Endothelial Activation Markers as Disease Activity and Damage Measures in Juvenile Dermatomyositis

Takayuki Kishi, Jonathan Chipman, Melvina Evereklian, Khanh Nghiem, Maryalice Stetler-Stevenson, Margaret E. Rick, Michael Centola, Frederick W. Miller, and Lisa G. Rider

ABSTRACT. ***Objective.*** Circulating endothelial cells (CEC), von Willebrand factor (vWF) antigen, P-selectin, and thrombomodulin are released from damaged endothelium, while decreases in circulating endothelial progenitor cells (CEPC) have been associated with poor vascular outcomes. We examined these markers in the peripheral blood of patients with juvenile dermatomyositis (JDM) and their correlations with disease assessments.

Methods. Peripheral blood endothelial cells and biomarkers were assessed in 20 patients with JDM and matched healthy controls. CEC and CEPC were measured by flow cytometry, while vWF antigen and activity, factor VIII, P-selectin, and thrombomodulin were measured in plate-based assays. Disease activity and damage, nailfold capillary density, and brachial artery flow dilation were assessed. Serum cytokines/chemokines were measured by Luminex.

Results. CEC, vWF antigen, factor VIII, and thrombomodulin, but not vWF activity, CEPC, or P-selectin, were elevated in the peripheral blood of patients with JDM. CEC correlated with pulmonary activity ($r_s = 0.56$). The vWF antigen correlated with Patient's/Parent's Global, cutaneous, and extramuscular activity ($r_s = 0.47$–0.54). CEPC negatively correlated with muscle activity and physical function ($r_s = -0.52$ to -0.53). CEPC correlated inversely with endocrine damage. The vWF antigen and activity correlated with interleukin 10 and interferon-gamma inducible protein-10 ($r_s = 0.64$–0.82).

Conclusion. Markers of endothelial injury are increased in patients with JDM and correlate with extramuscular activity. CEPC correlate inversely with muscle activity, suggesting a functional disturbance in repair mechanisms. (J Rheumatol First Release January 15 2020; doi:10.3899/jrheum.181275)

Key Indexing Terms:
JUVENILE DERMATOMYOSITIS ENDOTHELIAL FUNCTION DISEASE ACTIVITY
CIRCULATING ENDOTHELIAL CELLS ENDOTHELIAL PROGENITOR CELLS MYOSITIS

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The idiopathic inflammatory myopathies (IIM) are systemic autoimmune diseases characterized by chronic muscle inflammation[1]. An immune attack on muscle capillary endothelium, infiltration of plasmacytoid dendritic cells with a resulting type I interferon (IFN) response, and upregulation of major histocompatibility complex class I expression on the surface of myofibers appear to be central pathogenic events in adult and juvenile dermatomyositis (JDM)[2,3].

Circulating endothelial cells (CEC) are biomarkers of endothelial function, which represent the detachment of mature cells from the endothelial monolayer following damage. CEC are rarely found in the peripheral blood of healthy individuals, but plasma levels of CEC are increased in vascular disease and are believed to reflect the degree of endothelial damage or stress[4]. In a variety of inflammatory

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and autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic sclerosis, and antineutrophil cytoplasmic autoantibody–associated vasculitis, patients with active disease have increased numbers of CEC, which correlate with disease activity[5,6,7,8,9]. Circulating endothelial progenitor cells (CEPC), which are part of the mononuclear cell component of the blood, are produced in the bone marrow and migrate into blood vessels, and can differentiate into mature endothelial cells, which results in the formation of new blood vessels[10]. In response to vascular injury, the movement of CEPC to sites of injury increases and bone marrow CEPC pools are depleted, resulting in a decrease in the number of CEPC in the peripheral blood. In systemic rheumatic disease, CEPC are often decreased in numbers and function. They have several important roles, including maintaining endothelial function following inflammatory stress, protecting against atherosclerosis, and stimulating angiogenesis[11].

CEPC have been observed to be significantly less frequent in adult patients with polymyositis (PM) compared with healthy controls[12]. CEPC also demonstrated a decreased capacity to differentiate into mature endothelial cells in adult patients with dermatomyositis (DM)/PM, and this impairment was associated with type I interferon (IFN) and interleukin (IL)-18 serum activity[12]. Recently, Xu, *et al* reported no difference in the number of CEPC in patients with JDM compared to healthy control children, and CEPC did not correlate with JDM disease activity assessed by the Disease Activity Score (DAS) or with metabolic variables[13].

The purpose of our study was to further evaluate CEC, CEPC, and other peripheral blood endothelial biomarkers in patients with IIM compared to healthy controls, and to investigate the relationship between these endothelial markers with disease activity and damage measures in IIM.

