMTICS study (Schrag et al., 2019), a prospective observational cohort study that aimed to assess the contribution of genetic and environmental risk factors in CTD. Data were collected by 16 child and adolescent psychiatry and paediatric neurology outpatient clinics across Europe and in Israel. The project was based on two separate cohort studies: the COURSE study, including children and adolescents (3-16 years) with a diagnosis of CTD according to DSM-IV-TR (American Psychiatric Association, 2000), and the ONSET study, an at-risk cohort of first-degree relatives of children with CTD (3-10 years) without tics, OCD or trichotillomania. The ONSET cohort was followed up bimonthly for up to three years to assess the onset of tics according to study protocol. All unaffected children were recontacted and the majority were re-assessed via a brief telephone interview after the study ended. The regular study period took place between 2013-2018, with the final telephone reassessment concluded in May 2020.

The mean age of tic onset in the ONSET cohort was 7.9 years (SD 2.0, range 3.5-13.0); we therefore excluded those who were younger than 10-years-old at the time of their last assessment and had not developed any tics from the unaffected at-

risk comparison group to reduce the likelihood of including children who might still develop tics.

Exclusion criteria for both COURSE and ONSET included treatment with antibiotics during the last month (since a separate antibiotic study was part of the research plan), a serious medical or neurological illness or an inability to understand and comply with study procedures (Schrag et al., 2019).

The current study used data from a sub-sample of the EMTICS cohorts. The original research plan did not include *M. pneumoniae* as a factor of interest, rather it was added later as a secondary measure and analysed in remaining available serum samples. Therefore, the sub-sample in this study comprised all participants for whom we had baseline serum available to measure *M. pneumoniae* IgG. Rates of *M. pneumoniae* IgG positivity were compared across three groups: 302 participants with CTD (CTD cohort); 51 first-degree relatives who developed tics within the seven-year total study period (tic onset cohort); and 88 unaffected first-degree relatives who were ≥10 years old at time of their last assessment (unaffected cohort). The study was approved by the Institutional Review Boards of the participating centres. Parents and their child or children provided written informed consent or assent before entering the study.

Clinical Measures

For the COURSE cohort, an established diagnosis of TS, chronic motor tic disorder or chronic vocal tic disorder was confirmed by trained clinicians using DSM-IV-TR criteria at baseline (American Psychiatric Association, 2000), as was the possible presence of comorbid OCD and ADHD. Baseline tic severity was measured using the Yale Global Tic Severity Score (YGTSS) (Leckman et al., 1989), which

includes the Total Tic Severity Score (range 0-50) comprised of two subscales: YGTSS Motor Tic Severity Score (range 0-25) and YGTSS Vocal Tic Severity Score (range 0-25). In addition, the Clinical Global Impression Severity (CGI-S) Scale (Guy, 1976) was used to assess tic severity during the previous week. Participants in the ONSET cohort suspected of having developed tics were assessed by study clinicians and the onset of tics was confirmed according to DSM-IV-TR criteria (American Psychiatric Association, 2000). Use of psychotropic medication during the last two weeks was documented by the study clinicians.

Laboratory Measures

Samples were sent to the Department of Laboratory Medicine, Munich (LMU) and stored at -80°C until analysis. Serum IgG against *M. pneumoniae* were measured in the ISO 15189 accredited lab on a DiaSorin Liaison analyser using Chemiluminescence Immunoassay with paramagnetic microparticle solid phase (CLIA) technology; the analyser range was 1-200 AU/mL. *M. pneumoniae* elicits antibody responses after around one week of illness. This is initially an IgM response before seroconversion to IgG; serum IgG tends to peak at 3-6 weeks, before it gradually declines (Atkinson et al., 2008). IgG tends to remain elevated in children for around four years following infection but can also persist indefinitely (Daxboeck et al., 2003, Jacobs et al., 1986, Foy et al., 1977). Sera IgG ≥ 10 AU/ml was considered positive for previous infection with *M. pneumoniae* at any point in time and < 10 AU/ml as negative (Waris et al., 1998, Almasri et al., 2011). IgG positivity is most likely due to past infection with *M. pneumoniae*, rather than acute, and does not provide information on how recently the participant was infected.

