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ORIGINAL ARTICLE

Siglec-1, an easy and contributory inflammation marker in rheumatology

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Abstract

Objectives. Inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are poorly informative about interferon (IFN)-related disorders. In these conditions, the measure of the interferon score (IS), obtained by measuring the expression of IFN-stimulated genes, has been proposed. Flow cytometry-based assays measuring sialic-acid-binding Ig-like lectin 1 (Siglec-1) expression could be a more practical tool for evaluating IFN-inflammation. The study compared Siglec-1 measures with IS and other inflammatory indexes. We compared Siglec-1 measures with IS and other inflammatory indexes in real-world paediatric rheumatology experience. **Methods.** We recruited patients with immuno-rheumatological conditions, acute infectious illness and patients undergoing orthopaedic surgery as controls. Siglec-1 expression was measured in all samples, and IS, ESR and CRP were also recorded if available. **Results.** Overall, 98 subjects were enrolled in the study, with a total of 104 measures of Siglec-1. Compared with IS, Siglec-1 expression showed good accuracy (86.0%), specificity (72.7%) and sensitivity (85.7%). The measure of the percentage of Siglec-1-positive cells performed best at low levels of IFN-inflammation, while the measure of mean fluorescence intensity performed best at higher levels. *Ex vivo* studies on IFN-stimulated monocytes confirmed this behaviour. There was no link between Siglec-1 expression and either ESR or CRP, and positive Siglec-1 results were found even when ESR and CRP were normal. A high Siglec-1 expression was also recorded in subjects with acute infections. **Conclusion.** Siglec-1 measurement by flow cytometry is an easy tool to detect IFN-related inflammation, even in subjects with normal results of common inflammation indexes.

Keywords: acute infections, flow cytometry, inflammation indexes, interferon, paediatric rheumatology, Siglec-1

INTRODUCTION

Type I interferon (IFN) inflammation can be associated with a group of rare monogenic disorders called interferonopathies and with a wide spectrum of rheumatological disorders, encompassing undifferentiated connective tissue diseases (UCTD), dermatomyositis (DM), juvenile dermatomyositis (JDM), Sjögren syndrome (SS) and systemic lupus erythematosus (SLE).[1] However, inflammatory markers commonly used in clinical routine showed a poor correlation with IFN-related inflammation, and type I IFNs are not easily measurable in peripheral blood.[2–4] The measure of erythrocyte sedimentation rate (ESR) may correlate with inflammation in rheumatological or viral infections, but its significance does not reflect directly IFN inflammation but rather a set of factors including hyperfibrinogenemia, hypergammaglobulinemia and anaemia.[5,6] Indeed, it has been proposed that the ratio between ESR and C-reactive protein (CRP) may be useful to distinguish infection from flare in patients affected by SLE presenting with fever.[7] ESR elevation in rheumatological conditions might in part reflect IFN-induced hypergammaglobulinemia, but it does not correlate strictly with IFN inflammation.[4,8] Because of the insufficient sensitivity of methods to directly measure serum IFN, a number of indirect assays have been developed to measure soluble chemokines induced by IFNs or transcriptomic changes induced in leukocytes exposed to IFNs.[9,10] For example, recent studies in SLE and in monogenic interferonopathies disclosed the potential of calculating an 'interferon score' (IS) from the measure of expression of a set of interferon-stimulated genes (ISGs).[11–13] This assay reflects the exposure of blood cells to an IFN-driven environment. The measure of the IS is currently used in clinical practice as a screening tool for monogenic interferonopathies and is being evaluated to assist in the therapeutic stratification of rheumatological conditions. Indeed, the possibility of measuring the IS may be of specific value when considering the possible use of medications acting on the IFN pathway, such as antimalarials, JAK inhibitors and anifrolumab.

Unfortunately, even if improvements have been made in making the calculation of IS comparable among laboratories, the assay remains specialistic and largely based on in-house-developed protocols. Recent research has shown that it might be possible to get useful information about IFN inflammation with a simpler test that uses flow cytometry to measure the expression of sialic-acid-binding Ig-like lectin 1 (Siglec-1) (CD169) on monocytes. Siglec-1 is an adhesin expressed on the membrane of peripheral blood cells, especially monocytes and dendritic cells, involved in cell–cell adhesion and phagocytosis.[14] Its expression in monocytes is induced by IFNs and Toll-like Receptor 7 (TLR7) or 9 (TLR9) agonists.[15]

