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Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis

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Abstract

Objectives—Autoantibodies recognizing cytosolic 5′-nucleotidase 1A (NT5C1A) are found in adult patients with myositis and other autoimmune diseases. They are especially prevalent in adults with inclusion body myositis (IBM), in which they are associated with more severe weakness and higher mortality. This study was undertaken to define the prevalence and clinical features associated with anti-NT5C1A autoantibodies in juvenile myositis.

Methods—We screened sera from 380 juvenile myositis patients, 30 patients with juvenile idiopathic arthritis (JIA), and 92 healthy control children for anti-NT5C1A autoantibodies. Clinical characteristics were compared between myositis patients with and without anti-NT5C1A autoantibodies.

Results—Anti-NT5C1A autoantibodies were present in 102 of 380 (27%) patients with juvenile myositis and in 11 of 92 (12%) healthy control children (p=0.002) and 27% of children with JIA (p=0.05 vs. controls). Eighty-three of 307 (27%) juvenile dermatomyositis and 16 of 46 (35%) juvenile overlap myositis patients' sera were anti-NT5C1A autoantibody positive (p<0.01 vs. healthy controls for each), but only 3 of 27 (11%) sera from juvenile polymyositis patients were anti-NT5C1A positive. Juvenile myositis patients with and without anti-NT5C1A autoantibodies had similar clinical phenotypes. However, anti-NT5C1A autoantibody positive myositis patients had greater pulmonary symptoms at diagnosis (p=0.005), more frequent hospitalizations (p=0.01) and required a larger number of medications (p<0.001).

Conclusion—Anti-NT5C1A autoantibodies are present in more than one-quarter of children with juvenile myositis and JIA compared with only 12% of healthy children, suggesting NT5C1A is myositis-associated in children. As in adults with IBM, juvenile myositis patients with anti-NT5C1A autoantibodies have more severe disease.

Introduction

Myositis is a diverse group of autoimmune diseases that includes polymyositis (PM), dermatomyositis (DM), and inclusion body myositis (IBM).[1] Myositis patients frequently have autoantibodies associated with distinct clinical phenotypes.[2] Autoantibodies found exclusively in myositis patients are known as myositis-specific autoantibodies (MSAs), whereas those that are also found in other autoimmune conditions are known as myositis-associated autoantibodies (MAAs). Interestingly, while the same autoantibodies found in adult myositis are also common in juvenile myositis, they are not always seen in the same frequency or associated with the same clinical features in both age groups. For example, autoantibodies against p155/140 (transcriptional intermediary factor1; TIF-1) are highly associated with malignancy in adults, but not in children.[3]

Autoantibodies recognizing cytosolic 5′-nucleotidase 1a (NT5C1A) were initially described in adults with IBM. [4, 5] In these and subsequent studies, the reported prevalence of anti-NT5C1A autoantibodies in IBM patients has ranged from 33% to 80%, depending on the patient population, type of assay used, and the cut-offs chosen to define a positive result. [4-12]. Importantly, since all studies have shown that less than 10% of adults with PM are positive for anti-NT5C1A, detection of this autoantibody may be a potentially useful biomarker for distinguishing these two forms of myositis. Subsequent studies demonstrated that anti-NT5C1A autoantibodies are also found in about 10-15% of adult DM patients and in 4-36% of adult patients with lupus or Sjogren's syndrome.[8, 9, 12] Given that they are found in myositis as well as in other autoimmune diseases, anti-NT5C1A can be defined as an adult MAA.

In adults with DM, no distinguishing clinical features have been identified for patients with anti-NT5C1A autoantibodies.[8, 9] However, one study has shown that anti-NT5C1A autoantibody positive IBM patients are more likely to have dysphagia, facial weakness, reduced forced vital capacity, and to require assistive devices than those without these autoantibodies. [7] Consistent with the possibility that anti-NT5C1A autoantibodies confer a more severe disease phenotype, another recent paper showed that IBM patients with this serologic feature had a higher adjusted mortality risk than autoantibody negative IBM patients.[13]

As anti-NT5C1A autoantibodies have not been previously described in children with myositis, the purpose of the present study was to define the prevalence and clinical features of anti-NT5C1A autoantibodies in a large cohort of patients with juvenile myositis. We also examined whether anti-NT5C1A autoantibodies are present in another pediatric rheumatologic condition, juvenile idiopathic arthritis (JIA), towards determining if they are myositis-specific or -associated autoantibodies in children.

