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Original article

Siglec-1 expression on monocytes is associated with the interferon signature in juvenile dermatomyositis and can predict treatment response

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Abstract

Objective. JDM is a rare chronic immune-mediated inflammatory disease with a predominant role for type I IFN responses. We aimed to determine the potential of Siglec-1 expression on monocytes as a novel IFN-inducible biomarker for disease activity monitoring and prediction of treatment response in patients with JDM.

Methods. Siglec-1 was measured by flow cytometry on circulating monocytes of 21 newly diagnosed JDM patients before start of treatment and, for 10 of these, also during follow-up. The expression levels of five type I IFN-stimulated genes, MX1, IFI44, IFI44L, LY6E and IFIT3, were measured by RT-qPCR to determine the IFN signature and calculate an IFN score. IFN-inducible plasma proteins CXCL10 and galectin-9 were measured by multiplex immunoassay.

Results. Siglec-1 and IFN score were increased in JDM patients compared with controls and correlated with clinical disease activity. Stratification of patients by Siglec-1 expression at diagnosis identified those with high Siglec-1 expression as having a higher risk of requiring treatment intensification within the first 3 months after diagnosis (55% *vs* 0% of patients, $P = 0.01$). Siglec-1 expression strongly correlated with plasma levels of previously validated biomarkers CXCL10 ($r_s = 0.81$, $P < 0.0001$) and galectin-9 ($r_s = 0.83$, $P < 0.0001$), and was superior to the IFN score in predicting treatment response (area under the curve 0.87 *vs* 0.53, $P = 0.01$).

Conclusion. Siglec-1 on monocytes is a novel IFN-inducible biomarker in JDM that correlates with clinical disease activity and identifies patients at risk for a suboptimal treatment response. Further studies are required to validate these findings and their clinical potential.

Key words: Siglec-1, biomarkers, interferon signature, dermatomyositis, disease activity, predictor

BASIC SCIENCE

Rheumatology key messages

- Siglec-1 expression reflects the IFN signature and correlates with clinical disease activity in juvenile dermatomyositis.
- High levels of Siglec-1 at disease onset identify patients at risk for requiring treatment intensification.
- Siglec-1 significantly outperforms the type I IFN score in predicting suboptimal treatment response.

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Introduction

JDM is a rare paediatric chronic immune-mediated inflammatory disease typically characterized by symmetrical proximal muscle weakness and skin inflammation, including heliotrope rash, Gottron's papules and photosensitive rash [1]. A gap of knowledge concerning the appropriate treatment strategy based on the pathogenesis of this disease still poses a challenge for optimal clinical management of patients. Current treatment guidelines recommend immunosuppression for at least two years, combining prednisone and methotrexate. Considering the heterogeneity between patients in clinical presentation and disease course, a personalized treatment strategy could be beneficial in order to avoid over- and undertreatment [2]. Identification of biomarkers that can be used to stratify patients at disease onset and monitor disease activity and treatment response during follow-up is therefore crucial to personalize treatment and improve outcomes of patients with JDM.

Type I IFNs play an important role in the pathogenesis of various chronic immune-mediated inflammatory diseases such as SLE, DM, multiple sclerosis (MS), RA, primary SS (pSS) and SSc [3–5]. Therefore, targeting IFN is considered a promising therapeutic strategy in these diseases. IFNα is mainly secreted by plasmacytoid dendritic cells (pDCs), whereas IFNβ is predominantly produced by other cells, including myeloid cells, fibroblasts, phagocytes and epithelial cells [4]. Indirect measurements of type I IFN activity, using expression levels of IFN-stimulated genes (ISGs), have been proven to be more sensitive than direct detection of IFNs using conventional methods [6, 7]. Previous studies demonstrated that increased expression of type I ISGs, commonly referred to as 'the IFN signature', in blood cells and muscle tissue of juvenile and adult DM patients correlated with disease activity [8–13]. Furthermore, in longitudinal studies of adult and juvenile DM patients the type I IFN signature in blood changed with disease activity and therapy response [10, 14]. A phase 1b randomized placebo-controlled trial assessing sifalimumab, an anti-IFNα monoclonal antibody, in DM and polymyositis patients showed a positive correlation between neutralization of the IFN signature and improvement of manual muscle testing scores [15]. The type I IFN signature could, thus, possibly serve as a biomarker for monitoring disease activity and response to therapy in inflammatory myopathies such as JDM.

However, measurement of ISG expression is a relatively time-consuming and labour-intensive method, which hampers its suitability for diagnostic purposes and for guiding treatment in daily practice. For routine diagnostics, alternative, easily implementable methods may therefore be preferred, such as the measurement of IFN-inducible serum proteins or cell-bound proteins. Moreover, measures that are able to integrate both type I IFN activity and other disease-specific dominant inflammatory pathways may yield more comprehensive results to reliably monitor disease activity and predict treatment response.

One of the most strongly IFN-inducible markers is sialic acid-binding Ig-like lectin 1 (Siglec-1) (sialoadhesin, CD169). Siglec-1 is a monocyte/macrophage-restricted adhesion molecule that can bind to granulocytes, erythrocytes, B cells, and to CD43 on T cells [16, 17]. Increased Siglec-1 expression has been observed in SLE [7, 18], RA [19], MS [20] and pSS [21]. Furthermore, the frequency of Siglec-1 positive cells within $CD14^+$ blood monocytes correlated with disease activity in SLE patients [7] and was used to classify patients with a progressive form of MS [20]. Siglec-1 could thus be a suitable biomarker for monitoring disease activity in chronic immune-mediated inflammatory diseases. Currently, there are no data of Siglec-1 expression on blood monocytes and its correlations with disease activity, the type I IFN signature and other IFN-inducible biomarkers in JDM patients.

