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ORIGINAL RESEARCH

Inflammatory biomarkers predicting long-term remission and active disease in juvenile idiopathic arthritis: a population-based study of the Nordic JIA cohort

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ABSTRACT

Objectives To assess the ability of baseline serum biomarkers to predict disease activity and remission status in juvenile idiopathic arthritis (JIA) at 18-year follow-up (FU) in a population-based setting.

Methods Clinical data and serum levels of inflammatory biomarkers were assessed in the longitudinal population-based Nordic JIA cohort study at baseline and at 18-year FU. A panel of 16 inflammatory biomarkers was determined by multiplexed bead array assay. We estimated both univariate and multivariate logistic regression models on binary outcomes of disease activity and remission with baseline variables as explanatory variables.

Results Out of 349 patients eligible for the Nordic JIA cohort study, 236 (68%) had available serum samples at baseline. We measured significantly higher serum levels of interleukin 1β (IL-1β), IL-6, IL-12p70, IL-13, MMP-3, S100A9 and S100A12 at baseline in patients with active disease at 18-year FU than in patients with inactive disease. Computing receiver operating characteristics illustrating the area under the curve (AUC), we compared a conventional prediction model (gender, age, joint counts, erythrocyte sedimentation rate, C reactive protein) with an extended model that also incorporated the 16 baseline biomarkers. Biomarker addition significantly improved the ability of the model to predict activity/inactivity at the 18-year FU, as evidenced by an increase in the AUC from 0.59 to 0.80 (p=0.02). Multiple regression analysis revealed that S100A9 was the strongest predictor of inactive disease 18 years after disease onset.

Conclusion Biomarkers indicating inflammation at baseline have the potential to improve evaluation of disease activity and prediction of long-term outcomes.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Elevated levels of erythrocyte sedimentation rate (ESR) and baseline levels of granulocyte-macrophage colony-stimulating factor, interleukin 6 (IL-6), IL-17A and tumour necrosis factor have been associated with a higher probability of sustained disease activity at a 12-month follow-up.

WHAT THIS STUDY ADDS

⇒ Addition of validated inflammatory biomarkers to conventional clinical prognostic factors (gender, age, active joint counts, ESR and C reactive protein) significantly enhances prediction of disease activity/inactivity at 18 years of follow-up.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Early monitoring of validated inflammatory biomarkers, such as S100 proteins along with IL-1ß, IL-6, IL-12, IL-13 and MMP-3, could greatly influence the management of juvenile idiopathic arthritis.

⇒ Combining evaluation of biomarker levels with conventional clinical factors could potentially improve the identification of children who will not respond to conventional methotrexate therapy.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a heterogenous group of chronic arthritis with childhood onset and a fluctuating disease course. In a population-based Nordic JIA cohort, we reported that even 18 years after disease onset, almost 40% continued having active disease[1] as also reported in other long-term studies.[2 3] However, predicting the long-term severity of JIA and its potential remission through early retrieval of prognostic factors remains challenging.[2 4 5]

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A complex network of cytokines and inflammatory cells has shown to be essential to the pathogenesis of rheumatoid arthritis (RA) and JIA.[6 7] The role of key cytokines, including tumour necrosis factor alpha (TNFα), interleukin 1β (IL-1β), IL-6, Janus kinase-dependent cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF) and chemokines, is significant in RA pathogenesis. These cytokines may provide insight into disease pathogenesis and may inform tailored treatment plans, considering both their individual roles and the broader inflammatory tissue response.[6] Moreover, TNFα, IL-6, IL-10, IL-12 and IL-18 have been shown to correlate with disease activity in JIA.[8–11]