In a few studies on SLE and DM, the Siglec-1 measure by flow cytometry showed a good correlation with IS. However, there is no experience of the potential and limitations of this assay in the real world. Thus, we examined the concordance between Siglec-1 cytometry and IS in a series of subjects with various rheumatological conditions, acute infections or healthy controls. We further compared the significance of Siglec-1-positive results, with a special focus on subjects with normal values of CRP and ESR. [16–21]

RESULTS

Characteristics of the enrolled population

Overall, 98 subjects were enrolled in the study, with a total of 104 measures of Siglec-1. Thirty subjects were affected with various immuno-rheumatological conditions (24 females, average age 15.7 years), 41 were assessed for infectious conditions (20 females, average age 6.3 years) and 27 were controls (14 females, average age 14.6 years) (Figure 1). Patients with immuno-rheumatological disorders included the following: subjects with SLE, psoriatic arthritis, systemic and localised sclerosis, oligo and polyarticular juvenile idiopathic arthritis (JIA), COPA syndrome, JDM, tumor necrosis factor receptor-associated periodic syndrome (TRAPS), SS and primary immunodeficiencies. Most of these patients were already on treatment with immunomodulatory agents and had good control of the disease. Patients referred to the emergency room with a suspicion of acute infections had, in most cases, viral illnesses (e.g. adenovirus, respiratory syncytial virus, metapneumovirus, influenza B and Sars-CoV-2). However, a few of them also had bacterial infections such as nephrites. The control group was composed of healthy volunteers (adults), paediatric subjects without immunopathological problems undergoing blood sampling at the surgical department and children referred to the emergency room for traumas requiring intravenous analgesia.

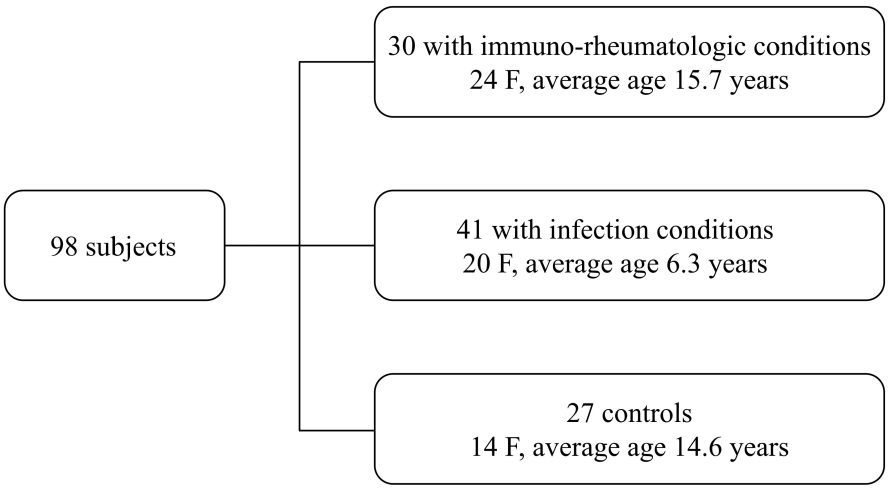

Figure 1. Flowchart of enrolled participants: 27 healthy controls, 41 subjects with infectious conditions and 30 with immuno-rheumatologic conditions.

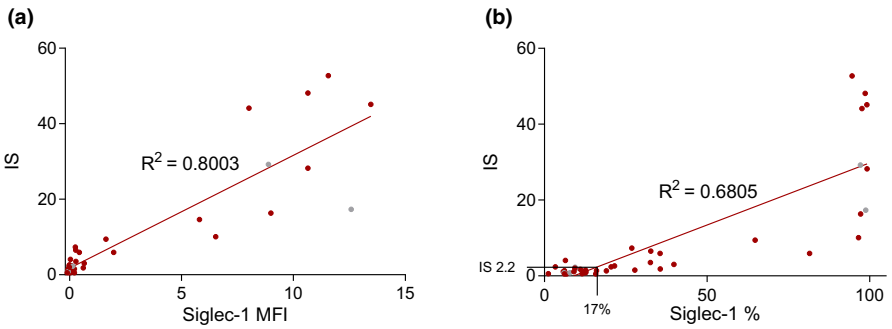

Figure 2. Correlation between interferon score (IS) and Siglec-1 expression on monocytes. **(a)** Siglec-1 expression expressed as mean fluorescence intensity (Siglec-1 MFI); **(b)** Siglec-1 expression expressed as percentage of Siglec-1-positive monocytes. Grey-filled circles: naive patients.

Siglec-1 expression on monocytes shows a good correlation with the IS

Overall, we obtained both the Siglec-1 measure and IS in 43 samples (28 from 26 patients with rheumatological complaints, 12 from 11 patients with acute infectious disorders and three from healthy controls).