MATERIALS AND METHODS

Subjects. Twenty patients with JDM fulfilled probable or definite Bohan and Peter criteria for IIM[14], and were enrolled in the US National Institute of Environmental Health Sciences myositis natural history study (institutional review board approval no. 94-E-0165). Patients provided written informed consent/assent according to the standards of the Declaration of Helsinki. Myositis-specific autoantibodies were identified in the sera of 20 patients using standard detection methods[15,16]. Median age at enrollment was 12.1 years, and the median time from diagnosis to enrollment was 23.2 months. JDM patient characteristics and their disease activity and damage measures[17] are shown in Table 1. Five patients underwent muscle biopsy to confirm a diagnosis of JDM; however, detailed muscle biopsy reports were available from only 2 patients. We also evaluated thigh and pelvis magnetic resonance imaging (MRI), including short-tau inversion recovery (STIR) muscle edema and T1 muscle atrophy and fatty infiltration, with scoring by 1 radiologist blinded to clinical status[18]. Clinical laboratory studies were adjusted based on age-defined upper limits of normal.

A healthy control group (n = 20) was recruited through the US National Institutes of Health's Office of Patient Recruitment, consisting of 13 females (65%) with a median enrollment age of 11.7 years [interquartile range (IQR) 9.7–15.9 yrs] and similar in racial composition to the patients with JDM. The controls had no evidence of autoimmune disease by history, physical

Table 1. Characteristics of 20 patients with juvenile dermatomyositis in the present study.

Characteristics	Values
Age at evaluation, yrs	12.1 (9.3–15.7)
Female sex	13 (65.0)
Race	
White	17 (85.0)
African American	1 (5.0)
Asian	1 (5.0)
White and Hispanic	1 (4.5)
Myositis-specific autoantibodies	
Anti-p155/140 (TIF1)	7 (35.0)
Anti-MJ (NXP2)	3 (15.0)
Anti-MDA5	6 (30.0)
Other MSA (Jo1, Mi-2, HMGCR)†	3 (15.0)
MSA-negative	1 (5.0)
Disease measures	
Physician's global activity score, 0–10 cm VAS	2.7 (1.6–4.4)
Patient's/parent's global activity score, 0–10 cm VAS	4.2 (2.1–5.6)
Physician's global damage, 0–10 cm VAS	1.9 (0.7–2.9)
Physical function	
HAQ or CHAQ, 0–3	0.4 (0.1–1.1)
CMAS, 0–52	45.5 (37.3–49.0)
Muscle strength	
MMT-8, 0–80	75.0 (68.5–78.8)
Muscle enzymes	
CK, 26–252 u/l	39.5 (29.0–66.0)
Aldolase, 1–7 u/l	5.8 (4.7–5.8)
AST, 0–34 u/l	30.5 (23.3–35.8)
LDH, 105–226 u/l	204 (170–241)
MDAAT VAS Organ System Scores‡	
Cutaneous, 0–10 cm VAS	2.6 (1.9–5.0)
Muscle, 0–10 cm VAS	2.1 (0.6–3.4)
Constitutional, 0–10 cm VAS	1.0 (0.4–1.4)
Pulmonary, 0–10 cm VAS	0.6 (0–1.0)
Skeletal, 0–10 cm VAS	0 (0–0.3)
Gastrointestinal, 0–10 cm VAS	0 (0–0.5)
Extramuscular VAS score, 0–60	4.6 (3.2–7.1)
Disease Activity Score	
Total, 0–20	10 (8–12)
Muscle, 0–11	5 (3–6)
Cutaneous, 0–9	6 (5–7)
Myositis Damage Index	
Total Extent of Damage score, 0–38	5.0 (3.0–8.5)
Total Severity of Damage score, 0–110	5.5 (3.3–8.0)
Muscle Severity of Damage, 0–10 cm VAS	1.5 (0.1–2.5)
Skeletal Severity of Damage, 0–10 cm VAS	1.5 (0.9–2.8)
Endocrine Severity of Damage, 0–10 cm VAS	0.6 (0–1.0)
Pulmonary Severity of Damage, 0–10 cm VAS	0 (0–0.9)
Other measures	
MRI T1 atrophy and fatty muscle infiltration score, 0–4	0 (0–0.5)
Brachial artery flow mediated dilation, % change	9.1 (8.0–14.8)
Periungual nailfold capillary density, mm	8.0 (6.8–10.0)
Delay to diagnosis, mos	4.0 (2.0–9.0)
Laboratory	
White blood cell count, 3.4–9.6, × 10^3/mcl	6.9 (4.7–8.5)
Platelet count, 161–380, × 10^3/mcl	300 (250–343)
Erythrocyte sedimentation rate, ULN 42, mm/h	19.0 (6.0–44.0)
Fasting serum insulin, mIU/l	15.1 (10.7–25.6)
LDL, ULN 159, mg/dl	118 (108–151)
Total cholesterol, ULN 240, mg/dl	202 (176–244)

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Table 1. Continued.

