**Serum protein signatures differentiate paediatric autoimmune/inflammatory disorders**

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**Abstract**

Because of their rarity, limited awareness among non-specialists, and significant overlaps in their clinical presentation, childhood autoimmune/inflammatory conditions represent a diagnostic and therapeutic challenge. Juvenile idiopathic arthritis (JIA), with its 7 sub-forms, is the most common paediatric “rheumatic” disease. Juvenile-onset systemic lupus erythematosus (jSLE) is a severe autoimmune/inflammatory disease that can affect any organ system and shares clinical features with JIA. To overcome issues around diagnostic approaches in the context of clinical overlap, we aimed at the definition of disease sub-form specific cytokine and chemokine profiles. Serum samples from patients with JIA (n=77) and jSLE (n=48), as well as healthy controls (n=30), were collected. Samples were analysed using the Meso Scale Discovery (MSD) U-PLEX Biomarker Group 1 (hu) panel. Distinct serum protein signatures associate with JIA vs jSLE disease groups. Proteins with high discriminatory ability include IL-23, MIP-1β, MCP-1, M-CSF and MDC. Furthermore, serum IL-18, MIF, MIP-5 and YKL-40 discriminate between systemic JIA and other JIA subtypes. Thus, simultaneous quantification of serum proteins in a panel format may provide an avenue for the diagnosis and monitoring of childhood autoimmune/inflammatory conditions.

**Keywords:** Juvenile idiopathic arthritis; Systemic lupus erythematosus; juvenile; Biomarker; Inflammation; Childhood; Paediatric.

**1. Introduction**

Autoimmune/inflammatory conditions represent a diagnostic and therapeutic challenge in pediatrics that is caused by a noticeable overlap in clinical features between inflammatory diseases, including joint swelling and pain, and the somewhat inconsistent presence of systemic symptoms (fevers, skin rashes, lymph node swelling, weight loss, fatigue, etc.) and/or autoantibodies. Limited awareness, especially among non-specialists, together with shared and overlapping features can complicate and delay diagnosis and treatment initiation, highlighting the need for improved diagnostic tools [1, 2] [3].

Juvenile idiopathic arthritis (JIA) is the most common paediatric autoimmune/inflammatory disease. It is characterised by chronic arthritis (for >6 weeks) of unknown cause that, by definition, presents before the 16th birthday [4, 5]. JIA is an umbrella term for a heterogeneous set of clinical conditions that, following International League of Associations for Rheumatology (ILAR) definitions, can be divided into 7 subgroups based on the number of joints involved (oligo- vs polyarticular) and the presence or absence of extra-articular manifestations and clinical markers [6]. The pathophysiology of JIA is complex, and likely varies between the 7 subforms, but cytokine dysregulation has been discussed as a key event [2, 7-9]. As currently no cure exists for JIA, “achievement of remission” is the therapeutic goal. Treatment options include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs; e.g. methotrexate), and biopharmaceutical drugs (e.g. anti-TNFα and anti-IL6 antibodies) [10].

Juvenile-onset SLE is a complex systemic autoimmune/inflammatory disease that can affect any organ system [3]. The disease is highly heterogeneous in its clinical presentation, severity and outcomes [11]. Notably, jSLE is typically more severe as compared to adult-onset SLE with higher disease activity, and increased organ involvement and damage [12]. The molecular pathophysiology of jSLE is incompletely understood. Especially in children, genetic pre-disposition plays a significant role, but in most individuals affected by SLE additional acquired mechanisms are necessary to result in disease expression [3, 13, 14]. Children, when compared to adult-onset SLE patients, more frequently experience musculoskeletal involvement, particularly at early disease stages, which can complicate the differentiation between JIA and SLE in individual cases [12, 15]. Treatment aims at limiting inflammation and damage; options include corticosteroids, anti-malarial drugs (usually hydroxychloroquine), DMARDs, cytotoxic drugs (cyclophosphamide), and biopharmaceutical drugs (e.g. anti-CD20 and anti-BLYS antibodies) [14].

Early diagnosis and initiation of treatment is associated with improved outcomes in both JIA [16] and jSLE [17]. Unfortunately, delays are common [18-20] as no single accurate diagnostic test currently exist for either disease. In the study presented, we used a multiplex chemoluminescence assay to analyse serum cytokine and chemokine profiles in JIA and jSLE patients as compared to matched healthy controls. Distinct protein signatures were detected across patient populations, highlighting the predictive diagnostic potential for serum proteins in paediatric autoimmune/inflammatory diseases.