Statistical Analysis

Clinical data of the three groups were described by mean values and standard deviations or percentages, according to the type of variable. The primary predictor variable for all our analyses was baseline serum *M. pneumoniae* IgG positivity (0 = absence, 1 = presence). We used generalised linear mixed models to assess whether IgG positivity was associated with i) having a CTD compared to the unaffected cohort; or ii) tic onset compared to the unaffected cohort. In addition, we used linear mixed-effects models to determine whether iii) IgG positivity was associated with tic severity within the CTD cohort. Since this is a multicentre study involving siblings, we used multilevel models with both site and family relatedness as cluster variables. Sex, age, the presence of a comorbidity (OCD and/or ADHD) and the use of psychotropic medication in the past two weeks were entered as covariates. Age and sex varied substantially between cohorts and both were significant predictors of tics, though not *M. pneumoniae* IgG positivity. We therefore also conducted a sensitivity analysis with age and sex matched subgroups (CTD versus unaffected n = 88; tic onset versus unaffected n=51) using propensity score matching in R (Randolph et al., 2014).

Table 1: Baseline Demographics and Clinical Characteristics

	CTD (n = 302)		**Tic onset (n = 51)**		**Unaffected (n = 88)**	
Age (range) (mean ± SD)	4.5-16.99	11.0 ± 2.7	3.2 - 10.6	6.9 ± 1.9	4.2 - 10.9	7.7 ± 1.8
Sex n (%)						

Male	235	77.8%	30	58.8%	36	40.9%
Female	67	22.2%	21	41.2%	52	59.1%
Tic Disorder n (%)						
Tourette Syndrome	274	90.7%				
Chronic Motor Tic Disorder	26	8.6%				
Chronic Vocal Tic Disorder	2	0.7%				
Tic severity (range) (mean ± SD)						
YGTTS Total	0-44	20.1 ± 8.3				
YGTTS Motor	0-23	12.8 ± 4.5				
YGTSS Vocal	0-21	7.4 ± 5.4				
CGI	1-6	3.6 ± 0.9				
Psychotropic drug treatment during the previous 2 weeks n (%)	108	35.8	2	3.9	2	2.3
Comorbidities n (%)						
OCD	86	28.5	2	3.9		
ADHD	76	25.2	4	7.8	10	11.4
M. pneumoniae IgG positivity n (%)	48	15.9	9	17.1	15	17.0

Abbreviations: CTD, chronic tic disorder; unaffected, did not develop tics and ≥ 10 years old at last assessment; OCD, obsessive-compulsive disorder; ADHD, attention deficit hyperactivity disorder; *M. pneumoniae* IgG, ≥ 10 AU/ml was considered positive

RESULTS

Study Cohort

Baseline demographic and clinical characteristics of participants are shown in **Table 1.** The vast majority of the CTD cohort were diagnosed with TS (n = 274, 90.73%), 26 (8.61%) had a chronic motor tic disorder and 2 (0.66%) a chronic vocal tic disorder. The average time between baseline and tic onset was 1.1 years (range 0.1 – 5.4 years) and therefore elevation of IgG would be expected to persist during this time. Increasing age was significantly associated with *M. pneumoniae* IgG seropositivity in the tic onset cohort (p = 0.04) but not in the CTD or unaffected cohorts and there was no significant association with sex among any of the groups (Supplementary materials, **Table 2** and **Graph 1** histograms show the distribution of seropositivity by age for each group).

Previous infection is not associated with higher odds of tics

M. pneumoniae IgG positivity was not associated with either having CTD when compared to the unaffected cohort ($p > 0.05$) (as shown in **Table 2)**, or tic onset when compared to the unaffected cohort ($p > 0.05$) (as shown in **Table 3)**. These results remained non-significant ($p > 0.05$) when we conducted a sensitivity analysis with age and sex matched groups (supplementary materials in **Table 1)**.

Table 2: GLM results comparing rates of seropositivity in CTD and unaffected cohort

Dependent variable (covariates)	OR	95% CI	*p*
CTD versus unaffected			
M. pneumoniae IgG positivity	0.71	0.29-1.75	0.45
Age	1.94	1.58-2.38	<0.01*
Sex	0.17	0.08-0.36	<0.01*
Comorbidity (OCD and/or ADHD)	3.23	1.23-8.49	0.02*
Psychotropic medication	3.89	0.82-18.41	0.09
Tic onset versus unaffected			
M. pneumoniae IgG positivity	1.74	0.61-4.97	0.30
Age	0.75	0.60-0.94	0.01*
Sex	0.54	0.24-1.17	0.12
Comorbid ADHD and/or OCD	0.54	0.13-2.17	0.38
Psychotropic medication	3.02	0.30-30.06	0.34

Note. GLM (generalised linear mixed model) with age, sex, comorbidity (OCD and/or ADHD) and psychotropic medication as covariates was used. The presence of either or both ADHD and/or OCD was entered as a single covariate (absence = 0, presence = 1). Unaffected: ONSET children who did not develop tics by end of the study or re-assessment and ≥ 10 years old; tic onset: ONSET children with tic onset. *$p < 0.05$.

