



Original article

Association of anti-HSC70 autoantibodies with cutaneous ulceration and severe disease in juvenile dermatomyositis

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Abstract

Objectives. JDM is an inflammatory myopathy characterized by prominent vasculopathy. AECAs are frequently detected in inflammatory and autoimmune diseases. We sought to determine whether AECAs correlate with clinical features of JDM, and thus serve as biomarkers to guide therapy or predict outcome.

Methods. Plasma samples from 63 patients with JDM, 49 patients with polyarticular JIA and 40 juvenile healthy controls were used to detect anti-heat shock cognate 71 kDa protein (HSC70) autoantibodies, a newly identified AECA, in ELISA assays. Clinical features were compared between JDM patients with and without anti-HSC70 autoantibodies.

Results. Anti-HSC70 autoantibodies were detected in 35% of patients with JDM, in 0% of patients with JIA ($P < 0.0001$) and in 0% of healthy donors ($P < 0.0001$). Both the presence of cutaneous ulcers (59% vs 17%, $P < 0.002$) and the use of wheelchairs and/or assistive devices (64% vs 27%, $P < 0.007$) were strongly associated with anti-HSC70 autoantibodies in JDM. High scores on the severity of myositis damage measures at the time of measurement of anti-HSC70 autoantibodies and an increased number of hospitalizations were also associated with anti-HSC70 autoantibodies. Intravenous immunoglobulin therapy was used more often in anti-HSC70 autoantibody-positive patients.

Conclusion. Anti-HSC70 autoantibodies are detected frequently in children with JDM and are novel myositis-associated autoantibodies correlating with disease severity.

Key words: JDM, vasculopathy, anti-endothelial cell antibodies, heat shock cognate 71 kDa protein, myositis-associated autoantibodies

Rheumatology key messages

- Anti-HSC70 autoantibodies were detected in 35% of North American JDM patients by ELISA using plasma.
- Anti-HSC70-positive patients with JDM show a marked increased tendency to develop cutaneous ulcers.
- The presence of anti-HSC70 autoantibodies significantly correlates with use of IVIG therapy in JDM patients.

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Introduction

JDM is the most common form of inflammatory myopathy in children [1]. Although relatively rare, JDM is associated with significant morbidity and, in some cases, mortality [2, 3]. One of the striking pathological features of involved tissues in JDM is the presence of prominent vascular and perivascular inflammation. In many cases, this inflammation is accompanied by thrombosis and vessel occlusion, which contributes directly to the observed clinical features [4, 5]. We recently reported that children with JDM have detectable autoantibodies to multiple antigens on human endothelial cells [6]. Among these antigens were molecular chaperones, such as heat shock cognate 71 kDa protein (HSC70; also known as HSPA8). Autoantibodies against HSC70 are more commonly detected in children with active, untreated disease than in healthy children. We also reported that these AECAs are rare in children with juvenile idiopathic arthritis, another chronic autoimmune disease affecting children. This has raised the question of whether an autoimmune response directed to the vasculature may play a role in the pathobiology of JDM.

A broad spectrum of autoantibodies has been detected in children with JDM, and many of these autoantibodies are linked to specific clinical features [1, 7]. In this study, we sought to determine whether anti-HSC70 autoantibodies, an AECA, might correlate with specific clinical features of JDM, and thus serve as surrogate biomarkers to guide therapy or predict outcome.

Methods

Patients and plasma samples

Plasma of JDM patients under treatment ($n=63$) were collected as part of natural history protocols at the National Institutes of Health (NIH) between 2003 and 2017. The median [interquartile range (IQR)] age of the patients with JDM (42 females and 21 males) was 11 (9–16) years. All except one patient without anti-HSC70 autoantibodies had the active disease at the time of measurement of the autoantibodies in JDM. Disease course was classified as monocyclic if the patient achieved remission without evidence of active disease within 2 years of diagnosis, based on the clinical features and laboratory testing; as polycyclic if the patient had recurrence of active disease after a definite clinical remission; and as chronic if active disease persisted for >2 years [8]. Active disease and remission were defined, respectively, as a physician global score of >0 and as clinically inactive disease for at least six continuous months of all treatment [9]. The presence or absence of symptoms at diagnosis in seven organ systems was examined using a total symptom score defined as the number of symptoms present divided by the number of symptoms evaluated (range, 0–1), as previously described [8]. For instance, pulmonary system score was evaluated using the five items (dysphonia, dyspnea