Here, we aimed to determine the potential of Siglec-1 expression on circulating monocytes as a novel biomarker for disease activity monitoring and prediction of treatment response in patients with JDM, as well as its correlation with the type I IFN response. These findings may aid understanding of the biologic basis of disease heterogeneity in JDM, help develop a personalized treatment strategy and substantiate the use of novel anti-IFN treatments.

Patients and methods

Participants

This is a multicentre study with six participating paediatric rheumatology centres in the Netherlands. A total of 21 JDM patients with probable or definite JDM according to Bohan and Peter criteria [22, 23] and/or the revised criteria for diagnosis of JDM [24, 25] were included between June 2015 and August 2019 at disease onset. Blood samples and clinical disease status were obtained from all patients at disease onset before start of treatment and in 10 of these 21 patients also during follow-up. JDM patients were classified into three groups based on clinical status at the time of blood sampling: patients with active disease before start of treatment ('onset'), patients with active disease while receiving medication ('active on medication') and patients with clinically inactive disease, as defined by the Paediatric Rheumatology International Trials Organisation (PRINTO) criteria for clinically inactive disease in JDM [26, 27], regardless of medication ('remission on/off medication'). All patients received initial treatment consisting of corticosteroids and methotrexate, in accordance with the SHARE guidelines [28], except for one patient who received no treatment due to very mild symptoms and one patient who received high-dose corticosteroids and IVIG but no methotrexate. Intensification of treatment was defined as an increase in dose of medication or the addition of new immunosuppressive medication within 3 months after diagnosis.

For the evaluation of type I ISG expression and Siglec-1 expression on monocytes, seven patients with

non-autoimmune muscle disease [Duchenne muscular dystrophy (DMD)] were included as disease controls. A healthy control group consisted of six children and nine adults. This study was approved by the institutional ethics committee of UMC Utrecht (METC 15–191) and conducted according to the Declaration of Helsinki. Age-appropriate written informed consent was obtained prior to study inclusion.

Disease activity measures

The Childhood Myositis Assessment Scale (CMAS; scale 0–52 [29]) was used to assess muscle disease activity and the physician's global assessment score (PGA; scale 0–10 [30]) was used to determine overall disease activity, including skin rash. In addition, Cutaneous Assessment Tool (CAT) scores measuring the severity of skin disease (scale 0–116) were recorded in the majority of patients [31]. Muscle enzymes, including creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were used as basic laboratory parameters for disease activity in JDM patients. ESR and CRP were also included as standard inflammation markers. Myositis-specific antibodies (MSA) were measured by line blot at disease onset.

CXCL10 and galectin-9 measurements

Blood was collected in sodium-heparin tubes. Plasma was spun down and aliquoted within 4 h after collection, and subsequently stored at −80°C until analysed. Multiplex immunoassay technology (xMAP, Luminex) was used to detect plasma levels of CXCL10 and galectin-9 simultaneously, as described previously [32].

Type I interferon-stimulated genes measurements

The expression levels of 5 type I IFN-stimulated genes, MX1, IFI44, IFI44L, LY6E and IFIT3 [33, 34], were measured by RT-qPCR to determine the IFN signature and calculate an IFN score. A detailed description of the methods can be found in Supplementary Data S1, available at *Rheumatology* online.

Siglec-1 expression on monocytes

Siglec-1 expression on monocytes was measured by flow cytometry, of which the protocol is provided in Supplementary Data S1, available at *Rheumatology* online.

Statistical analysis

The data were analysed by IBM SPSS statistics 25 and GraphPad Prism 8.3. Patient characteristics are presented as median, interquartile range (IQR) and percentages as appropriate. Correlations were determined by the Spearman's rank correlation coefficient. For comparisons between two groups, the Mann–Whitney *U* test was used for continuous variables and the Fisher's exact test for categorical variables. For comparisons between three or more groups, the Kruskal–Wallis test (unpaired data) and Friedman test (paired data) were used, with *post hoc* Dunn's test. Area under the receiver operating characteristic (ROC) curve (AUC) was used to determine the discriminative value of biomarkers for patients needing intensification of treatment. Cut-off values were determined based on the maximum Youden's Index and a minimum sensitivity of 80%. The method of DeLong *et al.* [35] was used for pairwise comparison of the AUCs. The 95% confidence intervals for sensitivity, specificity, negative predictive value and positive predictive value were calculated using Wilson's method. A two-sided *P*-value ≤ 0.05 was considered statistically significant.

Results

Patient characteristics

The characteristics of all 21 JDM patients and seven DMD patients are shown in Table 1. Median time elapsed from disease onset to sampling in the active on medication group was 74 days (IQR 42–113) and from disease onset to sampling in the remission on/off medication group 519 days (IQR 304–1137). The percentage of females in JDM patients was 57.1%. The median age of JDM patients in the onset group, the active on medication group and the remission on/off medication group was comparable (8.1, 8.7 and 9.1 years, respectively). The median age in the DMD group was 11.4 years. As expected, disease activity was highest in the onset group [median CMAS score 22.5 (IQR 15.3–34); median PGA score 7.5 (IQR 6–8); median CAT score 5 (IQR 2.8–7)], followed by the active on medication group [median CMAS score 38 (IQR 28.8–45.8); median PGA score 2 (IQR 1.5–3); median CAT score 1 (IQR 0–3.5)] and the remission on/off medication group [median CMAS score 52 (IQR 50–52); median PGA score 0 (IQR 0–0); median CAT score 0 (IQR 0–0)]. This pattern also applied to the standard laboratory markers. The frequency of prednisolone and methotrexate use was highest in the active on medication group (100%, 90%). In the remission on/off medication group, one patient was off medication. Five of the 21 JDM patients were positive for MSA, including one with anti-NXP2, one with anti-NXP2 and anti-Jo1, one with anti-PL7, one with anti-PL12 and one with anti-TIF1gamma.