Characteristics	Values
Medications	
Daily oral prednisone dose, mg/day	15.0 (6.4–23.1)
Oral prednisone usage	18 (90.0)
Intravenous methylprednisolone usage	16 (80.0)
Methotrexate usage	20 (100)
Intravenous immunoglobulin usage	12 (60.0)
Other immunosuppressive usage§	9 (45.0)
Hydroxychloroquine usage	15 (75.0)

Values are n (%) or median (interquartile range). †Other myositis-specific autoantibodies included 1 patient each with anti-Jo1, anti-Mi-2, and anti-HMGCR. ‡Cardiovascular MDAAT and gastrointestinal and peripheral vascular severity of damage scores were all 0. §Other immunosuppressives include azathioprine, mycophenolate mofetil, cyclosporine, cyclophosphamide, or antitumor necrosis factor therapy (etanercept and infliximab). AST: aspartate aminotransferase; (C)HAQ: (Childhood) Health Assessment Questionnaire; CK: creatine kinase; CMAS: Childhood Myositis Assessment Scale; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; LDH: lactate dehydrogenase; LDL: low-density lipoprotein; MDAAT: Myositis Disease Activity Assessment tool; MDA5: melanoma differentiation–associated gene 5; MMT: Manual Muscle Testing; MRI: magnetic resonance imaging; MSA: myositis-specific autoantibodies; NXP2: nuclear matrix protein 2; STIR: short-tau inversion recovery; TIF1: transcriptional intermediary factor 1; ULN: upper limit of normal; VAS: visual analog scale.

examination, and laboratory testing, and no infections, vaccines, or use of antiinflammatory medications within 2 months of enrollment.

Endothelial functional assessments. Patients with JDM and healthy controls underwent evaluation of periungual nailfold capillaries (NFC) and brachial artery flow mediated dilation (FMD; Table 1). NFC density was measured, blinded to patient clinical status, on the fourth digit of the right hand using a Nikon D810 digital camera (Nikon Inc.) with an 80-mm lens with ring light flash; mineral oil was applied to the periungual area for magnification, with a millimeter measuring tape used for reference in each photograph[19]. Brachial artery FMD was assessed by a standard protocol using a high-resolution ultrasonography (12.5-MHz linear-array transducer, model ATL HDI 5000, Advanced Technology Laboratories), as previously described[20].

Laboratory methods. All laboratory studies were performed by personnel blinded to patient characteristics and assessments. EDTA anticoagulated peripheral blood was processed within 4 h of collection. To detect CEC, whole blood lysis was performed using ammonium chloride, as previously described[21], prior to staining for 30 min at 4ºC with the following cocktail of antibodies (antibody concentration according to manufacturer's recommendations): CD31-FITC (Becton Dickinson), CD146-PE, (P1H12, Chemicon), and CD45–activated protein C (APC; Becton Dickinson). The 7-aminoactinomycin D Viability Dye (Beckman Coulter) was added 1 min prior to acquisition to discriminate live versus dead cells. Five million cells were acquired per tube using a FACSCalibur device (BD Biosciences). Live CEC were identified as 7AAD-negative, CD45-negative, CD146-positive, and CD31-positive cells.

CEPC were quantitated by flow cytometry of mononuclear cells isolated from buffy coat cells from EDTA anticoagulated peripheral blood, as previously described[22]. Each tube of aliquoted cells was stained with phycoerythrin (PE) or fluorescein isothiocyanate-conjugated CD34 monoclonal antibody and PerCPCy5.5-conjugated CD45 monoclonal antibody (BD Biosciences). Two additional monoclonal antibodies for CEPC and endothelial cell markers were also added to each tube of cells, including biotin-conjugated KDR (Sigma-Aldrich), and PE-conjugated CD133 or APC–conjugated CD133 (Miltenyi Biotec). Cell populations in subjects were also expressed as the number of circulating cells per volume of peripheral blood, based on the nucleated white blood cell count from the automated counter.

The von Willebrand factor (vWF) antigen assays were performed using Diagnostica Stago STA LIATEST vWF: antigen according to the manufacturer's directions. The vWF activity was measured in a Ristocetin cofactor assay as previously described[23]. Factor VIII activity was measured in a 1-stage activated partial thromboplastin time (aPTT) assay using George King Biomedical factor VIII deficient plasma and automated aPTT from Diagnostica Stago. P-selectin levels and thrombomodulin levels were measured in ELISA assays (R&D Systems Inc.). Serum levels of 23 cytokines and chemokines were measured using a bead-based immunofluorescence assay (Luminex Inc.) and multiplex cytokine reagents (Biosource International) as previously described[24]: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, CXCL8 (IL-8), IL-10, IL-12, IL-13, IL-15, IL-17, tumor necrosis factor-α, IFN-γ, IFN-α, granulocyte-macrophage colony-stimulating factor, macrophage inflammatory protein-1α (MIP-1α), MIP-1β, IFN-gamma inducible protein-10 (IP-10), CCL11 (eotaxin), RANTES, monocyte chemoattractant protein-1 (MCP-1), and IL-1 receptor antagonist. Sensitivity of the standards ranged from 1.95 pg/ml to 32,000 pg/ml.