on exertion, dyspnea at rest, interstitial lung disease, and pneumothorax or pneumomediastinum) or a minimum of four out of the five items and was determined based on the presence or absence of signs or symptoms prior to or at diagnosis. Severity of illness at onset, up to the time of diagnosis, was graded on a 4-point Likert scale as determined by the enrolling physician, and graded from mild to very severe disease activity [1]. The presence of ulceration was determined by pediatric and adult rheumatologists who enrolled patients into the study and was defined using the tools that we validated, including Myositis Disease Activity Assessment Tool and Cutaneous Assessment Tool. Plasma from patients with newly diagnosed, untreated (JIA; $n=49$, 39 females and 10 males) and healthy children (HC; $n=40$, 25 females and 15 males) were collected from children attending the General Pediatrics Clinics at the University of Oklahoma College of Medicine and the Women and Children's Hospital of Buffalo. The median (IQR) age of the patients with JIA and HC was 11 (7–14) and 12 (9–15) years, respectively. Children with JIA had the extended oligoarticular or polyarticular phenotypes as assessed using accepted international criteria [10]. Written informed consent, and, where appropriate, assent, was obtained from all participants prior to obtaining samples. This study was approved by the ethics committee of St. Marianna University School of Medicine (no. 1710), the University of Oklahoma Institutional Review Board (no. 12134), University at Buffalo Institutional Review Board (IRB no. MOD 00002155) and the National Institutes of Health Institutional Review Board (05E-N-200, 94-E-0165, 03-E-0099 and 11-E-0072).

Anti-HSC70 autoantibody detection

ELISA were conducted as described previously [6]. To detect autoantibodies in plasma to heat shock cognate 71 kDa protein encoded by the *HSPA8* gene, each well of an ELISA plate (Thermo Fisher Scientific, Waltham, MA, USA) was coated with 0.125 µg of HSC70 (StressMarq Biosciences Inc., Victoria, BC, Canada) in a carbonate buffer (50 mM sodium carbonate, pH 9.6). The plasma diluted 1:200 with 10% Block Ace was reacted with the coated recombinant proteins. Optical density at 490 nm was measured by a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The mean plus 3 s.d. among the group of healthy donors was defined as 100 arbitrary binding units (AU) and was used as a cut-off point for determining reactivity to HSC70.

Myositis autoantibodies and HLA typing

Sera were tested for myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) using validated immunoprecipitation and immunoprecipitation-immunoblotting methods at the Oklahoma Medical Research Foundation laboratory [1]. Anti-Ro52 autoantibodies were detected using ELISA for Ro52

[11]. Genotyping of HLA Class II (HLA DRB1 and DQA1) alleles was performed in Caucasian anti-MDA5 autoantibody-positive JDM patients [12].

Statistics

Plasma levels of autoantibodies were summarized as medians and IQR. Categorical variables were presented as percentages and were compared by Fisher's exact test. Continuous variables were compared by the Mann-Whitney *U*-test and expressed as medians and IQR. The frequencies of specific HLA alleles were compared between the subject groups using the χ^2 test or Fisher's exact test. Because we conducted an exploratory study, a two-tailed *P*-value of <0.05 was considered statistically significant and not corrected for multiple testing. Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

Results

Prevalence of anti-HSC70 autoantibodies in JDM compared with other rheumatic diseases and controls

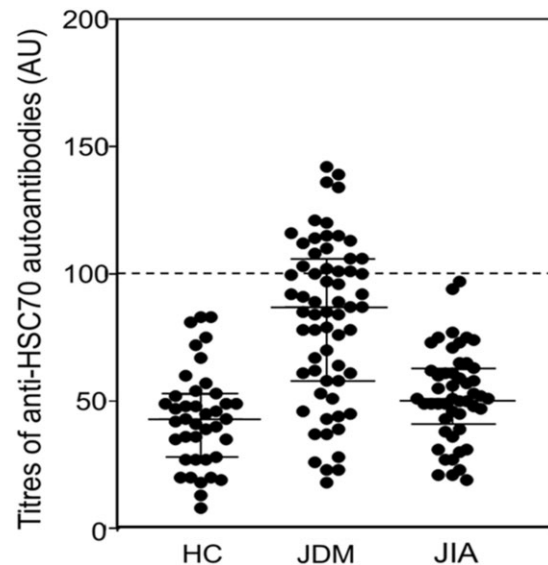
Significant differences in plasma levels and the frequency of autoantibodies to HSC70 were found between children with JDM and the other groups (Fig. 1).

Autoantibodies to HSC70 were detected in 35% (22/63) of patients with JDM, in 0% (0/49) of patients with JIA ($P < 0.0001$) and in 0% (0/40) of HC ($P < 0.0001$). Median (IQR) levels of autoantibodies to HSC70 were 86.6 (59.4–104.6) AU for JDM, 51.2 (43.5–62.3) AU for JIA and 43.2 (28.0–52.0) AU for HC. The median plasma levels of anti-HSC70 autoantibodies were higher in children with JDM than in children with JIA ($P < 0.0001$) and in HC ($P < 0.0001$). The ELISA assay exhibited no matrix effects and was shown to be highly reproducible.