Other blood biomarkers have been suggested to monitor JIA disease activity and facilitate prediction of clinical outcome. The phagocyte-related and proinflammatory S100A8/A9 and S100A12 proteins have been identified as important inflammatory markers in several conditions, including RA and JIA.[12 13] They are markedly elevated in systemic inflammatory conditions, such as systemic JIA[13 14] or Kawasaki disease,[15] and may enhance the potential of clinical characteristics for predicting disease outcomes.[16] In JIA, biomarkers, such as the S100 proteins, have facilitated identification of patients with unstable remission, patients at increased risk of relapse[17 18] and anti-TNFα responders in non-systemic JIA.[19]

Ganeva *et al* recently described the association of baseline serum biomarkers and inflammatory variables with the outcome of active JIA within the first year of diagnosis.[20] They showed that a high erythrocyte sedimentation rate (ESR) and high GM-CSF, IL-6, IL-17A and TNF baseline levels indicated an increased risk of ongoing disease activity after 12 months. Based on this, they discussed a strategy of including serum biomarkers as part of clinical management to ensure early identification of patients with JIA at risk of experiencing a severe outcome. The validity of these findings in a population-based cohort and the utility of serum biomarkers as predictors of long-term outcomes remain unknown.

This study aimed to assess the potential correlation between levels of serum biomarkers in early-stage JIA and disease activity, and to explore the role of these biomarkers in predicting disease activity and clinical outcome 18 years after disease onset.

METHODS

In this longitudinal study of the population-based Nordic JIA cohort,[1] we conducted a comparative analysis of serum levels of inflammatory biomarkers obtained both at baseline and at 18-year follow-up (FU) with their respective clinical data. Consecutive cases of newly diagnosed patients with JIA were recruited at disease onset from well-defined geographical areas of Denmark, Sweden, Norway and Finland. The recruitment period spanned from 1 January 1997 to 30 June 2000, with the objective of achieving a population-based representation, as previously described in detail.[1 21] A baseline visit was planned to take place 6 months (−1/+2 months) after disease onset.[21 22] The JIA categories were classified according to the International League of Associations for Rheumatology criteria.[23] At 18-year FU, an invitation was extended to all 510 participants originally enrolled in the Nordic JIA cohort. This invitation was extended regardless of disease activity, level of treatment and disease course, ensuring a non-selected setting.

At baseline and at the 18-year FU visit, we registered disease activity variables, including complete active joint count, physician's global assessment of disease activity (PhGA) on a 10 cm visual analogue scale (VAS), patient/parent's global assessment of well-being (PaGA), medication and blood tests (ESR, C reactive protein (CRP), antinuclear antibodies, rheumatoid factor, human leucocyte antigen B27 and biomarkers). In addition, the 18-year FU visit included an update of the demographic data. We applied the Juvenile Arthritis Disease Activity Score for 71 joints (JADAS71),[24] range 0–101. Additionally, scores on a 21-point circled VAS for PhGA and PaGA were collected, where 0 indicated no activity/best global assessment, and 10 indicated the maximum activity/poorest global assessment. We adopted the American College of Rheumatology 2011 provisional criteria for clinically inactive disease (CID)[25] and the preliminary Wallace criteria for clinical remission.[26] CID includes the following: (1) no active joints; (2) no fever, rash, serositis, splenomegaly or generalised lymphadenopathy attributable to JIA; (3) no active uveitis; (4) normal ESR and/or CRP level; (5) a PhGA that indicates no disease activity; and (6) a duration of morning stiffness of ≤15 min. For clinical remission on medication, the criteria for inactive disease on medication had to be fulfilled for a minimum of six continuous months.[26] To be in clinical remission off medication (CR), patients must have had inactive disease for a continuous period of 12 months as a minimum, during which they received no antiarthritis and/or antiuveitis medication.[26]

All samples were collected in serum tubes, centrifuged, aliquoted and stored at −80°C for biomarker analysis. The baseline samples were thawed no more than two times. The samples were diluted in Tris-buffered saline (TBS) 1:4 before analysis. We determined serum concentrations of S100A9 (as measurement of the S100A8/A9 complex), S100A12, IL-1β, IL-4, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-18, TNFα, matrix metalloproteinase 3 (MMP-3), myeloperoxidase (MPO), chemokine ligand 2 (CCL-2) and soluble CD25 (sCD25) using multiplexed bead array assay according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota, USA). Data acquisition and analysis were performed on a MAGPIX instrument (Merck Millipore, Darmstadt, Germany) using xPONENT V.4.2 software (Luminex). The analysing laboratory in Muenster, Germany, was blinded to the patients' clinical characteristics. The choice of these specific biomarkers over others is based on a combination of an explorative

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study and their established relevance to the disease process and treatment response.

