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The Vasculopathy of Juvenile Dermatomyositis: Endothelial Injury, Hypercoagulability, and Increased Arterial Stiffness

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Objective. Vasculopathy is considered central to the pathogenesis of juvenile dermatomyositis (DM) and is associated with severe extramuscular manifestations. We undertook this study to investigate the hypothesis that the vasculopathy of juvenile DM can be noninvasively tracked by examining biomarkers of endothelial injury, subclinical inflammation, hypercoagulability, and vascular arterial stiffness.

Methods. The study population was a UK cohort of children with juvenile DM. Circulating endothelial cells (CECs) and microparticles (MPs) were identified using immunomagnetic bead extraction and flow cytometry, respectively. Plasma thrombin generation was determined using a fluorogenic assay. Cytokine and chemokine levels were measured by electrochemiluminescence. Arterial stiffness was assessed using pulse wave velocity (PWV). Results were expressed as the median and interquartile range (IQR), and statistical significance was assessed using nonparametric analyses.

Results. Ninety patients with juvenile DM and 79 healthy control subjects were included. The median age of the patients was 10.21 years (IQR 6.68–13.40), and the median disease duration was 1.63 years (IQR 0.28–4.66). CEC counts were higher in all patients with juvenile DM compared to controls (median 96 cells/ml [IQR (40–192] and 12 cells/ml [IQR 8–24], respectively; $P < 0.0001$). Circulating MP numbers were also significantly higher in patients with active juvenile DM compared to controls (median 204.7×10^3/ml [IQR 87.9–412.6] and 44.3×10^3/ml [IQR 15.0–249.1], respectively; $P < 0.0001$). MPs were predominantly of platelet and endothelial origin. Enhanced plasma thrombin generation was demonstrated in patients with active juvenile DM compared to those with inactive disease ($P = 0.0003$) and controls ($P < 0.0001$). Carotid-radial PWV adjusted for age was increased in patients with juvenile DM compared to controls ($P = 0.003$).

Conclusion. We observed increased endothelial injury and increased levels of proinflammatory cytokines in patients with active juvenile DM. MP profiles reflected distinct disease activity status in juvenile DM and are markers of vascular pathology, platelet activation, and thrombotic propensity. Ongoing long-term vascular injury may result in increased arterial stiffness in patients with juvenile DM.

The views expressed herein are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

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INTRODUCTION

Vasculopathy is considered central to the pathogenesis of juvenile dermatomyositis (DM) and is associated with severe extramuscular manifestations (1–5). The nature of this vasculopathy is complex, with evidence of both a true inflammatory small vessel vasculitis during active phases of the disease (2,6) and a noninflammatory occlusive vasculopathy with capillary dropout later in the disease process (4,7). Notably, previous studies have indicated that the presence of severe vascular changes on muscle biopsy was predictive of a chronic disease course (8,9), suggesting that persistent vasculopathy is a poor prognostic factor and determinant of adverse outcome in juvenile DM (5). Moreover, in the longer term there may also be a systemic vasculopathy affecting larger arteries, potentially leading to accelerated atherosclerosis and premature cardiovascular morbidity later in adulthood (10,11).

A major hurdle to the study of the vasculopathy of juvenile DM has been a lack of noninvasively measurable biomarkers that reliably capture the full spectrum of the proposed pathogenesis (12,13). Therefore, defining disease activity trajectories related to persistent endothelial injury in juvenile DM historically has been challenging. We and others have previously described 2 methods for detecting endothelial cell components in blood that allow noninvasive assessment of vascular injury in systemic vasculitides: circulating endothelial cells (CECs) and endothelial-derived microparticles (EMPs) (14–22). We hypothesized that these noninvasively measured biomarkers of endothelial injury could be used to detect chronic vasculopathic injury and a putative prothrombotic state in juvenile DM. The present study was undertaken to examine biomarkers of endothelial injury, subclinical inflammation, hypercoagulability, and arterial stiffness in a UK cohort of patients with juvenile DM compared to age-similar healthy controls.

PATIENTS AND METHODS

Study design, subjects, and data collection. This was an observational comparative study, with ethical approval (MREC 1/3/022). The legal guardians of all subjects (or the subjects themselves if of legal age) provided written informed consent.

Patients with juvenile DM. For study inclusion, patients had to be age 2–19 years and have a diagnosis of juvenile DM (23). Patients were excluded from enrollment if they had any significant acute or chronic comorbidity that could cause acute endothelial injury, including intercurrent infection. Patients with juvenile DM were recruited from Great Ormond Street Hospital NHS Foundation Trust through the Juvenile Dermatomyositis Cohort and Biomarker Study (3,24) between September 2015 and January 2018 and were studied cross-sectionally. A subgroup of the patients studied cross-sectionally were also evaluated prospectively.

Definition of inactive juvenile DM. Patients were classified as having clinically inactive juvenile DM based on a modification of the Paediatric Rheumatology International Trials Organisation (PRINTO) criteria (25), as follows: absence of skin disease at the time of assessment, and at least 3 of the following 4 criteria: 1) creatine kinase (CK) ≤150 units/liter, 2) Childhood Myositis Assessment Scale (CMAS) score (26,27) ≥48/52, 3) Manual Muscle Testing 8 (MMT-8) score (28) ≥78/80, and 4) physician global assessment ≤0.2 (of a possible 10). Juvenile DM disease activity was ascertained by independent scrutiny of patients' medical records by 2 senior clinicians (MAO and DE); any discrepant cases were discussed to achieve consensus. All clinical and laboratory assessments were performed by one of the authors (CP), with blinding with regard to study group (healthy control or juvenile DM case), and juvenile DM disease status (active or inactive).

Healthy controls. Age-similar and sex-matched children who had no acute or chronic illnesses at the time of recruitment and were not regularly taking any medication at time of sampling were recruited as controls, with ethical approval (REC 11/LO/0330). These children were either healthy unaffected siblings of patients with Kawasaki disease recruited for another major study our group has undertaken in the past (29) or were recruited through the Versus Arthritis Centre for adolescent rheumatology young scientist days, where healthy adolescents were invited to spend a day in the laboratory and donate blood with written informed consent.

Demographic, clinical, and laboratory data. Data collected included: age, sex, age at disease onset, disease duration, clinical features at initial presentation, routine echocardiography results, histopathologic severity scores on muscle biopsy, presence and typing of myositis-specific antibodies (MSAs) (30), and treatments (past and current). Validated clinical tools and indices were used to capture the full extent of disease activity in a systematic manner, i.e., the CMAS, MMT-8, physician global assessment of disease activity using a 10-cm visual analog scale (VAS) (31), functional ability according to the Childhood Health Assessment Questionnaire (32), parent global assessment of the patient's overall well-being on a 10-cm VAS, and parent global assessment of the patient's pain on a 10-cm VAS. The following laboratory test results were also collected: complete blood cell count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), CK, alanine aminotransferase, and lactate dehydrogenase (LDH) levels, and antinuclear antibody status (33). Nailfold capillaroscopy was performed at the bedside with the use of a light, a 10× magnifying glass (otoscope), and a water-soluble gel (34) placed on the nailfold bed (of each of 8 fingers, excluding thumbs) to increase resolution; the result was considered abnormal in the presence of capillary loss with irregular capillary distribution, enlargement of capillary loops, changes in the capillary shape, or areas of hemorrhage (35).

Conventional cardiovascular risk factors. Age, height, weight, body mass index, and smoking status were recorded before vascular studies were performed. Echocardiography was performed in patients with juvenile DM as part of routine clinical practice at the time of recruitment. Resting (minimum 15 minutes) blood pressure and heart rate were measured at the brachial artery using an oscillometric manual sphygmomanometer (Greenlight 300; Accoson). Nonfasting total cholesterol and triglycerides were also measured.

Assessment of inflammation indices. High-sensitivity CRP (hsCRP), serum amyloid A (SAA), angiopoietin 1 and 2, soluble E-selectin, soluble intercellular adhesion molecules 1 and 3, soluble vascular cell adhesion molecule 1, soluble P-selectin, thrombomodulin, tumor necrosis factor (TNF), interleukin-1β (IL-1β), IL-6, IL-8, IL-10, monocyte chemotactic protein 1 (MCP-1), interferon-α (IFNα), IFNβ, IFNγ, IFN1, IFNγ-inducible 10-kd protein (IP-10), and TNF receptor II were assessed using a multiarray detection system based on electrochemiluminescence technology (Sector Imager 2400; Meso Scale Discovery) (29). Galectin-9 was assessed with a solid-phase enzyme-linked immunosorbent assay (R&D Systems) (36).