General features of JDM patients with and without anti-HSC70 autoantibodies

There were no significant differences in demographic characteristics, including age at diagnosis, age at enrolment in the NIH studies, gender, race, or delay to diagnosis (i.e. the time from onset of signs or symptoms of myositis to diagnosis), between JDM patients with and without anti-HSC70 autoantibodies (Table 1). Patients positive for anti-HSC70 autoantibodies more often had NT5C1A and/or anti-Ro52 autoantibodies (69% vs 29%, $P < 0.02$ and 43% vs 15%, $P < 0.03$, respectively). In contrast, there were no significant differences in the presence of any MSAs between JDM patients with and without anti-HSC70 autoantibodies. Furthermore, the presence of anti-HSC70 autoantibodies alone was more frequent in JDM patients with cutaneous ulcerations compared with that of anti-HSC70 autoantibodies co-occurring with anti-NT5C1A and/or anti-Ro52 autoantibodies [89% (8 out of 9) vs 38% (5 out of 13), $P = 0.031$].

Fig. 1 Results of ELISAs for anti-HSC70 autoantibodies



The dashed line of 100 arbitrary units indicates the cut-off values for anti-HSC70 autoantibody positivity. The average plus 3 s.d. among the group of healthy donors defined as 100 arbitrary units was 0.603 (optical density). The continuous lines indicate median and interquartile range of anti-HSC70 autoantibody levels. Plasma samples from 63 patients with JDM, 49 patients with JIA, and 40 sex- and age-matched healthy children (HC) were used for ELISA assays. Anti-HSC70 autoantibodies were detected in 35% of patients with JDM, in 0% of patients with JIA, and in 0% of HC.

Clinical features of JDM patients with and without anti-HSC70 autoantibodies

The presence of anti-HSC70 autoantibodies correlated with multiple clinical features of children with JDM. For example, cutaneous ulcers and malar erythema were associated with the presence of autoantibodies against HSC70 (59% vs 17%, $P < 0.002$ and 82% vs 54%, $P < 0.04$, respectively) (Table 2).

Furthermore, a history of fever was also associated with the presence autoantibodies against HSC70 (50% vs 17%, $P < 0.009$). Higher median serum levels of lactate dehydrogenase, which is reported to be released from endothelial cells in response to endothelial cytotoxicity [13], were found in JDM children with anti-HSC70 autoantibodies (median 488 in those with anti-HSC70 vs 266 in those without, $P < 0.002$). JDM patients with anti-HSC70 autoantibodies also had higher early pulmonary scores (median 0.20 vs 0, $P < 0.04$); however, they did not have an increased frequency of interstitial lung disease or dyspnea.

Clinical course and medications used in JDM patients with and without anti-HSC70 autoantibodies

The median number of hospitalizations was higher in JDM patients with anti-HSC70 autoantibodies than in

TABLE 1 General features of JDM patients with and without anti-HSC70 autoantibodies

	Anti-HSC70 positive (N = 22)	Anti-HSC70 negative (N = 41)	P-value
Age at diagnosis, median (IQR), years	8.6 (7.0–10.9)	8.0 (6.3–12.4)	0.8
Age at enrolment, median (IQR), years	10.7 (8.8–14.6)	11.2 (8.6–15.8)	0.9
Delay to diagnosis, median (IQR), months	3.5 (2.0–6.8)	5.0 (2.0–8.3) (N = 40)	0.3
Follow-up, median (IQR), years	2.8 (1.9–6.4)	3.1 (1.1–7.1)	0.8
Female, n/N (%)	12/22 (55)	30/41 (73)	0.2
Race, n/N (%)			
White	14/22 (64)	28/41 (68)	0.8
Black	3/22 (14)	4/41 (10)	0.7
Hispanic	1/22 (5)	2/41 (5)	1.0
Asian	0/22 (0)	1/41 (2)	1.0
Biracial or multiracial	4/22 (18)	6/41 (15)	0.7
Myositis-associated autoantibodies, n/N (%)	15/22 (68)	16/41 (39)	<0.04
Anti-NT5C1A	11/16 (69)	10/35 (29)	<0.02
Anti-Ro52	9/21 (43)	6/40 (15)	<0.03
Anti-Ro	6/22 (27)	4/41 (10)	0.08
Anti-U1RNP	1/22 (5)	2/41 (5)	1.0
Anti-PM-SCL	0/22 (0)	1/41 (2)	1.0
Myositis-specific autoantibodies, n/N (%)	20/22 (91)	34/41 (83)	0.5
Anti-p155/140 (TIF-1)	7/22 (32)	16/41 (39)	0.8
Anti-NXP-2	6/22 (27)	11/41 (27)	1.0
Anti-MDA5	6/22 (27)	3/41 (7)	0.06
Anti-Mi-2	1/22 (5)	1/41 (2)	1.0
Anti-KS	0/22 (0)	1/41 (2)	1.0
Anti-HMGCR	0/22 (0)	1/41 (2)	1.0
Anti-Jo1	0/22 (0)	1/41 (2)	1.0