Table 3: GLM results comparing rates of seropositivity in tic onset and unaffected cohort

Dependent variable (covariates)	OR	95% CI	*p*
Tic onset versus unaffected			
M. pneumoniae IgG positivity	1.74	0.61-4.97	0.30
Age	0.75	0.60-0.94	0.01*
Sex	0.54	0.24-1.17	0.12
Comorbid ADHD and/or OCD	0.54	0.13-2.17	0.38
Psychotropic medication	3.02	0.30-30.06	0.34

Note. GLM (generalised linear mixed model) with age, sex, comorbidity (OCD and/or ADHD) and psychotropic medication as covariates was used. The presence of either or both ADHD and/or OCD was entered as a single covariate (absence = 0, presence = 1). Unaffected: ONSET children who did not develop tics by end of the study or re-assessment and ≥ 10 years old; CTD, Chronic tic disorder: COURSE children. *$p < 0.05$.

Older age, male sex and the presence of a comorbidity (OCD and/ADHD) were all significantly associated with having a CTD compared to the unaffected cohort ($p < 0.01$) (**Table 2**), whilst older age and male sex were also significantly associated with the onset of tic compared to unaffected cohort ($p < 0.05$) (**Table 3**).

M. pneumoniae IgG positivity associated with tic severity in COURSE

The association between seropositivity and tic severity is shown in **Table 4.** As shown in **Figure 1**, the presence of IgG antibodies against *M. pneumoniae* was associated with a higher YGTSS Total Tic Severity Score (β = 2.64, s.e. = 1.15, p = 0.02). There was a significant association between *M. pneumoniae* IgG positivity on the YGTSS vocal subscale (β = 1.02, s.e. = 0.62, p = 0.03) but no association with the CGI-S (see **Table 4**).

Table 4: Results from LMM on an association between *M. pneumoniae* IgG positivity and tic severity scales

Independent variable (dependent variable)	β	SE	95% CI	p
M. pneumoniae IgG				
(YGTSS Total)	2.64	1.15	0.38-4.89	0.02*
(YGTSS Motor)	1.02	0.62	-0.21-2.24	0.10
(YGTSS Vocal)	1.71	0.78	0.18-3.24	0.03*
(CGI-S)	0.17	0.13	-0.09-0.44	0.20

Note. Linear mixed-effects models with age, sex, comorbidity (OCD and/or ADHD) and psychotropic medication as covariates were used. The presence of either or both ADHD and/or OCD was entered as a single covariate (absence = 0, presence = 1). YGTSS, Yale Global Tic Severity Scale [Total = motor + vocal tic severity score (range 0 – 50)]; CGI-S, Clinical Global Impression Severity Scale; *$p < 0.05$.

Insert Figure 1 – needs updating

DISCUSSION

The main finding of this multicentre study in children and adolescents with tic disorders was that *M. pneumoniae* IgG positivity was not associated with a diagnosis of CTD or tic onset in a prospective at-risk cohort. *M. pneumoniae* IgG positivity, however, was associated with higher tic severity in children and adolescents with established CTD.

In the current study, infection rates of *M. pneumoniae* were not higher among those with tics than those without. Seropositivity rates among all groups in this study were lower than those reported elsewhere in the general population (Atkinson et al., 2008, Tuuminen et al., 2000). The reasons for this observation are not clear. Spread of *M. pneumoniae* among children is thought to increase with early day care and school attendance, in spring and autumn, as well as during cyclic epidemics that tend to occur every 3-5 years (Atkinson et al., 2008). Reduced attendance as a result of medical appointments and fewer regional epidemics may partially account for the lower rates seen in our study population but neither fully explain the marked difference we observed. Another unexpected finding was that rates *M. pneumoniae* IgG positivity were fairly evenly distributed by age across the cohorts (see Supplementary materials **Graph 1**) and seropositivity was only associated with age in the tic onset group and not the CTD or unaffected cohorts. *M. pneumoniae* IgG persists in the serum for a long-time following infection (estimates of around 4 years but can be indefinite (Daxboeck et al., 2003, Jacobs et al., 1986, Foy et al., 1977)), therefore, rates IgG positivity tend to increase with age during childhood: one population-based study found the incidence IgG positivity to increase from 12% in pre-school to 50-60% in adolescents (Tuuminen et al., 2000). One reason why we may not have observed an association with age in this study might have been because only a relatively small