Statistical analysis. Analyses were performed using JMP for Windows, version 11.0.0 (SAS Institute Inc.). Wilcoxon-rank sums tests were used to compare median values of endothelial markers between patients and healthy controls. Data were expressed as median (IQR). Spearman rank correlations (Spearman ρ or r_s) assessed plausible relationships among the endothelial markers, and between endothelial markers and disease activity or damage measures, as well as among the endothelial functional assessments. This investigative analysis defines possible association at the $\alpha = 0.05$ level and significant association at the $\alpha = 0.01$ level.

RESULTS

The median number of CEC was significantly higher in patients with JDM compared to healthy controls [median (IQR): JDM 0.85 (0.52–1.95) vs healthy controls 0.18 (0.11–0.33) cells/ml; Figure 1A]. Levels of thrombomodulin [JDM 7.0 (2.4–10.1) vs healthy controls 1.9 (0.68–3.5) ng/ml], vWF antigen [JDM 142.0 (90.0–251.8) vs healthy controls 97.0 (81.0–131.0) IU/dl], and factor VIII [JDM 202 (152–254) vs healthy controls 137 (124-170) IU/dl] were also significantly higher in patients with JDM compared to healthy control subjects (Figure 1B–D). There were no significant differences between patients with JDM and healthy control subjects in vWF activity [JDM 154 (104–196), healthy controls 121 (97–150)%; Figure 1E], P-selectin levels [JDM 31.0 (24.0–41.5), healthy controls 24.0 (24.0–38.0) ng/ml; data not shown], or in the number of CEPC [JDM 35.0 (24.8–81.1), healthy controls 34.7 (29.0–92.3) cells/ml; Figure 1F]. There were no significant differences in endothelial cells or markers between patients who received intravenous methylprednisolone, intravenous immunoglobulin, hydroxychloroquine, or other immunosuppressive therapies compared to patients not receiving these medications. Corticosteroid dose did not correlate with any endothelial cells or markers. All patients studied received methotrexate; therefore effects of this medication could not be evaluated.

We assessed correlations among endothelial markers, and found some endothelial cells and markers correlated with each other. VWF antigen, vWF activity, and factor VIII all highly correlated with each other ($rs = 0.82–0.90$, $p < 0.001$).

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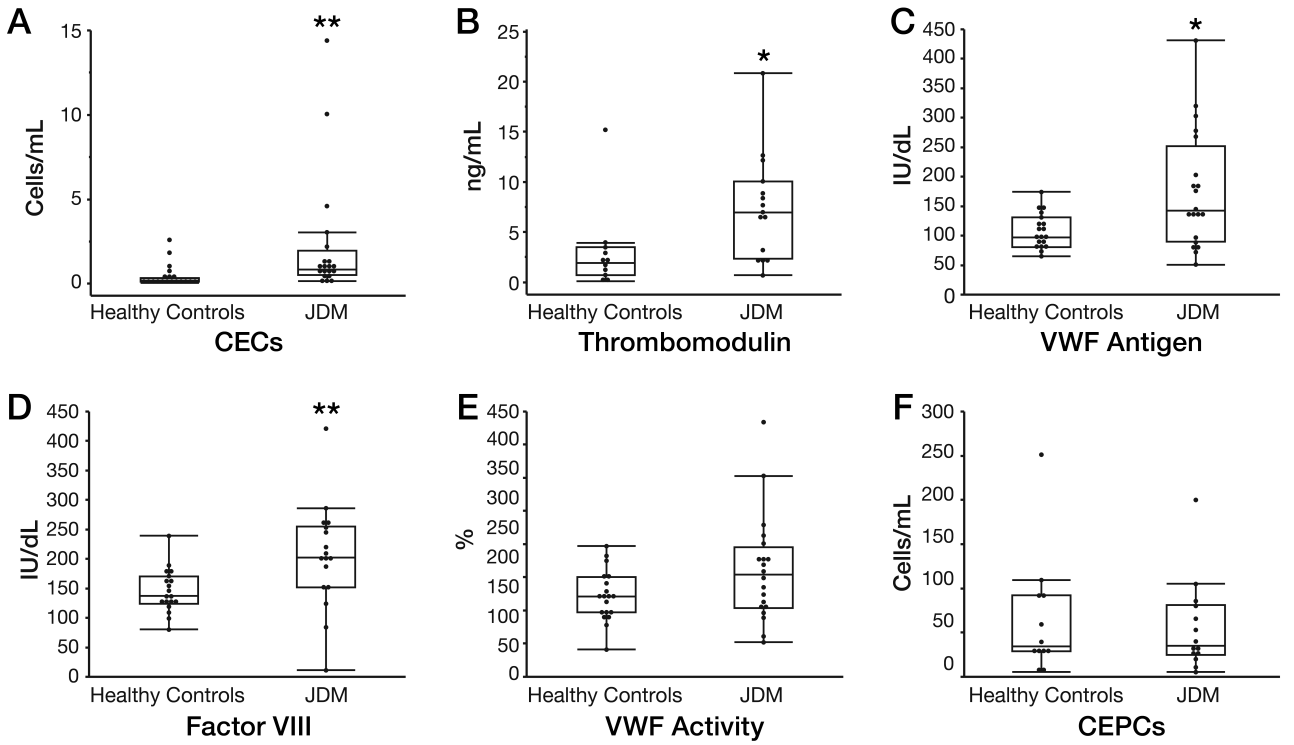