Statistics

The biomarker concentrations exhibited a non-normal distribution, as evaluated by Q-Q plot and Kolmogorov-Smirnov statistic. Therefore, the results are presented as median with 1st–3rd IQR or 95% CI and compared using the Mann-Whitney U test for continuous data and Fisher's exact test for dichotomous variables. For paired comparison, Wilcoxon signed-rank test was used. In addition, Spearman's rank-order correlation was used to evaluate the correlation between the biomarkers and JADAS.

We used both univariate and multivariate logistic regression models to estimate binary outcomes of disease activity and remission with baseline variables as explanatory variables. The predicted probabilities (fitted values) of the logistic regressions were used to compute the receiver operating characteristic (ROC) curve and the area under the curve (AUC) to evaluate model performance.

Analysis of variance (ANOVA) was used to compare nested multivariate logistic regression models. For the goodness of fit, the omnibus test of model coefficients and the Hosmer and Lemeshow test were performed.

RESULTS

At baseline, 510 patients were enrolled in the study. In this study, the Finnish patients (n=161) did not have access to sample storage and accordingly, they were excluded to ensure a population-based setting ending up with a total cohort of 349 eligible participants. Of these, blood samples from 113 patients were no longer available. Therefore, serum analysis for baseline biomarkers was performed on 236 (67.6%) of the remaining 349 patients from the other Nordic countries (figure 1). From the 434 patients registered at the 18-year FU, 329 attended a clinical visit of which 284 had blood samples available for biomarker analysis. From the patients with blood samples at baseline (n=236) clinical data at the 18-year FU were recorded for 199 patients. 150 patients had paired samples taken both at the baseline visit and at the 18-year FU.

Demographic data and clinical characteristics for the 236 patients registered at the baseline visit and at 18-year FU are shown in table 1. Median JADAS71 at baseline was 5.0 (IQR: 2.0–11.0) compared with 2.0 (IQR: 0.0–6.4) at FU. CID at 18-year FU was observed in 58% and CR in 39.4% of the patients. At the baseline visit, 180 of the 236 patients were disease-modifying antirheumatic drug (DMARD) treatment naïve (76.3%), 11 had previously