Patients and Methods

Patients and serum samples

Patients from the Childhood Myositis Heterogeneity Collaborative Study with probable or definite myositis by Bohan and Peter criteria [14] with a serum sample available for NT5C1A autoantibody testing were included in the study. Serum samples, stored at -80 degrees Celsius from 1-27 years, were available from 380 children with myositis, 30 with JIA and 92 healthy control children. The myositis patients included 307 (81%) with juvenile DM (JDM), 27 (7%) with juvenile PM (JPM) and 46 (12%) with juvenile connective tissue disease-myositis overlap (JCTM) syndromes. The JCTM subgroup included 7 with juvenile idiopathic arthritis, 14 with juvenile systemic lupus, 11 with systemic sclerosis, and 14 with various other rheumatic conditions. Healthy control children enrolled in the same studies and were often age, gender and race-matched to myositis patients. They had no family history of autoimmune disease in first degree relatives, no history of infections or immunizations within the two months prior to enrollment, and no history of chronic inflammatory diseases.

All subjects were enrolled in investigational review board-approved natural history studies from 1990 to 2016, as previously described,[15] and all provided informed consent. A standardized physician questionnaire captured demographics, clinical features, laboratory features, environmental exposures at illness onset or diagnosis, as well as therapeutic usage and responses.[15] Seven organ system symptom scores at diagnosis, defined as the number of symptoms present divided by the number of symptoms assessed, and an overall clinical symptom score as the average of the seven individual organ symptom scores, were calculated as previously described.[16, 17] Complete clinical response and remission were defined as at least 6 months of inactive disease on or off therapy, respectively.[17] A course of treatment was defined as a single episode from beginning of administration of a given medication to the termination of treatment with that medication, or combination of medications, in each patient. The majority of patients had verification of the data via medical record review. HLA typing of DRB1 and DQA1 alleles was performed as previously described [18]. Sera from a control group of healthy children was obtained in the same protocols, and sera from 39 patients with JIA were obtained from the NIEHS Twin Sibling study. [19]

Myositis autoantibody assays

Anti-NT5C1A autoantibody detection—As previously described, lysates of HEK 293 cells transfected with NT5C1A and non-transfected HEK 293 cell lysates were electrophoresed on sodium dodecyl sulfate–polyacrylamide gels, transferred to nitrocellulose membranes, and immunoblotted with either a positive control rabbit polyclonal antibody recognizing NT5C1A (Applied Biological Materials) or human sera diluted 1:1,000 for 1 hour. To achieve uniformity between assays, each assay run included a positive control (i.e., rabbit anti-NT5C1A immunoblotting non-transfected vs. NT5C1A-transfected HEK 293 lysates). [8] Exposures of immunoblots in which the positive control lanes were of equivalent intensity were used for scoring. Human sera that recognized the 43-kd NT5C1A protein in NT5C1A transfected cells but not in untransfected cells were considered to be positive for anti-NT5C1A autoantibodies. All blots were independently scored by two readers (RY and ALM, who were blinded to sample identity) as being positive or negative for anti-NT5C1A reactivity. The interrater reproducibility for the positivity of this study was excellent, with an agreement of 96.1% and a Cohen's kappa coefficient of 0.90. In the few cases where there was disagreement, a third blinded reader (IPF) adjudicated.