**2. Materials and Methods**

*2.1. Patients and healthy controls*

Serum samples were collected from JIA patients (n=77), jSLE patients (n=48), as well as healthy controls (n=30). Samples were collected through the UK jSLE Cohort Study [11]. jSLE patients were followed between 2006 and 2018, aged ≤16 years at the time of diagnosis and classified according to the revised American College of Rheumatology classification criteria [21]. Patient/parent reported ethnicity information was collected using the UK National Census categorisations. Disease activity was measured by SLE disease activity index scores, and defined as “very high” (SLEDAI ≥10), “high” (SLEDAI 5-9) or “moderate” (SLEDAI ≤4). The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the National Research Ethics Service North West, Liverpool East (REC reference 06/Q1502/77). Informed consent was obtained from all subjects involved in the study.

*2.2. Detection and quantification of serum proteins*

Serum samples were analysed using the Meso Scale Discovery (MSD) U-PLEX Biomarker Group 1 (hu) 71-Plex assays. The antibody-set contains biotinylated capture antibodies and corresponding detection antibodies for 71 cytokines and chemokines involved in multiple biological processes. Assays were performed following manufacturer’s instructions using MSD 96-well 10-spot plates and recommended diluents, with all serum samples being vortexed thoroughly before use. Where experimental measurements were below detection limits, concentrations were considered as “0”.

*2.3. Bioinformatic approach and statistical analysis*

Demographic data analysis: Pearson’s Chi-square test was used to assess for differences in demographic factors between binary data and Mann-Whitney U test for continuous data. Bonferroni adjustment was applied to account for multiple testing.

Protein data analysis: All data analysis were undertaken in the statistical software R [22] and visualisations with the package ggplot2 [23]. Data processing was undertaken for all samples by removing outliers, identified using Principal Component Analysis (PCA) and defined outside three times the interquartile range of the sample distribution. Missing values were imputed via k-NN (k-nearest neighbours) method. Data were normalised using probabilistic quotient normalisation (PQN) and batch correction due to plate variation was undertaken by taking a ratio derived from control samples present in all plates. Univariate statistical tests were undertaken using a Kolgomorov-Smirnov test for two-sample tests and a Kruskal-Wallis test for three or more groups. Benjamini-Hochberg adjustment of resultant p-values was used to correct for multiple testing. Multivariate analyses included PCA and the supervised Partial least squares – discriminant analysis (PLS-DA) modelling (package mixOmics [24]). This modelling approach was used to find differences between JSLE, JIA and healthy control samples using a 2/3 train-test split. The optimum number of PLS components was computed using 5-fold cross-validation. Performance metrics were computed for the mean of five models for reproducibility and VIP scores were extracted to determine the most important variables.

**3. Results**

*3.1. Study population*

The study population comprised patients with JIA (n=77), jSLE (n=48), as well as healthy controls (n=30). Clinical and demographic information are summarised in Table 1. Between groups, differences were seen in relation to ethnicity. The proportion of White Caucasian patients was significantly higher among JIA patients and healthy controls as compared to jSLE patients. Conversely, the proportion of (south) Asian patients was significantly higher in the jSLE population when compared with JIA patients and healthy controls.

**Table 1.** Demographic and clinical information at time of sampling.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Healthy controls (n=30) | JIA (n=77) | jSLE (n=48) | pc-value |
| **Female (%)** | 19 (63%) | 56 (73%) | 33 (69%) | ns |
| **Age, years [range]** | 13 [8-15] | 13 [9-15] | 14 [12-16] | ns |
| **Male (%)** | 11 (37%) | 21 (27%) | 15 (31%) | ns |
| **Age, years [range]** | 8 [8-15] | 15 [13-17] | 11 [7-15] | ns |
| **Ethnicity** |  |  |  |  |
| **Caucasian (%)** | 27 (90%) | 75 (97%) | 23 (48%) | pc<0.001 |
| **Asian (%)** | 1 (3%) | 2 (3%) | 18 (38%) | pc<0.001 |
| **Black (%)** | 2 (7%) | - | 3 (6%) | - |
| **Other (%)** | - | - | 2 (4%) | - |
| **Unknown (%)** | - | - | 2 (4%) | - |

Data are presented as total and fractions (%) for categorical variables. Median values with interquartile ranges [25-75 IQR] are presented for continuous variables. Continuous variables were analysed using Mann Whitney U tests; categorical variables were tested using Pearson’s Chi-square tests. ns = not significant. pc = p-value adjusted for false discovery rate.