Figure 1. Endothelial cells and endothelial markers in patients with JDM and healthy control subjects. Box and whisker plots show the median values. Interquartile range (25–75%) within the boxes and the 5% and 95% are also shown, in control subjects vs patients with JDM for the following endothelial cells and markers: (A) CEC, (B) thrombomodulin, (C) vWF antigen, (D) factor VIII, (E) vWF activity, and (F) CEPC. *p < 0.05, **p < 0.01 for JDM vs controls. JDM: juvenile dermatomyositis; CEC: circulating endothelial cells; vWF: von Willebrand factor; CEPC: circulating endothelial progenitor cells.

There was no significant correlation of the number of CEC, CEPC, P-selectin levels, or thrombomodulin levels with any of the other endothelial markers.

We assessed the relationship of endothelial cells and markers with myositis disease activity measures and selected significant associations as shown in Figure 2. The number of CEC significantly correlated with the Myositis Disease Activity Assessment Tool (MDAAT) pulmonary visual analog scale (VAS) activity, which involved assessment of dyspnea, dysphonia, and interstitial lung disease, including pulmonary function testing ($r_s = 0.56$, $p = 0.001$; Figure 2A). The number of CEPC correlated inversely with MDAAT muscle VAS activity, and physical function, as assessed by the (Childhood) Health Assessment Questionnaire (CHAQ; $r_s = –0.52$ to $–0.53$, $p = 0.054–0.055$; Figures 2B–2C). The vWF antigen correlated with patient's/parent's global activity and extramuscular VAS activity, as well as with cutaneous VAS activity ($r_s = 0.47–0.54$, $p = 0.014–0.046$; Figure 2D–2F). P-selectin correlated with the serum levels of aldolase ($r_s = 0.60$, $p = 0.019$).

These measures did not correlate with the number of endothelial cells (CEC, CEPC) or with circulating levels of endothelial markers assessed: Manual Muscle Testing-8, Childhood Myositis Assessment Scale, Muscle DAS, Skin DAS, and STIR MRI muscle edema scores. None of the endothelial markers correlated with NFC density.

Regarding laboratory data, erythrocyte sedimentation rate correlated with vWF antigen and activity, and factor VIII ($r_s = 0.63–0.82$, $p = 0.0002–0.017$), and correlated inversely with P-selectin ($r_s = –0.56$, $p = 0.048$). White blood cell count and platelet count did not correlate with endothelial cells or markers.

There was no correlation of Physician Global Damage or of Myositis Damage Index Extent or Severity of Damage scores with any of the endothelial cells or markers. Patients with a longer delay to diagnosis (> 4 mos) had higher numbers of CEC than patients with a shorter delay [1.12 (0.73–3.83) vs 0.56 (0.22–0.91), $p < 0.040$]. The number of CEPC inversely correlated with endocrine damage severity in the Myositis Damage Index ($r_s = –0.56$, $p = 0.039$). MRI T1 muscle damage scores inversely correlated with CEPC cells ($r_s = –0.53$, $p = 0.051$). None of the other endothelial markers correlated with T1 MRI. Brachial artery FMD did not differ between patients with JDM and healthy controls, and did not correlate with endothelial markers in patients with JDM (data not shown). Fasting serum insulin positively correlated with vWF antigen and factor VIII level ($r_s = 0.50–0.68$, $p = 0.039–0.005$). Total and low-density lipoprotein (LDL) cholesterol levels significantly inversely correlated with CEPC ($r_s = –0.68$ to $–0.71$, $p = 0.007–0.010$).

These variables were significantly increased in patients with JDM compared with healthy controls (Figures 3A–C): serum IL-10 [JDM 37.1 (26.0-64.6), healthy controls 19.8

