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SIGLEC1 (CD169) is a sensitive biomarker for the deterioration of the clinical course in childhood systemic lupus erythematosus

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Abstract

Background: To analyse the validity of membrane-bound SIGLEC1 (CD169) as a sensitive biomarker for monitoring disease activity in pediatric systemic lupus erythematosus (SLE).

Methods: 27 children and adolescents with SLE were followed for a mean of 13.5 months. During consecutive routine visits SLEDAI-2k, C3, C4 and ds-DNA values were determined. Additionally, expression of SIGLEC1 on monocytes was determined by flow cytometry. The amount of PE-labelled CD169 mAb bound per monocyte was analyzed using QuantiBRITE™ PE tubes. Associations between biomarkers and the clinical course were investigated by regression analysis.

Results: In general, SIGLEC1 expression is high on SLE-derived monocytes (mean 6 359 (SD 6 056) molecules/monocyte, cut-off 2 500 molecules/monocyte), all patients with newly diagnosed SLE exhibit elevated expression (mean 13366 (SD 7 750) molecules/monocyte). Changes (Δ) in SIGLEC1 levels during the clinical course is the only biomarker that significantly correlates with the change in SLEDAI-2k ($beta_{ST} = 0.28$, $p = 0.001$). At follow-up visit, a clinically important worsening was experienced by 47.6% of patients with a Δ SIGLEC1 $>$ 2 151 molecules/cell (OR 5.31) and 72.4% with a Δ SIGLEC1 $>$ 756 molecules/cell (OR 8.90). Conversely, 36.4% of patients with a Δ SIGLEC1 $<$ -2 818 molecules/cell (OR 4.16, percentiles as cut-off criteria) and 50.0% of patients with a Δ SIGLEC1 $<$ -1 370 molecules/cell (OR 3.55, application of Youden index) showed clinical improvement. SIGLEC1 expression correlates inversely with the amount of therapeutically applied hydroxychloroquine ($p < 0.001$).

Conclusions: SIGLEC1 expression on monocytes is a sensitive biomarker for adjusting disease activity in childhood SLE and represents a promising and easily applicable tool for disease monitoring.

Keywords

Pediatric systemic lupus erythematosus, childhood, SIGLEC1, SLEDAI, biomarker, hydroxychloroquine, type 1 interferon

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Background

Disease flares characterize childhood systemic lupus erythematosus (cSLE). The subsequent improvement

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of symptoms and biomarkers is often a result of intensified drug therapy. Assessment of disease activity relies on the evaluation by the experienced care-giving physician and the use of biomarkers.[1] A detailed recording of the disease activity is crucial in order to estimate the prognosis and to manage medical treatment in a personalized way.

The pathogenesis of systemic lupus erythematosus (SLE) is multifactorial, involving the innate and the adaptive immunity by disturbing apoptotic cell clearance, cytokine synthesis as well as B- and T-cell immunity.[2] It is now well established, that type I interferons (IFN) play an important role in the initiation and perpetuation of the inflammatory processes leading to acute symptoms and chronic damages in patients with SLE.[3,4]

Traditionally, changes in anti-ds-DNA-antibody titres and complement factor levels have been used as marker for disease activity.[5,6] More recently, studies investigated whether abnormalities within the type I IFN pathways can be used as sensitive biomarkers for disease activity. Binding of type I IFNs to the type I IFN receptor (IFNAR1) initiates signalling cascades through multiple pathways involving the Janus activated kinase (JAK) signal transducers which in turn leads to phosphorylation of signal transducer and activator of transcription (STAT) proteins, resulting in the transcription of several hundreds of type I IFN-stimulated genes (ISGs).[7] However, when analysing changes within the expression levels of the ISGs in longitudinal observations of adult SLE patients, these IFN signatures failed to predict changes in disease activity.[8,9] In contrast, IFN-α directly measured in sera from patients with SLE correlated with disease activity (radioimmunoassay,[10] dissociation-enhanced lanthanide fluorescent immunoassay[11]). In line with this observation, IFN-regulated chemokines (e.g. the interferon-inducible protein-10 (IP-10)) correlated well with disease severity in longitudinal studies.[12,13]

Transcriptome analysis of monocytes from adult SLE patients revealed a dominant type I IFN signature with sialic acid-binding immunoglobulin-like lectin 1 (SIGLEC1, sialoadhesin) being a very prominent candidate for disease monitoring.[14] Members of the SIGLEC protein family are transmembrane proteins that bind sialic acid and C2-set immunoglobulin domains immune responses and regulate innate and adaptive immune cell functions.[15] SIGLEC1 is rapidly upregulated by inflammatory macrophages and modulates T cell function and activation in various inflammatory disease models (reviewed in[15]). The expression of SIGLEC1 on monocytes is increased in adult SLE patients with a first diagnosis of systemic lupus erythematosus or during active SLE disease.[14,16] Furthermore, SIGLEC1 expression on monocytes was shown to reflect lupus flares with high sensitivity and specificity and correlates with disease activity over time.[17] However, data of pediatric populations is lacking.

In the current study we analyzed the predictive value of SIGLEC1 expression as a biomarker for disease activity in an independent cohort of 27 children with SLE, which were followed over a mean of 13.5 months.