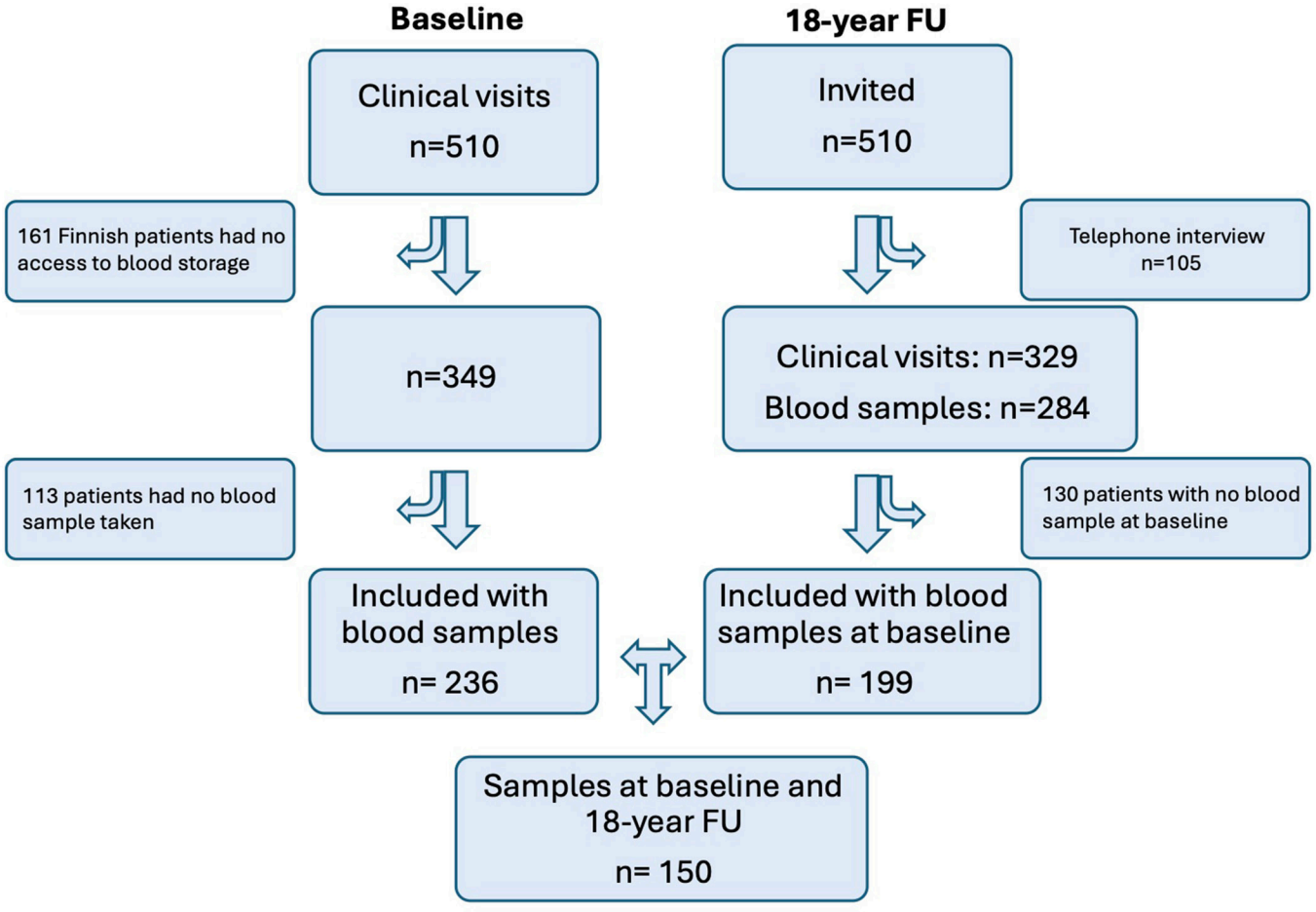

Figure 1 Flow chart of study population. FU, follow-up.

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Table 1 Demographic and clinical characteristics of participants with baseline samples from the Nordic juvenile idiopathic arthritis (JIA) cohort (n=236)

	n	Baseline (n=236)	n	18-year follow-up (n=199)
Females		164 (69%)		
Age at onset (years)	236	6.0 (2.9–10.4)		
ANA positive	208	82 (39.4)		
HLA-B27 positive	229	44 (19.0)		
Age at last follow-up (years)			199	23.6 (20.5–27.6)
Disease duration (years)			199	17.7 (16.8–18.6)
ESR, mm/hour	194	12 (6.0–24.0)	125	6.0 (3.0–9.0)
ESR>20, mm/hour	194	54 (27.8)	125	9 (7.2)
CRP, mg/L	196	0.0 (0.0–10.0)	155	4.0 (1.7–5.0)
CRP>8, mg/L	196	52 (26.5)	155	15 (9.7)
Active joint count	236	1.0 (0–3.0)	199	0.0 (0.0–0.0)
Cumulative joints	236	3.0 (1.0–7.0)	199	8.0 (3.0–16.0)
JADAS71	159	5.0 (2.0–11.0)	184	2.0 (0.0–6.4)
CHAQ/(baseline)/HAQ	198	0.3 (0.0–0.925)	188	0.0 (0.0–0.25)
Remission off medication		NA	193	76 (39.4)
Inactive disease		NA	193	112 (58.0)
Systemic JIA		10 (4.2%)		7 (3.5%)
Oligoarticular persistent		113 (47.9%)		45 (22.6%)
Oligoarticular extended		8 (3.4%)		42 (21.1%)
Polyarticular RF positive		5 (2.1%)		2 (1.0%)
Polyarticular RF negative		46 (19.5%)		28 (14.1%)
Psoriatic arthritis		2 (0.8%)		9 (4.5%)
Enthesitis-related arthritis		20 (8.5%)		22 (11.1%)
Undifferentiated		32 (13.6%)		44 (22.1%)