Other myositis autoantibodies were tested by validated methods, including protein and RNA immunoprecipitation (IP) using radiolabeled HeLa or K562 cell extracts and double immunodiffusion.[15] For anti-p155/140, anti-MJ (Nuclear Matrix Protein 2, NXP2), and anti-melanoma-differentiation-associated gene 5 (MDA5) autoantibodies, serum samples were screened by IP, with confirmation testing by IP-immunoblotting.[15] Anti-3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) autoantibodies were screened by enzyme-linked immunosorbent assay (ELISA) and confirmed by immunoprecipitation using ^{35}S-methionine-labeled HMGCR protein produced by in-vitro transcription and translation as previously described.[20]

Analysis

Dichotomous variables were expressed as percentages and absolute frequencies, and continuous features were reported as means and standard deviations (SD). Pairwise comparisons for categorical variables between groups were made using chi-square test or Fisher's exact test, as appropriate, while continuous variables were compared using Student's t-test. Logistic and linear regression were used to adjust the comparisons for possible confounding variables, including the year of diagnosis, length of follow-up, and myositis autoantibodies. Creatine kinase, a highly positively skewed variable, was expressed as median, first, and third quartile for descriptive purposes and transformed through a base-10 logarithm for analysis. All statistical analyses were performed using Stata/MP 14.1. As this was an exploratory study, a 2-sided p value of 0.05 or less was considered statistically significant except for the HLA analyses, in which the Benjamini and Hochberg method to correct for multiple comparisons was performed.

Results

Anti-NT5C1A autoantibodies were more prevalent in juvenile myositis patients than in healthy control children (27% vs. 12%; p=0.002) (Table 1). Sera from 27% of JDM, 11% of JPM, and 35% of JCTM patients had anti-NT5C1A autoantibodies. Among these clinical subgroups, the prevalence of anti-NT5C1A autoantibodies was significantly increased in JDM (p=0.003) and JCTM patients (p=0.001), including those with lupus-myositis overlap (p=0.02), compared to healthy controls. Patients with JCTM had a higher prevalence of anti-NT5C1A autoantibodies than those with JPM (p=0.03); otherwise there were no significant differences in autoantibody prevalences among the JDM, JPM, and JCTM subgroups. Of note, the prevalence of anti-NT5C1A autoantibodies was significantly increased in the children with JIA compared to healthy controls (27% vs. 12%; p=0.02) (Table 1).

There were no significant differences in gender or race between juvenile myositis patients with and without anti-NT5C1A autoantibodies (Table 2). There was an association between the presence of anti-NT5C1A and anti-p155/140 autoantibodies, and fewer of the anti-NT5C1A autoantibody positive patients were negative for other MSAs. Specifically, 34% of the anti-p155/140-positive patients were also positive for anti-NT5C1A compared with just 24% of anti-p155/140-negative patients (p=0.04). Conversely, patients with anti-NT5C1A autoantibodies more frequently had co-existing anti-p155/140 autoantibodies than those who were negative for anti-NT5C1A autoantibodies (42% vs. 31%, p= 0.04). Of note, anti-NT5C1A autoantibody positive patients were more likely to have shorter follow-up times (4.4 vs. 6.2 years, p=0.01) compared to anti-NT5C1A autoantibody negative patients (Table 2). Given these differences, subsequent multivariate analyses were adjusted for follow-up duration and the presence of myositis autoantibodies (including anti-p155/140 autoantibodies) as well as the year of diagnosis (considering that treatment strategies may have changed over time).

In the multivariate analysis comparing their clinical features, anti-NT5C1A autoantibody-positive patients were more likely to have Raynaud's phenomenon and V-sign or shawl rashes compared to anti-NT5C1A autoantibody-negative patients (Table 3). The prevalences of dysphonia and photosensitivity were increased among anti-NT5C1A autoantibody

positive patients in the univariate analysis but not in the multivariate analysis, and there was a trend towards more frequent interstitial lung disease in those with anti-NT5C1A autoantibodies (Table 3). There were no other significant differences in the prevalences of the main muscle, lung, gastrointestinal, constitutional involvement, or other cutaneous manifestations between these two groups in either univariate or multivariate analyses (Table 3).