Patients and methods

27 children and adolescents with the diagnosis of SLE fulfilling at least four criteria for SLE of the American College of Rheumatology (ACR)[18] were recruited from the rheumatology section and nephrology department of the children's hospital University Medicine Charité, Berlin from October 2014 to March 2017. Patients with clinical signs of active infections were excluded. The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2k)[19,20] was determined by S L vS. Levels of SIGLEC1 on monocytes in disease control groups were retrospectively analyzed from previously collected samples. All available samples from patients with (i) arthralgias ($n = 22$) and (ii) raynaud phenomena without increase of antinuclear antibodies or association with other autoimmune phenotypes ($n = 11$), (iii) familial mediterranean fever (FMF) ($n = 7$), (iv) juvenile idiopathic arthritis (JIA) other than the systemic JIA with available JADAS-10 ($n = 20$) and (v) systemic JIA ($n = 8$) were included. If SIGLEC1 was determined several times in one patient, the first value was included in the analysis. For the analysis of patients with or without kidney involvement four newly diagnosed children with SLE were additionally recruited.

Flow cytometric detection of SIGLEC1 on monocytes

Expression of SIGLEC1 on monocytes was determined by flow cytometry using a highly standardized quantitative assay. Briefly, 25 µl of EDTA-anticoagulated whole blood was incubated with 10 µl of mouse-anti-human antibody cocktail containing phycoerythrin (PE)-labelled anti-CD169 monoclonal antibody (mAb) (labelled with a fluorochrome/protein ratio of 1:1), allophycocyanine (APC)-labelled anti-CD14 mAb and Krome Orange-labelled anti-CD45 mAb for 15 min at room temperature (RT) in the dark (all antibodies from Beckman Coulter, Krefeld, Germany). Red blood cells were then lysed by addition of 500 µl of Versa-Lysis solution (Beckman Coulter) to each reaction tube. After incubation for 30 min at RT in the dark, samples were centrifuged for 5 min at $200 \times g$ at RT. Samples were then washed once with

1000 µl PBS containing 2% fetal calf serum (FCS) and centrifuged again for 5 min at 200 × g at RT. Stained samples were acquired on a 10-color flow cytometer (Navios, Beckman Coulter) and analyzed using the Navios software.

During each analytical run, QuantiBRITE™ PE tubes (BD Biosciences) were used to convert the fluorescent channel 2 (FL2) mean fluorescent intensity (MFI) signals on CD14+ monocytes to monoclonal antibodies bound per cell (mAb/cell) values. FL2 MFI values and absolute values of PE molecules (as given by the manufacturer) for each QuantiBRITE™ bead population were used to perform linear least square regression analysis to determine the best calibration value which then was used to convert the FL2 MFI values of monocytes in the analytical sample into the amount of PE-labelled CD169 mAb bound per monocyte (mAb/cell).

The reference range for the expression of SIGLEC1 in healthy controls was determined to be less than 2 500 SIGLEC1 molecules/monocyte.

Table 1. Characteristics of pediatric SLE patients.

Characteristics	SLE patients (n = 27)
age, mean (range)	14 (2–18)
sex, n (%)	
female	24 (89%)
male	3 (11%)
ethnicity, n (%)	
Caucasian	19 (70%)
Others (not caucasian, asian, hispanic)	8 (30%)
ACR criteria at diagnosis, mean (range)	5 (4–8)
SLEDAI-2k, mean (range)	7.9 (0–44)
disease duration, mean years (range)	14 (3–19)
follow-up period, mean days (range)	412 (0–857)
organ involvement	
kidney, n (%)	7 (26%)
APS, n (%)	6 (22%)
neuropsychiatric, n (%)	2 (7%)
chilblain	1 (0%)
medication during follow-up	
oral prednisolone, n, mean dose (range)	19 (70%), mean 16 mg/day (0–200 mg/day)
methylprednisolone pulse therapy, n (%)	11 (40%)
hydroxychloroquine, n (%)	23 (85%)
azathioprine, n (%)	4 (15%)
mycophenolate mofetil, n (%)	13 (48%)
plasmapheresis, n (%)	1 (5%)
cyclophosphamide, n (%)	4 (15%)
cyclosporine, n (%)	1 (5%)

ACR – American College of Rheumatology, SLE – systemic lupus erythematosus, SLEDAI – SLE Disease Activity Index,.

Statistical analysis

Differences between SIGLEC-1 expression between disease group were calculated by the Mann-Whitney U-Test. The correlation between SIGLEC1 and parameters for disease activity (JADAS-10 for JIA patients without sJIA, CRP for sJIA and FMF) was calculated by Spearman correlation coefficients. Linear mixed models were applied to analyse the association between biomarker levels and the change in biomarkers (Δ) to adjust for multiple visits per patient (Table 2A and 2B). The biomarkers were standardized in order to get standardized regression coefficients. The standardized regression coefficients can be interpreted similarly as