*3.2. Serum proteins discriminate between disease groups*

Analytes difficult to accurately quantify included IL-2, IL-12p70, IL-1α and GM-CSF, where the serum concentrations were either below the assay detection limit or dynamic range in more than half of the samples. For analysis, results below the lower detection limit were set to a numerical value of “0”.

First, differential serum protein levels were investigated across disease groups and healthy controls. Significant differences were seen in several proteins, many of which have previously been implicated in the pathogenesis of JIA and/or jSLE. Partial least squares discriminant analysis (PLSDA) models of two components were trained to discriminate between samples from healthy controls, JIA or jSLE patients. This bioinformatic model allowed discrimination between the three groups with ~90% accuracy (Figure 1a). Average model metrics are shown in Tables 2 (training sets) and 3 (testing sets). Variable importance in projection (VIP) scores of the model detailing the contribution of each feature (i.e. protein) towards each of the components in the PLSDA model were used to predict potential biomarkers. The top 60% VIP proteins are shown in Figure 1b, including IL-23, MIP-1β, MCP-1 and M-CSF as most promising candidates (Fig. 1b-2).

To estimate the minimum number of proteins necessary to quantify in order to accurately discriminate between groups, PLSDA models with various different feature counts and selection features were produced. As shown in Figure 3, reduction of proteins to 27 did not significantly impact on the accuracy of the model (Table 4). Reducing the number of analytes below 27, however, reduced the performance of the model.

**Table 2.** Mean performance metrics of trained PLSDA model on training set.

|  |  |  |  |
| --- | --- | --- | --- |
|  | jSLE | JIA | Healthy controls |
| **Accuracy** | 0.92 | 0.98 | 0.92 |
| **Precision** | 0.91 | 0.97 | 0.78 |
| **Recall** | 0.82 | 1.00 | 0.84 |
| **F1** | 0.86 | 0.98 | 0.81 |

Metrics are reported for each sample type. Accuracy reports the fraction of samples correctly classified. Precision reports the fraction of samples predicted as a class that were actually that class. Recall reports the fraction of samples that were actually that class that were classified as that class. F1 is a weighted mean between precision and recall.

**Table 3.** Mean performance metrics of trained PLSDA model on testing set.

|  |  |  |  |
| --- | --- | --- | --- |
|  | jSLE | JIA | Healthy controls |
| **Accuracy** | 0.91 | 0.94 | 0.89 |
| **Precision** | 0.90 | 0.94 | 0.72 |
| **Recall** | 0.80 | 0.95 | 0.80 |
| **F1** | 0.84 | 0.94 | 0.76 |

Metrics are reported for each sample type. Accuracy reports the fraction of samples correctly classified. Precision reports the fraction of samples predicted as a class that were actually that class. Recall reports the fraction of samples that were actually that class that were classified as that class. F1 is a weighted mean between precision and recall.