A number of differences between anti-NT5C1A autoantibody-positive and -negative patients suggested that patients with these autoantibodies had more severe disease. In the multivariate analysis, anti-NT5C1A-positive patients had higher pulmonary symptom scores ($p=0.003$) and showed a trend towards higher total symptom score ($p=0.09$) at the time of diagnosis (Table 3). Anti-NT5C1A autoantibody-positive patients had a higher mean number of hospitalizations (1.6 vs. 1.1, $p=0.01$) than those without this autoantibody. Furthermore, anti-NT5C1A autoantibody-positive patients required a greater number of medication treatments per year (4.8 vs. 3.6, $p=<0.001$) with a higher percentage of patients requiring intravenous pulse steroids (78% vs. 47%, $p<0.001$), intravenous immunoglobulin (67% vs. 24%, $p<0.001$), and use of other immunosuppressive medications (33% vs. 20%, $p=0.04$) (Table 4). Anti-NT5C1A autoantibody-positive patients had a trend for a more severe functional class at last assessment, but this did not reach statistical significance in the multivariate analysis (29% vs. 18% functional class 2, $p=0.08$).

The multivariate analyses were adjusted for the presence or absence of each MSA. Consequently, our finding that anti-NT5C1A autoantibodies are associated with more severe disease is independent of the MSA status of the patient. Furthermore, when analyzed separately, MSA negative, anti-p155/140-positive, and anti-NXP2-positive patients all had evidence of more severe disease when they were also anti-NT5C1A-positive (data not shown).

To determine whether there is an immunogenetic association with anti-NT5C1A autoantibodies, we compared the prevalence of HLA DRB1 and DQA1 alleles between Caucasian juvenile myositis patients with and without this autoantibody. We also compared Caucasian patients with anti-NT5C1A autoantibodies to healthy Caucasian subjects and all anti-NT5C1A-positive patients to all anti-NT5C1A-negative patients. However, after multiple correction adjustment, no statistically significant associations between anti-NT5C1A autoantibodies and these class II MHC alleles were found in any of these comparisons.

Discussion

In adults, autoantibodies recognizing NT5C1A are considered to be MAAs rather than MSAs because they are found not only in patients with myositis, but also in patients with lupus and Sjogren's syndrome.[8, 9] In this study, we show that anti-NT5C1A autoantibodies are also MAAs in children since they are found not only in juvenile myositis patients, but also in those with JIA. As expected for a MAA, we found that anti-NT5C1A autoantibodies were frequently found in association with other MSA, especially with anti-p155/140 autoantibodies.

The prevalence of anti-NT5C1A autoantibodies varies among adults with different myositis subtypes. They occur most frequently in those with IBM, less frequently in DM, and least frequently in those with PM. Here, we demonstrate that the prevalence of these autoantibodies in children also varies among myositis subtypes, with 27% of JDM but only 11% of JPM patients testing positive for anti-NT5C1A autoantibodies. In the current study, we also found that anti-NT5C1A autoantibodies are present in 35% of children with myositis-overlap syndromes. Although the number of patients in each myositis overlap subgroup was small, the autoantibodies were found most commonly in those with myositis associated with juvenile lupus (36%), less frequently in myositis associated with juvenile systemic sclerosis (27%), and least frequently in those with myositis associated with juvenile idiopathic arthritis (14%) patients. In adults, the prevalence of anti-NT5C1A autoantibodies was less than 5% in adults with myositis-scleroderma overlap,[9] but future studies will be required to define the prevalence of anti-NT5C1A in adults with other forms of myositis-overlap.

In adults with IBM, anti-NT5C1A autoantibodies have been associated with more severe muscle disease [7] and increased mortality.[13] However, no evidence for an association between anti-NT5C1A autoantibodies and more severe disease in adults with other forms of myositis has been reported. Here, we demonstrate that in patients with juvenile myositis these autoantibodies are associated with more frequent hospitalizations, higher pulmonary symptoms, and increased number of medications used. The underlying reasons for the association of anti-NT5C1A autoantibodies with more severe disease remain unclear, but may relate to a direct effect of the autoantibodies on myofiber protein degradation, as demonstrated in a study of passive transfer of anti-NT5C1A autoantibodies into mice. [21] Another possibility is that a more severe inflammatory response in juvenile myositis predisposes patients to the development of additional immunoreactivities such as that against NT5C1A.