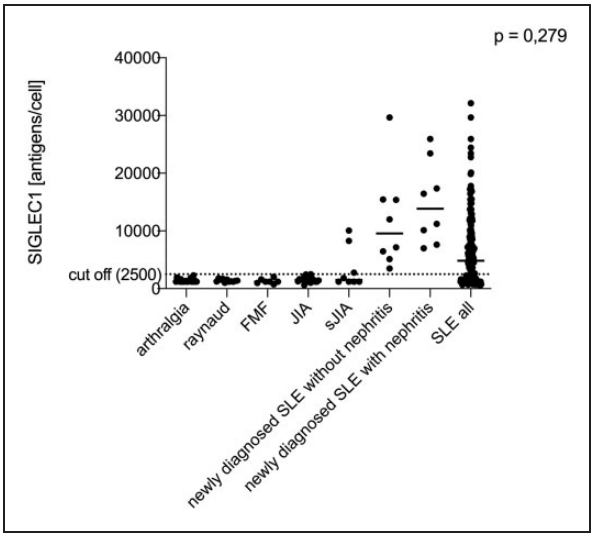

Figure 1. SIGLEC1 levels in disease control groups and children with SLE. The levels of SIGLEC1 expression on monocytes derived from the first analysed sample of children with arthralgias (n = 22, mean age 12 years [range 4–18 years], mean SIGLEC1 1385 molecules/cell [range 1200–2300 molecules/cell, SD 305], mean CRP 0,82 mg/l [range 0,3–1,7 mg/l]), Raynaud phenomena (n = 11, mean age 16 years [10–18 yrs.], mean SIGLEC1 1390 molecules/cell [range 1032–1789 molecules/cell, SD 237], mean CRP 0,4 mg/l [range 0,3-0,5 mg/l]), familial mediterranean fever (n = 7, mean age 11 yrs. [11–17 yrs.], mean SIGLEC1 1285 molecules/cell [range 731–2061 molecules/cell, SD 430], mean CRP 17,5 mg/l [range 1,4–47,8 mg/l]), JIA others than sJIA (n = 20, mean age 12 yrs. [2–17 yrs], mean SIGLEC1 1544 molecules/cell [range 601–2474 molecules/cell, SD 461], mean CRP 16,5 mg/l [0,3–82,3 mg/l]) and sJIA (n = 8, mean age 11 yrs [6–18 yrs], mean SIGLEC1 3469 molecules/cell [range 1200–10077 molecules/cell, SD 3597], mean CRP 47,2 mg/l [range 2–124 mg/l]) is shown. Additionally, SIGLEC1 levels of children with newly diagnosed untreated SLE with or without kidney involvement (total n = 16, mean age 14 yrs [3–17 yrs], mean SIGLEC1 13366 molecules/cell [range 3498-29658 molecules/cell, SD 7750), mean SLEDAI-2k 22 [4-44]) as well as all samples from patients with SLE captured in this study (n = 28, for clinical characteristics see Table 1) are depicted. SIGLEC1 expression did not differ between the group of newly diagnosed children with or without kidney involvement (p = 0.279).

correlation coefficients. Correlation coefficients in the order of 0.10 are interpreted as small; those of 0.25 as medium, and those of 0.40 as large in terms of magnitude of effect sizes[21] (Supplementary Figure 1). Two sensitivity analyses were performed. First, an adapted SLEDAI-2k was calculated with the exclusion of decreased complement (C3 and/or C4) and increased ds-DNA-ab domain. The analysis of the biomarker association was repeated with the adapted SLEDAI-2k in a sensitivity analysis. Second, the association analysis was repeated by cutting off the distributions of biomarkers at the 5th and 95th percentile to exclude extreme values that may bias the standardized regression coefficients. Mixed effects logistic regression models were applied to model the likelihood of an increase or decrease of the SLEDAI-2k by 2, 3 or 4 points including SIGLEC1 as continuous and categorical predictor variable, respectively. SIGLEC1 distribution was categorized by two different approaches: (i) the 20th or 80th percentiles were used as cut-off, and (ii) the cut-off was determined by means of the Youden index.[22] The time until the first decrease by at least 2 points in SLEDAI-2k was analysed by a Cox-proportional hazard model including SIGLEC1 as predictor variable. Statistical analyses were conducted with SAS 9.4. A p-value of less than 0.05 was considered to be statistically significant.