**Table 4.** Proteins required for optimal model performance.

|  |  |  |  |
| --- | --- | --- | --- |
| **Protein** | **HC, median (range), pg/mL** | **jSLE, median (range), pg/mL** | **JIA, median (range), pg/mL** |
| **Macrophage-derived chemokine (MDC)** | 3402 (2020-8233) | 2275 (709.4-4622) | 1804 (487.7-3405) |
| **Tumour necrosis factor α (TNF-α)** | 3.788 (2.538-240.8) | 3.89 (2.062-14.08) | 46.12 (1.473-1703) |
| **Macrophage colony-stimulating factor (M-CSF)** | 14.09 (7.893-33) | 18.86 (10.2-77.45) | 12.69 (5.234-29.19) |
| **I-309** | 23.75 (14.5-43.27) | 29.4 (9.422-129.2) | 19.67 (7.812-119.8) |
| **Erythropoietin (EPO)** | 86.57 (26.01-134.2) | 89.7 (20.76-394) | 67.92 (21.76-183.2) |
| **Interferon–inducible T Cell Alpha Chemoattractant (I-TAC)** | 23.17 (13.11-222.4) | 38.34 (10.83-806) | 19.49 (0.5347-95.78) |
| **Monocyte chemoattractant protein 4 (MCP-4)** | 66.23 (16.96-300) | 91.3 (9.884-509) | 61.99 (7.088-458.9) |
| **Granulocyte colony-stimulating factor (G-CSF)** | 17.49 (1.464-52.37) | 19.23 (8.745-60.66) | 23.94 (11.26-70.31) |
| **Cutaneous T-cell-attracting chemokine (CTACK)** | 658.1 (343.8-1598) | 479.4 (96.38-1156) | 509.9 (0.327-2425) |
| **Epithelial neutrophil activating peptide-78 (ENA78)** | 1718 (495.9-4202) | 859.3 (52.39-5726) | 987.4 (3.874-3563) |
| **Interleukin-1 receptor antagonist (IL-1RA)** | 249.5 (154.9-3280) | 317.7 (31.64-1514) | 263.8 (73.89-5303) |
| **Interleukin-17B (IL-17B)** | 7.433 (4.094-13.67) | 4.457 (0.722-51.63) | 4.505 (1.44-13.57) |
| **Interleukin-17D (IL-17D)** | 261.1 (78.83-423.4) | 120.8 (25.28-535.4) | 149.2 (42.57-386.4) |
| **Stromal cell-derived factor 1a (SDF-1a)** | 403.8 (187-957.3) | 389.1 (143.4-988.4) | 285 (111.6-537.7) |
| **Interferon β (IFN-β)** | 78.62 (15.66-207.5) | 28.95 (0.6304-107.4) | 44.33 (6.397-696.1) |
| **Interleukin-13 (IL-13)** | 11.57 (7.051-39.24) | 9.421 (4.083-23.07) | 12.21 (0.09224-33.7) |
| **Interleukin-17 (IL-17)** | 10.87 (4.217-19.46) | 6.527 (0.9047-22.88) | 6.474 (0.8072-41.77) |
| **Interferon α2a (IFN-α2a)** | 0.4074 (0.09143-1.701) | 0.9409 (0.1313-43.92) | 2.239 (0.2069-33.36) |
| **Monocyte chemoattractant protein 1 (MCP-1)** | 421.1 (265.9-1067) | 437.6 (156.1-1529) | 293.7 (65.26-667.5) |
| **Interleukin-23 (IL-23)** | 3.35 (0.3941-18.74) | 2.594 (0.03093-10.82) | 12.16 (2.782-41.95) |
| **Interleukin-12 p40 subunit (IL-12p40)** | 271.9 (9.484-582.6) | 192 (11.84-876.6) | 174.7 (26.96-459) |
| **Tumour necrosis factor β (TNF-β)** | 0.2853 (0.03169-0.454) | 0.2601 (0.06578-8.24) | 0.2849 (0.09782-6.902) |
| **Interleukin-6 (IL-6)** | 0.2808 (0.01559-0.845) | 0.5338 (0.02133-6.879) | 0.463 (0.06897-13.04) |
| **Macrophage Inflammatory Protein 1α (MIP-1α)** | 87.86 (56.16-373.9) | 76.63 (49.24-3292) | 52.3 (26.41-109.9) |
| **Interleukin-17A/F (IL-17A/F)** | 0.9779 (0.06293-6.728) | 1.49 (0.3796-10.23) | 0.8596 (0.0236-2.372) |
| **Interleukin-27 (IL-27)** | 441.8 (258.3-4074) | 418.6 (105.1-1388) | 904.7 (375.5-2167) |
| **Monocyte chemoattractant protein 3 (MCP-3)** | 3.402 (1.427-6.176) | 5.182 (1.076-20.67) | 2.778 (0.9923-13.29) |

*3.3. Patient subgroup analysis*

To evaluate whether differences in serum protein levels allow for the prediction of JIA subforms, samples were divided into 4 categories: oligoarticular, polyarticular, psoriatic, and systemic arthritis (sJIA). While no differences were seen between the oligoarticular (n=29), polyarticular (n=34) or psoriatic (n=3) JIA groups, samples in the sJIA group (n=11) exhibited significantly elevated levels of IL-18, and reduced levels of macrophage migration inhibitory factor (MIF), macrophage inflammatory protein 5 (MIP-5) and YKL-40 (Fig. 4a).