The measure of Siglec-1 expression on monocytes by flow cytometry showed a good correlation with the IS. Overall, median fluorescence intensity (MFI) had a stronger relationship with IS than the percentage of positive cells. This is likely because MFI was better at telling the difference between higher values of IS (R^2 for MFI = 0.8003, for percentage 0.6805) (Figure 2a). On the contrary, the correlation between percentages of Siglec-1-positive cells and IS tend to perform better than the MFI at lower levels of interferon inflammation (Figure 2b). These results support the possible utility of both parameters to track the response of monocytes to IFNs. Indeed, when the Siglec-1 MFI is plotted against the percentage of positive cells, it is clear that percentages have a greater discriminative power at low intensities of fluorescence, while MFI works better when almost all the monocytes are positive for Siglec-1. The relationship between Siglec-1 percentage and MFI can be resumed by a one-phase decay curve that can be described by the equation $x = -\ln(1 - y/100)/0.6$ (Figure 3a). This graph could mean that when IFN levels are low, it tends to increase the expression of Siglec-1 in a growing number of monocytes. Conversely, when IFN levels are high, the stimulation only makes the

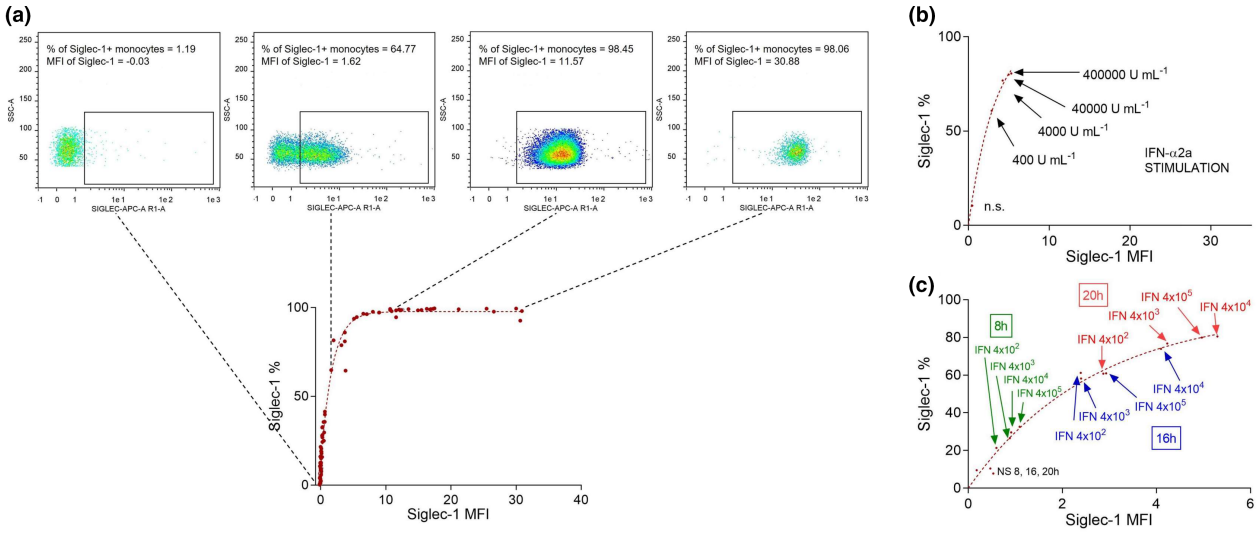

Figure 3. Correlation between Siglec-1 mean fluorescence intensity (MFI) and Siglec-1%. **(a)** Relationship between Siglec-1 MFI and the percentage of Siglec-1 positive cells, showing that subjects expressing Siglec-1 on almost all monocytes can still be differentiated on the basis of Siglec-1 MFI; we also included four dot-plot to better described this correlation. **(b)** Relationship between percentage and MFI of Siglec-1 expression in healthy control monocytes stimulated *ex vivo* for 20 h with increasing concentrations of IFN-α2a. **(c)** Further studies *ex vivo* highlighted the dependence of Siglec-1 percentage and MFI after increasing doses and timing of IFN-α2a stimulation. Grey-filled circles: naive patients.

expression of Siglec-1 stronger in cells that were already positive. *Ex vivo* studies tended to reproduce such behaviour, with increased recruitment of Siglec-1-positive cells at low stimulation levels (low dosage of IFN or low stimulation time) and an increase only in MFI at stronger stimulations (higher IFN dose, Figure 3b and longer stimulation time, Figure 3c). Interferon scores assessed on *in vitro* IFN-α2a-stimulated monocytes tend to have the same behaviour: Values increase depending on time or IFN-α2a dose (data not shown).