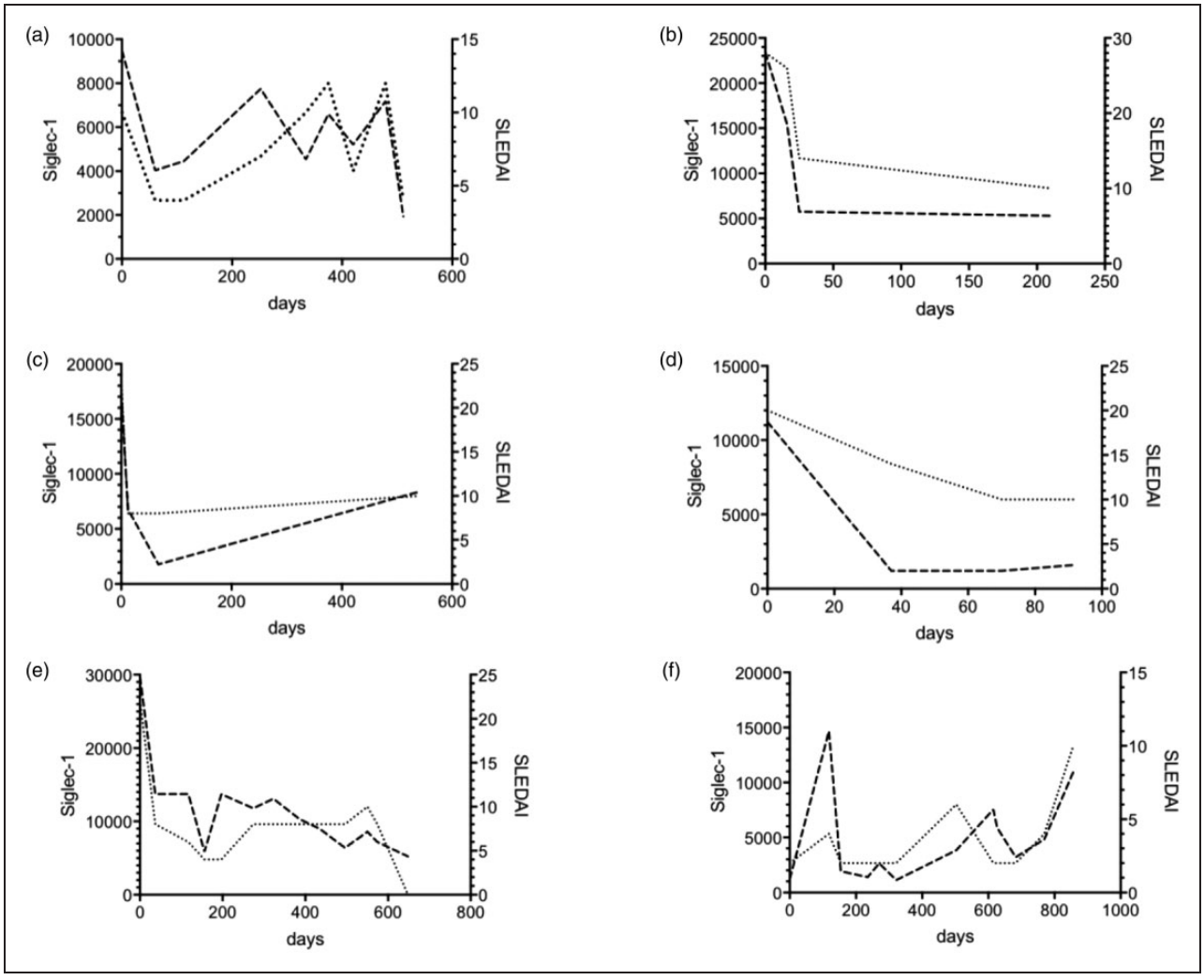

Figure 2. Individual clinical courses showing disease activity versus time SIGLEC1 levels. In patient A SIGLEC1 expression initially decreased due to initiation of treatment. Later on both, SLEDAI-2k and SIGLEC1 increased again because of discontinuation of most medication due to pregnancy (day 252). In patient B – D the high elevated SIGLEC1 levels at first manifestation declined parallel to the SLEDAI-2k due to the dramatic effect of high doses methylprednisolone on SIGLEC1 expression. In patient E initial methylprednisolone pulse therapy led to decrease in SIGLEC1-expression, due to persistent activity azathioprine was added at day 117. In patient F increase of oral steroid therapy at day 600 led to a decrease of disease activity and SIGLEC1-expression. After follow-up the patient was treated with increased doses of mycophenolate mofetil. SIGLEC1 - levels dashed line, SLEDAI – darkened line.

Results

SIGLEC1 is elevated in cSLE and correlates with the clinical course

27 children and adolescents with cSLE were followed over a mean period of 13.5 months. In total 164 visits were captured. Seven patients suffered from nephritis, two from neuropsychiatric cSLE, and six had additionally signs of antiphospholipid syndrome (further details see Table 1). The overall mean SIGLEC1 expression was 6 359 molecules/monocyte (SD 6056) (Figure 1). In untreated newly diagnosed cases, SIGLEC1 was elevated in all samples (n = 16 including 4 additionally recruited patients, mean 13 366 (SD 7750) molecules/monocyte). In this patient group no difference of SIGLEC1 expression was found when comparing patients with (n = 8, mean 14896 (SD7119)

Table 2. Correlation of biomarkers with clinical course.

A

Parameter at visit	SLEDAI-2k	SIGLEC1	C3	C4	ds-DNA ab
SLEDAI	1				
SIGLEC1					
n	159	1			
$beta_{ST}$	0.40				
p-value	<0.0001				
C3					
n	146	149	1		
$beta_{ST}$	–0.32	–0.43			
p-value	0.002	<0.0001			
C4					
n	145	148	149	1	
$beta_{ST}$	–0.46	–0.36	0.68		
p-value	<0.0001	0.001	<0.0001		
ds-DNA ab					
n	145	148	139	139	1
$beta_{ST}$	0.23	0.15	–0.27	–0.20	
p-value	0.008	0.050	<0.0001	0.002	

B

Change of SLEDAI and biomarker (Δ $visit_{t-1}$ and $visit_t$)	Δ SLEDAI-2k	Δ SIGLEC1	Δ C3	Δ C4	Δ ds-DNA ab
Δ SLEDAI	1				
Δ SIGLEC1					
n	130	1			
$beta_{ST}$	0.28				
p-value	0.001				
Δ C3					
n	111	113	1		
$beta_{ST}$	–0.07	–0.16			
p-value	0.444	0.114			
Δ C4					
n	109	111	113	1	
$beta_{ST}$	–0.02	–0.06	0.58		
p-value	0.832	0.579	<0.0001		
Δ ds-DNA ab					
n	108	111	98	98	1
$beta_{ST}$	0.11	0.03	–0.26	–0.24	
p-value	0.241	0.770	0.003	0.007	

Correlation of single biomarker levels at time of visit with the clinical course (SLEDAI-2k) (A) and of change of biomarker from previous to current visit (Δ $visit_{t-1}$ and $visit_t$) with the change in the clinical course (SLEDAI-2k).

$beta_{ST}$: standardized beta-coefficient; n: number of analyzed visits; ds-DNA ab: double-stranded DNA antibodies.

Table 3. Change of SIGLEC1 expression predicts minimal clinically changes in disease activity.

A

Prediction of minimal clinically important worsening (SLEDAI-2k $\geq$ 2 points)

Disease course	No worsening		Minimal clinical worsening						
	change of SLEDAI-2k $<$ 2 btw. $visit_{t-1}$ and $visit_t$		SLEDAI-2k $\geq$ 2 btw. $visit_{t-1}$ and $visit_t$ points						
	114		21						
visits (n)	mean (SD)	n (%)	mean (SD)	n (%)	OR	95%CI	p value	AUC	95%CI
Δ SIGLEC1 (change of 500 antigens/cell) btw. $visit_{t-1}$ and $visit_t$	−1250 (5378)		2290 (3505)		1.08	1.02 – 1.14	0.006	0.74	0.63 – 0.85
Δ SIGLEC1 btw. $visit_{t-1}$ and $visit_t$ $>$ 2151*		17 (14.9)		10 (47.6)	5.31	1.87 – 15.09	0.002		
Δ SIGLEC1 btw. $visit_{t-1}$ and $visit_t$ $>$ 756**		25 (21.9)		15 (72.4)	8.90	3.13 – 25.32	$<$0.001		