The type I IFN signature is upregulated in JDM and decreases upon treatment

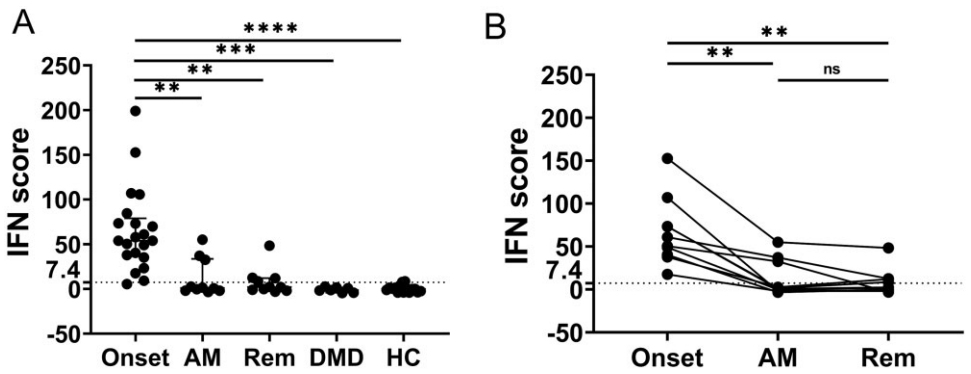
To first establish the type I IFN signature within our cohort, RNA expression of five type I ISGs was measured in total PBMCs. The integrated IFN score as well as expression of the individual genes (MX1, IFI44, IFI44L, LY6E and IFIT3) were significantly higher in the onset group compared with the active on medication group, the remission on/off medication group, the DMD disease-control group and healthy controls (Fig. 1A and Supplementary Fig. S1, available at *Rheumatology*

Table 1 Patient characteristics

Characteristics	JDM ($n = 21$)			Duchenne muscular dystrophy ($n = 7$)
	Onset ($n = 21$)	Active on medication ($n = 10$)	Remission on/off medication ($n = 10$)	
Age (years), median (IQR)	8.1 (4.0–12.0)	8.7 (4.4–14.5)	9.1 (5.8–16.9)	11.4 (7.9–15.9)
Sex, *n* (%) female	12 (57.1)	5 (50)	5 (50)	0 (0)
CMAS score (scale of 0–52), median (IQR)	22.5 (15.3–34.0)	38 (28.8–45.8)	52 (50.0–52.0)	NA
Physician's global assessment (scale of 0–10), median (IQR)	7.5 (6.0–8.0)	2 (1.5–3.0)	0 (0–0)	NA
CAT score (scale of 0–116), median (IQR)	5.0 (2.8–7.0)	1.0 (0–3.5)	0 (0–0)	NA
Medication, *n* (%)				
Prednisolone	0	10 (100)	1 (10)	0
Prednisolone dosage (mg/kg/day), median (IQR)	0 (0–0)	0.6 (0.50–1.06)	0.68 (0.68–0.68)	0 (0–0)
Methotrexate	0	9 (90)	8 (80)	0
Hydroxychloroquine	0	1 (10)	1 (10)	0
Rituximab	0	1 (10)	0	0
IVIG	0	1 (10)	0	0
CK (IU/litre), median (IQR)	2536 (243.5–5379.0)	56 (35.5–160.0)	94 (65.5–129.0)	NA
LDH (IU/litre), median (IQR)	637 (343.3–884.3)	286 (223.3–435.3)	245 (154.3–277.5)	NA
AST (IU/litre), median (IQR)	157.5 (50.0–316.8)	32 (19.8–41.0)	34 (24.5–41.0)	NA
ALT (IU/litre), median (IQR)	63.0 (23.5–116.0)	29 (21.0–59.3)	23 (15.3–36.0)	NA
CRP (mg/litre), median (IQR)	1.7 (0.5–5.0)	0.50 (0.2–0.8)	0.8 (0.7–1.3)	NA
ESR (mm/h), median (IQR)	18.5 (7.0–33.0)	5 (2.0–9.30)	5 (2.8–8.3)	NA

ALT: alanine transaminase; AST: aspartate transaminase; CAT: Cutaneous Assessment Tool; CK: creatine kinase; CMAS: Childhood Myositis Assessment Scale; IQR: interquartile range; LDH: lactate dehydrogenase; NA: not applicable.

Fig. 1 Type I IFN signature in JDM patients and controls



(**A**) The IFN score in the onset group, AM group, Rem group, DMD group, and HCs. (**B**) Changing of the IFN score during longitudinal follow-up. Horizontal lines in (**A**) represent medians and interquartile ranges. The dotted line in (**A**) and (**B**) depicts the mean + 2SD of the IFN score in healthy controls. Connecting lines in (**B**) represent individual patients. Multiplicity-adjusted *P*-values were determined by Kruskal–Wallis test (**A**) and Friedman test (**B**) with Dunn's *post hoc* test for multiple comparisons. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; AM: active on medication; DMD: Duchenne muscular dystrophy; HC: healthy control; ns, not significant; Rem: remission on/off medication.

online). At disease onset, one JDM patient had a negative IFN score (5.4). This patient had mild muscle involvement (CMAS score 48) and low global disease activity (PGA score 1). Negative IFN scores were found in all DMD patients. One adult and one child in the healthy control group had borderline positive IFN scores (8.6 and 7.8). Among JDM patients, the IFN score decreased over time after start of treatment (Fig. 1B). Expression levels of

single ISGs over time all showed a similar pattern of decline under treatment (Supplementary Fig. S2, available at *Rheumatology* online).