In some instances, individual autoantibodies have significant associations with specific HLA alleles. However, in this study, we did not find an association between the presence of anti-NT5C1A autoantibodies and any class II HLA alleles. This is consistent with a recent report by Limaye, showing no HLA associations in adult IBM patients with anti-NT5C1A autoantibodies. [11]

In the current study, we were surprised to find that 11 out of 94 (12%) healthy children were positive for anti-NT5C1A autoantibodies detected by a previously validated immunoblotting assay. [7] We have demonstrated that 61% of adult IBM patients and only 5% of adult PM patients were anti-NT5C1A autoantibody positive using the immunoblotting method, findings which are in agreement with an established dot blot technique [4] and an established immunoprecipitation technique. [5] In our previous study, we also found that 5% of healthy adult controls were anti-NT5C1A-positive by immunoblotting. [7] While another study reported that none of 32 adult healthy controls were anti-NT5C1A positive by immunoprecipitation [5], the prevalence of anti-NT5C1A autoantibodies in healthy adult controls has not been reported for the dot blot assay [4] or either of two different ELISA assays.[6] Nonetheless, taken together, these comparisons indicate that the anti-NT5C1A immunoblot assay performs as well as other detection methods and does not have an

unacceptably high false positive rate. Of note, high titer autoantibodies against Ro52, another common target of the immune system in various systemic autoimmune diseases, are also found in healthy controls as well as in patients with more severe disease manifestations. [22]

When comparing the prevalence of anti-NT5C1A autoantibodies in children and adults within the same clinical groups using the same immunoblotting detection method, younger subjects consistently are more likely to have reactivity to these autoantibodies. For example, 27% of JDM but only 15% of adult DM [7] patients are anti-NT5C1A autoantibody positive ($p = 0.03$). Similarly, 11% of JPM but only 5% of adult PM patients [7] have these autoantibodies. Given that the same pattern is also observed among healthy children and healthy adults, we hypothesize that in both diseased and healthy groups, immunoreactivity against NT5C1A may decrease with age.

The current study has several limitations. First, the cohort of juvenile myositis patients had some data collected retrospectively, resulting in some missing data, and was collected over more than 20 years, with potential chronology bias. However, we adjusted the variables of this study for the year of diagnosis and tested the distribution of missing values across groups and did not find evidence of a significant bias (data not shown). Second, we only screened a small number of patients with one other systemic autoimmune disease, JIA; the small sample size in this group of patients did not allow us to study differences in severity between patients with and without anti-NT5C1A autoantibodies in a reliable way. Future studies will be needed to determine the full range of pediatric rheumatologic conditions in which anti-NT5C1A autoantibodies are found and to determine whether their presence correlates with disease severity or other clinical features. Third, there is no widely accepted “gold standard” for detecting anti-NT5C1A autoantibodies. However, we have developed and validated an immunoblotting detection method. This technique has a sensitivity (61%) and specificity (95%) for detecting anti-NT5C1A autoantibodies in adult IBM patients that is consistent with most other methods that utilize full-length NT5C1A protein (sensitivity range 47-80% and specificity range 95-100%). [4-12] Of note, one published study utilizing an ELISA method detected anti-NT5C1A autoantibodies in only 37% of IBM patients. However, this assay used 3 short peptides rather than the full length NT5C1A protein and the authors of the study acknowledged that it may have poor sensitivity since it cannot detect reactivity to conformational epitopes. [9]

These limitations notwithstanding, this study shows that anti-NT5C1A autoantibodies are present in approximately one-quarter of patients with juvenile myositis as well as JIA. Furthermore, as shown for adults with IBM, juvenile myositis patients with anti-NT5C1A autoantibodies have more severe disease than those without these autoantibodies. Additional studies will be required to confirm the association with disease severity in JM and to determine whether anti-NT5C1A autoantibodies are a result or a cause of the more severe clinical manifestations seen in the juvenile myositis and adult IBM patients who have them.