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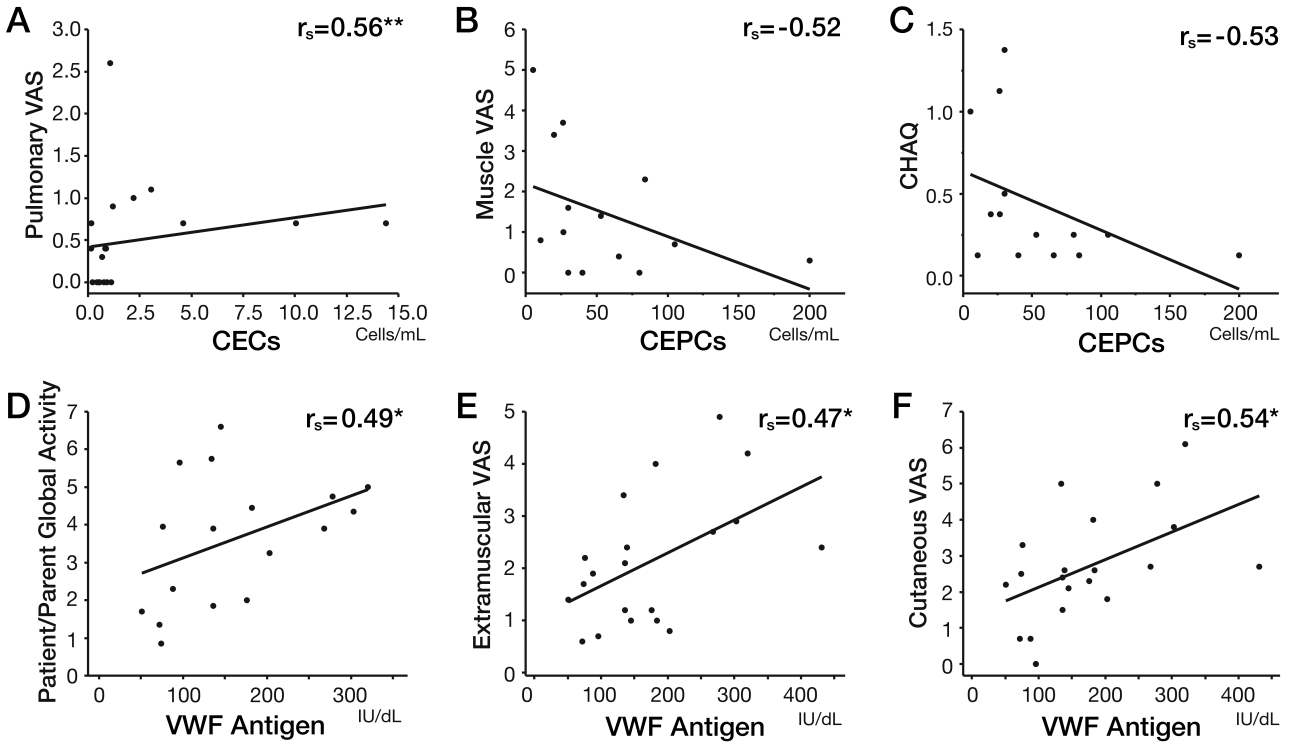

Figure 2. Correlations between endothelial cells/markers and disease activity measures in patients with juvenile dermatomyositis. Spearman rank correlations (r_s) between (A) CEC and pulmonary VAS activity, (B) CEPC and muscle VAS activity, (C) CEPC and CHAQ, (D) vWF antigen and patient's/parent's global activity score, (E) vWF antigen and extramuscular VAS activity, and (F) vWF antigen and cutaneous VAS activity. *p < 0.05. **p < 0.01. CEC: circulating endothelial cells; CEPC: circulating endothelial progenitor cells; CHAQ: Childhood Health Assessment Questionnaire; VAS: visual analog scale; VWF: von Willebrand factor.

(14.5–23.1) pg/ml], IP-10 [JDM 100.7 (53.1–182.7), healthy controls 18.4 (12.7-35.5) pg/ml], and MCP-1 [JDM 918.4 (296.8–1303.0), healthy controls 383.6 (257.5–558.1) pg/ml]. These cytokines/chemokines correlated with disease activity measures. IL-10 correlated with physician's global activity (r_s = 0.53, p = 0.019), patient's/parent's global activity (r_s = 0.47, p = 0.043), MDAAT extramuscular activity (r_s = 0.52, p = 0.023), serum alanine aminotransferase (ALT; r_s = 0.50, p = 0.031), and total DAS (r_s = 0.52, p = 0.026). IP-10 correlated with MDAAT Muscle VAS (r_s = 0.52, p = 0.021), MDAAT extramuscular activity (r_s = 0.52, p = 0.021), total DAS (r_s = 0.51, p = 0.030), patient's/parent's global activity (r_s = 0.46, p = 0.048), CHAQ (r_s = 0.49, p = 0.048), and serum ALT (r_s = 0.47, p = 0.041). MCP-1 correlated with total DAS (r_s = 0.48, p = 0.043). Other serum cytokines/chemokines were not significantly different between JDM and controls, including levels of serum IFN-α (43.5 pg/ml vs 25.7 pg/ml, p = 0.63). The vWF antigen significantly correlated with IL-10 (r_s = 0.81, p = 0.0001; Figure 3D) and with eotaxin, IP-10, and MCP-1 (r_s = 0.65–0.76, p = 0.0006–0.007), and vWF activity significantly correlated with IL-10 (r_s = 0.82, p < 0.0001), IP-10 (r_s = 0.64, p = 0.007; Figure 3E), eotaxin, and MCP-1 (r_s = 0.66–0.70, p = 0.002–0.003). CEC and CEPC did not correlate with any cytokines/chemokines. Thrombomodulin correlated with IL-4 (r_s = 0.52, p = 0.046).