In conclusion, JDM patients have significantly elevated IFN scores at disease onset, which decrease after start of treatment, coinciding with decreasing disease activity.

Siglec-1 expression on $CD14^+$ monocytes is increased in JDM and decreases upon treatment

Because Siglec-1 expression on monocytes was previously shown to be related to ISGs in SLE [18], we assessed Siglec-1 expression on $CD14^+$ monocytes of JDM patients. Illustrative FACS plots obtained from two JDM patients and one healthy control are shown in Fig. 2A, indicating strongly upregulated Siglec-1 expression on monocytes at JDM disease onset. Overall, Siglec-1 median fluorescent intensity (MFI) on $CD14^+$ cells was significantly elevated in JDM patients in the onset group compared with the active on medication group, the remission on/off medication group, the DMD group and the healthy controls (Fig. 2B). The frequency of Siglec-1^+ cells within $CD14^+$ monocytes showed a similar pattern (Fig. 2B). After start of treatment, both Siglec-1 MFI and frequency of Siglec-1^+ cells within $CD14^+$ monocytes decreased over time (Fig. 2C).

As expected, Siglec-1 MFI was significantly associated with the IFN score in JDM patients ($r_s = 0.88$, $P < 0.0001$; Fig. 2D). Although a decrease in Siglec-1 levels and IFN scores was observed in all patients upon treatment, we noticed that two out of 10 JDM patients with longitudinal follow-up still had clearly elevated levels of Siglec-1 MFI (929 and 602) with positive IFN scores (48 and 12) during clinically inactive disease. These two patients did not have any clinical symptoms at time of blood sampling or afterwards, but did receive a higher total dose of methotrexate and prednisolone in the initial phase of the disease compared with the other patients in the remission on/off medication group. One patient was positive for anti-NXP2.

In conclusion, JDM patients have significantly elevated Siglec-1 expression at disease onset, which decreases after start of treatment and correlates with the IFN signature.

Siglec-1 expression correlates with clinical disease activity

To evaluate whether Siglec-1 expression is related to clinical disease activity, we assessed its correlations with clinical disease activity parameters CMAS, PGA and CAT.

Siglec-1 MFI strongly correlated with the CMAS ($r_s = -0.66$, $P < 0.0001$), PGA ($r_s = 0.75$, $P < 0.0001$) and CAT score ($r_s = 0.65$, $P = 0.0002$) (Fig. 3A). As expected, the IFN score also correlated with the CMAS, PGA and CAT score (Supplementary Fig. S3, available at *Rheumatology* online).

In conclusion, Siglec-1 (as well as the IFN score) significantly correlates with clinical disease activity.

Siglec-1 expression at onset identifies JDM patients at risk for suboptimal treatment response

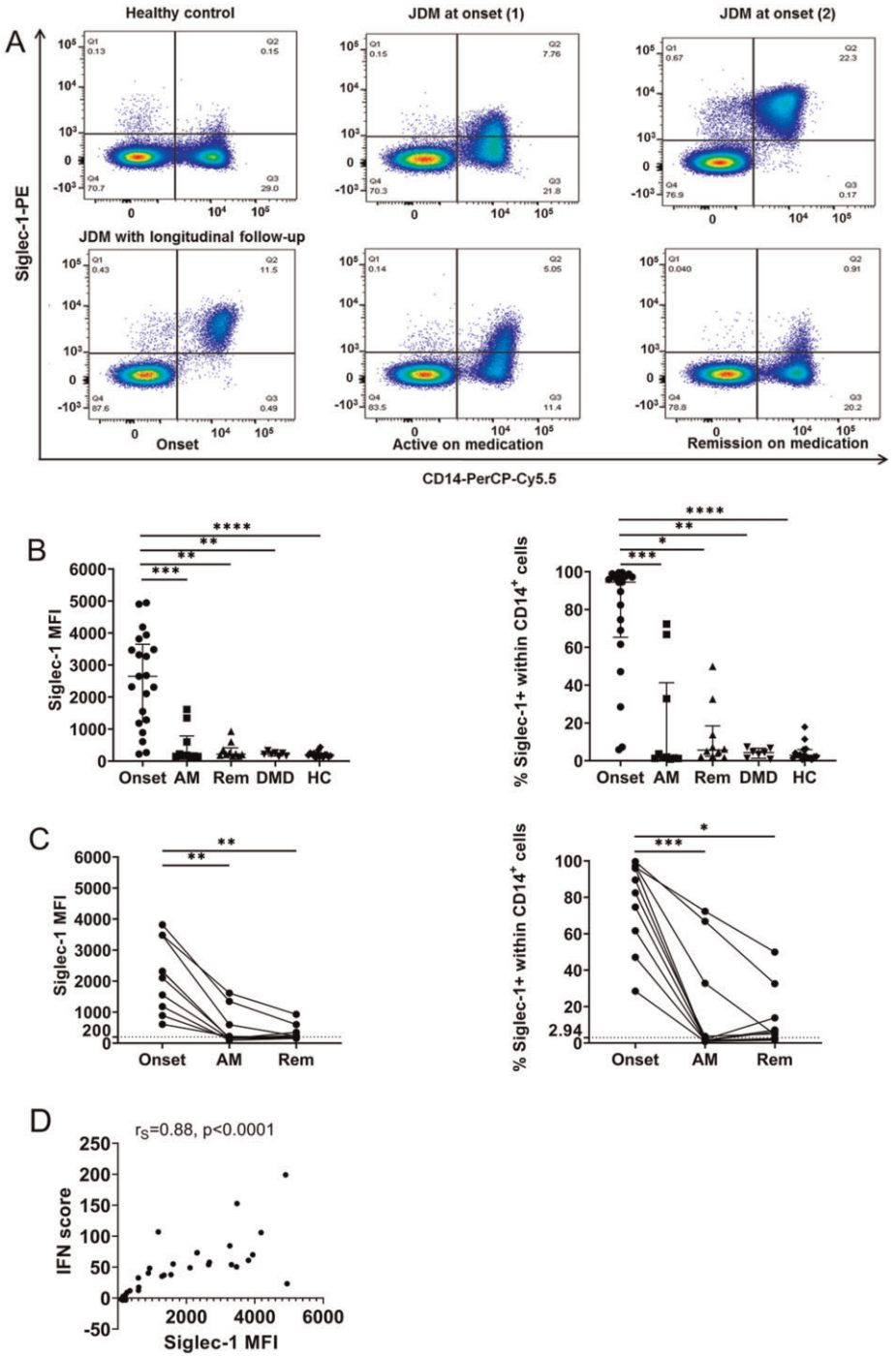
We noted that not only over time, but also at disease onset, Siglec-1 expression showed a remarkable variation among JDM patients, although in all patients, onset values were higher than the median healthy control value (MFI of 200). We therefore assessed whether high levels of Siglec-1 expression at onset could be related to and predictive for response to treatment and/or disease severity.