Considering the good correlation between Siglec-1 expression and IS, we further assessed the concordance of positive and negative results between the two assays. Given that Siglec-1 percentage was more reliable at low levels of IFN inflammation, we used it to establish the cut-off of positivity, while MFI was only used to describe the intensity of the Siglec-1 signal when almost all cells were positive. Thus, in this work, we will refer to Siglec-1 MFI when considering continuous variables, while we will rely on Siglec-1% for dichotomous values such as positive or negative. The threshold for positivity was chosen at the percentage of 17% of cells positive for Siglec-1, which intercepts the correlation line with IS at the cut-off value of 2.2.

Considering this cut-off value, we analysed discordant results obtained between Siglec-1 cytometry and transcriptomic IS (Table 1). The percentage of Siglec-1-positive cells was concordant with IS in 37 of 43 measures. Considering IS as the golden standard, the flow cytometry assay had an 86.0% accuracy. The specificity was 72.7%, and the sensitivity was 85.7%. Three measures were mildly positive for IS (IS = 2.3, 2.4, 4.1) and negative for Siglec-1. Specifically, there was a boy with deficiency of adenosine deaminase 2 disease, a boy with TRAPS outside an inflammatory flare and a boy with likely infectious bronchopneumopathy. Conversely, three measures (all with infections) were positive for Siglec-1 percentage (19.1%, 27.8% and 35.6%) and negative for IS.

Distribution of Siglec-1 values in subjects with immuno-rheumatological conditions and acute infections compared with controls

We analysed the results of 104 measures of Siglec-1 in the 98 subjects divided into three nosologic groups (35 measures in 30 subjects with immuno-rheumatological conditions, 42 measures in

Table 1. Analysis of discordant positivity with Siglec-1 cytometry and transcriptomic interferon score. For some patients more than one sample was analysed.

Siglec-1% > 17%	Interferon score > 2.2	Analysed samples (no. of patients)	Diagnoses
+	+	18 (16)	14 Subjects with active immuno-rheumatological disorders; 2 infectious diseases
−	+	3	1 TRAPS, 1 DADA2, 1 infection
+	−	3	3 Infections
−	−	19	10 With immuno-rheumatologic conditions; 6 with infections; 3 controls
Total		43	

DADA2, deficiency of adenosine deaminase 2 disease; TRAPS, TNF receptor-associated periodic syndrome.

41 patients assessed for acute infections and 27 measures in healthy volunteers of surgical controls).

In this case, to compare Siglec-1 positivity as a continuous variable, we chose to rely on the MFI, which has a better correlation with IS at high values of inflammation. Both immuno-rheumatological and infectious groups displayed significantly higher expression of Siglec-1 compared with controls ($P = 0.0012$ for the immuno-rheumatological group and $P = 0.0003$ for the infectious group), and the group of patients with acute infections tends to display a higher Siglec-1 expression (average MFI of 8.0) compared with patients with immuno-rheumatological conditions (average MFI of 3.4, $P = 0.1285$) (Figure 4).

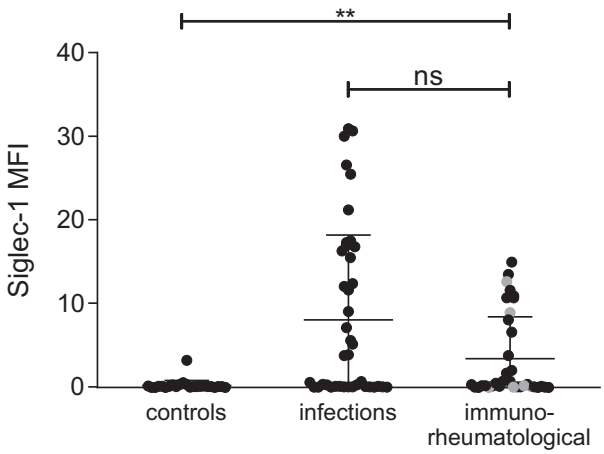

Figure 4. Distribution of the expression of Siglec-1 in the three analysed groups: controls ($n = 27$), infections ($n = 41$) and immuno-rheumatologic ($n = 30$). Grey-filled circles: naive patients. $^{**}P < 0.005$.