B

Prediction minimal clinically important improvement (SLEDAI-2k $\leq$ 2 points)

Disease course	No improvement		Minimal clinical improvement						
	change of SLEDAI-2k $>$ -2 btw. $visit_{t-1}$ and $visit_t$		SLEDAI-2k $\leq$ - 2 btw. $visit_{t-1}$ and $visit_t$ points						
	91		44						
visits (n)	mean (SD)	n (%)	mean (SD)	n (%)	OR	95%CI	p value	AUC	95%CI
Δ SIGLEC1 (change of 500 antigens/cell) btw. $visit_{t-1}$ and $visit_t$	235 (4714)		−2632 (5890)		0.95	0.91 – 0.98	0.006	0.66	0.56 – 0.77
Δ SIGLEC1 btw. $visit_{t-1}$ and $visit_t$ $<$ -2818***		11 (12.1)		16 (36.4)	4.16	1.72 – 10.02	0.002		
Δ SIGLEC1 btw. $visit_{t-1}$ and $visit_t$ $<$ -1370**		20 (22.0)		22 (50.0)	3.55	1.64 – 7.68	0.001		

Application of different cut-offs for the prediction of minimal clinically important worsening (A) and clinically important improvement (B).
*$>$ 80th percentile of Δ SIGLEC1 distribution, **optimal cut-off according to maximum Youden-index (sensitivity + specificity − 1), ***$<$ 20th percentile of Δ SIGLEC-1 distribution.

molecules/monocyte) or without kidney involvement (n = 8, mean 11836 (SD 8526) molecules/monocyte) (p = 0.279) (Figure 1).

In comparison SIGLEC1 was not elevated in children with arthralgias (n = 22, mean 1385, SD 305), raynaud phenomena (n = 11, mean 1390, SD 237), familial mediterranean fever (n = 7, mean 1285, SD 431) and JIA other than sJIA (n = 20, mean 1544, SD 461). In three out of eight samples from patients with sJIA levels of SIGLEC1 (mean 3469, SD 3597) were elevated (Figure 1). In patients with JIA other than sJIA disease activity was measured by calculating the JADAS-10 (mean 9.6, SD 8.8). In patients with sJIA and FMF disease activity was estimated by measuring the CRP levels (mean 47.2 mg/l, SD 44.2 and mean 8,3 mg/l, SD 17.5, respectively). No correlation between JADAS-10 or CRP levels and the level of SIGLEC1 expression was found (correlation of SIGLEC1 and JADAS-10 in patients with JIA other than sJIA: r = −0,03, p = 0,89; correlation of SIGLEC1 and CRP in patients with sJIA and FMF: r = 0,10, p = 0,82 and r = 0,74, p = 0.07, respectively).

In Figure 2, six courses of disease are demonstrated by plotting against the SLEDAI-2k as well as the SIGLEC1 expression. These graphs show that the number of expressed SIGLEC1 molecules on monocytes reflects disease activity.

Correlation of biomarker with SLEDAI-2k

When analysing C3, C4, ds-DNA-ab and SIGLEC1 at time of single visit, these biomarkers significantly correlated with the SLEDAI-2k (Table 2A; SIGLEC1: $beta_{ST} = 0.40$, $p < 0.001$; C3: $beta_{ST} = -0.32$, $p = 0.002$; C4: $beta_{ST} = -0.46$, $p < 0.0001$; ds-DNA-ab: $beta_{ST} = 0.23$, $p = 0.008$). A sensitivity analysis was performed by calculating an adapted SLEDAI-2k with the exclusion of decreased complement (C3 and/or C4) and increased ds-DNA-ab domain. The correlations were slightly lower (SIGLEC1: $beta_{ST} = 0.39$, $p < 0.001$; C3: $beta_{ST} = -0.21$, $p = 0.04$; C4: $beta_{ST} = -0.38$, $p < 0.0001$; ds-DNA-ab: $beta_{ST} = 0.21$, $p = 0.018$) as compared to SLEDAI-2k. For correlation of SIGLEC1 with SLEDAI-2k, C3, C4 and dSLEDAI-2k see Supplementary Figure 1.

Since the clinical courses and the levels of biomarkers are dynamic processes, we correlated changes (Δ of biomarker level between $visit_{t-1}$ and $visit_t$) of a single biomarker with the changes of the clinical severity (Δ of SLEDAI between $visit_{t-1}$ and $visit_t$). In this analysis (Table 2B), only the change in SIGLEC1 levels (Δ SIGLEC1) correlated significantly with the change in SLEDAI-2k ($beta_{ST} = 0.28$, $p = 0.001$).

These observations demonstrate that SIGLEC1 can be another sensitive biomarker for the evaluation of disease activity in cSLE.