Assessment of endothelial injury. *Circulating endothelial cells and microparticles.* CECs were identified using CD146-immunomagnetic bead extraction as previously described (37). Circulating MPs were identified by flow cytometry (BD LSRII). The MP population was defined as particles that were <1.1 μm in size and bound to annexin V (AnxV). Platelet-derived MPs (PMPs) were defined as AnxV+CD42a+ particles. The AnxV+CD42a– MP population was then used to further characterize EMPs (AnxV+CD62E+CD42a–), B cell–derived MPs (CD19+AnxV+CD42a–), T cell–derived MPs (CD3+AnxV+CD42a–), and tissue factor (TF)–positive monocyte-derived MPs (TF+CD14+AnxV+CD42a–). MPs were stained with BV421 (BioLegend)–conjugated AnxV for binding with phosphatidylserine that is present in all MPs, phycoerythrin-conjugated mouse anti-human CD62E (clone 68-5H11; BioLegend) for defining endothelial-derived MPs, BV711-conjugated mouse anti-human CD19 (clone HIB19; BD OptiBuild) for identifying B cell–derived MPs, BV605-conjugated mouse anti-human CD14 (clone M5E2; BioLegend) for identifying monocyte-derived MPs, and allophycocyanin-conjugated mouse anti-human CD3 (clone UCHT1; BioLegend) for identifying T cell–derived MPs. Additional labeling with PerCP-conjugated mouse anti-human CD42a (BD PharMingen) was done to exclude MPs of platelet origin. To assess TF expression on monocyte-derived MPs, samples were stained with fluorescein isothiocyanate–conjugated mouse anti-human TF (clone VD8; American Diagnostica). All samples were analyzed on an LSR II flow cytometer with FACSDiva software (BD Biosciences).

Plasma thrombin generation assay. To assess the prothrombotic tendency of plasma, a thrombin generation assay was performed in recalcified citrated platelet-poor plasma (PPP), as previously described (16,38). PPP (40 μl) was incubated with 50 μl fluorogenic substrate (0.5 m*M* Z-G-G-R-AMC/7.5 m*M* Ca^{2+}) and the reaction monitored by excitation/emission (360/460 nm) at 1-minute intervals for 90 minutes with an Optima Fluorescence plate reader (BMG Labtech). Lag time, peak thrombin (n*M*), peak time, velocity index, and endogenous thrombin potential (ETP) were quantified using a Technothrombin kit according to the protocol recommended by the manufacturer (DiaPharma).

Assessment of arterial stiffness. Carotid-femoral pulse wave velocity (PWV) and carotid-radial PWV were used as markers of arterial stiffness, measured by oscillometry using a Vicorder device (Skidmore Medical) in accordance with American Heart Association recommendations (39).

Statistical analysis. Descriptive statistics were reported as the median and interquartile range (IQR) for continuous variables and as the absolute frequency and percentage for categorical variables. The significance of the differences between groups was assessed by Mann-Whitney U test (for 2 groups) or Kruskal-Wallis test (for multiple groups), and correlations between variables were assessed using Spearman's rank correlation coefficient. Categorical data were compared by chi-square test, or by Fisher's exact test in

Table 1. Characteristics of the patients with juvenile dermatomyositis (DM) and the healthy controls*

	Juvenile DM patients	Healthy controls
Female, no. (%)	57 (63.3)	48 (58.5)
Smoking, no. (%)	0 (0.0)	0 (0.0)
Body mass index, kg/m^2	19.5 (15.7–22.7)	20.5 (17.0–23.2)
Systolic blood pressure in relation to age slope, mm Hg/year, y-intercept when x = 0.0	90.80–104.5	88.88–110.9
r^2	0.15	0.10
Diastolic blood pressure in relation to age slope, mm Hg/year y-intercept when x = 0.0	49.93–58.79	49.19–64.16
r^2	0.11	0.10
Triglycerides, mmoles/liter	0.96 (0.67–1.20)	0.77 (0.58–1.06)
Cholesterol, mmoles/liter	3.7 (3.4–4.2)†	4.4 (3.7–4.8)

* Except where indicated otherwise, values are the median (interquartile range).
† $P = 0.003$ versus healthy controls, by Mann-Whitney U test.

Table 2. Demographic characteristics, laboratory parameters, and juvenile DM disease activity measures in the 90 patients*

	All patients (n = 90)	Patients with active juvenile DM (n = 64)	Patients with inactive juvenile DM (n = 26)	Difference or OR (95% CI) [*P*]†
Female, no. (%)	57 (63.3)	48 (75.0)	9 (34.6)	5.667 (2.182, 15.00) [0.0003]
Disease duration, years	1.63 (0.28–4.66)	0.87 (0.02–3.99)	4.14 (1.80–7.03)	3.265 (1.080, 3.400) [0.0005]
Age at study recruitment, years	10.21 (6.68–13.40)	10.21 (6.00–14.04)	10.56 (6.94–12.14)	0.3530 (−2.230, 2.070) [0.9894]
Age at disease onset, years	5.48 (3.4–9.25)	5.72 (3.80–9.99)	4.44 (2.72–6.76)	−1.28 (−3.34, −0.08) [0.0339]
Vascular domain score on initial diagnostic muscle biopsy	1.0 (0.0–2.0)	1.0 (0.0–2.0)	0.00 (0.0–0.5)	1.0 (0.0, 1.0) [0.0491]
Hemoglobin, gm/liter	124 (116–131)	121 (113–127)	127 (120–137)	5.0 (3.0, 13.0) [0.0025]
Leukocytes, $\times 10^9$/liter	6.47 (5.30–7.90)	6.56 (5.21–8.43)	6.40 (5.51–7.21)	−0.165 (−1.02, 0.83) [0.8353]
Neutrophils, $\times 10^9$/liter	3.51 (2.58–4.46)	3.61 (2.59–4.54)	3.47 (2.52–4.16)	−0.14 (−0.82, 0.52) [0.6743]
Lymphocytes, $\times 10^9$/liter	1.94 (1.32–2.55)	1.85 (1.30–2.59)	2.31 (1.61–2.55)	0.46 (−0.16, 0.69) [0.1729]
Platelets, $\times 10^9$/liter	310 (247–356)	307 (245–355)	321 (255–373)	14.0 (−28.0, 44.0) [0.7535]
ESR, mm/hour (normal <10)	9 (418)	10 (5–20)	4 (3–14)	−6.0 (8.0, 0.0) [0.0330]
CRP, mg/liter (normal <20)	5 (5–5)	5 (5–5)	5 (5–6)	0.0 (0.0, 0.0) [0.6314]
CK, units/liter (normal 6–330)	89 (69–138)	84 (66–220)	93 (78–122)	9.0 (−26.0, 22.0) [0.7821]
LDH, units/liter (normal 450–770)	651 (560–809)	694 (583–829)	581 (540–653)	−113.5 (−214.0, −52.0) [0.0008]
ALT, units/liter (normal 10–35)	26 (15–42)	29 (17–54)	23 (11–30)	−6.0 (−18.0, 0.0) [0.0550]
Cholesterol, mmoles/liter	4.0 (3.4–4.3)	3.8 (3.4–4.1)	4.2 (3.55–4.45)	0.4 (−0.2, 0.7) [0.204]
Triglycerides, mmoles/liter	1.0 (0.6–1.5)	0.97 (0.60–1.47)	1.14 (0.60–1.71)	0.17 (−0.32, 0.50) [0.698]
Systolic blood pressure in relation to age slope, mm Hg/year, y-intercept	97.13	96.84	97.28	– [0.8321]
r^2	1.25	0.29	0.61	– [–]
BMI, kg/m^2	17.4 (15.5–21.4)	17.4 (15.4–21.7)	17.4 (16.2–20.7)	−0.014 (−1.958, 1.568) [>0.99]
MMT-8	78 (67–80)	74 (59–80)	80 (78–80)	6.0 (1.0, 12.0) [0.0001]
CMAS	50 (44–52)	48 (37–52)	52 (50–52)	4.0 (1.0, 7.0) [0.0005]
C-HAQ	0.125 (0.000–0.625)	0.25 (0.000–1.000)	0.000 (0.000–0.125)	−0.25 (−0.375, 0.000) [0.0027]
Physician global assessment	1.1 (0.2–2.7)	2.0 (0.70–3.10)	0.2 (0.0–0.2)	−1.8 (−2.5, −1.1) [<0.0001]
Parent/patient global assessment	0.7 (0.0–4.0)	1.9 (0.0–5.0)	0.0 (0.0–0.60)	−1.9 (−2.0, 0.0) [0.0009]
Pain global assessment	0.2 (0.0–1.8)	0.9 (0.0–3.0)	0.0 (0.0–0.2)	−0.95 (−1.00, 0.00) [0.006]
ANA positive, no. (%)	57 (63.3)	43 (67.2)	14 (53.8)	1.755 (0.6915, 4.363) [0.2339]
MSA positive, no. (%)‡	33 (67.3)	27 (64.3)	6 (85.7)	0.300 (0.025, 2.319) [0.2630]
Anti-SRP	4 (12.1)	4 (14.8)	0 (0.0)	– [–]
Anti–NXP-2	10 (30.3)	7 (25.9)	3 (50.0)	– [–]
Anti-TIF1γ	8 (24.2)	8 (29.6)	0 (0.0)	– [–]
Anti–MDA-5	4 (12.1)	3 (11.1)	1 (16.7)	– [–]
Treatment at time of recruitment, no. (%)§	56 (62.2)	40 (62.5)	16 (61.5)	1.042 (0.4116, 2.542) [0.93]
Prednisolone	28 (31.1)	24 (37.5)	4 (15.4)	1.042 (1.029, 9.616) [0.04]