Siglec-1 expression does not correlate with ESR and CRP and can identify IFN inflammation in subjects with negative ESR and CRP

To assess the correlation between the expression of Siglec-1 and the values of ESR or CRP as continuous variables, we performed a linear regression analysis. No correlation was found between Siglec-1 MFI and either ESR or CRP, as demonstrated by very low R^2 values (Figure 5). Similarly, there was no correlation when comparing Siglec-1 percentage with ESR or CRP (data not shown).

To assess the potential utility of the Siglec-1 measure in clinical practice, we compared Siglec-1 percentage, ESR and CRP as dichotomous variables with positive or negative results. For ESR and CRP, we relied on the reference ranges of our local laboratory (20 mm h^{-1} for ESR and 5 mg L^{-1} for CRP). The cut-off for the percentage of Siglec-1-positive cells was 17%, chosen based on the comparison with border-line IS (IS > 2.2). Discordant values were analysed descriptively, without performing specific statistical analysis (Table 2). We included data from 60 samples for which ESR and CRP were available in addition to Siglec-1 measures.

Forty samples tested positive for Siglec-1. Seventeen of these samples, from 16 patients affected in most cases by infections, were also positive for ESR and CRP. In 13 samples, both Siglec-1 and ESR were high, while CRP was low. These samples came from six patients with SLE (five measures), one with UCTD, one with COPA syndrome (two measures), one with SS, one with hypogammaglobulinemia and three with infections. In 10 cases, only Siglec-1 was positive, and these were all immuno-rheumatological conditions except for two patients who were suspected of having infections and one control. These patients also had high IS values (median IS = 9.4, range 5.9–52.7). Six cases, all representing samples of patients with suspected infections, displayed positive ESR and CRP with negative Siglec-1.

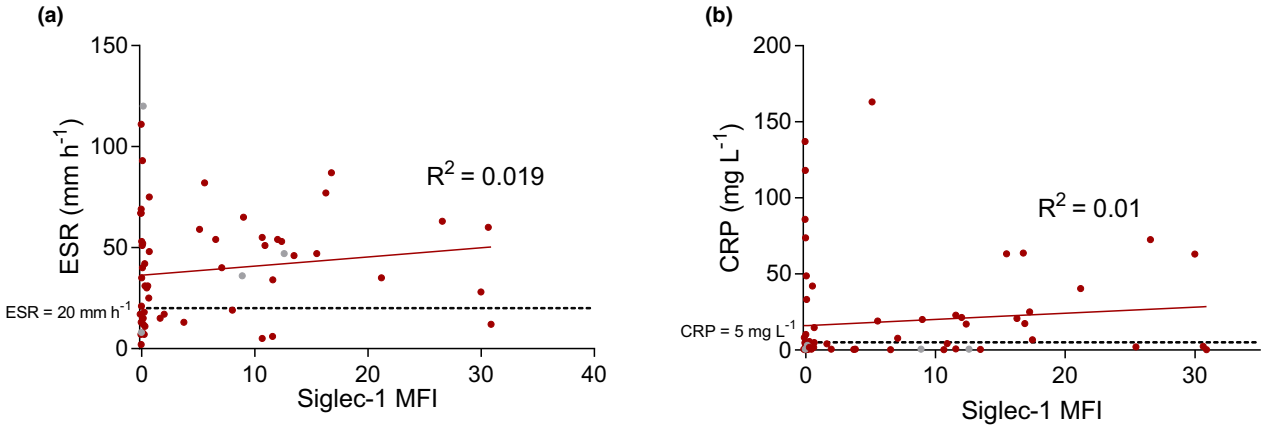

Figure 5. Correlation between Siglec-1 mean fluorescence intensity (MFI) and common markers of inflammation. **(a)** Siglec-1 MFI and erythrocyte sedimentation rate (ESR); **(b)** Siglec-1 MFI and C-reactive protein (CRP). Several samples display high Siglec-1 expression in spite of negative ESR or CRP values (respectively, below 20 mm h^{-1} and below 5 mg L^{-1}). Grey-filled circles: naive patients.

Table 2. Description of discordant cases for Siglec-1 mean fluorescence intensity positivity, erythrocyte sedimentation rate and C-reactive protein. For some patients more than one sample was analysed.