Association of minimal clinically important differences with changes in SIGLEC1 expression

The minimal clinically important difference (MCID) is defined as the smallest difference in a score that mandates change in the patient's management. In childhood an increase or decrease of SLEDAI-2k

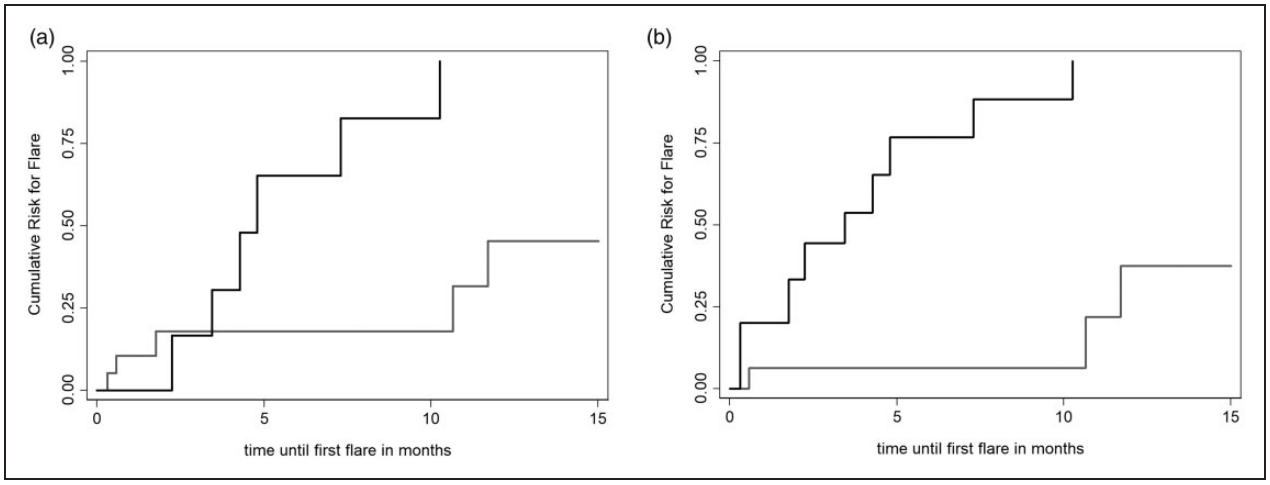

Figure 3. Prediction of minimal clinically worsening of disease activity according to Δ SIGLEC1/SLEDAI-2k at visit. Shown is the cumulative rate of minimal clinically disease worsening (SLEDAI-2k ≥ 2) at the different time points. In (A) the cut-off was determined by the upper distribution of the Δ SIGLEC1 (> 80th percentile, grey line Δ SIGLEC1 ≤ 2151, black line > 2151), in (B) optimal cut-off according to maximum Youden-index was applied (grey line Δ SIGLEC1 ≤ 756, black line > 756).

by 2 can be considered as a clinically significant difference.[23]

We therefore analysed whether Δ SIGLEC1 is associated with an MCID in SLEDAI-2k. The applied cut-offs were defined either by the 20th and 80th percentile of Δ SIGLEC1 distribution or by the optimum cut-off according to the maximum Youden-index.

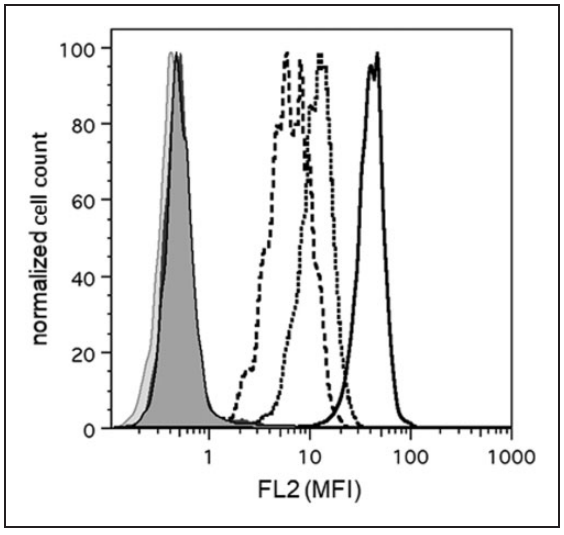

Figure 4. Representative flowcytometric analysis of SIGLEC-1 expression. Analysis of quantified SIGLEC1 expression on monocytes derived from a newly diagnosed patient with cSLE at time of diagnosis (black line, mIgG1 isotype black tinted) and five (dotted line, isotype dark grey tinted) as well as nine days after methylprednisolone pulse therapy (dashed line, isotype light grey tinted). During each analytical run, QuantiBRITE™ PE tubes were used to convert mean fluorescent intensity (MFI) signals on CD14+ monocytes to monoclonal antibodies bound per cell (mAb/cell) values. MFI – mean fluorescence intensity.

Table 3 compares patients with an increase or decrease of SLEDAI-2k ≥2 to all patients with no clinical worsening or improvement, respectively. In general, patients with an increase of SLEDAI-2k ≥2 had a mean Δ SIGLEC1 of 2290 (SD 3505) molecules/monocyte (Table 3A). The odds ratio for a clinical worsening in the case of increase of Δ SIGLEC1 was 1.08 (95%CI: 1.02; 1.14), the AUC was calculated with 0.74, meaning three out of four patients with clinical worsening had high Δ SIGLEC1. 47.6% of patients with a Δ SIGLEC1 > 2151 molecules/cell (percentiles as cut-off) and 72.4% of patients with a Δ SIGLEC1 > 756 molecules/cell (application of Youden index), respectively, showed an MCID with a SLEDAI ≥2 at time of $visit_t$ (Table 3A). This corresponds to an odds ratio of 5.31 (95%CI: 1.87; 15.09) and 8.90 (95%CI: 3.13; 25.32) for an increase of Δ SIGLEC1 in respect to clinical worsening.

When analysing only patients with an increase of SLEDAI-2k ≥3 and ≥4, odds ratio for clinical worsening was 4.16 (95%CI: 1.22; 14.22) or 5.13 (95%CI: 1.56; 16.91) and 3.86 (95%CI: 1.08; 13.80) or 5.48 (95%CI: 1.50; 20.03), respectively.