To this end, we stratified patients by median Siglec-1 MFI level (Siglec-1 high, MFI $\geq$2650 and Siglec-1 low, MFI $<$2650) and compared treatment response between these two groups. Importantly, patients with high Siglec-1 expression at diagnosis had a higher frequency of receiving treatment intensification within 3 months after diagnosis when compared with patients with low Siglec-1 expression (55% *vs* 0% of patients, $P = 0.01$; Fig. 3B). In contrast to Siglec-1, stratifying patients by median IFN score (IFN score high, score $\geq$53.96 and IFN score low, score $<$53.96) did not identify patients at risk for treatment intensification within 3 months (27% *vs* 30% of patients, $P = 1.00$; Fig. 3B).

When comparing clinical disease activity between the two groups, patients with high Siglec-1 expression trended towards having more severe muscle involvement [median CMAS score 17 (IQR 7.5–30.0) *vs* median score 28.5 (IQR 21.5–43.5), $P = 0.08$], and PGA was comparable [median score 8 (IQR 6–9) *vs* median score 7 (IQR 3–8), $P = 0.20$; Fig. 3C], although all patients with high Siglec-1 levels had a PGA score $\geq$5. Severity of skin disease was also comparable between the Siglec-1 high group and the Siglec-1 low group [median CAT score 6.0 (IQR 2.8–7.3) *vs* median score 4.5 (IQR 2.3–5.0), $P = 0.35$, respectively; Fig. 3C]. CK, LDH, AST and ALT were all significantly higher in the Siglec-1 high group [median CK 4126 (IQR 1385–7334) *vs* median CK 270.0 (IQR 142.0–3074) IU/litre, $P = 0.04$, median LDH 865.0 (IQR 652.5–1314) *vs* median LDH 355.0 (IQR 261.5–592.5) IU/litre, $P = 0.003$, median AST 278.5 (IQR 117.8–445.0) *vs* median AST 59.5 (IQR 40.5–208.3) IU/litre, $P = 0.02$, and median ALT 79.0 (IQR 63.0–127.0) *vs* median ALT 24.0 (IQR 20.8–68.5) IU/litre, $P = 0.03$]. The Siglec-1 high group also had a significantly higher IFN score than the Siglec-1 low group [median score 61.0 (IQR 53.9–105.7) *vs* median score 39.1 (IQR 15.4–73.2), $P = 0.05$; Fig. 3D], as expected. Within the Siglec-1 high group, patients who received treatment intensification had more severe muscle and skin involvement at disease onset than patients who did not receive treatment intensification, while PGA scores were comparable (Supplementary Fig. S4, available at *Rheumatology* online).