Categorical variables were analysed by Fisher's exact tests and are presented as count/total (percentage). Continuous variables were analysed by the Mann-Whitney *U*-test and are expressed as median (IQR [Q1–Q3]). Myositis-associated autoantibodies (*n*) include anti-Ro (10), anti-Ro52 (15), anti-NT5C1A (21), anti-U1RNP (3) and anti-polymyositis-scleroderma antibodies (1). Myositis-specific autoantibodies (*n*) include anti-p155/140 (23), anti-NXP-2 (17), anti-MDA5 (9), anti-Mi-2 (2), anti-KS (1), anti-HMGCR (1) and anti-Jo1 (1) autoantibodies. HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; IQR: interquartile range; Jo1: anti-histidyl tRNA synthetase; MDA5: melanoma differentiation associated protein-5; NT5C1A: cytosolic 5'-nucleotidase 1A; NXP-2: nuclear matrix protein-2; PM-SCL: polymyositis-scleroderma; TIF-1: transcription intermediary factor 1.

those without (median 2.0 vs 1.0, $P < 0.04$) (Table 3). Use of wheelchairs and/or assistive devices was associated with the presence of anti-HSC70 autoantibodies (64% vs 27%, $P < 0.007$), as was the use of wheelchairs in the observation period and at last follow-up (55% vs 22%, $P < 0.02$ and 18% vs 2%, $P < 0.05$, respectively). Lastly, JDM patients with anti-HSC70 autoantibodies had higher Myositis Damage Index severity of damage scores than those without at the time of measurement of the autoantibodies (median 8.0 vs 5.0, $P = 0.01$).

JDM patients with anti-HSC70 autoantibodies more often received IVIG compared with those without (95% vs 73%, $P < 0.05$). There was no association of the presence of anti-HSC70 autoantibodies with disease course, other medications or response to treatment (Table 3). It is notable that few patients with JDM achieved complete clinical response or remission in this study.

Differences in human leucocyte antigen allele associations in JDM patients with and without anti-HSC70 autoantibodies

A weak association was observed between HLA class I or II alleles and the presence of anti-HSC70 autoantibodies.

The presence of autoantibodies against HSC70 was associated with the presence of HLA-C*04 [16.7% vs 0%, odds ratio (OR) not calculable, $P = 0.04$] and a decrease in the frequency of HLA-DPB1*0401 [17.7% vs 47.1%, OR 0.24 (95% CI: 0.07, 0.91), $P = 0.04$].

Discussion

The presence of prominent vascular and perivascular inflammation is a notable feature of JDM and contributes directly to the clinical manifestations of the disease [2, 4]. We have recently reported that, similar to other vasculitides, patients with JDM have autoantibodies directed against a broad range of antigens in and/or on endothelial cells [6]. We also found that autoantibodies to an endothelial cell antigen, heat shock protein cognate 70 (HSC70), were more common and detected in higher titres in children with newly diagnosed, untreated disease than in children who had achieved remission. Here we sought to explore clinical associations with anti-HSC70 autoantibodies and found that anti-HSC70 autoantibodies occur frequently in JDM and are associated with multiple features of disease severity.

TABLE 2 Clinical features of JDM patients with and without anti-HSC70 autoantibodies

Clinical features, ever present	Anti-HSC70 positive (N = 22)	Anti-HSC70 negative (N = 41)	P-value
Skin involvement, n/N (%)			
Cutaneous ulcer	13/22 (59)	7/41 (17)	<0.002
Periungual capillary dilatation	22/22 (100)	38/41 (93)	0.5
Malar erythema ^a	18/22 (82)	22/41 (54)	<0.04
Oedema	9/22 (41)	7/41 (17)	0.07
Calcinosis	12/22 (55)	16/41 (39)	0.3
Systemic involvement, n/N (%)			
Weight loss	11/22 (50)	21/41 (51)	1.0
Fever	11/22 (50)	7/41 (17)	<0.009
Muscle involvement, n/N (%)			
Distal weakness	16/22 (73)	27/41 (66)	0.8
Proximal and distal weakness	16/22 (73)	27/41 (66)	0.8
Muscle atrophy	12/22 (55)	16/41 (39)	0.3
Lung involvement, n/N (%)			
Dyspnea on exertion	13/22 (59)	15/41 (37)	0.1
Dyspnea at rest	1/22 (5)	9/41 (22)	0.1
Interstitial lung disease	4/22 (18)	2/41 (5)	0.2
Joint involvement, n/N (%)			
Arthralgia	16/22 (73)	33/41 (80)	0.5
Arthritis	14/22 (64)	24/41 (59)	0.8
Gastrointestinal involvement, n/N (%)			
Regurgitation	8/22 (36)	11/41 (27)	0.6
Abdominal pain ^a	13/22 (59)	13/39 (33)	0.06
Early total symptom score, median (IQR)	0.33 (0.23–0.38)	0.28 (0.20–0.35)	0.2
Early muscle score	0.43 (0.29–0.57)	0.43 (0.29–0.57)	0.5
Early joint score	0.50 (0.50–1.0)	0.50 (0.50–1.0)	0.9
Early cutaneous score	0.38 (0.33–0.44)	0.38 (0.31–0.44)	0.9
Early gastrointestinal score	0.13 (0–0.25)	0 (0–0.13)	0.09
Early pulmonary score	0.20 (0–0.20)	0 (0–0.20)	<0.04
Early constitutional score	0.38 (0.25–0.69)	0.25 (0.25–0.5)	0.6
Muscle enzymes (highest value), median (IQR), IU/l			
AST	122 (48–202)	85 (46–127)	0.3
ALT	58 (38–107)	54 (30–127)	0.7
LDH ^b	488 (346–808)	266 (207–377)	<0.002
CK	1154 (286–3537)	800 (278–3082)	0.7
Severity at onset, median (IQR)	2.0 (2.0–3.0)	2.0 (2.0–3.0)	1.0