number of participants from each group were found to be seropositive, thus reducing the power and likelihood of observing a significant association. Nonetheless, given the strong association between age and seropositivity reported in the literature and a significant association in the tic onset group, we adjusted for age in all our models and conducted sensitivity analysis with age and sex matched subgroups.

We observed no association between *M. pneumoniae* IgG positivity and the presence of a CTD or the first development of tics. The only previous study of *M. pneumoniae* in TS found elevated IgA, but not IgG, in 29 individuals with TS (6 to 60 years of age) compared to healthy controls (Müller et al., 2004). The authors proposed that this may be because *M. pneumoniae* IgA is particularly elevated in extra-pulmonary manifestations of *M. pneumoniae* infection (Müller et al., 2004). Due to lack of available serum for analysis, IgA was not measured in this study and should be considered in future research of suspected *M. pneumoniae* involvement in neuropsychiatric disease. Nonetheless, our findings do not suggest that children with tics or children who will later go on to develop tics are particularly susceptible to *M. pneumoniae* infection or mount an unusually sustained response to the pathogen compared to people without tics.

However, *M. pneumoniae* IgG positivity was associated with a higher YGTSS total tic severity score (range 0-50) of 2.64 points on average among participants with CTD after adjustments for age, sex, the presence of comorbid OCD and/or ADHD and use of psychotropic medication. This association between seropositivity and greater tic severity could reflect either a direct or indirect relationship. As this is a cross-sectional study that only measured IgG antibodies, indicative of past infection, at baseline, a single point in time, we cannot determine whether previous infection is associated with tic onset; whether infection coincides with a worsening of symptoms; or whether a

worsening of symptoms increases the risk of infection. An understanding of this timeline using prospective longitudinal studies that measure IgG, IgM and IgA antibodies and multiple measures of tic severity are needed to determine whether there is a direct relationship between tic severity and *M. pneumoniae*.

Alternatively, seropositivity and greater tic severity may be epiphenomena of a shared underlying mechanism, with neither directly influencing the other. Several studies have indicated that individuals with tic disorders have enhanced immune-inflammatory responses, including stronger antibody responses (Bombaci et al., 2009, Martino et al., 2011, Krause et al., 2010), as well as positive correlations between greater tic severity and interleukin-2 (IL-2) (Bos-Veneman et al., 2011) and the expression of genes that control immune-modulating neurotransmitters (Tian et al., 2011a, Tian et al., 2011b, Gunther et al., 2012). In a recent review of immunological mechanisms in brain development and tic disorders, Martino and colleagues suggest that abnormal immune priming, most likely driven by a genetic predisposition possibly interacting with environmental factors, may alter both the maturation of neural networks and immune regulatory mechanisms (Martino et al., 2020). Thereby, resulting in the co-occurrence of neurodevelopmental disorders (such as tic disorders) and hypersensitive immune responses to pathogens (such as to *M. pneumoniae*) (Martino et al., 2020). Psychosocial stress, a strong predictor of greater tic severity, may also influence immune responses to infection via activation of the hypothalamic-pituitary-adrenal axis (Martino et al., 2020), although this mechanism is yet to be fully elucidated. Finally, behavioural patterns, which may be accentuated in young people with greater tic severity and/or a higher comorbid neuropsychiatric burden, could increase the risk of exposure to infectious pathogens (i.e., “reverse causation”)

(Martino et al., 2020) and, thus, also indirectly contribute the association observed in thus study.

Limitations and future directions

This study has a number of strengths: EMTICS recruited a large population of children and adolescents with CTD from multiple centres across Europe and in Israel. It is unique in its assessment of first-degree relatives as prospective at-risk cohort and these children were extensively followed up for a period of up to seven years to assess the possible onset of tics.