DISCUSSION

The findings of our study demonstrate that endothelial biomarkers are frequently altered in the peripheral blood of patients with JDM and are associated with myositis disease activity. The number of CEC (a marker of endothelial damage[4]), levels of thrombomodulin (an angiogenic factor[25]), and vWF antigen and factor VIII, which are associated with endothelial dysfunction[26], are increased in patients with JDM compared to healthy individuals. Increased numbers of CEC and increased levels of other endothelial activation markers have also been observed in patients with other inflammatory and systemic rheumatic diseases[5,6,7,8,9,27,28]. Plasma vWF antigen has previously been reported to be elevated in the peripheral blood of adult and JDM patients who have active disease[29], and serum P-selectin levels, related to leukocyte recruitment at sites of vascular injury, was significantly increased in adult patients with DM[30], although we did not see an elevation in our JDM population. We also observed a correlation of some endothelial markers with each other, including a strong relationship among vWF antigen, vWF activity, and factor VIII, which are in the same activation pathway[31].

The number of CEPC was not altered in patients with JDM compared to healthy subjects in our study, or in another report of JDM[13]. We also did not examine the functional

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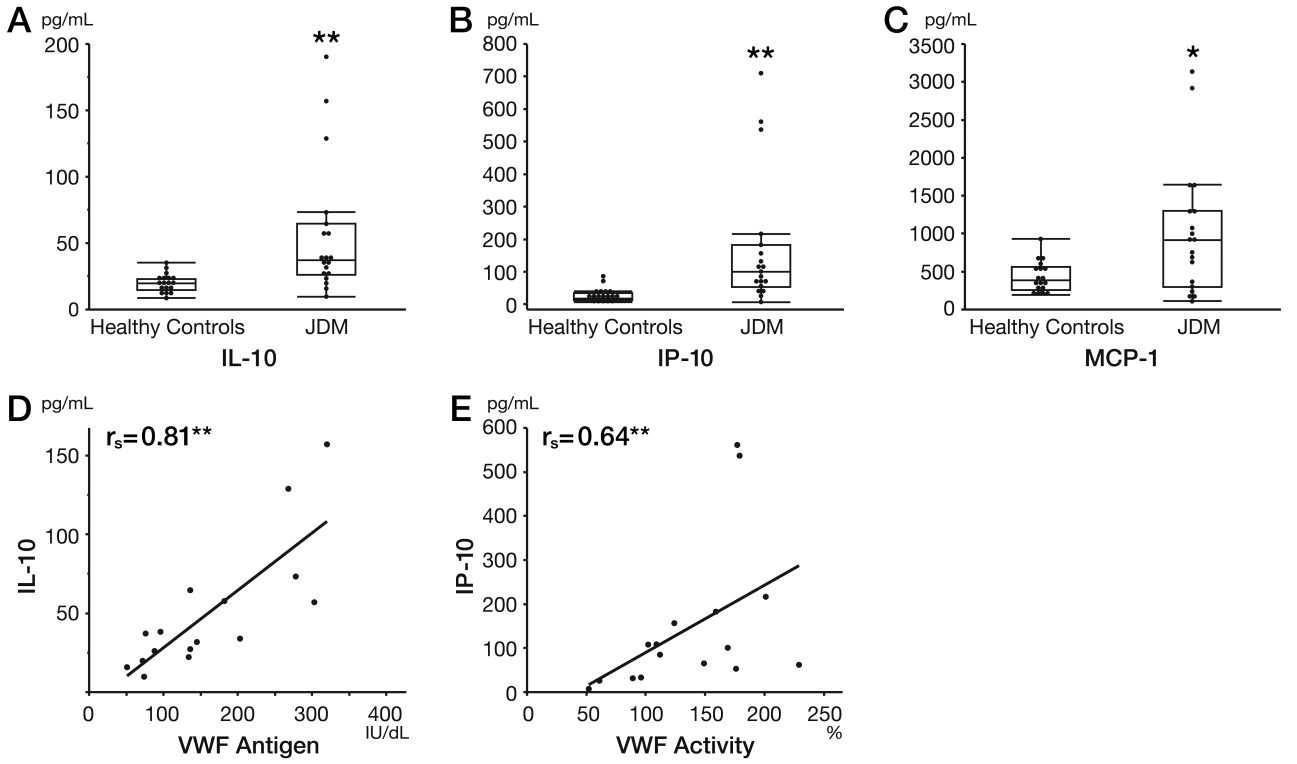

Figure 3. Cytokines/chemokines in patients with JDM and healthy control subjects and correlations between endothelial cells/markers and cytokines/chemokines. Box and whisker plots show the median values. Interquartile range (25–75%) within the boxes and the 5% and 95% are also shown, in healthy control subjects vs patients with JDM for the following cytokines and chemokines: (A) IL-10, (B) IP-10, and (C) MCP-1. *p < 0.05 and **p < 0.01 for JDM vs controls. Spearman's rank correlations (r_s) among endothelial cells/markers vs cytokines and chemokines in patients with JDM: (D) vWF antigen and IL-10, and (E) vWF activity and IP-10. **p < 0.01 for Spearman rank correlation coefficients. JDM: juvenile dermatomyositis; CEC: circulating endothelial cells; vWF: von Willebrand factor; CEPC: circulating endothelial progenitor cells; IL-10: interleukin 10; IP-10: interferon-gamma inducible protein-10; MCP-1: monocyte chemotactic protein-1.