Conversely, 36.4% of patients with a Δ SIGLEC1 < -2818 molecules/cell (percentiles as cut-off criteria) and 50.0% with a Δ SIGLEC1 < -1370 molecules/cell (application of Youden index), experienced a minimal clinically important improvement at time of $visit_t$ (Table 3B). In this case, the corresponding odds ratios for decreased Δ SIGLEC1 for prediction of MCID were 4.16 (95%CI: 1.72; 10.02) and 3.55 (95%CI: 1.64; 7.68), respectively. The prediction for improvement performed on a lower level (AUC 0.66).

In a next step, we analysed the time to the first occurrence of MCID in SLEDAI-2k worsening as a function of Δ SIGLEC1. In the case of a Δ SIGLEC1

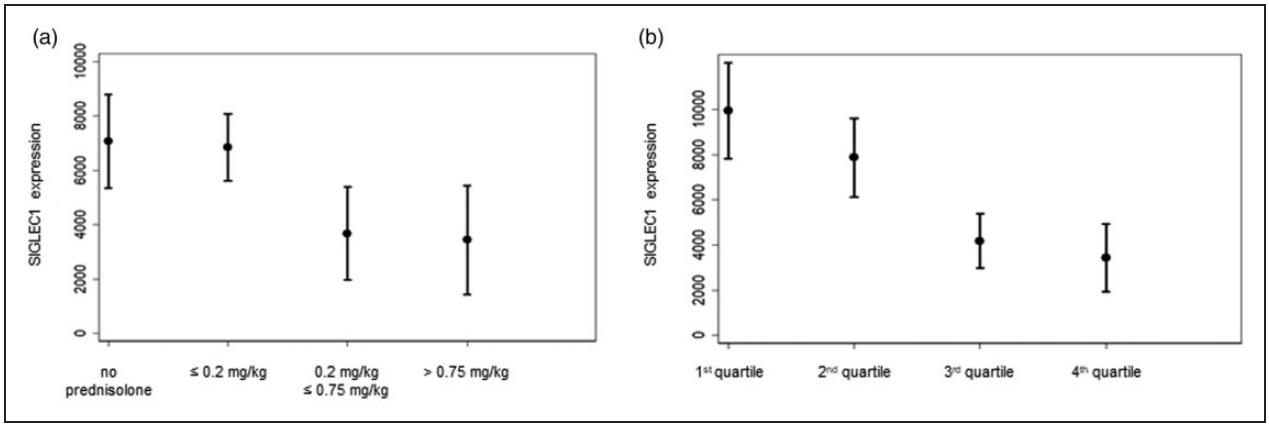

Figure 5. Association of SIGLEC1 expression with medication. Patients were grouped according to the daily applied prednisolone dose (no (n = 71), ≤ 0.2 mg/kg/day (n = 73), 0.2 - ≤ 0.75 mg/kg/day (n = 9) and > 0.75 mg/kg/day (n = 10)) (a) as well as the applied hydroxychloroquine dose given in quartiles (1^{st} quartile (n = 45): 0 mg, 2^{nd} quartile (n = 42): ≤ 3.33 mg/kg/day, 3^{rd} quartile (n = 45): > 3,33 – 4 mg/kg/day, 4^{th} quartile (n = 32): > 4.0 mg/kg/day) (b). On the y-axis the SIGLEC1 expression is shown.

>2151 molecules/cell the odds ratio was 7.02 (95%CI 1.63 30.32, p = 0.009) and for Δ SIGLEC1 > 756 molecules/cell OR = 22.3 (95%CI 2.73; 182.77, p = 0.004). The cumulative risk for MCID over time is shown in Figure 3.

Influence of medication on SIGLEC1 expression

An exploratory analysis was conducted to investigate the association between medication and SIGLEC1 expression. As shown in Figure 2, an association of glucocorticoid treatment on SIGLEC1 expression can be observed in individual cases. In one patient close meshed analysis of SIGLEC1 expression was performed after methylprednisolone pulse therapy (MMP) (Figure 4). In this single patient a decrease in SIGLEC1 expression was observed within the first 9 days with a rapid increase over the next three and a half weeks (SIGLEC1 molecules/monocyte: before MPP 29 658, after MMP: 5th day 16 844, 9th day 3 336, 13th day 4 571, 36th day 13 749).

When correlating SIGLEC1 expression with orally applied prednisolone doses, the highest SIGLEC1 values were observed in patients without steroid therapy. There was no statistical difference between patients treated with different doses of prednisolone (p = 0.176, Figure 5(a)).

In contrast, patients treated with higher doses of hydroxychloroquine had a statistically significant lower SIGLEC1 expression than patients treated with no or low dosages of hydroxychloroquine (p < 0.001, Figure 5(b)) in univariate analysis.

Conclusions

For the first time we were able to demonstrate that SIGLEC1 expression on monocytes is a sensitive biomarker for the evaluation of disease activity in cSLE. In contrast to changes in C3, C4 and double-stranded DNA antibodies, only Δ SIGLEC1 correlated with an improvement or deterioration of disease activity measured by the change of the SLEDAI-2k. Three out of four patients with a clinical worsening had a high Δ SIGLEC1. Using different cut-offs of Δ SIGLEC1, a clinical worsening was predicted with an odds ratio of 5.31 and 8.90, being superior in comparison to other biomarkers.

The sensitivity of this biomarker in SLE patients can be explained by the fact that - after IFI27 - SIGLEC1 is the most interferon-upregulated gene in monocytes[14] and in whole blood.[24] Furthermore, it is well demonstrated that an ongoing activation of the type I interferon system contributes to the SLE disease process.[4] The potential role of SIGLEC1 expression on monocytes as a monitoring tool has been demonstrated in adult patients with SLE.[14,16,17] Furthermore, soluble SIGLEC1 correlates well with the membrane-bound form and is associated with low serum C3 and increased frequency of renal complications in adult patients with SLE.[25] Young age and short disease duration are general risk factors for the probabilities of flares.[16,26] Age is inversely correlated with IFNα activity in female SLE patients and increased IFNα serum levels are further risk factors for disease flares.[16,27] Additionally, SLE patients with disease manifestation during childhood tend to experience a more severe disease course compared to adult-onset patients.[28–30] These observations explain the potential of SIGLEC1 as a biomarker for disease activity in cSLE observed in the present study.