(Continued)

Table 2. (*Cont'd*)

	All patients (n = 90)	Patients with active juvenile DM (n = 64)	Patients with inactive juvenile DM (n = 26)	Difference or OR (95% CI) [*P*]†
Methotrexate	41 (45.6)	28 (43.7)	13 (50.0)	0.7778 (0.3203, 1.883) [0.59]
IV immunoglobulin	4 (4.4)	3 (4.7)	1 (3.8)	– [1.00]
Cyclophosphamide	1 (1.1)	1 (1.6)	0 (0.0)	– [–]
Rituximab	1 (1.1)	1 (1.6)	0 (0.0)	– [–]
TNF inhibitor	10 (11.1)	6 (9.4)	4 (15.4)	0.5690 (0.1623, 1.935) [0.47]
Other	5 (5.6)	4 (6.2)	1 (3.8)	– [–]

* For some parameters, data were not available for all 90 patients, as follows: for vascular domain score on initial diagnostic muscle biopsy, n = 46 (37 and 9, patients with active juvenile dermatomyositis [DM] and patients with inactive juvenile DM, respectively); for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and creatine kinase (CK) levels, Childhood Myositis Assessment Score (CMAS), and physician global assessment, n = 89 (63 and 26, respectively); for lactate dehydrogenase (LDH) levels, n = 87 (63 and 24, respectively); for alanine aminotransferase (ALT) levels, n = 88 (64 and 24, respectively); for cholesterol and triglyceride levels, n = 44 (27 and 17, respectively); for Manual Muscle Testing 8 (MMT-8) score, n = 88 (62 and 26, respectively); for Childhood Health Assessment Questionnaire (C-HAQ), n = 79 (53 and 26, respectively); for parent/patient global assessment, n = 81 (56 and 25, respectively); for pain global assessment, n = 79 (54 and 25, respectively); for myositis-specific antibodies (MSAs), n = 49 (42 and 7, respectively). Except where indicated otherwise, values are the median (interquartile range). 95% CI = 95% confidence interval; BMI = body mass index; anti-SRP = anti–signal recognition particle; anti–NXP-2 = anti–nuclear matrix protein 2; anti-TIF1γ= anti–transcription intermediary factor 1γ; anti–MDA-5 = anti–melanoma differentiation–associated gene 5; TNF = tumor necrosis factor.
† Odds ratios (ORs) are shown for categorical values, i.e., number (%) female, antinuclear antibody (ANA) positive, MSA positive, and treatment at the time of recruitment. *P* values were determined by chi-square test, Fisher's exact text, or Mann-Whitney U test.
‡ Other MSAs, found in smaller numbers of patients, were as follows: anti–PL-7 (2 patients), anti–PL-12 (2 patients), anti–small ubiquitin-like modifier activating enzyme (1 patient), and anti–Mi-2 (1 patient).
§ Doses were as follows: prednisolone 1–2 mg/kg/day tapered over 6–9 months, subcutaneous (SC) methotrexate 15 mg/m^2/week, intravenous (IV) immunoglobulin 2 gm/kg over 48 hours every 4 weeks, IV cyclophosphamide 350–500 mg/m^2 for 5–6 monthly doses, IV rituximab 750 mg/m^2 for 2 doses 14 days apart, IV infliximab 6 mg/kg every 4–8 weeks, and SC adalimumab 20 mg every 2 weeks if body weight <30 kg and 40 mg every 2 weeks if body weight ≥30 kg. Other treatments were azathioprine 1–2 mg/kg/day and mycophenolate mofetil 600 mg/m^2 twice daily.

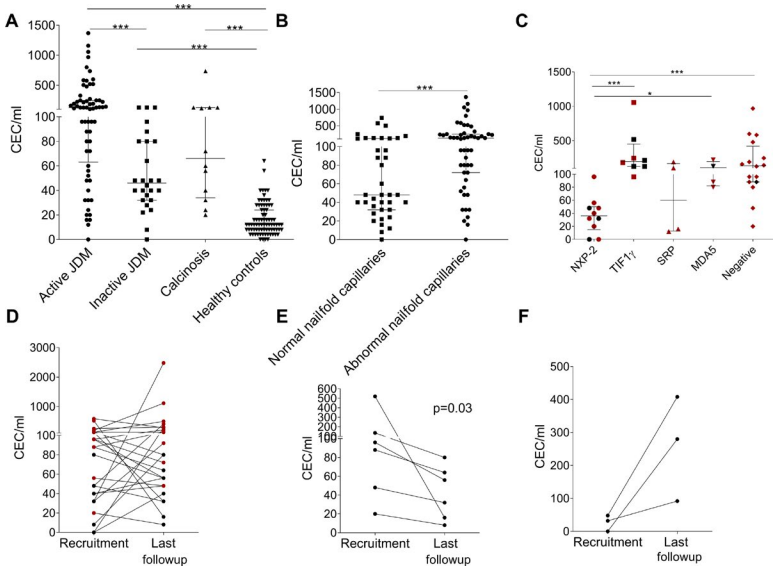

Figure 1. Circulating endothelial cells (CECs) in patients with juvenile dermatomyositis (JDM). **A**, CEC numbers were higher among the 90 patients with juvenile DM compared to 79 healthy controls. CEC numbers also differed significantly between patients with active juvenile DM (n = 64) and those with inactive juvenile DM (n = 26). **B**, Patients with juvenile DM with abnormal nailfold capillaries (n = 52) had higher CEC numbers compared to patients with normal nailfold capillaries (n = 38). **C**, Patients with juvenile DM with transcription intermediary factor 1γ (TIF1γ) antibodies (n = 8) had higher CEC numbers compared to patients with nuclear matrix protein 2 (NXP-2) antibodies (n = 10). Data are not shown for 1 patient with small ubiquitin-like modifier activating enzyme, 2 patients with PL-7, 2 patients with PL-12, and 1 patient with Mi-2 antibodies, due to low numbers. Red symbols represent juvenile DM patients with active disease. Horizontal and vertical bars in **A–C** show the median and interquartile range. **D**, CEC levels were assessed prospectively in 25 patients with juvenile DM. Red symbols represent active disease at the time of the assessment. **E** and **F**, There was a significant decrease in CEC levels in the 6 patients who had active juvenile DM at the time of recruitment and inactive disease at the last follow-up (**E**), while CEC levels increased in the 3 patients who had inactive juvenile DM at the time of recruitment and active disease at the last follow-up (**F**). * = $P < 0.05$; *** = $P < 0.001$. SRP = signal recognition particle; MDA-5 = melanoma differentiation–associated gene 5.

Table 3. Cytokines/chemokines and other inflammatory molecules in the patients with juvenile DM and the healthy controls*