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Table 1
Prevalence of anti-NT5C1A in the sera of pediatric patients

	Total (n=502) % (N)
Juvenile myositis (n=380)	27% (102) **
Juvenile Dermatomyositis (n= 307)	27% (83) **
Juvenile Polymyositis (n= 27)	11% (3)
Juvenile myositis overlap syndromes (n=46)	35% (16) **
Juvenile myositis overlapping with Juvenile systemic lupus erythematosus (n=14)	36% (5) *
Juvenile myositis overlapping with Juvenile systemic sclerosis (n=11)	27% (3)
Juvenile myositis overlapping with Juvenile idiopathic arthritis (n=7)	14% (1)
Juvenile myositis overlapping with other pediatric autoimmune diagnosis (n=14)	50% (7) **
Juvenile idiopathic arthritis (n=30)	27% (8) *
Healthy pediatric controls (n=92)	12% (11)

*<0.05;

**<0.01;

***<0.001

%= percentage positive for anti-NT5C1A autoantibodies, N=number of sera testing positive for anti-NT5C1A autoantibodies.

Table 2

Demographic and myositis autoantibody features of juvenile myositis patients according to anti-NT5C1A autoantibody status

	Anti-NT5C1A Ab+ (n=102)	Anti-NT5C1A Ab- (n=278)	p-value
Age at diagnosis (years)	9.5 (4.4)	8.8 (4.3)	0.2
Age at enrollment (years)	11.8 (6.0)	12.7 (7.4)	0.3
Delay to diagnosis (years)	0.69 (1.00)	0.71 (1.23)	0.9
Follow-up (years)	4.4 (4.6)	6.2 (6.8)	0.01
Female	70% (71/102)	72% (200/278)	0.7
Race			
White	65% (66/102)	65% (182/278)	0.9
Black	12% (12/102)	17% (47/278)	0.2
Hispanic	7% (7/102)	6% (17/278)	0.8
Other races	17% (17/102)	12% (32/278)	0.2
Myositis autoantibodies			
Anti-p155/140	42% (42/100)	31% (82/268)	0.04
Anti-NXP2	22% (22/101)	21% (58/274)	0.9
Anti-MDA5	10% (10/102)	8% (22/275)	0.6
Anti-synthetase autoantibodies	4% (4/96)	4% (11/273)	1.0
Anti-SRP	0% (096)	3% (7/273)	0.2
Anti-Mi2	4% (4/96)	3% (9/267)	0.8
Anti-HMGCR	1% (1/102)	1% (3/278)	1.0
MSA negative	19% (19/102)	29% (77/269)	0.05

Dichotomous variables were represented as percentage (count/total) and continuous variables as mean (SD).

Table 3
Clinical features according to anti-NT5C1Aautoantibody status

	Ever present			
	Anti-NT5C1A Ab+ (n=102)	**Anti-NT5C1A Ab - (n=278)**	**univariate p-value**	**multivariate p-value**
Muscle involvement				
Proximal weakness	99% (101/102)	100% (277/278)	0.5	0.6
Distal weakness	50% (51/101)	45% (122/271)	0.3	0.4
Muscle atrophy	35% (36/102)	38% (105/274)	0.6	0.8
Myalgia	66% (67/101)	63% (172/271)	0.6	0.5
Falling	44% (45/102)	45% (122/274)	0.9	0.8
Dysphonia	40% (40/101)	29% (80/275)	0.05	0.2
Lung involvement				
Interstitial lung disease	13% (13/102)	7% (20/276)	0.09	0.06
Dyspnea on exertion	35% (36/102)	27% (74/273)	0.1	0.2
Joint involvement				
Arthritis	57% (58/102)	49% (137/277)	0.2	0.5
Arthralgia	66% (67/102)	64% (177/276)	0.8	0.7
Joint contractures	68% (69/102)	57% (159/277)	0.07	0.4
Skin involvement				
Heliotrope rash	84% (85/101)	77% (214/277)	0.1	0.6
Gottron's papules	87% (89/102)	81% (223/277)	0.1	0.5
Calcinosis	27% (28/102)	31% (86/278)	0.5	0.5
Raynaud's phenomenon	17% (17/102)	14% (39/276)	0.5	0.03
Mechanic's hands	9% (9/100)	7% (18/275)	0.4	0.2
V or Shawl sign rash	43% (44/102)	26% (72/276)	0.001	0.02
Malar rash	81% (83/102)	66% (184/278)	0.004	0.1
Photosensitivity	62% (61/99)	43% (116/272)	0.001	0.09
Linear extensor erythema	42% (42/101)	34% (91/271)	0.2	0.5
Gastrointestinal involvement				
Dysphagia	44% (45/102)	39% (109/277)	0.4	0.5
Regurgitation	25% (26/102)	19% (52/277)	0.2	0.4
Systemic involvement				
Weight loss	44% (44/101)	42% (115/277)	0.7	0.9
Fever	30% (30/100)	31% (83/267)	0.8	0.6
Early symptom scores	0.27 (0.12)	0.23 (0.11)	0.005	0.1
Total				
Muscle	0.38 (0.19)	0.38 (0.20)	0.8	0.9
Joint	0.52 (0.41)	0.43 (0.42)	0.06	0.4
Cutaneous	0.29 (0.15)	0.24 (0.13)	0.002	0.3
Gastrointestinal	0.09 (0.13)	0.07 (0.10)	0.03	0.06
Pulmonary	0.13 (0.17)	0.08 (0.15)	0.004	0.005