However, there were also some notable limitations. The main limitation is the lack of longitudinal data or IgA measurements which preclude any analysis of a temporal relationship or any inference regarding causality. As mentioned, measurements of *M. pneumoniae* IgA, in addition to IgG antibodies, would be useful given previous reports of particular elevation of IgA in extra-pulmonary manifestations of *M. pneumoniae* infection (Müller et al., 2004). There was also only a small number of available serum samples for the tic onset and unaffected cohorts and only a small proportion of each group were *M. pneumoniae* IgG positive, which reduced the likelihood of observing significant differences between groups.

Conclusion

This is the largest cross-sectional study of *M. pneumoniae* infection in children and adolescents with CTD to date and the first to prospectively investigate *M. pneumoniae* infection in first-degree relatives (siblings) in relation to the onset of tics. We found no evidence of an association between previous *M. pneumoniae* IgG positivity and the presence of CTD or first onset of tics. However, we did observe an

association between *M. pneumoniae* IgG positivity and greater tic severity. It is possible that *M. pneumoniae* infection influences tic severity in CTD. Alternatively, greater disease severity may increase the risk of infection. However, it seems more likely that this observation reflects a propensity toward enhanced immune responses in people with tic disorders (as has been previously reported) and that greater tic severity and *M. pneumoniae* seropositivity share underlying mechanisms rather than a causal relationship. This is the first study to report this finding and further research into the relationship between infections and CTD may shed light on the mechanisms underlying disease onset, severity and course.

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SUPPLEMENTAL MATERIAL

Table 1: Sensitivity Analysis – age and sex matched subgroup: seropositivity and the odds of having CTD or tic onset

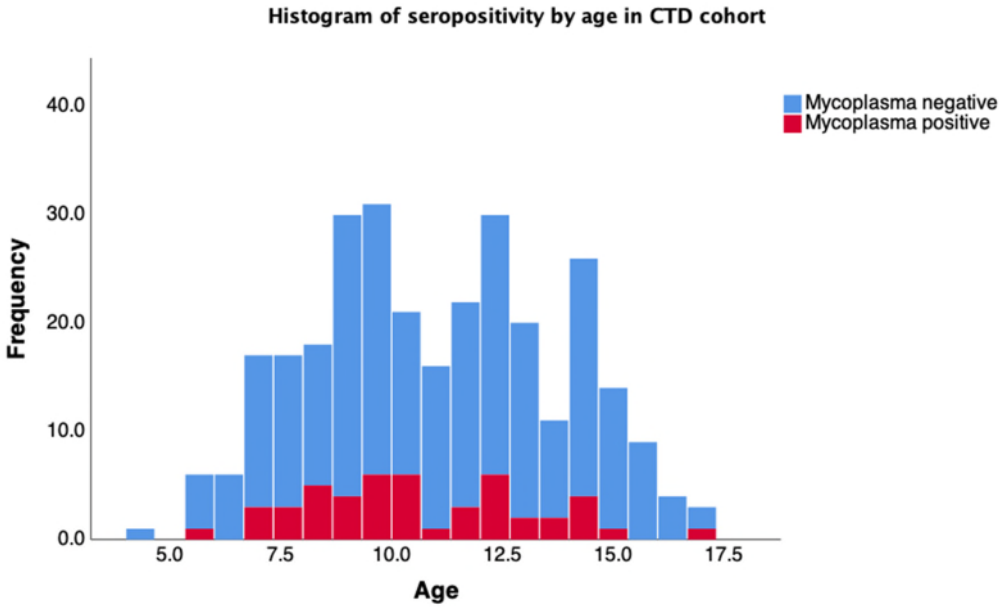
Predictor variable (dependent variable)	OR	95% CI	*p*
M. pneumoniae IgG			
(Unaffected versus CTD) (n=88)	0.51	0.15-1.73	0.28
(Unaffected versus tic onset) (n=51)	1.26	0.42-3.75	0.68