In previous studies the expression of SIGLEC1 was assessed by the number of SIGLEC1-positive cells[14] or the relative mean fluorescence intensities.[16,17] Using quantification beads, we now introduce a method to quantify the number of expressed SIGLEC1 molecules on the cell surface of monocytes. This method will help to ensure that this biomarker can be used both in studies and in clinical practice with a high resolution.

In our cohort, severe disease worsening, e.g. SLEDAI-2k > 3[31] or SLEDAI > 5[32] was rare. Thus, we primarily analysed the correlation of changes in SIGLEC1 expression with the "minimal clinically important differences". MCID is defined as the smallest difference in a score of a disease measure of interest that patients perceive as beneficial and that would mandate, in the absence of side-effects, a change in the patient's management.[33]

The SLEDAI-2k, which also captures persistent activity in rash, mucous membrane ulcers, alopecia, and proteinuria, is equivalent to SLEDAI in describing changes in disease activity from one visit to the next.[19] In childhood SLE, an increase or decrease of SLEDAI-2k by 2 can be clinically significant.[23] In the present study, especially the worsening of disease activity as determined by an increase in SLEDAI-2k by at least 2 correlated well with an increase in Δ SIGLEC1, pointing to the potential use of SIGLEC1 as a biomarker for disease management.

In our pediatric cohort, an elevation of monocytic SIGLEC1 expression above the regular cut-off was detected in all patients with newly diagnosed cSLE with or without kidney involvement. Furthermore, a similar upregulation of SIGLEC1 was detected in children with Aicardi-Goutières syndrome, a rare monogenic autoinflammatory disorder characterized by severe neurological complications, inflammatory symptoms mimicking SLE and high expression of ISG (manuscript in preparation). Therefore, in larger upcoming studies, it should be investigated whether SIGLEC1 expression on monocytes is suitable as a simple, rapidly

available biomarker for the prompt diagnosis of interferon-mediated diseases that are initially difficult to diagnose in a timely manner.

Expression of type 1 interferons is central for disease pathology in SLE and correlates with autoantibody production and disease activity.[34] In SLE patients, the effect of glucocorticoids on interferon pathways is reduced by the pathological stimulation of plasmacytoid dendritic cells through recognition of self nucleic acids by the toll-like receptors TLR7 and TLR9. Therefore, only high dosages of intravenously applied glucocorticoids normalize the interferon signature, which is most likely caused by a reduction of circulating plasmacytoid dendritic cells.[35] The inhibition of the IFN-signature by pulse therapy is transient, returning to pre-pulse levels by day 8.[35] In one of our patients, glucocorticoid pulse therapy also led to a decrease of SIGLEC1 expression with a minimum at day 9 followed by subsequent re-expression. The highest SIGLEC1 levels were observed in patients not receiving glucocorticoids. But detailed analysis on the influence of glucocorticoids on SIGLEC1 levels during the disease course is hampered by the fact that patients received various combination therapies.

Hydroxychloroquine decreases interferon production by the inhibition of toll-like receptors in plasmacytoid dendritic cells.[36] Furthermore, low whole-blood HCQ concentrations are associated with SLE disease activity and are predictors of disease exacerbation.[37] In line with this observation, we observed an inverse correlation between the dose of applied hydroxychloroquine and the level of SIGLEC1 expression. However, one has to consider that this association is the result of an exploratory analysis. There may exist other confounding parameters influencing the association between hydroxychloroquine and SIGLEC1 expression.

Although the results have to be confirmed in another independent cohort, our study provides first evidence that SIGLEC1 expression on monocytes can be used as a sensitive biomarker for disease activity in cSLE, thus being a promising tool to monitor drug therapy.

Abbreviations

ACR: American College of Rheumatology
AUC: area under the curve
C3: serum complement component 3
C4: serum complement component 4
CI: confidence interval
CIC: circulating immune complexec
SLE: childhood systemic lupus erythematosusds
DNA: double-strandedDNA
FL2: fluorescent channel 2
FMF: familial mediterranean fever
HCQ: hydroxychloroquine
IFN: interferons
IFNAR1: interferon alpha receptor 1
IFI27: interferon alpha inducible protein 27
JAK: janus activated kinase
JIA: juvenile idiopathic arthritis
ISGs: IFN-stimulated genes
IP 10: interferon-inducible protein-10 mAB: monoclonal antibody mAb/cell: monoclonal antibodies bound per cell
MCID: minimal clinically important difference
MFI: mean fluorescent intensity
MPP: methylprednisolone pulse therapy
PE: phycoerythrin
RT: room temperature
SIGLEC1: sialic acid-binding immunoglobulin-like lectin 1
sJIA: systemic juvenile idiopathic arthritis
SLE: systemic lupus erythematosus
SLEDAI: SLE Disease Activity Index
STAT: signal transducer and activator of transcription
TLR: toll-like receptors

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Due to the retrospective analysis of data derived from the routine care, no consultation by the ethic committee was demanded.

Consent for publication

Due to the retrospective analysis of data derived from the routine care, no consent form was demanded.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Contributorship

SL v S, TK, ML and JT were the care giving physicians; CM and NU performed the flow cytometric analysis, SL v S, TK, JK, RB, CM and NU were responsible for study conception and design as well as data acquisition. All authors were involved in manuscript drafting and revising it critically for intellectual content and approved the final version.

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Supplemental material

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