Categorical variables were analysed by Fisher's exact tests and are presented as count/total (percentage). Continuous variables were analysed by the Mann–Whitney *U*-test and are expressed as median (IQR [Q1–Q3]). ^aIn those with the history during the follow-up period after onset of first symptom. ^bAll values of LDH were adjusted for the NIH upper limit of normal for LDH in adults, which is 226 U/l. ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase; IQR: interquartile range; LDH: lactate dehydrogenase.

We found that anti-HSC70 autoantibodies are seen in 35% of children with JDM, which is higher than what we previously observed [6] and may reflect a larger number of patients with severe disease in the current cohort or a difference in the ethnogeographic distribution of these autoantibodies in JDM patients. Importantly, the presence of anti-HSC70 autoantibodies was also associated with cutaneous ulceration, a manifestation of vasculopathy in JDM [5], raising the question of whether these autoantibodies may be associated with other forms of vasculopathy and/or vasculitis. However, there were no significant associations between anti-HSC70 autoantibodies and calcinosis, which may be driven by smouldering vascular disease in JDM [4, 14], nor was there an association

with periungual capillary dilatation, a predominant vasculopathic feature, which was present in almost all of the JDM patients in our cohort. Furthermore, anti-HSC70 autoantibodies have also been observed in chronic inflammatory illnesses without vasculitis, for instance, myasthenia gravis [15].

Anti-HSC70 autoantibodies were also associated with multiple measures of disease severity in JDM, including use of wheelchairs and/or devices, number of hospitalizations and scores on the severity of myositis damage measures, which highlights the potential of anti-HSC70 autoantibodies as a predictor of disease severity and poor prognosis in JDM. Patients with anti-HSC70 autoantibodies were also more likely to have been treated with IVIG than patients who were autoantibody-negative.

TABLE 3 Disease outcomes and medications used in JDM patients with and without anti-HSC70 autoantibodies

Disease outcomes and medications, ever present	Anti-HSC70 positive (N = 22)	Anti-HSC70 negative (N = 41)	P-value
Ever hospitalized, n/N (%)	17/22 (77)	24/41 (59)	0.2
Median number of hospitalizations, median (IQR)	2.0 (0.5–4.5) (N = 19)	1.0 (0–1.5) (N = 39)	<0.04
Wheelchair/device ^a use, n/N (%)	14/22 (64)	11/41 (27)	<0.007
Wheelchair use, n/N (%)	12/22 (55) ^c	9/41 (22) ^c	<0.02
	4/22 (18) ^d	1/41 (2) ^d	<0.05
Disease activity at final assessment, median (IQR), median (IQR)	1.0 (1.0–2.0)	1.0 (1.0–1.0)	0.1
Physician global activity score ^b , median (IQR)	2.3 (1.5–3.9)	2.5 (1.7–3.5)	0.7
Physician global damage assessment ^b , median (IQR)	2.2 (1.0–3.8) (N = 21)	1.4 (0.7–2.7) (N = 30)	0.07
Myositis Damage Index severity of damage score ^b , median (IQR)	8.0 (5.0–14.0) (N = 21)	5.0 (2.0–7.0) (N = 29)	0.01
CHAQ score ^b , median (IQR)	2.1 (1.0–4.7)	2.6 (1.1–4.7)	0.6
MMT8 score (left) ^b , median (IQR)	74.0 (66.0–80.0) (N = 21)	72.5 (66.5–77.3) (N = 32)	0.6
MMT8 score (right) ^b , median (IQR)	74.0 (66.0–80.0) (N = 21)	75.0 (66.5–78.0) (N = 32)	0.9
CMAS score ^b , median (IQR)	42.5 (34.8–48.3) (N = 20)	44.0 (38.3–49.0) (N = 30)	0.4
Disease course, n/N (%)			
Monocyclic course	0/16 (0)	2/27 (7)	0.5
Polycyclic course	2/16 (13)	3/27 (11)	1.0
Chronic continuous course	14/16 (88)	22/27 (81)	0.7
Response to treatment			
Complete clinical response, n/N (%)	1/22 (5)	7/40 (18)	0.2
Remission, n/N (%)	0/22 (0)	1/40 (3)	1.0
Prednisone discontinuation ^e , n/N (%)	0/22 (0)	3/40 (8)	0.5
Total number of medications used, median (IQR)	7.0 (5.3–8.0) (N = 22)	6.0 (4.0–7.3) (N = 40)	0.2
Medications received, n/N (%)			
Oral steroids	22/22 (100)	40/40 (100)	1.0
Intravenous pulsed steroids	20/22 (91)	30/40 (75)	0.2
Methotrexate	21/22 (95)	39/40 (98)	1.0
Intravenous immunoglobulin	21/22 (95)	29/40 (73)	<0.05
Other DMARDs	22/22 (100)	39/40 (98)	1.0