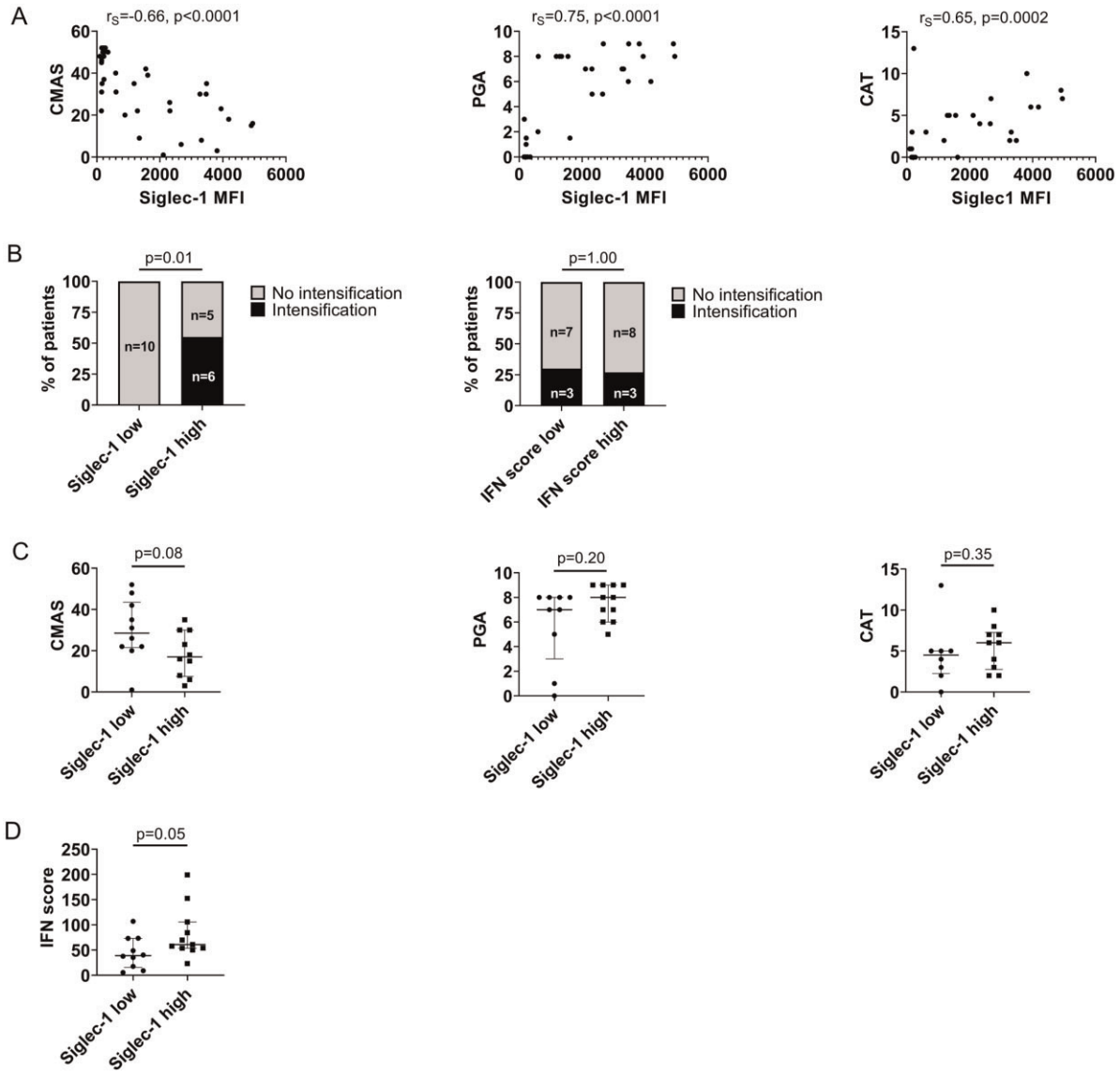
In summary, high Siglec-1 expression at onset identifies a clinically relevant subgroup of patients at risk for failure to standard treatment.

Fig. 2 Siglec-1 expression in JDM patients and controls



(**A**) Illustrative FACS plots of Siglec-1 expression on $CD14^+$ cells acquired from a healthy control (MFI 122), two JDM patients at disease onset (MFI 899 and 4903), and one JDM patient during longitudinal follow-up (onset, MFI 3469; active on medication, MFI 597; remission on medication, MFI 229). (**B**) Siglec-1 MFI on $CD14^+$ cells and the frequency of Siglec-1^+ cells within $CD14^+$ cells in the onset group, AM group, Rem group, DMD group, and HCs. (**C**) After start of treatment, both Siglec-1 MFI and frequency of Siglec-1^+ cells within $CD14^+$ cells decreased over time. (**D**) Spearman's rank correlation between Siglec-1 MFI and IFN score. Horizontal lines in (**B**) represent medians and interquartile ranges. The dotted lines in (**C**) depict the median MFI and median frequency of Siglec-1^+ cells within $CD14^+$ cells in healthy controls. Connecting lines in (**C**) represent individual patients. Multiplicity-adjusted *P*-values were determined by Kruskal–Wallis test (**B**) and Friedman test (**C**) with Dunn's *post hoc* test for multiple comparisons. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. AM: active on medication; DMD: Duchenne muscular dystrophy; HC: healthy control; MFI, median fluorescence intensity; Rem: remission on/off medication; Siglec-1, sialic acid binding Ig like lectin-1.

FIG. 3 Siglec-1 correlates with clinical disease activity and identifies a clinically relevant patient subgroup