Next, samples were grouped based on age at sample collection, dividing patients into pre- (<10 years) vs peri-/post-pubertal (>10 years) sub-cohorts. Among JIA patients, serum IL2-Ra levels were reduced in post-pubertal patients (n=52) when compared with pre-pubertal (n=25) individuals (Fig. 4b), while in jSLE patients IL-17B was reduced in post-pubertal patients (n=27) compared to pre-pubertal (n=21) individuals (Fig. 4c).

Finally, cytokines in jSLE sera were tested for differences based on disease activity at sample collection. Serum IL-33 levels were elevated in jSLE patients with “very high” (SLEDAI ≥ 10) (n=7) when compared to patients with “high” (SLEDAI 5-9) (n=14) or “moderate” (SLEDAI ≤4) (n=24) disease activity (Fig. 4d).

**4. Discussion**

The molecular pathophysiology of JIA [4] and jSLE [3, 13, 14] are largely unknown. Based on findings from this study, the analysis of serum cytokine and chemokine profiles may provide insight into the pathology of these autoimmune/Inflammatory conditions and deliver target proteins for the establishment of diagnostic and/or prognostic biomarkers.

As expected, a number of cytokines/chemokines tested here were elevated in JIA and/or jSLE as compared to healthy controls. Furthermore, several also displayed significant differences between the two systemic inflammatory conditions. Of the ten proteins with the highest ability to discriminate between disease conditions, eight (IL-23, MIP-1β, SDF-1a, MCP-1, IL-17F, MIP-1α, IP-10 and M-CSF) have a pro-inflammatory function, while two (IL-27 and MDC) have a dual pro- and anti-inflammatory functions [25, 26]. In this, several key observations from this study support previous reports on the laboratory phenotype and molecular pathophysiology of SLE and JIA.

***4.1. SLE***

The IL-17 family of proteins consist of six isoforms (IL-17A-F) [27] that are primarily produced by effector Th17 cells [28]. Pathologically elevated IL-17 production is a hallmark feature of SLE [29]. Pro-inflammatory IL-17A and IL-17F isoforms exhibit high homology and are both capable of forming hetero- and homodimers [30]. IL-17A has previously been linked with CREMα-mediated epigenetic regulation in SLE [31]. Consistent with previous reports from our group, serum levels of IL-17A/F were elevated, while IL-17F levels were reduced in jSLE serum samples when compared to healthy controls [31-33]. Somewhat surprising, median levels of IL-17A homodimer measured in this study were slightly lower in jSLE patients, although a very broad range of values were observed in the disease groups whereas the distribution in healthy controls was more uniform. Serum concentrations of all IL-17 isoforms measured in this study are presented in supplementary figure 1. This high degree of interpatient variability (also seen in the JIA group) may be due to pathophysiological differences between individual patients. Indeed, we and others reported on more “autoinflammatory” phenotypes in jSLE cohorts when compared to adult-onset SLE patients [34-36]. A majority of jSLE patients with genetic forms of SLE or SLE-like disease carry mutations in genes associated with type I interferon responses and not T or B lymphocyte activation. Thus, at least in early disease stages, effector T cell responses and cytokine expression patterns may play a secondary role.

Although, unintuitively, IL-17F was reduced in jSLE serum samples when compared with healthy controls, the A/F heterodimer abundance was increased. While IL-17F is typically expressed in higher levels, the A isoform is considered to be of higher inflammatory potency and therefore more involved in autoimmune/inflammatory disease [37]. As mentioned above, in SLE, T cellular production of IL-17F is reduced [33], thereby tilting the A/F ratio towards a more proinflammatory phenotype with potential pathogenic consequences. Another member of the IL17-family, IL-17E, was reduced in the serum of jSLE patients as compared to healthy controls. Notably, IL-17E (also known as IL-25) has anti-inflammatory effects by acting as a receptor antagonist to IL-17A function [38]. Furthermore, we found that IL-17B, C and D were on average reduced in jSLE serum compared with healthy controls, although the jSLE group also contained the individual patients with the highest levels. These observations are of key relevance and somewhat expected, as several drugs targeting IL-17A (secukinumab, ixekizumab and bimekizumab) or IL-17 receptor A (brodalumab) have been developed for treatment of autoimmune disease. While none of them are currently approved for the use in SLE, their use is subject to discussion [39] and supported by a clinical report of a patient with refractory lupus nephritis complicated by psoriasis vulgaris, who was successfully treated with secukinumab [40].