Categorical variables are analysed by Fisher's exact tests and are presented as count/total (percentage). Continuous variables are analysed by the Mann–Whitney *U* test and are expressed as median (IQR [Q1–Q3]). ^aWheelchair, bed bound and other assistive devices are included. ^bEvaluation at the time of measurement of anti-HSC70 autoantibodies. ^cIn the observation period. ^dAt last follow-up. ^eDiscontinuation of prednisone in first 12 months of treatment. CHAQ: childhood HAQ; IQR: interquartile range; CMAS: Childhood Myositis Assessment Scale.

Although it is reported that cutaneous ulcers occur in patients with JDM whose disease is more resistant to immunosuppressive therapy [16], there was no significant difference between histories of cutaneous ulcers and the use of IVIG regardless of whether JDM patients had autoantibodies against HSC70 (data not shown). This suggests that patients with anti-HSC70 autoantibodies may show poor response to standard first-line measures of glucocorticoids plus MTX because of more tenacious disease independent of cutaneous ulceration. This finding raises the question of whether anti-HSC70 autoantibodies might be used as a biomarker to select patients likely to have a more severe or intractable disease. In addition, the question arises as to whether autoantibodies to HSC70 interfere with MTX therapy as HSC70 binds MTX and is involved in its transport into the cells [17, 18].

We report that anti-HSC70 autoantibodies are a novel MAA that are not associated with any particular MSA but co-occur frequently with the MAA anti-NT5C1A and anti-Ro52. We did not see a significant association with

anti-MDA5 autoantibodies, even though anti-MDA5 positive patients frequently have cutaneous ulceration [19]. Anti-NT5C1A autoantibodies occur more often in patients with pulmonary symptoms at diagnosis and have the potential to be a predictor of severe disease [20]. Similarly, the presence of anti-Ro52 autoantibodies has relevance to interstitial lung disease and has the potential to be used as a predictor of disease severity and poorer prognosis [11]. Interestingly, anti-HSC70 autoantibodies are not associated with pulmonary manifestations of disease. Here, questions remain as to the association between anti-HSC70 autoantibodies and anti-Ro52 autoantibodies. Ro52 ELISA assay may be affected by matrix effects (i.e. high levels of IgG, cholesterol and rheumatoid factor) and/or lead to false positives in the presence of other autoantibodies, such as anti-SS-A/Ro, anti-SS-B/La and anti-Jo1 autoantibodies. These effects were not investigated in the prior study of Ro52 in juvenile myositis [11]. Future analyses utilizing direct comparison of these and other autoantibodies are

required to determine which are the most reliable and clinically useful in predicting disease severity.

Our study also raises the question of the extent to which anti-HSC70 autoantibodies contribute to the observed pathology in JDM, and specifically to the vasculopathy. Complement and immunoglobulin deposits in vessel walls are documented features of JDM [21] and of DM [21, 22], and it is tempting to speculate that autoantibodies directed to endothelial cells could directly contribute to the vascular injury. However, it is just as plausible that AECAs are formed in response to the vascular injury, while still playing a role in the ongoing inflammatory process. We are becoming increasingly aware that cellular and tissue damage by itself can drive autoantibody formation [23]. For example, in a mouse model of experimental myositis, tissue damage was caused by CD8⁺ T cells and type I interferons, but not by CD4⁺ T cells, B cells or autoantibodies, and anti-transcriptional intermediary factor 1 (TIF1) γ autoantibody production followed the development of experimental myositis [24]. Interestingly, HSC70 is also known to modulate viral infectivity and its presence correlates with virus-induced host immunoprotective responses [25]. As infectious diseases have also the potential to be a significant risk factor for JDM pathophysiology, further studies are needed to assess the roles of anti-HSC70 autoantibodies in infection events in JDM [26, 27].