Siglec-1 positive cells > 17%	Erythrocyte sedimentation rate	C-reactive protein	Analysed samples (no. of patients)	Diagnoses
+	+	+	17 (16)	14 Infections, 2 immuno-rheumatological
+	+	–	13 (10)	3 Systemic Lupus Erythematosus, 1 UCTD, 1 COPA, 1 Sjögren Syndrome, 1 hypogammaglobulinemia, 3 infections
+	–	+	0	–
+	–	–	10 (10)	1 Systemic Lupus Erythematosus, 1 DNase2 deficiency, 1 UCTD, 2 Juvenile Dermatomyositis, 1 hypogammaglobulinemia, 1 STAT1 GOF, 2 infections, 1 healthy control
–	+	+	6 (6)	6 Infections
–	+	–	4 (4)	2 Infections, 1 TRAPS, 1 Juvenile Idiopathic Arthritis
–	–	+	0	–
–	–	–	10 (9)	7 Infections, 1 scleroderma, 1 Histiocytosis H
Total			60	

COPA, COPI coat complex subunit alpha autoinflammatory syndrome; STAT1 GOF, immune deficiency associated with gain of function of STAT1; TRAPS, TNF receptor-associated periodic syndrome; UCTD, undifferentiated connective tissue sisorder.

A high ESR with negative Siglec-1 and CRP was found in four subjects: two with suspected infections, one with TRAPS and 1 with JIA. Apart from the subjects with TRAPS, who had a border line IS of 2.3, all the subjects in this group had negative IS. The girl with JIA also presented persistent hyperimmunoglobulinemia G (1690 mg dL^{-1}).

DISCUSSION

We showed that the measure of Siglec-1 expression is a valuable, easier and cheaper alternative to the IS in subjects with rheumatological conditions. Our results confirm and widen those from previous studies conducted on specific diseases, such as SLE, systemic sclerosis, rheumatoid arthritis and DM.[16–21] However, our study is the first to compare Siglec-1 expression with IS in a real-world setting, comparing various rheumatological conditions with acute infectious illnesses and healthy controls. Furthermore, we also give some hints on how to best interpret the results of flow cytometry in this setting. Indeed, flow cytometry can allow measuring a cell marker in distinct ways, either by calculation of the MFI or by determination of the percentage of positive cells. The measurement of MFI requires robust calibration methods to make results repeatable.

For example, we used the instrument calibration beads and normalised our result against the background signal of the isotype control to have more repeatable results, while others used calibration beads to convert the MFI in each assay to the number of antibodies bound per cell.[21] Alternatively, it is possible to measure the percentage of Siglec-1-positive cells, which is less dependent on instrument calibration. The choice of whether to prefer MFI or the percentage of positive cells mainly depends on the biological significance of the two phenomena.

We found a good correlation between IS and Siglec-1 expression, both when it was calculated as the MFI and as the percentage of positive cells. The result is not surprising, since Siglec-1 is also one of the genes whose expression is measured to calculate IS.[22] However, the correlation between the percentage of positive cells and IS tended to worsen for higher values of IS, as the percentage of Siglec-1-positive cells approximated 100%. This effect was clearer when we plotted the Siglec-1 MFI and the percentage of positive cells together. This showed that the percentage of positive cells worked better when IFN exposure was low, while the MFI was better at telling the difference when inflammation levels were high. We clarified this phenomenon through *ex vivo* studies on peripheral blood monocytes. We showed that greater changes in the percentage of Siglec-1-positive cells occurred at low intensity of IFN stimulation (both as concerns the amount of IFN and the duration of stimulation), while the MFI tended to change more linearly with higher levels of IFN stimulation. Based on these results, we proposed to rely on the percentage of positive cells as the screening tool for its optimal sensitivity, and to refer to Siglec-1 MFI to measure the higher levels of inflammation and their changes over time.

A few discrepancies remained between flow cytometry and IS, but they might also depend on the difference in the type of sample analysed. Indeed, cytometry is based on the Siglec-1 expression on monocytes, while IS is usually carried out on whole blood. As a result, the variable distribution of the leukocyte formula can have an impact on IS, making it more challenging to understand the meaning of the findings. The calculation of the IS on sorted cell populations can overcome this issue, yet it makes even more complex and costly to routinely use in clinics. For this reason, anti-inflammatory treatments acting on distinct cell subsets may differentially affect the two assays. Whether this is a limit or a benefit of IS is unclear, but it makes it more difficult to assign a unique meaning to the results of IS based on the huge variations in the different cells present in whole blood.

The real-world setting of our study was at the same time a limitation and an added value. It was a limitation, as patients were consecutively enrolled only when a deep investigation of inflammation markers was required based on the judgement of the clinician. No patient underwent blood sampling for the study alone. Furthermore, samples from treatment-naïve patients were rarely available. Conversely, the real-word setting was an added value as it allowed comparing a large variety of conditions as concerns diagnosis and treatments.