	All patients with juvenile DM	Healthy controls	Difference (95% CI) [*P*]†	Patients with active juvenile DM	Patients with inactive juvenile DM	Difference (95% CI) [*P*]†
hsCRP, mg/liter	0.6 (0.2–2.8) [82]	0.5 (0.2–1.3) [38]	−0.1 (−0.4, 0.1) [0.4598]	0.8 (0.3–3.5) [56]	0.3 (0.1–1.3) [26]	−0.6 (−0.8, 0.03) [0.0803]
SAA, pg/ml	981,099 (513,087–3,754,459) [82]	1,185,209 (567,991–2,557,865) [38]	204,110 (−395,979, 555,845) [0.8474]	1,693,738 (473,854–6,415,952) [56]	707,571 (510,506–1,649,307) [26]	−986,167 (−1,400,334, 99,169) [0.1559]
Ang-1, pg/ml	56,119 (40,956–68,409) [82]	43,856 (34,874–51,798) [79]	−12,262 (−17,605, −7,625) [<0.0001]	50,676 (38,522–67,004) [56]	62,335 (52,997–69,594) [26]	11,659 (−663, 18,152) [0.0719]
Ang-2, pg/ml	5,332 (3,228–7,658) [82]	2,880 (2,128–4,063) [79]	−2,343 (−2,812, −1,330) [<0.0001]	5,525 (3,187–8,071) [56]	4,777 (3,316–6,101) [26]	−748.2 (−2,264, 509.9) [0.2952]
E-selectin, pg/ml	15.53 (11.15–19.38) [40]	13.00 (10.43–18.95) [68]	−2.532 (−3.826, 1.288) [0.3034]	16.94 (12.15–20.37) [29]	13.39 (8.037–16.57) [11]	−3.555 (−7.659, 1.149) [0.1180]
P-selectin, pg/ml	132.6 (111.3–169.8) [40]	75.86 (59.41–109.7) [68]	−56.78 (−71.44, −39.63) [<0.0001]	130.6 (111.2–167.6) [29]	139.5 (110.7–183.8) [11]	8.84 (−28.00, 34.20) [0.7427]
sICAM-1, pg/ml	466,272 (300,922–850,713) [82]	258,000 (223,015–327,902) [79]	−208,272 (−278,348, −124,988) [<0.0001]	460,709 (324,064–802,161) [56]	493,339 (237,039–982,812) [26]	32,630 (−151,430, 140,617) [0.7476]
sICAM-3, pg/ml	0.4868 (0.3347–0.7612) [40]	0.4100 (0.3610–0.54958) [37]	−0.07685 (−0.1852, 0.01246) [0.1025]	0.5495 (0.3143–0.7812) [29]	0.4570 (0.3249–0.6269) [11]	−0.09256 (−0.2603, 0.1145) [0.6814]
sVCAM-1, pg/ml	700,174 (434,162–1,114,190) [82]	409,113 (358,698–532,000) [79]	−29,1061 (−398,169, −177,236) [<0.0001]	729,902 (458,852–1,122,167) [56]	694,383 (402,496–1,053,256) [26]	−35,519 (−218,017, 156,561) [0.7178]
TM, pg/ml	4.978 (3.985–6.065) [40]	3,800 (3,261–5,084) [68]	−1.178 (−1.434, −0.3485) [0.0012]	4.998 (3.805–6.233) [29]	4.798 (4.032–5.867) [11]	−0.1995 (−0.8694, 0.9446) [0.9762]
TNFRII, pg/ml	6,258 (4,055–9,433) [84]	5,048 (3,748–6,354) [73]	−1,210 (−2,228, −360.4) [0.004]	6,272 (4,246–11,283) [58]	4,803 (3,626–6,840) [26]	−1,469 (−2,833, 138.5) [0.0760]
Galectin-9, ng/ml	76.3 (52.4–134.0) [86]	38.4 (27.1–50.8) [72]	−37.93 (−53.56, −29.49) [<0.0001]	81.53 (59.24–166.2) [60]	51.09 (31.55–69.66) [26]	−30.44 (−73.45, −13.67) [0.0019]
IL-10, pg/ml	0.5 (0.3–0.9) [90]	0.3 (0.2–0.5) [54]	−0.2395 (−0.345, −0.117) [<0.0001]	0.5957 (0.3662-0.9598) [64]	0.2980 (0.2111–0.6643) [26]	−0.2977 (−0.3918, −0.06151) [0.0057]
IL-1β, pg/ml	0.09 (0.05–0.13) [90]	0.06 (0.02–0.08) [54]	−0.03214 (−0.048, −0.009) [0.003]	0.09074 (0.05444–0.1427) [64]	0.9462 (0.04930–0.1263) [26]	0.003883 (−0.02646, 0.02643) [0.9947]
IL-6, pg/ml	0.8 (0.3–1.6) [90]	0.3 (0.2–0.6) [54]	−0.4763 (−0.636, −0.183) [<0.0001]	0.8723 (0.3760–1.644) [60]	0.2919 (0.1903–0.7435) [25]	−0.5804 (−0.7128, −0.1282) [0.0016]
IL-8, pg/ml	12.0 (7.1–21.6) [90]	5.7 (4.3–11.0) [54]	−6.366 (−7.840, −2.631) [<0.0001]	11.88 (7.219–22.75) [64]	11.17 (6.968–16.37) [26]	−0.7047 (−5.191, 2.535) [0.6109]

(Continued)

Table 3. (*Cont'd*)

	All patients with juvenile DM	Healthy controls	Difference (95% CI) [*P*]†	Patients with active juvenile DM	Patients with inactive juvenile DM	Difference (95% CI) [*P*]†
TNF, pg/ml	2.4 (1.8–4.3) [90]	1.7 (1.3–2.1) [54]	−0.7383 (−1.36, −0.500) [<0.0001]	2.550 (1.719–4.873) [64]	2.115 (1.549–3.171) [26]	−0.4355 (−1.326, 0.1656) [0.1778]
IP-10, pg/ml	152.6 (66.8–449.7) [90]	100 (62.12–169.6) [54]	−52.58 (−116.4, −12.79) [0.007]	278.8 (140.2–1,223) [64]	63.3 (42.7–129.1) [26]	−215.5 (−345.5, −108.2) [<0.0001]
MCP-1, pg/ml	365.7 (226.3–571.5) [90]	210.1 (163.2–306.9) [54]	−155.7 (−204.8, −73.36) [<0.0001]	422.3 (236.4–763.9) [64]	297.7 (216.9–432.8) [26]	−124.6 (−233.1, −0.6691) [0.0442]
IFNγ, pg/ml	4.6 (2.4–8.8) [88]	2.3 (1.1–4.9) [54]	−2.310 (−3.100, −0.7846) [0.001]	4.857 (2.062–9.686) [62]	3.810 (2.415–7.055) [26]	−1.047 (−2.452, 1.118) [0.5529]
IFNα, pg/ml	0.82 (0.73–0.99) [90]	0.40 (0.00–0.74) [40]	−0.4260 (−0.6449, −0.3047) [<0.0001]	0.82 (0.73–1.03) [64]	0.84 (0.72–0.98) [26]	0.01648 (−0.1205, 0.07203) [0.5576]
IFNβ, pg/ml	5.2 (4.2–6.7) [88]	5.3 (3.8–6.7) [37]	0.1321 (−0.8971, 1.113) [0.8685]	5.3 (4.4–7.7) [65]	4.8 (2.7–6.5) [23]	−0.4731 (−2.243, 0.3097) [0.1764]
IFNλ1, pg/mln	3.8 (2.9–5.0) [90]	2.5 (0.7–3.8) [34]	−1.346 (−2.416, −0.5438) [0.0004]	4.0 (3.5–5.0) [64]	2.3 (1.3–5.2) [26]	−1.686 (−2.499, −0.7997) [0.0033]

* Values are the median (interquartile range) [number tested]. DM = dermatomyositis; 95% CI = 95% confidence interval; hsCRP = high-sensitivity C-reactive protein; SAA = serum amyloid A; Ang-1 = angiopoietin 1; sICAM-1 = soluble intercellular adhesion molecule 1; sVCAM-1 = soluble vascular cell adhesion molecule 1; TM = thrombomodulin; TNFRII = tumor necrosis factor receptor II; IL-10 = interleukin-10; IP-10 = interferon-γ (IFNγ)–inducible 10-kd protein; MCP-1= monocyte chemotactic protein 1.
† *P* values were determined by Kruskal-Wallis test followed by Mann-Whitney U test.

the case of expected frequencies of <5. Differences between medians with 95% confidence intervals (95% CIs) of the differences were calculated. The Wilcoxon matched pairs signed rank test was used to compare variables at initial presentation and at latest follow-up for patients who were studied prospectively. Analysis of covariance was used to compare the slope of blood pressure versus age and PWV versus age between groups, using linear regression. *P* values less than 0.05 were considered significant (2-sided for CEC analyses; analysis of all the other indices was considered exploratory, and therefore no adjustments were made for multiple comparisons). Tibco Statistica, release 13.3 (StatSoft) and GraphPad Prism version 4.0 were used for data analyses.

RESULTS

Demographic characteristics of the study subjects. Ninety-patients with juvenile DM (median age 10.21 years [IQR 6.68–13.40]) were studied cross-sectionally. Fifty-seven (63.3%) were female. Seventy-nine healthy control children and adolescents were included in the final analysis; 3 additional control subjects had been enrolled but were subsequently excluded (due to severe eczema, upper respiratory tract infection, and ongoing medication treatment, respectively). The median age of the healthy controls was 16.7 years (IQR 10.7–17.4). There was no significant difference between the juvenile DM and healthy control groups in demographic characteristics, body mass index, or blood pressure (Table 1). In addition to the cross-sectional study, 25 children with juvenile DM (median age 11.22 years [IQR 8.16–14.05]) were studied prospectively, with data collected at baseline and during at least 1 follow-up visit (median follow-up time 0.86 years [IQR 0.42–1.53]).

Clinical features, juvenile DM disease activity measures, and routine laboratory parameters. Presenting clinical features, laboratory results, and disease activity according to various juvenile DM scoring tools are summarized in Table 2. The median age at disease onset in the 90 patients was 5.48 years (IQR 3.40–9.25), with a median time from disease onset to diagnosis of 0.34 years (IQR 0.17–0.69). At the time of recruitment, the median duration of disease was 1.63 years (IQR 0.28–4.66). Sixty-four of the 90 patients had clinically active juvenile DM according to the modified PRINTO criteria at the time of recruitment, and 12 had calcinosis.

Of the 49 patients tested, 33 (67.3%) were positive for MSAs, with nuclear matrix protein 2 (NXP-2) being the predominant type (n = 10). Most of the children with active disease were female (*P* = 0.0003 versus those with inactive disease), and the group with active disease was older at disease onset (*P* = 0.0339) and had a shorter disease duration (*P* = 0.0005) compared to children with inactive disease. They also had higher ESR (*P* = 0.0330) and LDH levels (*P* = 0.0008) compared to the group with inactive disease, whereas CK and CRP levels did not differ. Echocardiography was performed in 66 of the patients with juvenile DM. Results were normal in 58 patients, and a small pericardial effusion was detected in 3. The remaining 5 patients had tricuspid regurgitation, aortic regurgitation, mild concentric left ventricular hypertrophy, mildly reduced right ventricular systolic function, and patent foramen ovale (1 patient each).