It is reported that anti-HSC70 autoantibodies are detected in other autoimmune and inflammatory diseases, including Behçet's disease, systemic sclerosis, Kawasaki disease, inflammatory bowel diseases, myasthenia gravis and immune thrombocytopenic purpura [15, 28–32]. Associations with disease severity in other autoimmune diseases is currently unknown. For instance, in myasthenia gravis, decreased titres of anti-HSC70 autoantibodies have been associated with clinical improvement; however, the presence of anti-HSC70 autoantibodies was not correlated with disease severity [15]. To clarify whether anti-HSC70 autoantibodies are associated with clinical features and treatment response and/or are involved in the pathophysiology of other autoimmune and inflammatory diseases is important.

It is reported that HLA-C polymorphisms have shown independent associations with specific subsets of RA, specifically RA patients with vasculitis [33]. Among RA patients with vasculitis, the frequency of HLA-C*04 was significantly diminished. Furthermore, the incidence of granulomatosis with polyangiitis was significantly associated with HLA-DPB1*0401 allele frequency [34]. Thus, the presence of anti-HSC70 autoantibodies and their association with HLA alleles in other forms of vasculopathy may link these observations. These associations deserve further investigation. Since high throughput next generation genotyping has been implemented, HLA data at high resolution will be increasingly available and allow a more detailed analysis of potential associations with implicated HLA antigens in larger patient cohorts.

A limitation of the current study is our small patient sample size, few patients with inactive disease or remission, few patients with low disease severity and the

small number of patients with specific co-morbidities such as retinitis or gastrointestinal ulcers. Thus, it was impossible to determine whether there was a true association between anti-HSC70 autoantibodies and these rare clinical complications. Furthermore, this is a retrospective study, which may be insufficient for biomarker studies. In addition, no correction for multiple testing was performed. Further studies using larger numbers of patients and understanding of JDM-related disease phenotypes, including other vasculitic symptoms in particular, and comparative studies between races could more clearly determine the clinical significance of anti-HSC70 autoantibodies in patients with JDM.

In conclusion, we have demonstrated that anti-HSC70 autoantibodies are detected in more than a third of children with JDM. These autoantibodies correlate with multiple measures of disease severity, suggesting that they may serve as useful clinical biomarkers to detect children who will require therapies to augment or complement standard first-line treatment with glucocorticoids and methotrexate.