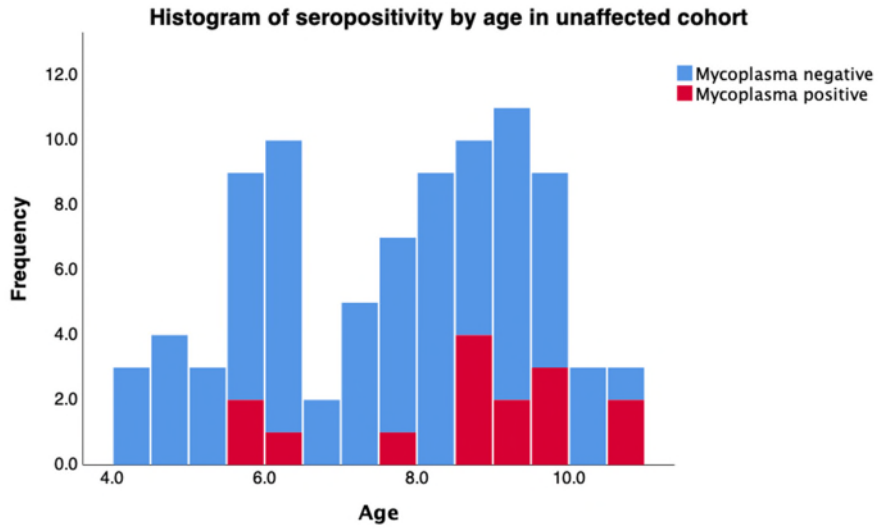
Note. Propensity score matching. Unaffected (did not develop tics by end of the study or re-assessment and ≥ 10 years old); CTD, Chronic tic disorder.

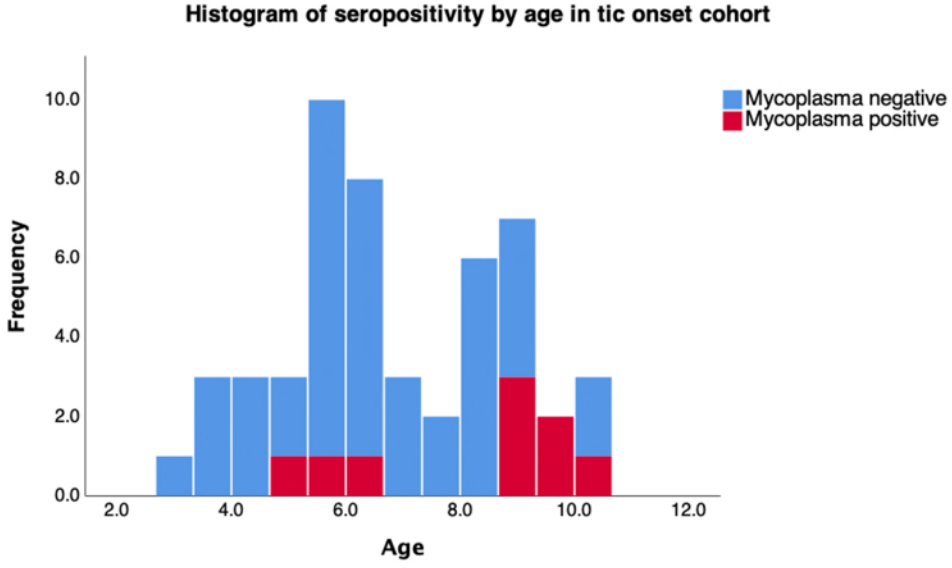
Table 2: GLM *M. pneumoniae* seropositivity association with age and sex in each cohort

M. pneumoniae seropositivity (predictors)	OR	95% CI	*p*
CTD cohort			
Age	0.94	0.84-1.06	0.33
Sex	1.41	0.69-2.90	0.35
Tic onset cohort			
Age	1.59	1.01-2.51	0.04*
Sex	1.76	0.37-8.36	0.47
Unaffected cohort			
Age	1.48	1.00-2.18	0.05
Sex	1.21	0.34-4.30	0.77

Note. GLM (generalised linear mixed model) with age and sex entered as covariates. Unaffected: ONSET children who did not develop tics by end of the study or re-assessment and ≥ 10 years old; CTD, Chronic tic disorder: COURSE children. *$p < 0.05$.


Histogram of seropositivity by age in CTD cohort
Mycoplasma negative
Mycoplasma positive
Frequency
40.0
30.0
20.0
10.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
Age


Histogram of seropositivity by age in unaffected cohort
Mycoplasma negative
Mycoplasma positive
Frequency
12.0
10.0
8.0
6.0
4.0
2.0
0.0
4.0
6.0
8.0
10.0
Age


Histogram of seropositivity by age in tic onset cohort
Mycoplasma negative
Mycoplasma positive
Frequency
10.0
8.0
6.0
4.0
2.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Age

APPENDIX 2. EMTICS AUTHORSHIPS

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