One of the interesting things about our study is that the control groups included kids who had serious infections. This helped us address the challenging interpretation of data showing IFN inflammation without ruling out an infection at the same time. It is worth noting that we include only children undergoing blood sampling for acute infections, which is not the rule and may account for the selection of a high proportion of unusual or severe infections. We found that IFN-mediated inflammation may be even higher in these patients than in people with immuno-rheumatological conditions. This means that a single high value of Siglec-1, or IS, should be taken with caution. However, we should also consider that, on the one hand, some subjects with suspected infections actually had no active inflammation and, on the other hand, some of the immuno-rheumatological conditions investigated may not be associated with IFN inflammation. We previously demonstrated that only a small proportion of children with JIA have high IS.[23] Unfortunately, we cannot tell whether the result of Siglec-1 positivity obtained in single samples from these patients is related to inflammatory conditions or to some unrecognised intercurrent infection. Thus, we highly recommend carrying out multiple measures to track the IFN inflammation in rheumatological disorders.

A last original finding in our work is the comparison between Siglec-1 positivity and the most commonly used inflammation indexes currently used in clinical practice in rheumatology, the ESR and CRP. We showed that the assay can detect IFN-related inflammation in a not negligible proportion of subjects who had normal ESR and CRP results. This can be of valuable importance for the detection of inflammation in

patients in the early stages of rheumatological conditions such as UCTD, SS and SLE.[24] Even if there is no clear recommendation for therapy in these patients, the finding of IFN inflammation together with constitutional symptoms may pave the way to a trial of hydroxychloroquine or to a closer follow-up. It is interesting to note that Siglec-1 was positive in two cases of monogenic interferonopathies (a case of Aicardi–Goutières syndrome and a case of DNase2 deficiency), both with high IS, even though ESR and CRP tests were normal. This suggests that the cytometry-based assay might be a good way to screen for interferonopathies. Our study also highlighted a small group of subjects with increased ESR but normal CRP and the Siglec-1 index. We speculated that in these cases, the high ESR might just reflect hypergammaglobulinemia and not a true inflammatory phenomenon (as in two patients with clinically silent TRAPS and JIA).

Finally, our results show that Siglec-1 expression on peripheral monocytes can be used as a sign of inflammation. This could be useful in rheumatology, along with other consolidated signs of inflammation. In particular, this can be a useful tool for checking for monogenic interferonopathies in children or finding early signs of rheumatologic conditions like UCTD. Furthermore, it can also have a role in the therapeutic stratification and follow-up of patients with other rheumatological conditions, especially in the spectrum of SLE, DM, scleroderma and SS. The assay can be easily and rapidly reproduced in every flow cytometry laboratory, even if we still miss reliable procedures for inter-laboratory validation of results. However, shared protocols and calibration measures can be used in future to have more reliable results and to establish reference values suitable for clinical use. Further studies are needed to disclose the potential of routine use of Siglec-1 cytometry at disease onset, before the start of medication, which can influence results. Furthermore, similarly to what is already known for other inflammatory indexes, the possibility of an infectious disease underpinning a high value of Siglec-1 expression must always be considered.

METHODS

This is a real-world cross-sectional study performed on a third-level paediatric rheumatology unit. The aim was to evaluate the feasibility and reliability of the Siglec-1 measure on peripheral blood monocytes by flow cytometry as a correlate of IFN-related inflammation. The study was conducted on subjects undergoing blood draws for any clinical reason not directly related to the project. Specifically, subjects consecutively referred to the Pediatric Rheumatology Unit of the IRCCS Burlo Garofolo from June 2022 to May 2023 were enrolled in the study if they needed a thorough assessment of systemic inflammation according to the judgement of the caring physician. Thus, this is a convenience series. Subjects referred to the paediatric emergency room with infectious symptoms were included if a blood draw was performed for any reason. Control cases included children referred to the emergency room for traumas requiring intravenous analgesia, patients without known immuno-rheumatological conditions referred to the hospital for orthopaedic surgery and healthy adult volunteers.

For all participants, written informed consent was obtained as per protocol RC30/22, approved by the local Institutional Board Review.

Information on the diagnosis and current treatments was collected from the clinical sheets and recorded in a structured database together with laboratory data, including IS, blood cell count and differential, ESR and CRP values, when available for the same blood draw.