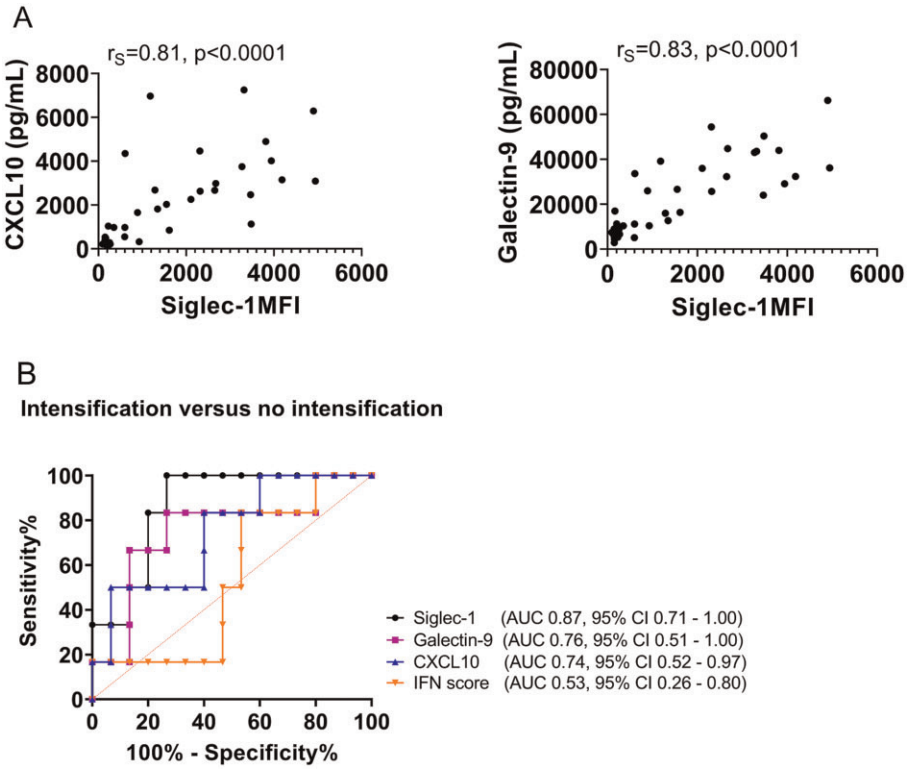
(**A**) Spearman's rank correlations between Siglec-1 MFI on $CD14^+$ cells and clinical disease activity. (**B**) Frequency of receiving treatment intensification within 3 months after diagnosis in the Siglec-1 low/high groups and the IFN score low/high groups. (**C**) Clinical disease activity in the Siglec-1 low group and the Siglec-1 high group. (**D**) IFN scores in the Siglec-1 low group and the Siglec-1 high group. Horizontal lines in (**C**) and (**D**) represent medians and interquartile ranges. *P*-values were determined by Fisher's exact test (**B**) and Mann–Whitney *U* test (**C** & **D**). CAT: Cutaneous Assessment Tool; CMAS: Childhood Myositis Assessment Scale; MFI: median fluorescence intensity; PGA: physician's global assessment; r_S: Spearman's rank correlation coefficient; Siglec-1: sialic acid binding Ig like lectin-1.

Siglec-1 expression strongly correlates with CXCL10 and galectin-9 biomarker levels, and is superior to the IFN score in predicting treatment response

We previously identified and validated IFN-inducible markers CXCL10 and galectin-9 as sensitive biomarkers for disease activity in JDM patients, with a prognostic value for response to treatment [36, 45]. We therefore assessed whether Siglec-1 showed a correlation to CXCL10 and galectin-9 (all JDM patients' samples were included in the analysis). The Siglec-1 MFI indeed significantly correlated with CXCL10 and galectin-9 ($r_s = 0.81$, $P < 0.0001$ and $r_s = 0.83$, $P < 0.0001$, respectively; Fig. 4A).

Next, we evaluated the performance and determined the optimal cut-off values of Siglec-1, CXCL10, galectin-9 and the IFN score at onset of disease for identifying JDM patients requiring treatment intensification within 3 months after diagnosis. Siglec-1 showed the best

Fig. 4 Performance of Siglec-1, galectin-9, CXCL10 and the IFN score for identifying JDM patients requiring treatment intensification



(**A**) Spearman's rank correlations between Siglec-1 MFI on $CD14^+$ cells and CXCL10 (left) and galectin-9 (right). (**B**) Area under the receiver operating characteristic (ROC) curve (AUC) of Siglec-1, galectin-9, CXCL10 and the IFN score at disease onset for identifying JDM patients requiring treatment intensification within 3 months after diagnosis. The dashed diagonal line represents the curve of a predictor with no discriminative ability (AUC 0.5). MFI, median fluorescence intensity; r_S, Spearman's rank correlation coefficient; Siglec-1, sialic acid binding Ig like lectin-1.

performance (AUC 0.87, 95% CI 0.71, 1.0), followed by galectin-9 (AUC 0.76, 95% CI 0.51, 1.0), CXCL10 (AUC 0.74, 95% CI 0.52, 0.97) and the IFN score (AUC 0.53, 95% CI 0.26, 0.80; significantly different from Siglec-1, $P = 0.01$) (Fig. 4B). A cut-off value of 2,663 for Siglec-1 MFI yielded a high sensitivity (100%) and a high negative predictive value (100%), which ensures reliable identification of patients at risk for suboptimal treatment response. Sensitivity, specificity, negative predictive value and positive predictive value of the determined cut-off values for Siglec-1, galectin-9, CXCL10 and the IFN score are shown in Table 2.

In conclusion, Siglec-1 strongly correlates with IFN-inducible markers CXCL10 and galectin-9, and outperforms the IFN score in identifying patients at risk for a suboptimal response to standard treatment.

Discussion

In this study, we have shown that in JDM patients: (i) increased Siglec-1 expression on circulating monocytes reflects the type I IFN signature and correlates with clinical disease activity; (ii) high levels of Siglec-1 at disease onset identify a subgroup of patients at risk for requiring treatment intensification; and (iii) Siglec-1 significantly outperforms the IFN score in predicting suboptimal treatment response.