In previous studies, we showed elevated secretion of IL-6 from PBMCs isolated from jSLE patients [32], which was elevated in serum samples from jSLE patients tested here. Furthermore, immune regulatory IL-10 was increased in sera from jSLE patients, which is consistent with previous work showing elevated levels jSLE plasma [41] and serum [42]. Notably, elevated IL-10 levels correlate with disease activity in adult SLE patients [43-50], and IL-10 expression in SLE T-cells correlate with disease activity [51]. Inactivating anti-IL-10 antibodies also improved disease activity scores in otherwise treatment refractory patients [52].

Elevated levels of type I interferons are associated with multiple autoimmune inflammatory diseases, including SLE [53], but low basal levels of circulating proteins typically make them difficult to accurately quantify. Here we detected significantly elevated serum levels of IFN-α2a (as well as type II IFN-γ), but reduced levels of IFN-β in jSLE patients compared with healthy controls. In SLE, IFN-α appears to be the major stimulus of interferon-related gene expression as the upregulation of interferon-inducible genes from treatment of model epithelial cells with SLE plasma can be modulated by anti-IFN-α antibodies, but not by antibodies against IFN-β or IFN-γ [54]. While pro-angiogenic cytokine VEGF has previously been reported to be elevated in jSLE serum [55], this study did not deliver differential serum levels between jSLE patients and healthy controls. Why this is can only be speculated, but may be related to the specific patient characteristics in this study, e.g. a higher degree of ethnic diversity in the jSLE group. It should also be noted that the range of concentrations detected among jSLE patients was much larger compared to controls.

Lastly, among jSLE patients, IL-33 was elevated in patients with “very high” as compared to “high” or “moderate” disease severity. This is in agreement with previous studies that linked elevated IL-33 serum concentrations with jSLE [56, 57]. In a separate study, no significant difference was seen, but IL-33 levels were below the detection limit in the vast majority of samples in this study [58]. However, the authors noted significantly elevated levels of the soluble form of IL-33 receptor ST2, which also correlated with SLE disease activity [58], suggesting that it could potentially be used as a surrogate marker for IL-33.

***4.2. SLE and JIA***

Serum levels of pro-inflammatory IL-6 and TNF-α have been reported elevated in JIA patients. Indeed, IL-6 was suggested to have potential as a marker of disease activity as it correlates with disease activity during treatment with the TNF inhibitor etanercept [59]. In this study, we detected elevated TNF-α serum levels in JIA but not jSLE patients, when compared to healthy controls. In agreement with previous reports, serum IL-6 levels were elevated in both JIA and jSLE patients when compared to controls.

***4.3. JIA***

Increased circulating levels of IL-4, IL-10, and MIF have all been previously associated with JIA [7, 60], while, in this study no significant differences were seen. Previous studies suggested that IL-10 vary between subtypes of JIA, with hereditary predisposition to reduced IL-10 being linked to extended oligoarticular JIA and sJIA [61-65]. Likely caused by relatively small sample size and multiple comparisons between groups, we did not detect variable IL-10 serum levels between JIA subgroups in this study. Concentrations of MCP-1 (also known as CCL2), MIP-1α (also known as CCL3), eotaxin (also known as CCL11) and MDC (also known as CCL22) were all reduced in JIA samples tested in this study when compared with healthy controls. This is in contrast to a previous study on JIA plasma that showed all four being elevated [7], and therefore requires further investigation. MDC modulates adaptive immune responses by promoting dendritic cell contact with regulatory T-cells (Tregs) through their CCR4 receptor [66], a requirement for Treg-mediated suppression of adaptive immunity. While MDC-deficient mice showed an enhanced immune response induced by vaccination, they were also more susceptible to inflammatory disease [66], suggesting an important role for MDC in immune regulation.