Measure of Siglec-1 on peripheral blood monocytes

All samples underwent flow cytometry to perform the Siglec-1 measure. Briefly, 100 μL of whole blood was stained with VioBlue-conjugated anti-CD14 antibody to mark monocytes and APC-conjugated anti-Siglec-1 antibody or APC-conjugated Mouse-IgG1 isotype control to assess the background signal (all the antibodies were from Miltenyi Biotec, Bergisch Gladbach, Germany). The samples were analysed on the MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec) and processed with the FlowLogic software (Inivai, Mentone, VIC, Australia). Monocytes were gated based on their CD14 expression and the side scatter parameter. The expression of Siglec-1, both as the MFI or as the percentage of cells with a positive signal, is then expressed as the signal obtained with anti-Siglec-1 after subtracting the value of the isotype control.

Measure of the interferon score (IS)

Peripheral blood was collected in PaxGene RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland), and stored at −20°C until processing. Total RNA was purified using the dedicated kit (PreAnalytiX).

The High Pure miRNA Isolation Kit (Roche, Basel, Switzerland) was used to purify RNA from peripheral blood lymphomonocytes for *ex vivo* experiments (see below).

Collected RNA was quantified and retro-transcribed with the Transcriptor first-strand cDNA synthesis kit (Meridian Bioscience, Cincinnati, OH, USA).

Relative expression of 6 ISGs (*IFI27*, *IFI44L*, *IFIT1*, *ISG15*, *RSAD2* and *SIGLEC1*) was conducted by real-time PCR using the CFX Opus 96 instrument (BioRad, Hercules, CA, USA), TaqMan Gene Expression Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) and TaqMan probes (Thermo

Fisher Scientific). The quantities of target genes were normalised with the expression levels of two housekeeping genes, *HPRT1* and *G6PD*. Relative quantitative gene expression analysis was conducted using the $2^{-\Delta\Delta C_t}$ method compared with the control group.[22] The IS, corresponding to the median of the relative quantifications of the six genes analysed, was calculated for each subject, and the threshold value for determining the positivity or negativity of the test was determined by the mean of the IS of the healthy subjects +2 standard deviations (giving a cut-off value of 2.2).

Statistical analysis

Siglec-1 expression on monocytes, measured either as the MFI or as the percentage of cells with a positive signal (higher than the background signal of the isotypic control), was correlated with other reference parameters (IS, ESR or PCR values) by regression analysis using the statistical software Prism GRaphPad8 (La Jolla, CA, USA). Only cases with the availability of complete data were included in each analysis. The R^2 coefficient, whose value is closer to 1 the tighter the correlation, describes the goodness of correlation.

The diagnostic accuracy of the Siglec-1 measure compared with IS was calculated as: (true positive results + true negative results)/overall series.

A comparison of Siglec-1 expression in different groups was made by comparing the two disease groups (immuno-rheumatologic or infectious) with each other or towards the control group using the Mann–Whitney test for unpaired groups, without assuming a Gaussian distribution. Differences between the two groups are assumed to be statistically significant with a P-value < 0.05.

Effect of *ex vivo* stimulation of monocytes with IFN-α2a on Siglec-1 expression

Peripheral blood lymphomonocytes were obtained from healthy volunteers. Aliquots of 1×10^6 cells were either unstimulated or treated with human recombinant IFN-α2a (Miltenyi Biotec) at increasing concentrations from 0 to 4×10^5 U mL^{-1} in RPMI 5% AB serum and incubated at 37°C for 8, 16 or 20 h. After incubation, half of the cells were used to measure the IS (see above), and the other half were stained with anti-CD14 and anti-Siglec-1 antibodies as previously described, and finally fixed. Samples were acquired with a MACSQuant Analyzer 10 Flow Cytometer (Miltenyi Biotec), and analysed with the FlowLogic software. The expression of Siglec-1, both when assessed as MFI and percentage of positive cells, was normalised against the background signal of the isotype control.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Valentina Boz: Data curation; investigation; writing – original draft. **Alessandra Tesser:** Conceptualization; formal analysis; investigation; methodology; writing – review and editing. **Francesca Burlo:** Data curation; investigation. **Nicola Donadel:** Investigation; writing – review and editing. **Serena Pastore:** Data curation; investigation; resources. **Alessandro Amaddeo:** Investigation; resources; writing – review and editing. **Francesca Vittoria:** Investigation; resources. **Matteo Padovan:** Investigation; resources. **Marianna Di Rosa:** Data curation; investigation. **Alberto Tommasini:** Funding acquisition; project administration; supervision; writing – review and editing. **Erica Valencic:** Conceptualization; methodology; project administration; supervision; writing – review and editing.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

ETHICS APPROVAL

The study has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the Independent Review Board of the IRCCS Burlo Garofolo (approval no. 07/2022).

CONSENT TO PARTICIPATE

Patients were requested to sign an informed consent for acceptance of project no. RC30/22.

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