	Ever present			
	Anti-NT5C1A Ab+ (n=102)	**Anti-NT5C1A Ab - (n=278)**	**univariate p-value**	**multivariate p-value**
Cardiac	0.03 (0.08)	0.03 (0.08)	0.8	0.6
Constitutional	0.41 (0.26)	0.39 (0.27)	0.4	0.8

Abbreviations: NT5C1A, cytosolic 5′-nucleotidase 1A; Ab, autoantibody. Dichotomous variables were represented as percentage (count/total), continuous variables as mean (SD) and the creatine kinase was presented as median (Q1-Q3).

Table 4

Disease outcomes and medications received according to anti-NT5C1A autoantibody status

	Anti-NT5C1A Ab+ (n=102)	Anti-NT5C1A Ab – (n=278)	Univariate p-value	Multivariate p-value
Disease Course				
Monocyclic course	16% (12/76)	23% (53/229)	0.2	0.2
Polycyclic course	18% (14/76)	24% (56/229)	0.3	0.6
Chronic continuous course	66% (50/76)	52% (120/229)	0.04	0.2
Steinbrocker functional class at final assessment (mean)	1.5 (0.8)	1.4 (0.8)	0.6	0.6
Muscle enzymes				
Peak creatine kinase, IU/L	1010 (296-3971)	672 (252-5460)	0.7	0.2
Peak aldolase, IU/L	16.9 (23.9)	20.8 (37.2)	0.3	0.7
Severity at onset (mean, 0-4)	2.1 (1.4)	2.2 (0.9)	0.4	0.3
Mortality	4% (4/102)	3% (9/278)	0.8	0.3
Hospitalized	65% (62/96)	55% (148/268)	0.1	0.1
Number of hospitalizations	1.6 (2.3)	1.1 (1.7)	0.04	0.01
Wheelchair use	21% (21/100)	18% (47/268)	0.4	0.1
Response to treatment				
Complete clinical response	22% (19/87)	33% (74/225)	0.06	0.6
Remission	15% (13/88)	27% (63/232)	0.02	0.5
Total # of medications used	4.8 (2.0)	3.6 (2.0)	< 0.001	< 0.001
Medications received				
Oral steroids	99% (87/88)	99% (230/232)	1.0	0.6
Intravenous pulsed steroids	78% (69/88)	47% (110/232)	< 0.001	< 0.001
Methotrexate	83% (73/88)	71% (164/232)	0.03	0.4
Intravenous immunoglobulin	67% (59/88)	24% (55/232)	< 0.001	< 0.001
Other DMARDs	33% (29/88)	20% (46/232)	0.01	0.04

Abbreviations: NT5C1A, cytosolic 5′-nucleotidase 1A; Ab, autoantibody; DMARDs, disease modifying anti-rheumatic drugs.

Dichotomous variables were represented as percentage (count/total), continuous variables as mean (SD) and the creatine kinase was presented as median (Q1-Q3).