Of the 25 patients with juvenile DM studied prospectively, 17 had active disease at the time of recruitment, and 8 had inactive disease. Three of the patients initially classified as having inactive disease had a disease flare (mainly affecting the skin) at the last follow-up visit.

Endothelial injury. *CECs.* CEC numbers were higher in patients with juvenile DM (median 96 cells/ml [IQR 40–192]) compared to healthy controls (median 12 [IQR 8–24]) (difference –84 [95% CI –100.0, –56.00]; *P* < 0.0001) (Figure 1A). Patients with active juvenile DM had higher numbers of CECs than those with inactive juvenile DM (difference –82 [95% CI –40.00, –128.00]; *P* < 0.0001). Previous studies have suggested that vasculopathy may play a role in the pathogenesis of calcinosis in juvenile DM (1,5), and we noted higher numbers of CECs in juvenile DM patients with calcinosis compared to healthy controls (difference –54 [95% CI –100.00, –28.00]; *P* < 0.0001) (Figure 1A), but no significant difference between the patients with and those without calcinosis (*P* = 0.5). We also observed that 10 of the 12 patients with calcinosis had active juvenile DM. Further analysis of specific disease features pertinent to the vasculopathy of juvenile DM showed that CEC numbers were higher among patients who had nailfold capillary changes (median 128 cells/ml [IQR 72–248]) compared to patients with normal nailfold capillaries (median 48 cells/ml [IQR 32–119]) (difference –80 [95% CI –104.0, –24.00]; *P* = 0.0006) (Figure 1B). As noted above, 49 patients had been tested for MSAs. Among the 8 patients who were positive for transcription intermediary factor 1γ antibodies, CEC numbers were higher compared to the 10 patients with NXP-2 antibodies (median 200 cells/ml [IQR 128–452] versus 36 cells/ml [IQR 15–56]) (difference 164 [95% CI 88.00, 472.0]; *P* < 0.0001) (Figure 1C).

Among the 25 patients studied prospectively, there was no significant difference between the number of CECs at the time of recruitment (median 88 cells/ml [IQR 36–128]) and at the time of the last follow-up (median 80 cells/ml [48-280]) (*P* = 0.25) (Figure 1D). Seventeen of these patients (68%) had active juvenile DM at the time of recruitment and 14 (56%) had active juvenile DM at the last follow-up. There was a decrease in CEC numbers among patients whose disease status changed from active at baseline to inactive at the last follow-up (n = 6) (median difference –32 [95% CI –504, –12]; *P* = 0.03) and an increase among patients who had inactive disease at baseline and active disease at the last follow-up (n = 3) (median difference 280 [95% CI 60, 360]; *P* = 0.25) (Figures 1E and F).

Circulating levels of inflammation markers. Overall, there was a significant difference in circulating levels of inflammation markers between patients with juvenile DM and controls, and between patients with active juvenile DM and those with inactive juvenile DM

Table 4. Circulating microparticle profile and thrombin generation parameters in the patients with juvenile DM and the healthy controls*

	Juvenile DM patients (n = 90)	Healthy controls (n = 56)	Difference (95% CI) [*P*]†	Juvenile DM patients with active disease (n = 64)	Difference (95% CI) [*P*], patients with active juvenile DM vs. controls†	Juvenile DM patients with inactive disease (n = 26)	Difference (95% CI) [*P*], patients with inactive juvenile DM vs. controls†	Difference (95% CI) [*P*], patients with active juvenile DM vs. patients with inactive juvenile DM†
Total AnxV+ MPs	204.7 (87.9–412.6)	44.3 (15.0–249.1)	179.6 (71.2, 191.9) [<0.0001]	300.1 (186.3–584.6)	−256.7 (−281.5, −153.8) [<0.0001]	81.3 (33.8–190.5)	−36.9 (−55.1, 14.1) [0.24]	219.7 (−276.5, −130.9) [<0.0001]
EMPs (AnxV+CD62E+CD42a−), $\times 10^3$/ml	4.7 (1.3–13.8)	2.3 (0.5–5.1)	2.3 (0.3, 3.8) [0.0075]	6.1 (20.3–2.5)	−3.8 (−6.2, −1.4) [0.0001]	1.8 (0.3–6.8)	0.6 (−0.9, 1.3) [0.79]	−4.4 (−6.8, −1.2) [0.0004]
PMPs (AnxV+CD42a+), $\times 10^3$/ml	82.4 (26.5–173.6)	18.1 (6.3–98.3)	64.3 (13.1, 67.0) [0.0006]	123.1 (50.2–198.3)	−104.9 (−102.9, −38.7) [0.0001]	28.3 (8.0–47.7)	−10.2 (−15.9, −10.1) [0.88]	−94.7 (−107.9, −43.7) [<0.0001]
TF+CD14+AnxV+CD42a−, $\times 10^3$/ml	0.4 (0.0–2.9)	0.08 (0.0–0.6)	0.3 (0.0, 0.5) [0.001]	0.6 (0.1–4.3)	−0.5 (−0.8, −0.1) [0.0002]	0.2 (0.0–1.5)	−0.09 (−0.2, 0.0) [0.16]	−0.4 (−0.7, 0.0) [0.06]
CD19+AnxV+CD42a−, $\times 10^3$/ml	3.6 (1.2–15.4)	1.8 (0.5–6.3)	1.7 (0.3, 2.9) [0.008]	5.2 (1.7–24.6)	3.4 (−5.0, −1.0) [0.0002]	1.3 (0.6–5.4)	0.5 (−0.9, 1.0) [0.99]	−3.9 (−5.3, −0.9) [0.0014]
CD3+AnxV+Cd42a−, $\times 10^3$/ml	1.2 (0.2–3.9)	0.5 (0.1–1.9)	0.7 (0.0, 0.8) [0.06]	1.5 (0.4–5.2)	−0.9 (−1.3, −0.1) [0.006]	0.6 (0.0–1.5)	−0.1 (−0.3, 0.3) [0.98]	−0.9 (−1.6, −0.05) [0.02]
Peak thrombin, n*M*	151.5 (111.4–228.2)	44.8 (22.8–78.6)	106.7 (87.3, 124.8) [<0.0001]	182.1 (126.7–270.6)	−137.3 (−157.7, −104.7) [<0.0001]	128.2 (90.4–166.8)	−83.4, (−94.4, −55.3) [<0.0001]	−53.8 (−89.7, −28.6) [<0.0001]
ETP, n*M*/minute	3,286 (2,593–3,924)	1,000 (387–1,945)	2,286 (1,737, 2,391) [<0.0001]	3,540 (2,862–4,154)	−2,539 (−2,676, −1,963) [<0.0001]	2,904 (2,175–3,480)	−1,903 (−2,034, −1,270) [<0.0001]	−636 (−1,026, −321) [0.0003]
Lag time, minutes	21.0 (15.2–30.5)	35.0 (24.9–51.0)	−14.0 (−17.0, −9.0) [<0.0001]	20.0 (15.1–28.1)	15.0 (10.0, 18.5) [<0.0001]	26.0 (15.5–32.7)	9.0 (5.0, 16.5) [<0.0001]	6.0 (−0.5, 7.5) [0.07]
Velocity index, n*M*/minute	18.7 (10.7–35.5)	2.8 (0.6–7.2)	15.9 (10.7, 17.9) [<0.0001]	23.6 (12.6–37.7)	20.8 (−23.9, −13.3) [<0.0001]	13.9 (9.1–22.7)	−11.1 (−12.8, −7.3) [<0.0001]	−9.7 (−14.6, −2.9) [0.002]

* Values are the median (interquartile range). DM = dermatomyositis; 95% CI = 95% confidence interval; AnxV = annexin V; MPs; = microparticles; EMPs = endothelial-derived MPs; PMPs = platelet-derived MPs; TF = tissue factor; ETP = endogenous thrombin potential (corresponding to the area under the curve for thrombin generation assay curves).
† *P* values were determined by Kruskal-Wallis test followed by Mann-Whitney U test.

(Table 3). Patients with active disease had higher levels of IL-10, IL-6, IFNλ1, MCP-1, IP-10, and galectin-9 compared to patients with inactive disease or healthy controls. As endothelial cells are the main source of galectin-9 (40), we then examined the correlation between levels of galectin-9 and CECs; a strong correlation was identified (r = 0.48, $P < 0.0001$). Patients with juvenile DM with abnormal nailfold capillaries had higher levels of galectin-9 (median 105.1 ng/ml [IQR 54.8–196.7]) compared to patients with normal nailfold capillaries (median 57.6 ng/ml [IQR 35.1–67.1]) (difference –47.5 [95% CI –81.1, –22.3]; $P = 0.0004$).

Circulating MPs and plasma thrombin generation. Total AnxV+ MP numbers were significantly increased among patients with juvenile DM compared to patients with inactive juvenile DM and healthy controls (both $P < 0.0001$) (Table 4). MPs were mainly of platelet and endothelial origin. B cell–derived MPs were the third most common MP population. Total AnxV+ MP numbers correlated with CEC numbers (r = 0.42, $P < 0.0001$) and with galectin-9 levels (r = 031, $P = 0.01$). (Figures 2A and B). CD62E+ MP counts also correlated strongly with CEC counts (r = 0.20, $P = 0.027$) (Figure 2C).