Data are expressed as median (IQR) or number of patients (%).
ANA, antinuclear antibodies; CHAQ, Childhood Health Assessment Questionnaire; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; HLA-B27, human leucocyte antigen B27; JADAS71, Juvenile Arthritis Disease Activity Score for 71 joints; NA, not applicable; RF, rheumatoid factor.

been treated with DMARDs and 45 (19.1%) were still on DMARDs. None of the patients had received biologics at the time of the baseline visits (data not shown). At the 18-year FU, 29 out of the 199 (14.6%) participants were treated with DMARDs, and 31 (15.6%) were treated with biologics, with 18 of them receiving a combination of both. We found no significant differences in clinical characteristics between the groups of patients for whom baseline serum samples (n=236) were available and those for whom blood samples were not available (n=113) (online supplemental table S1).

The levels of IL-18, S100A9 and S100A12 at baseline were significantly higher in patients with systemic JIA than in those with non-systemic JIA (p<0.001) (table 2). The baseline levels of serum biomarkers for all JIA categories are shown in online supplemental figure S1. At the baseline visit, eight patients were classified as having extended oligoarthritis with significantly higher baseline levels of S100A12 than the 113 patients with persistent oligoarthritis (median 2195 pg/mL (95% CI 1056 to 6247) vs median 889 pg/mL (95% CI 409 to 1595), p=0.029). Of the 121 patients with oligoarticular JIA at baseline, 45 were classified as having persistent oligoarticular JIA and 61 as not having the persistent type (42 extended oligoarthritis, 3 psoriatic arthritis, 5 enthesitis-related arthritis, 11 undifferentiated) at the 18-year FU. 15 were missing. Baseline MMP-3 was significantly lower in patients who had persistent oligoarticular JIA at 18-year FU compared with those with a non-persistent subtype (2654 pg/mL (1060–11 982 pg/mL) vs 6949 pg/mL (1868–21 735 pg/mL), p=0.028). For the other biomarkers we found no significant difference.

Paired comparison of serum analyses from the same individuals obtained both at baseline and at the 18-year FU showed that the levels of IL-1β, IL-6, IL-10, IL-17A, IL-18 and sCD25 obtained at baseline ranged from 1.4 to 5.5 times higher than at the 18-year FU (p<0.001) (table 3). Levels of IL-1β, IL-4, IL-6, IL-10, IL-13, MMP-3, S100A9 and S100A12 were significantly lower in DMARD-naïve patients at baseline than in non-naïve DMARD

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Table 2 Serum levels of biomarkers at baseline comparing patients with systemic JIA (n=10) versus non-systemic JIA (n=226)