Not only in JDM, but also in other diseases Siglec-1 expression has been related to disease activity and severity. In SLE patients, Siglec-1 expression correlated with disease activity and could be used to monitor response to treatment [7, 18]. Moreover, Siglec-1 had prognostic value for identifying SLE patients at risk for developing renal complications [37], and was used to classify patients with a progressive form of MS [20]. In pSS, Siglec-1 expression could characterize patients with extraglandular involvement and high disease activity [21].

In line with previous studies, we found an increased type I IFN score in the blood of patients with JDM, which strongly correlated with disease activity during longitudinal follow-up of patients [9, 10].

Because Siglec-1 expression has previously been related to the IFN signature in chronic immune-mediated inflammatory diseases such as SLE [18] and SSc [38], we hypothesized that Siglec-1 expression may be used

TABLE 2 Sensitivity, specificity, NPV and PPV of biomarkers for identifying JDM patients requiring treatment intensification

Markers	Cut-off value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)	PPV (%) (95% CI)
Siglec-1 MFI	2663	100.0 (61.0, 100.0)	73.3 (48.0, 89.1)	100.0 (74.1, 100.0)	60.0 (31.3, 83.2)
Galectin-9	36 042 pg/mL	83.3 (36.5, 99.1)	73.3 (44.8, 91.1)	91.7 (59.8, 99.6)	55.6 (22.7, 84.7)
CXCL10	2833 pg/mL	83.3 (43.6, 97.0)	60.0 (35.7, 80.2)	90.0 (59.6, 98.2)	45.5 (21.3, 72.0)
IFN score	49.65	83.3 (43.6, 97.0)	46.7 (24.8, 69.9)	87.5 (52.9, 97.8)	38.5 (17.7, 64.5)

Cut-off values for Siglec-1, galectin-9, CXCL10 and the IFN score were determined based on the maximum Youden's Index and a minimum sensitivity of 80%. The 95% CIs were calculated using Wilson's method. MFI: median fluorescent intensity; NPV: negative predictive value; PPV: positive predictive value; Siglec-1: sialic acid binding Ig like lectin-1.

as a surrogate marker for an activated IFN system in JDM. Siglec-1 expression indeed correlated strongly with the IFN score. Moreover, Siglec-1 correlated with multiple measures of disease activity and the longitudinal dynamics of Siglec-1 were shown to hold potential value for disease monitoring in the individual patient. In addition, we show here for the first time that Siglec-1 may also have prognostic value for response to treatment, outperforming the IFN score. The higher performance of Siglec-1 over the IFN score might be explained by its more integrated response to stimuli: Siglec-1 induction is related to type I as well as type II IFNs and various other inflammatory stimuli and cytokines [38–42], whereas the used IFN score is more restricted to type I IFN responsive genes. Siglec-1 may thus represent the inflammatory disease processes in JDM more comprehensively than the IFN signature.

In this study, there were two patients with remarkable levels of Siglec-1 expression and positive IFN scores during clinically inactive disease and both of these patients received higher doses of medications than the other patients. This suggests that elevated Siglec-1 levels and/or IFN scores under treatment could be a sign of ongoing sub-clinical immune activation/inflammation. As this inflammation is seemingly not resolved by the standard treatment regimen consisting of prednisone and methotrexate, these patients might rather benefit from more targeted therapies such as anti-IFN antibodies (e.g. sifalimumab) or JAK-inhibitors (e.g. ruxolitinib). Ruxolitinib was recently proposed as a new mechanism-based treatment as it led to both clinical and biological improvement in four patients with refractory DM [43] and one patient with refractory JDM [44].

Siglec-1 expression also correlated strongly with plasma levels of CXCL10 and galectin-9, which we previously validated as reliable biomarkers for disease activity in JDM and could serve as prognostic tools for treatment response [36, 45]. This current study presents Siglec-1 as an additional highly sensitive marker for disease activity and identifying patients at risk for treatment intensification. Newly diagnosed JDM patients with Siglec-1 expression above a set cut-off value may, thus, potentially benefit from more intense monitoring in the initial treatment phase to detect suboptimal response to standard treatment as soon as possible, or from more aggressive or rather targeted initial treatment. Because of this potential prognostic value at onset, and the high correlation between Siglec-1 and the type I IFN score, Siglec-1 expression may help to select patients that are most likely to benefit from novel anti-IFN treatment strategies.

Measuring surface expression of Siglec-1 by flow cytometry is less labour-intensive and time-consuming than quantification of the IFN signature by gene expression analysis, which makes it a more suitable candidate for routine diagnostics. Clinical implementation of Siglec-1 as a tool to monitor disease activity and guide treatment could enable a personalized treatment strategy in JDM patients. This study has several strengths. First, this is a prospective multicentre study, in which patients were included prior to start of treatment in order to avoid medication effects. Second, all inflammatory biomarkers were measured simultaneously in all samples. Third, with longitudinal blood samples we were able to study the relationship between biomarkers, disease activity and response to treatment in time. Despite the relatively small number of patients, especially those followed up longitudinally, due to the rarity of the disease, we were able to obtain significant results. However, future studies are required to confirm the prognostic value of Siglec-1 at onset of disease, including its cut-off value, and to assess its possible value in predicting disease flares.

In conclusion, Siglec-1 may serve as a relevant additional biomarker for monitoring disease activity and predicting treatment response in JDM. High levels of Siglec-1 expression at disease onset identified a subgroup of patients at risk for a suboptimal treatment response. Siglec-1 may, therefore, help to stratify patients for more intense treatment regimens, to identify potential candidates for anti-IFN therapy and guide anti-IFN treatment strategies.

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Data availability statement

All data and protocols of this study are available to the scientific community upon reasonable request.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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