Enhanced plasma-mediated thrombin generation, ETP, lag time, and velocity index were demonstrated in patients with active juvenile DM compared to patients with inactive juvenile DM and controls (Table 4). TF+CD14+ MP counts were strongly associated with ETP, a single summative parameter of thrombin generation (41) (r = 0.21, $P = 0.02$) (Figure 2D). ETP was also correlated with total AnxV+ MP numbers (r = 023, $P = 0.02$) and with numbers of EMPs (r = 0.23, $P = 0.01$) and CD19+Anx V+ MPs (r = 0.23, $P = 0.01$). No significant correlation between ETP and numbers of CD3+AnxV+ MPs or PMPs was observed (r = 0.18, $P = 0.06$ and r = 0.17, $P = 0.069$, respectively).

Arterial stiffness. We confirmed a strong positive association between age and carotid-femoral and carotid-radial PWV (both $P < 0.0001$) (Figures 3A and B). The slope for carotid-radial PWV in relation to age among patients with juvenile DM (0.44 m/second/year, y-intercept 3.414) differed significantly from that among healthy controls slope (0.12 m/second/year, y-intercept 5.903) ($P = 0.003$) (Figure 3C), indicating significantly increased arterial stiffness among patients with juvenile DM. No significant difference in carotid-femoral PWV was observed ($P = 0.12$) (Figure 3D).

DISCUSSION

We conducted a large cross-sectional study of patients with juvenile DM and explored biomarkers to monitor the vasculopathy of this disease. Our data provide evidence of increased endothelial injury in children with active juvenile DM, associated with proinflammatory cytokines, high levels of circulating MPs with a propensity to drive thrombin generation and potentially increase occlusive vasculopathy, and increased arterial stiffness in patients with juvenile DM compared to controls. These noninvasively measured vascular indices provide unique insight into the pathogenesis of vascular injury in this disease and could be used for clinical monitoring of the vasculopathy of juvenile DM.

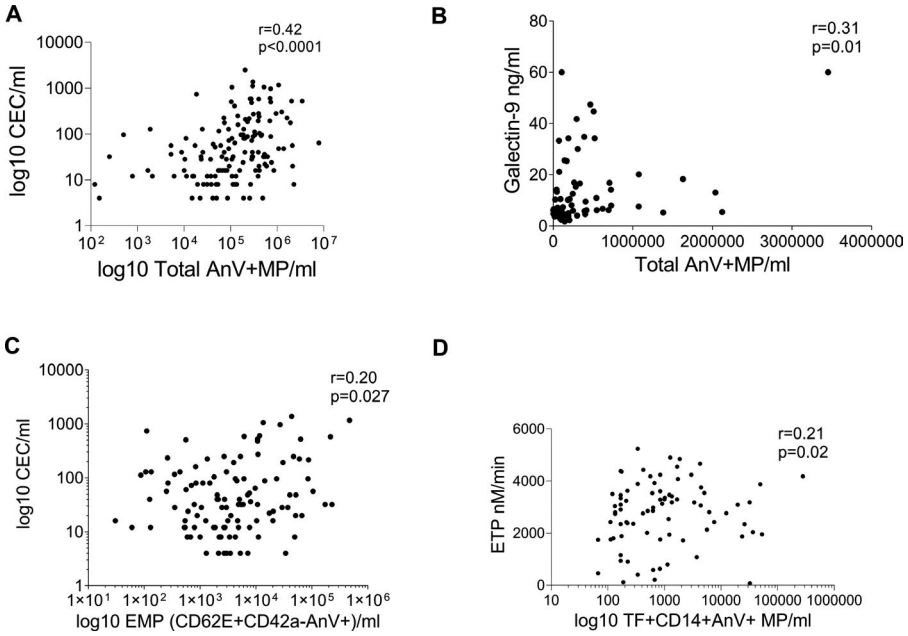

Figure 2. Correlation of circulating microparticle (MP) levels with other indices of endothelial injury and thrombin generation in patients with juvenile DM. **A** and **B**, Total annexin V (AnV)–positive MP counts correlated with CEC counts (**A**) and galectin-9 levels (**B**). **C**, Endothelial MP (EMP) counts correlated with CEC counts. **D**, Circulating tissue factor (TF)–positive MP counts correlated with plasma endogenous thrombin potential (ETP) values. Correlations were assessed with Spearman's rank correlation coefficient. See Figure 1 for other definitions.

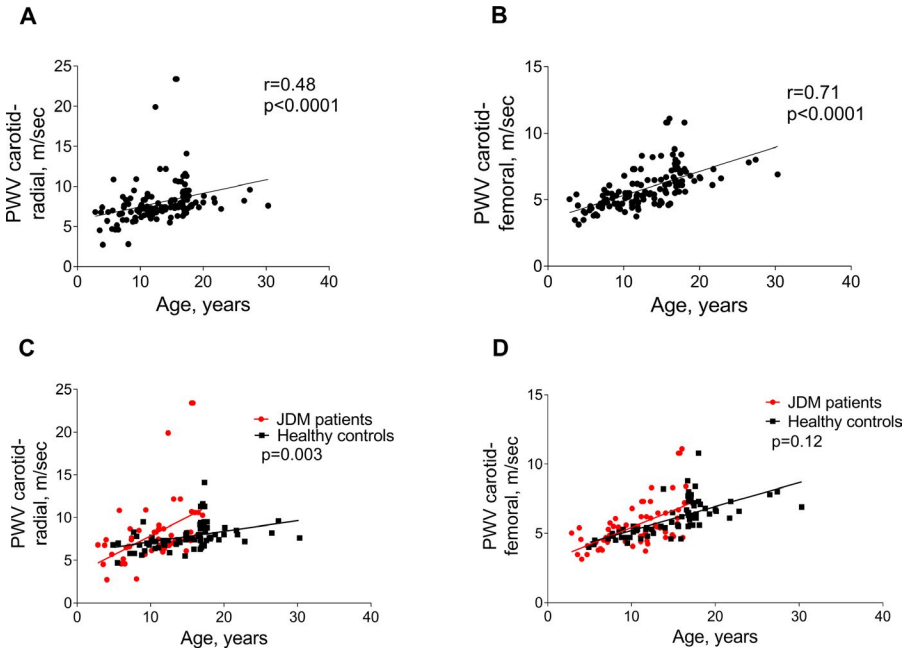

Figure 3. Carotid-radial and carotid-femoral pulse wave velocity (PWV) in patients with juvenile dermatomyositis (JDM) and healthy controls. **A** and **B**, Both carotid-radial PWV (**A**) and carotid-femoral PWV (**B**) correlated with age in the group of all subjects combined (patients with juvenile DM and controls). **C** and **D**, The slope for carotid-radial PWV in relation to age in patients with juvenile DM differed significantly from the slope in healthy controls (**C**), whereas a difference was not observed for carotid-femoral PWV (**D**), by analysis of covariance.

CECs are mature cells that have detached from the vessel wall in response to endothelial injury (17,22,42,43). We demonstrated increased levels of CECs in juvenile DM, in accordance with another recent study that also demonstrated increased CEC counts in juvenile DM, despite the use of a different method (flow cytometry) for cell enumeration (44). CEC numbers also strongly correlated with other biomarkers of endothelial injury such as galectin-9 levels and EMP counts, thus supporting the robustness of these endothelial injury indices. CEC counts were also found to be elevated in patients with juvenile DM whose disease was considered to be clinically inactive. This raises the possibility that in some patients with juvenile DM there is ongoing subclinical endothelial injury and disease activity that is not captured by laboratory parameters and disease activity measures that are currently routinely used. In addition, the demonstration of elevated CEC counts in patients with abnormal nailfold capillaries supports the notion that this finding is indeed a clinical sign of active juvenile DM vasculopathy.

We did not demonstrate any differences in traditional markers of systemic inflammation as assessed by hsCRP or SAA or any differences in routine cardiovascular risk factors (45) to account for the elevated CEC or MP counts we observed (Tables 1 and 3). We did, however, detect consistently higher levels of endothelial activation–related cell adhesion molecules, cytokines, and chemokines in patients with juvenile DM compared to healthy controls. This observation likely indicates a chronic disturbance in endothelial cell homoeostasis in patients with juvenile DM, including in some patients with apparently quiescent clinical disease activity as assessed using routine clinical tools. Juvenile DM is considered an interferonopathy, and therefore, not surprisingly, we detected high levels of IFN-driven cytokines/chemokines (IFNα, IFNλ1, MCP-1, IP-10) (46,47) in all patients with juvenile DM compared to controls, especially in patients with active disease, though we do note that other cytokines also correlated with active disease.