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References

- Shah M, Mamyrova G, Targoff IN *et al.*; Childhood Myositis Heterogeneity Collaborative Study Group. The clinical phenotypes of the juvenile idiopathic inflammatory myopathies. *Medicine* 2013;92:25–41.
- Banker BQ, Victor M. Dermatomyositis (systemic angiopathy) of childhood. *Medicine (Baltimore)* 1966;45:261–89.
- Huber AM, Mamyrova G, Lachenbruch PA *et al.*; Childhood Myositis Heterogeneity Collaborative Study Group. Early illness features associated with mortality in the juvenile idiopathic inflammatory myopathies. *Arthritis Care Res (Hoboken)* 2014;66:732–40.
- Crowe WE, Bove KE, Levinson JE, Hilton PK. Clinical and pathogenetic implications of histopathology in childhood polydermatomyositis. *Arthritis Rheum* 1982;25:126–39.
- Gitiaux C, Antonio MD, Aouizerate J *et al.* Vasculopathy-related clinical and pathological features are associated with severe juvenile dermatomyositis. *Rheumatology (Oxford)* 2016;55:470–9.
- Karasawa R, Tamaki M, Sato T *et al.* Multiple target autoantigens on endothelial cells identified in juvenile dermatomyositis using proteomics. *Rheumatology (Oxford)* 2018;57:671–6.
- Rider LG, Shah M, Mamyrova G, Huber AM *et al.*; Childhood Myositis Heterogeneity Collaborative Study Group. The myositis autoantibody phenotypes of juvenile idiopathic inflammatory myopathies. *Medicine (Baltimore)* 2013;92:223–43.
- Habers GE, Huber AM, Mamyrova G *et al.*; Childhood Myositis Heterogeneity Study Group. Association of myositis autoantibodies, clinical features, and environmental exposures at illness onset with disease course in Juvenile myositis. *Arthritis Rheumatol* 2016;68:761–8.
- Oddis CV, Rider LG, Reed AM *et al.*; International Myositis Assessment and Clinical Studies Group. International consensus guidelines for trials of therapies in the idiopathic inflammatory myopathies. *Arthritis Rheum* 2005;52:2607–15.
- Petty RE, Southwood TR, Manners P *et al.*; International League of Associations for Rheumatology. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision. *J Rheumatol* 2004;31:390–2.
- Sabbagh S, Pinal-Fernandez I, Kishi T *et al.* Anti-Ro52 autoantibodies are associated with interstitial lung disease and more severe disease in patients with juvenile myositis. *Ann Rheum Dis* 2019;78:988–95.
- O'Hanlon TP, Carrick DM, Arnett FC *et al.* Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -DRB1 and -DQA1 allelic profiles and motifs define clinicopathologic groups in Caucasians. *Medicine (Baltimore)* 2005;84:338–49.
- Yu S, Xia T, Xuehong W, Hui F. Vascular protection of salicin on IL-1 β -induced endothelial inflammatory response and damages in retinal endothelial cells. *Artif Cells Nanomed Biotechnol* 2019;47:1995–2002.
- Hoeltzel MF, Oberle EJ, Robinson AB, Agarwal A, Rider LG. The presentation, assessment, pathogenesis, and treatment of calcinosis in juvenile dermatomyositis. *Curr Rheumatol Rep* 2014;16:467.
- Munakata S, Chen M, Aosai F *et al.* The clinical significance of anti-heat shock cognate protein 71 antibody in myasthenia gravis. *J Clin Neurosci* 2008;15:158–65.
- Gupta A, Paliana RK, Prasad VD, Guleria S. Extensive skin ulcers in a child with juvenile dermatomyositis. *BMJ Case Rep* 2018;bcr-2017-222915.
- Liu T, Dean A, Ashwini S *et al.* Identification and characterization of a 66-68-kDa protein as a methotrexate-binding protein in murine leukemia L1210 cells. *Cell Stress Chaperones* 2013;18:223–34.
- Liu T, Singh R, Rios Z *et al.* Tyrosine phosphorylation of HSC70 and its interaction with RFC mediates methotrexate resistance in murine L1210 leukemia cells. *Cancer Lett* 2015;357:231–41.
- Tansley SL, Betteridge ZE, Gunawardena H *et al.*; UK Juvenile Dermatomyositis Research Group. Anti-MDA5 autoantibodies in juvenile dermatomyositis identify a distinct clinical phenotype: a prospective cohort study. *Arthritis Res Ther* 2014;16:R138.
- Yeker RM, Pinal-Fernandez I, Kishi T *et al.* Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis. *Ann Rheum Dis* 2018;77:714–9.
- Whitaker JN, Engel WK. Vascular deposits of immunoglobulin and complement in idiopathic inflammatory myopathy. *N Engl J Med* 1972;286:333–8.
- Lahoria R, Selcen D, Engel AG. Microvascular alterations and the role of complement in dermatomyositis. *Brain* 2016;139:1891–903.
- Mackay IR, Natasha VL, Noel RR. Cell damage and autoimmunity: a critical appraisal. *J Autoimmun* 2008;30:5–11.
- Okiyama N, Ichimura Y, Shobo M *et al.* Immune response to dermatomyositis-specific autoantigen, transcriptional intermediary factor 1 γ can result in experimental myositis. *Ann Rheum Dis* 2021;80:1201–8.
- Wang Z, Li Y, Yang X *et al.* Mechanism and complex roles of HSC70 in viral infections. *Front Microbiol* 2020;11:1577.

- 26 Gan L, O'Hanlon TP, Lai Z *et al.* Gene expression profiles from disease discordant twins suggest shared antiviral pathways and viral exposures among multiple systemic autoimmune diseases. *PLoS One* 2015;10:e0142486.
- 27 Manlhiot C, Liang L, Tran D *et al.* Assessment of an infectious disease history preceding juvenile dermatomyositis symptom onset. *Rheumatology (Oxford)* 2008;47:526–9.
- 28 Zheng Z, Sohn S, Ahn KJ, Bang D, Cho SB. Serum reactivity against herpes simplex virus type 1 UL48 protein in Behcet's disease patients and a Behcet's disease-like mouse model. *Acta Derm Venereol* 2015;95:952–8.
- 29 Fujimoto M, Sato S, Ihn H, Takehara K. Autoantibodies to the heat-shock protein hsp73 in localized scleroderma. *Arch Dermatol Res* 1995;287:581–5.
- 30 Zhou Y, Cui J, Hu H *et al.* Identification of a novel anti-heat shock cognate 71 kDa protein antibody in patients with Kawasaki disease. *Mol Med Rep* 2020;21:1771–8.
- 31 Jarjour WN, Jeffries BD, Davis JS IV *et al.* Autoantibodies to human stress proteins. A survey of various rheumatic and other inflammatory diseases. *Arthritis Rheum* 1991;34:1133–8.
- 32 Xiao C, Chen S, Yuan M *et al.* Expression of the 60 kDa and 71 kDa heat shock proteins and presence of antibodies against the 71 kDa heat shock protein in pediatric patients with immune thrombocytopenic purpura. *BMC Blood Disord* 2004;4:1.
- 33 A. Siegel RJ, Bridges SL Jr, Ahmed S. HLA-C: an accomplice in rheumatic diseases. *ACR Open Rheumatol* 2019;1:571–9.
- 34 Richard AW, Alex JM, Sarah LM. HLA allele variation as a potential explanation for the geographical distribution of granulomatosis with polyangiitis. *Rheumatology (Oxford)* 2015;54:359–62.