	All	Systemic JIA (sJIA)	Non-systemic JIA (non-sJIA)	P value sJIA versus non-sJIA
n	236	10	226	
IL-1β	16.5 (6.7–30.5)	19.2 (11.6–32.1)	16.5 (6.2–30.5)	0.462
IL-4	88.6 (55.4–132.7)	94 (5–191)	89 (57–133)	0.951
IL-6	6.7 (3.2–13.0)	11.5 (3.6–46.0)	6.7 (3.2–12.6)	0.254
IL-10	5.3 (2.3–7.1)	6.7 (2.7–10.5)	5.3 (2.3–7.1)	0.180
IL-12p70	13.4 (0.5–72.5)	31.2 (0.5–108.4)	13.4 (0.5–72.5)	0.524
IL-13	497 (387–621)	576 (461–719)	497 (387–621)	0.080
IL-17	10.3 (0.8–16.2)	16.7 (5.2–36.1)	9.7 (0.8–16.0)	0.161
IL-18	205 (136–291)	2377 (594–8409)	198 (136–273)	<0.001**
TNFα	9.2 (1.7–16.6)	6.7 (1.0–27.3)	9.2 (22–16.6)	0.932
MMP-3	5174 (1747–17 493)	12 368 (2706–65 192)	5064 (1727–16 424)	0.172
CCL-2	180 (112–251)	135 (49–172)	183 (112–255)	0.102
sCD25	587 (420–766)	709 (427–891)	581 (418–760)	0.394
GM-CSF	0.4 (0.4–3.8)	3.2 (0.4–13.5)	0.4 (0.4–3.7)	0.059
MPO	2294 (1579–3572)	2367 (1953–5161)	2289 (1533–3565)	0.449
S100A9	511 (314–1277)	2786 (949–5643)	505 (309–1054)	0.006*
S100A12	924 (434–1610)	1947 (1253–10 124)	904 (431–1601)	0.007*

Values are in picograms/millilitre (pg/mL) expressed as median (IQR). *P<0.01; **p<0.001.
CCL-2, chemokine ligand 2; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; JIA, juvenile idiopathic arthritis; MMP-3, matrix metalloproteinase 3; MPO, myeloperoxidase; S100A9, S100 calcium-binding protein A9; S100A12, S100 calcium-binding protein A12; sCD25, soluble CD25; TNFα, tumour necrosis factor alpha.

patients (online supplemental table S2). Correspondingly, JADAS71 measured at baseline was significantly lower for the DMARD-naïve group than for the DMARD-treated group (4.2 (1.7–8.1) vs 11.5 (4.9–18.2), respectively; p<0.001).

Patients with active disease at the 18-year FU had significantly higher levels of IL-1β, IL-6, IL-12p70, IL-13, MMP-3, S100A9 and S100A12 at baseline than had patients with inactive disease at FU (figure 2A, online supplemental table S3). Patients who had achieved remission off medication at the 18-year FU had significantly lower levels of IL-1β, IL-12p70, IL-13 and MMP-3 measured at baseline than the remaining cohort had (non-achievement of remission off medication) (figure 2B, online supplemental table S3). These results were not affected by the exclusion of patients with systemic JIA from the cohort (online supplemental table S4).

Using 'active disease' as the response variable as opposed to 'inactive disease', we investigated the ORs of developing active/inactive disease at the time of the 18-year FU as a function of the following clinical variables registered at baseline (gender, age at onset, number of active and cumulative joints, JADAS71, Childhood Health Assessment Questionnaire, ESR and CRP) as well as the baseline serum biomarkers. ROC curves and AUCs from the logistic model predictions were computed for the prediction of 'inactive disease' and 'remission off medication' by the Wallace definition,[25 26] no active joints and JADAS71≤1 at the 18-year FU (table 4). For each variable, univariately, table 4 lists the AUC of the ROC for the predicted probabilities, the 95% CI of the AUC and associated p values. The associated p values listed in table 4 are in reference to the null hypothesis that the AUC of the ROC is 0.5. Our findings demonstrated for the prediction of inactive disease predictive capability for IL-1β, IL-6, IL-12p70, IL-13, MMP-3, S100A9 and S100A12, as well as ESR and active and cumulative joints. The AUC for the prediction of remission off medication at 18-year FU showed acceptable predictive ability for IL-1β, IL-6, IL-12p70, IL-13 and MMP-3 (table 4). Computing for the prediction of JADAS71>1 revealed predictive capability for IL-1β, IL-6, IL-12p70, IL-13, MMP-3, S100A9 and S100A12, as well as ESR and active and cumulative joints. The AUC for the prediction of remission off medication at 18-year FU showed acceptable predictive ability for IL-1β, IL-6, IL-12p70, IL-13 and MMP-3 (table 4). Computing for the prediction of JADAS71>1 revealed predictive capability for IL-1β, IL-4, IL-6, IL-12, IL-13, IL-17, TNFα, MMP-3, S100A9 and S100A12 (table 4).