In agreement with previous reports, IL-18 was significantly elevated in sJIA patients when compared to other forms of JIA [67-69]. A previous study had noted two distinct subset of sJIA [70], one IL-6 dominant and one IL-18 dominant profile, which may explain the broad range in concentrations seen in the sJIA patients in our study. Although no statistically significant IL-6 elevation was noted in sJIA patients here, again a broader range of IL-6 concentrations were noted in this form of the disease (data not shown). Both IL-6 and IL-18 pathways are involved in the pathophysiology of sJIA [71, 72] and treatment targets, as several drugs targeting IL-6 (sirukumab, siltuximab, olokizumab, clazakizumab) or its receptor (tocilizumab and sarilumab), as well as IL-18 (tadekinig alfa) exist or are in development [73, 74]. Thus, differential cytokine levels within various form of JIA suggests that molecular phenotyping through e.g. cytokine profiling may be used to inform treatment.

Overall, differences between disease groups (jSLE vs JIA vs healthy controls) were more prominent than differences between subgroups within the same patient population (sub-forms of JIA).

As gender in combination with age (puberty) affects inflammatory responses, disease expression and outcomes in JIA and SLE [4, 14], we analysed serum protein expression in relation to puberty status. Indeed, differences between peri/post- and pre-pubertal patients in both JIA and jSLE were seen in relation to soluble IL2Ra, which was reduced in post-pubertal JIA patients, and IL-17B that was reduced in post-pubertal jSLE patients. While this is interesting, the pathophysiological and mechanistic meaning remains unknown. Thymic involution is triggered by puberty with subsequent reduction in T-cell production, however, while IL-2 receptor expression is abundant in regulatory and effector T-cells [75], IL-17B is not produced by activated T cells [76].

While offering new insights into serum chemokine and cytokine expression across paediatric autoimmune/inflammatory conditions, this study has limitations. While the JIA and healthy control groups were comprised almost entirely of White Caucasian patients, the jSLE group included a noticeably proportion of ethnic diversity. In some of the samples, certain analytes could not be detected. However, it is not clear whether this was due to the actual sample levels being reduced, or a limitation of assay sensitivity as the physiological levels of many inflammatory cytokines are typically very low. Based on clinical information available and the limited number of patients per group, we were unable to correlate protein patterns with disease activity in JIA and/or treatment at sample collection. Lastly, findings will have to be validated in larger, independent multi-ethnic groups with targeted panels, including e.g. the minimum set of 27 target candidates.

**5. Conclusions**

Distinct cytokine/chemokine signatures, including a minimum of 27 serum proteins, associate with paediatric autoimmune/inflammatory diseases, and discern between JIA and jSLE. Some of these proteins may correlate with disease activity in jSLE (IL-33) or associate with sub-forms of JIA (IL-18, MIF, MIP5, YKL40). Individual proteins or their combination may therefore be used for future diagnostic approaches, assessment of disease activity or to inform treatment decisions. However, prospective studies in larger unrelated and multi-ethnic cohorts are needed to further determine the potential value of candidate items as diagnostic and/or prognostic biomarkers.

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**Figure legends**

**Figure 1.** Serum cytokine signatures differ between patients with JIA, jSLE and controls. (**a**) PLSDA model score plots for two components. Model based on serum protein concentrations in JIA, jSLE, and HC. (**b**) Analytes with “highest importance” in the model (VIP scores) for the two components.

**Figure 2.** Serum concentrations of cytokines discriminating between disease groups. (**a**) Relative abundances of serum cytokines with highest potential for discriminating between JIA, jSLE, and HC patients. (**b**) Relative abundances of serum cytokines discriminating between subforms of JIA between JIA, jSLE, and HC patients. Boxplots show median and IQR for relative abundance with individual sample values as black dots. \*, pc<0.05; \*\*, pc<0.01.

**Figure 3.** Impact of reduction of proteins included in the panel on model performance. PLSDA models with various different feature counts and selection of features were produced to evaluate how the accuracy of the model was degraded by removal of proteins.

**Figure 4.** Serum proteins associate with patient subgroups. (**a**) Relative serum concentrations of IL-18, MIF, MIP-5 and YKL-40 in JIA patients grouped by disease type. (**b**) Relative serum concentrations of IL2-Ra in JIA patients grouped by pubertal status. (**c**) Relative serum concentrations of IL-17B in jSLE patients grouped by pubertal status. (**d**) Relative serum concentrations of IL-33 in jSLE patients grouped by disease severity. Boxplots show median and IQR for relative abundance with individual values as black dots.