Additionally, we detected elevated levels of circulating endothelial, platelet, monocyte, and B cell–derived MPs that are highly prothrombotic (48) in patients with juvenile DM. We have previously demonstrated enhanced MP-mediated thrombin generation in children with active vasculitis (16), potentially explaining some of the excess thrombotic risk associated with vasculitis. Similarly, we detected elevated levels of MPs, including highly prothrombotic TF+ MPs, and enhanced plasma thrombin generation in patients with active juvenile DM. This increased prothrombotic propensity, mediated by MPs among other prothrombotic factors, might contribute to occlusive vasculopathy and organ injury in juvenile DM. The exact mechanism by which different types of MPs may promote endothelial dysfunction and thrombogenicity in juvenile DM remain to be established.

We also showed that children with juvenile DM have enhanced carotid-radial PWV, consistent with increased arterial stiffness compared to healthy children. This increased PWV may suggest a generalized secondary systemic vasculopathy, ultimately leading to accelerated atherosclerosis. Other factors such as sedentary lifestyle, long-term treatment with glucocorticoids, and ongoing systemic inflammation may

also contribute to this finding. It is not yet known whether premature cardiovascular morbidity occurs later in adulthood in patients with juvenile DM, but our data strongly suggest that this could be a future concern, and indeed has been observed in adults with juvenile DM (49,50). Prospective studies to evaluate changes of PWV over time in patients with juvenile DM are needed.

No differences in carotid-femoral PWV between patients with juvenile DM and controls were demonstrated in this study. Carotid-radial PWV mainly reflects the peripheral arterial stiffness of upper limb muscular arteries (branchial and radial arteries), while carotid-femoral PWV is a marker of central arterial (aortic, i.e., elastic artery) stiffness (51,52). Previous studies have suggested that the variation in elastin–collagen smooth muscle proportions within different arterial segments determines the observed arterial stiffness in response to various cardiovascular risk factors (53). It is therefore perhaps not surprising that inflammatory processes such as juvenile DM may also have a different effect on the arterial stiffness of separate parts of the arterial tree. In addition, other studies have suggested that carotid-radial PWV mainly reflects microvascular endothelial dysfunction (54,55), and therefore it could be the case that juvenile DM induces such microvascular changes rather than larger structural arterial changes.

The present findings have multiple potential implications with regard to therapy. In patients with ongoing vasculitic endothelial injury, prolonged immunosuppressive treatment and/or consideration of novel directed therapeutic strategies may be needed to target the vasculopathy of juvenile DM. We observed an up-regulation of several proinflammatory cytokines/chemokines that could potentially provide a therapeutic target. Of particular interest is the up-regulation of IFN-driven cytokines/chemokines that could be contributing to driving endothelial injury. Notably, several recent transcriptomic studies (56,57) have shown up-regulation of IFN-stimulated genes within the capillaries of the muscle and disruption of vascular network organization upon exposure of endothelial cells to IFN, highlighting the involvement of this pathway in the vasculopathy of myositis (58). Based on these observations, targeting IFN-related endothelial injury with JAK inhibition has therefore emerged as a novel therapeutic strategy for myositis (59,60). In that context, we have reported the use of CECs to monitor the rapid response of endothelial injury to JAK inhibition in a patient with juvenile DM (60). MP profiling and thrombin generation assays could provide a novel means of assessing prothrombotic risk in patients with juvenile DM, allowing improved risk stratification and potential targeting of primary thrombosis prevention (61). Finally, if larger prospective studies confirm increased arterial stiffness in children with juvenile DM, formal therapeutic lifestyle interventions may be considered, in order to reduce this risk of accelerated cardiovascular morbidity.

Our study has several limitations. It was a single-center study of a heterogeneous cohort of patients with juvenile DM. At the time of recruitment, patients were receiving a variety of treatments, although they were treated in accordance with published clinical guidelines (62). MSA testing to better understand the potential relevance of these antibodies to vascular phenotype and influence on circulating IFN and galectin-9 levels was not available in all patients. Our control population was age similar, but not exactly age matched, due to of lack of availability of control samples from very young healthy children.

In conclusion, we have demonstrated dynamic changes in biomarkers of endothelial injury (MPs and CECs) in children with juvenile DM. Future studies could also explore these indices in the context of clinical trials, to better understand the use of more targeted therapeutic strategies on vascular phenotype in juvenile DM.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Papadopoulou had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Papadopoulou C, McCann LJ. The vasculopathy of juvenile dermatomyositis [review]. Front Pediatr 2018;6:284.
2. Whitaker JN, Engel WK. Vascular deposits of immunoglobulin and complement in idiopathic inflammatory myopathy. N Engl J Med 1972;286:333–8.
3. McCann LJ, Juggins AD, Maillard SM, Wedderburn LR, Davidson JE, Murray KJ, et al. The Juvenile Dermatomyositis National Registry and Repository (UK and Ireland): clinical characteristics of children recruited within the first 5 yr. Rheumatology (Oxford) 2006;45:1255–60.
4. Crowe WE, Bove KE, Levinson JE, Hilton PK. Clinical and pathogenetic implications of histopathology in childhood polydermatomyositis. Arthritis Rheum 1982;25:126–39.
5. Gitiaux C, De Antonio M, Aouizerate J, Gherardi RK, Guilbert T, Barnerias C, et al. Vasculopathy-related clinical and pathological features are associated with severe juvenile dermatomyositis. Rheumatology (Oxford) 2016;55:470–9.
6. Baechler EC, Bauer JW, Slattery CA, Ortmann WA, Espe KJ, Novitzke J, et al. An interferon signature in the peripheral blood of dermatomyositis patients is associated with disease activity. Mol Med 2007;13:59–68.
7. Emslie-Smith AM, Engel AG. Microvascular changes in early and advanced dermatomyositis: a quantitative study. Ann Neurol 1990;27:343–56.
8. Mamyrova G, Kleiner DE, James-Newton L, Shaham B, Miller FW, Rider LG. Late-onset gastrointestinal pain in juvenile dermatomyositis as a manifestation of ischemic ulceration from chronic endarteropathy. Arthritis Rheum 2007;57:881–4.