capacity of CEPC to differentiate into mature cells. In contrast, in adult PM, CEPC numbers have been observed to be decreased[12], similar to patients with SLE[9,11,32], and to have decreased ability to differentiate into mature endothelial cells[12,32,33]. This decrease in CEPC numbers, maturation, and function correlates with type I IFN and IL-18 serum activity[9,12,32,33]. The lack of decrease of CEPC in most patients with myositis, despite a type I IFN response, as evidenced by increases in serum type I IFN-inducible cytokines and chemokines, may relate to deposition of CEPC in affected muscle tissue[34], or to a combination of anti- and proangiogenic factors in the muscle tissue and periphery that may affect CEPC migration and detection[3,35,36].

Both CEC and vWF antigen are increased in JDM peripheral blood and both correlated with extramuscular disease activity in our study, which mainly consisted of pulmonary and cutaneous features, but they did not correlate with measures of muscle activity or damage. Higher vWF levels have been previously associated with some adult DM symptoms, including weakness, fatigue, fever, and elevated muscle enzymes[37] and with disease flare in JDM[29]. Plasma thrombomodulin levels were previously found to be higher in adult patients with DM with interstitial lung disease[38]. These reports suggest that endothelial markers may be associated with disease activity and vascular inflammation of DM, and our results also indicated that these endothelial markers correlated with extramuscular disease activity of JDM, including in the skin.

In contrast, the number of CEPC also inversely correlated with functional disability measured by the CHAQ, as well as with MDAAT muscle VAS. The inverse relationship of CEPC with muscle function and disease activity suggests blood vessel regeneration may be diminished or there may be a functional disturbance in repair mechanisms during active disease. The correlation of CEPC with measures of disease activity was not observed in the study by Xu, *et al*[13], which may be related to differences in the subsets of CEPC examined or to differences in disease duration or therapy. The lack of increased CEPC in the peripheral blood, but correlation of circulating endothelial activation markers with skin and extramuscular activity, is consistent with findings in the muscle of patients with JDM observed by Baumann, *et al*[39]. In JDM muscle, endothelial cell activation is associated with early myogenesis, but an absence of increased endothelial progenitor cells suggests they are not contributing to the vascular repair process[39].

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CEPC also correlated inversely with endocrine and MRI muscle damage, which contrasted to childhood SLE in which CEPC did not correlate with clinical damage[32]. In patients with JDM, we found CEPC also inversely correlated with metabolic variables, including serum lipids, but did not relate to brachial artery FMD, a functional measure of vascular damage. The number of CEPC has been reported to inversely correlate with conventional cardiovascular risk factors, including total and LDL cholesterol. CEPC correlate with coronary atherosclerosis, but are inversely correlated with metabolic syndrome in patients with SLE[32,40]. These metabolic factors, by modulating the levels of oxidative stress, nitric oxide activity, or other physiologic processes, could directly influence the mobilization or half-life of CEPC and lead to depletion of a presumed finite supply of CEPC[41].

Type I IFN have an important role in IIM pathogenesis and the severity of vasculopathic changes in affected tissues[3,35]. IL-10, IP-10 (CXCL10), and MCP1 (CCL2) are significantly increased in patients with JDM compared with healthy controls, as previously reported[42,43]. The vWF antigen and its activity positively correlated with type I IFN cytokines/chemokines, which also have been reported to correlate with IIM extramuscular disease activity[43,44]. Our data did not show a difference in other serum cytokines, between JDM and healthy controls, including IFN-α, probably because of a lack of sensitivity of the assay used. Type I IFN induces antiangiogenic properties in CEPC, through an IFN/IL-18 axis[12], consistent with several studies demonstrating toxic, antiproliferative, and antiangiogenic effects of type I IFN toward endothelial cells[45]. On the other hand, the role of the proangiogenic activity of myogenic progenitor cells appears to be driven by type I IFN, resulting in the stimulation of vessel remodeling and muscle recovery in JDM[46].

There are some limitations to our study. The number of patients with JDM was relatively small. We could not evaluate these endothelial markers by myositis autoantibody status, because the number of patients was too small. Second, a single study visit precluded evaluation of the progression of vascular damage or changes in these biomarkers over time. Patients included in our study were not new-onset patients and received varying therapies; therefore, we could not evaluate the effects of treatment. Finally, endothelial activation markers could not be correlated with muscle biopsy microvascular changes, because only 2 patients in our study had a muscle biopsy available at the time of diagnosis with information on their biopsy features, and these were prevalent cases.

Markers of endothelial function, including CEC, vWF, and thrombomodulin, were increased in patients with JDM and correlate with extramuscular disease activity. CEPC were not increased in the peripheral blood of patients with JDM, but CEPC possibly correlate inversely with muscle activity measures, and with muscle and endocrine damage. These findings suggest a functional disturbance in repair mechanisms.

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