9. Pachman LM, Hayford JR, Chung A, Daugherty CA, Pallansch MA, Fink CW, et al. Juvenile dermatomyositis at diagnosis: clinical characteristics of 79 children. J Rheumatol 1998;25:1198–204.
10. Tisseverasinghe A, Bernatsky S, Pineau CA. Arterial events in persons with dermatomyositis and polymyositis. J Rheumatol 2009;36:1943–6.
11. Zaller B, Li X, Sundquist J, Sundquist K. Risk of subsequent coronary heart disease in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden. PLoS One 2012;7:e33442.
12. Brogan PA, Davies R, Gordon I, Dillon MJ. Renal angiography in children with polyarteritis nodosa. Pediatr Nephrol 2002;17:277–83.
13. Brogan P, Eleftheriou D, Dillon M. Small vessel vasculitis. Pediatr Nephrol 2010;25:1025–35.
14. Clarke LA, Shah V, Arrigoni F, Eleftheriou D, Hong Y, Halcox J, et al. Quantitative detection of circulating endothelial cells in vasculitis: comparison of flow cytometry and immunomagnetic bead extraction. J Thromb Haemost 2008;6:1025–32.
15. Clarke LA, Hong Y, Eleftheriou D, Shah V, Arrigoni F, Klein NJ, et al. Endothelial injury and repair in systemic vasculitis of the young. Arthritis Rheum 2010;62:1770–80.
16. Eleftheriou D, Hong Y, Klein NJ, Brogan PA. Thromboembolic disease in systemic vasculitis is associated with enhanced microparticle-mediated thrombin generation [letter]. J Thromb Haemost 2011;9: 1864–7.
17. Eleftheriou D, Ganesan V, Hong Y, Klein NJ, Brogan PA. Endothelial injury in childhood stroke with cerebral arteriopathy: a cross-sectional study. Neurology 2012;79:2089–96.
18. Koga H, Sugiyama S, Kugiyama K, Watanabe K, Fukushima H, Tanaka T, et al. Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol 2005;45:1622–30.
19. Pericleous C, Clarke LA, Brogan PA, Latchman DS, Isenberg DA, Ioannou Y, et al. Endothelial microparticle release is stimulated in vitro by purified IgG from patients with the antiphospholipid syndrome. Thromb Haemost 2013;109:72–8.
20. Clancy RM. Circulating endothelial cells and vascular injury in systemic lupus erythematosus. Curr Rheumatol Rep 2000;2:39–43.
21. Haubitz M, Woywodt A. Circulating endothelial cells and vasculitis. Intern Med 2004;43:660–7.
22. Woywodt A, Streiber F, de Groot K, Regelsberger H, Haller H, Haubitz M. Circulating endothelial cells as markers for ANCA-associated small-vessel vasculitis. Lancet 2003;361:206–10.
23. Bohan A, Peter JB. Polymyositis and dermatomyositis: part 1 [review]. N Engl J Med 1975;292:344–7.
24. Martin N, Krol P, Smith S, Murray K, Pilkington CA, Davidson JE, et al. A national registry for juvenile dermatomyositis and other paediatric idiopathic inflammatory myopathies: 10 years' experience; the Juvenile Dermatomyositis National (UK and Ireland) Cohort Biomarker Study and Repository for Idiopathic Inflammatory Myopathies. Rheumatology (Oxford) 2011;50:137–45.
25. Lazarevic D, Pistorio A, Palmisani E, Miettunen P, Ravelli A, Pilkington C, et al. The PRINTO criteria for clinically inactive disease in juvenile dermatomyositis. Ann Rheum Dis 2013;72:686–93.
26. Lovell DJ, Lindsley CB, Rennebohm RM, Ballinger SH, Bowyer SL, Giannini EH, et al, in cooperation with The Juvenile Dermatomyositis Disease Activity Collaborative Study Group. Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies. II. The Childhood Myositis Assessment Scale (CMAS): a quantitative tool for the evaluation of muscle function. Arthritis Rheum 1999;42:2213–9.
27. Huber AM, Feldman BM, Rennebohm RM, Hicks JE, Lindsley CB, Perez MD, et al. Validation and clinical significance of the Childhood Myositis Assessment Scale for assessment of muscle function in the juvenile idiopathic inflammatory myopathies. Arthritis Rheum 2004;50:1595–603.
28. Rider LG, Koziol D, Giannini EH, Jain MS, Smith MR, Whitney-Mahoney K, et al. Validation of manual muscle testing and a subset of eight muscles for adult and juvenile idiopathic inflammatory myopathies. Arthritis Care Res (Hoboken) 2010;62:465–72.
29. Shah V, Christov G, Mukasa T, Brogan KS, Wade A, Eleftheriou D, et al. Cardiovascular status after Kawasaki disease in the UK. Heart 2015;101:1646–55.
30. Tansley SL, Simou S, Shaddick G, Betteridge ZE, Almeida B, Gunawardena H, et al. Autoantibodies in juvenile-onset myositis: their diagnostic value and associated clinical phenotype in a large UK cohort. J Autoimmun 2017;84:55–64.
31. Rider LG, Feldman BM, Perez MD, Rennebohm RM, Lindsley CB, Zemel LS, et al, in cooperation with the Juvenile Dermatomyositis Disease Activity Collaborative Study Group. Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies. I. Physician, parent, and patient global assessments. Arthritis Rheum 1997;40:1976–83.
32. Singh G, Athreya BH, Fries JF, Goldsmith DP. Measurement of health status in children with juvenile rheumatoid arthritis. Arthritis Rheum 1994;37:1761–9.
33. Guzman J, Petty RE, Malleson PN. Monitoring disease activity in juvenile dermatomyositis: the role of von Willebrand factor and muscle enzymes. J Rheumatol 1994;21:739–43.
34. Feldman BM, Rider LG, Reed AM, Pachman LM. Juvenile dermatomyositis and other idiopathic inflammatory myopathies of childhood. Lancet 2008;371:2201–12.
35. Hasegawa M. Dermoscopy findings of nail fold capillaries in connective tissue diseases. J Dermatol 2011;38:66–70.
36. Chihara M, Kurita M, Yoshihara Y, Asahina A, Yanaba K. Clinical significance of serum galectin-9 and soluble CD155 levels in patients with systemic sclerosis. J Immunol Res 2018;2018:9473243.
37. Woywodt A, Blann AD, Kirsch T, Erdbruegger U, Banzet N, Haubitz M, et al. Isolation and enumeration of circulating endothelial cells by immunomagnetic isolation: proposal of a definition and a consensus protocol. J Thromb Haemost 2006;4:671–7.
38. Bidot L, Jy W, Bidot C Jr, Jimenez JJ, Fontana V, Horstman LL, et al. Microparticle-mediated thrombin generation assay: increased activity in patients with recurrent thrombosis. J Thromb Haemost 2008;6:913–9.
39. Urbina EM, Williams RV, Alpert BS, Collins RT, Daniels SR, Hayman L, et al. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. Hypertension 2009;54:919–50.
40. O'Brien MJ, Shu Q, Stinson WA, Tsou PS, Ruth JH, Isozaki T, et al. A unique role for galectin-9 in angiogenesis and inflammatory arthritis. Arthritis Res Ther 2018;20:31.
41. Tripodi A. Thrombin generation assay and its application in the clinical laboratory. Clin Chem 2016;62:699–707.
42. Kluz J, Kopec W, Jakobsche U, Prajs I, Adamiec R. Vasculitis in systemic lupus erythematosus (SLE)–assessment of peripheral blood mononuclear cell activation and the degree of endothelial dysfunction: initial report. Postepy Hig Med Dosw (Online) 2007;61:725–35.
43. Kutlay S, Calayoglu R, Boyvat A, Turkcapar N, Sengul S, Keven K, et al. Circulating endothelial cells: a disease activity marker in Behcet's vasculitis? Rheumatol Int 2008;29:159–62.
44. Kishi T, Chipman J, Evereklian M, Nghiem K, Stetler-Stevenson M, Rick ME, et al. Endothelial activation markers as disease activity and damage measures in juvenile dermatomyositis. J Rheumatol 2020;47:1011–8.

45. Wahezi DM, Liebling EJ, Choi J, Dionizovik-Dimanovski M, Gao Q, Parekh J. Assessment of traditional and non-traditional risk factors for premature atherosclerosis in children with juvenile dermatomyositis and pediatric controls. Pediatr Rheumatol Online J 2020;18:25.
46. Bilgic H, Ytterberg SR, Amin S, McNallan KT, Wilson JC, Koeuth T, et al. Interleukin-6 and type I interferon–regulated genes and chemokines mark disease activity in dermatomyositis. Arthritis Rheum 2009;60:3436–46.
47. Reed AM, Peterson E, Bilgic H, Ytterberg SR, Amin S, Hein MS, et al. Changes in novel biomarkers of disease activity in juvenile and adult dermatomyositis are sensitive biomarkers of disease course. Arthritis Rheum 2012;64:4078–86.
48. Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E, et al. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. J Exp Med 2003;197:1585–98.
49. Eimer MJ, Brickman WJ, Seshadri R, Ramsey-Goldman R, McPherson DD, Smulevitz B, et al. Clinical status and cardiovascular risk profile of adults with a history of juvenile dermatomyositis. J Pediatr 2011;159:795–801.
50. Silverberg JI, Kwa L, Kwa MC, Laumann AE, Ardalan K. Cardiovascular and cerebrovascular comorbidities of juvenile dermatomyositis in US children: an analysis of the National Inpatient Sample. Rheumatology (Oxford) 2018;57:694–702.
51. Liu J, Zhang Y, Cao TS, Duan YY, Yuan LJ, Yang YL, et al. Preferential macrovasculopathy in systemic sclerosis detected by regional pulse wave velocity from wave intensity analysis: comparisons of local and regional arterial stiffness parameters in cases and controls. Arthritis Care Res (Hoboken) 2011;63:579–87.
52. Niiranen TJ, Kalesan B, Larson MG, Hamburg NM, Benjamin EJ, Mitchell GF, et al. Aortic-brachial arterial stiffness gradient and cardiovascular risk in the community: the Framingham Heart Study. Hypertension 2017;69:1022–8.
53. Van der Heijden-Spek JJ, Staessen JA, Fagard RH, Hoeks AP, Boudier HA, van Bortel LM. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. Hypertension 2000;35:637–42.
54. McCall DO, McGartland CP, Woodside JV, Sharpe P, McCance DR, Young IS. The relationship between microvascular endothelial function and carotid-radial pulse wave velocity in patients with mild hypertension. Clin Exp Hypertens 2010;32:474–9.
55. Badhwar S, Chandran DS, Jaryal AK, Narang R, Deepak KK. Regional arterial stiffness in central and peripheral arteries is differentially related to endothelial dysfunction assessed by brachial flow-mediated dilation in metabolic syndrome. Diab Vasc Dis Res 2018;15:106–13.
56. Greenberg SA, Pinkus JL, Pinkus GS, Burleson T, Sanoudou D, Tawil R, et al. Interferon-α/β-mediated innate immune mechanisms in dermatomyositis. Ann Neurol 2005;57:664–78.
57. Suarez-Calvet X, Gallardo E, Nogales-Gadea G, Querol L, Navas M, Diaz-Manera J, et al. Altered RIG-I/DDX58-mediated innate immunity in dermatomyositis. J Pathol 2014;233:258–68.
58. Ladislau L, Suarez-Calvet X, Toquet S, Landon-Cardinal O, Amelin D, Depp M, et al. JAK inhibitor improves type I interferon induced damage: proof of concept in dermatomyositis. Brain 2018;141:1609–21.
59. Aeschlimann FA, Fremond ML, Duffy D, Rice GI, Charuel JL, Bondet V, et al. A child with severe juvenile dermatomyositis treated with ruxolitinib. Brain 2018;141:e80.
60. Papadopoulou C, Hong Y, Omoyinmi E, Brogan PA, Eleftheriou D. Janus kinase 1/2 inhibition with baricitinib in the treatment of juvenile dermatomyositis. Brain 2019;142:e8.
61. Lee CJ, Ansell JE. Direct thrombin inhibitors. Br J Clin Pharmacol 2011;72:581–92.
62. Enders FB, Bader-Meunier B, Baildam E, Constantin T, Dolezalova P, Feldman BM, et al. Consensus-based recommendations for the management of juvenile dermatomyositis. Ann Rheum Dis 2017;76:329–40.