Logistic regression was performed to assess the impact of a number of biomarkers (IL-12p70, IL-13, MMP-3, S100A9 and S100A12) and conventional variables (ESR, active and cumulative joints) on the likelihood of predicting the outcome of active/inactive disease at the

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Table 3 Comparison of serum biomarker levels obtained at baseline and at 18-year FU

	Baseline—all	18-year FU—all	Paired samples Baseline	18-year FU	P value (Wilcoxon)
n	236	284	150	150	
IL-1β	16.5 (6.7–30.5)	11.8 (8.6–18.0)	16.9 (3.8–30.5)	12.5 (8.2–19.7)	<0.001
IL-4	88.6 (55.4–132.7)	83.0 (66.2–112.8)	81.9 (54.0–121.0)	80.0 (63.0–125.0)	0.010
IL-6	6.7 (3.2–13.0)	4.5 (3.3–6.2)	6.4 (3.2–13.6)	4.5 (3.6–6.0)	<0.001
IL-10	5.3 (2.3–7.1)	0.7 (0.0–2.3)	5.0 (1.4–7.1)	0.9 (0.3–2.8)	<0.001
IL-12	13.4 (0.5–72.5)	58.8 (22.8–92.4)	33.6 (0.5–88.4)	72.5 (42.0–105.0)	<0.001
IL-13	497 (387–621)	448 (363–498)	502 (389–621)	467 (392–529)	0.249
IL-17	10.3 (0.8–16.2)	1.9 (0.7–3.3)	8.0 (0.8–15.2)	1.9 (0.7–5.6)	<0.001
IL-18	205 (136–291)	149 (106–219)	190 (126–282)	139 (98–211)	<0.001
TNFα	9.2 (1.7–16.6)	8.2 (3.9–12.8)	6.7 (1.0–14.6)	6.9 (3.6–12.3)	0.537
MMP-3	5174 (1747–17 493)	6557 (4656–10 098)	6095 (1954–16 424)	6600 (4874–10 758)	0.466
CCL-2	180 (112–251)	182 (143–231)	166 (108–251)	187 (150–240)	0.066
CD25	587 (420–766)	780 (442–1554)	540 (413–737)	336 (264–418)	<0.001
GM-CSF	0.4 (0.4–3.8)	0.4 (0.4–3.6)	0.5 (0.4–4.8)	1.7 (0.4–4.7)	0.638
MPO	2294 (1579–3572)	4010 (1925–6178)	2299 (1494–3414)	4522 (2967–6231)	<0.001
S100A9	511 (314–1277)	780 (442–1554)	549 (305–1191)	782 (445–1553)	0.009
S100A12	924 (434–1610)	922 (591–1423)	976 (443–1637)	922 (576–1456)	0.407

Values are in picograms/millilitre (pg/mL) expressed as median (IQR). P values were calculated using Wilcoxon signed-rank test for paired comparison.
*Paired serum analyses from the same individuals taken both at baseline and at 18-year FU, n=150.
CCL-2, chemokine ligand 2; FU, follow-up; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MMP-3, matrix metalloproteinase 3; MPO, myeloperoxidase; S100A9, S100 calcium-binding protein A9; S100A12, S100 calcium-binding protein A12; TNFα, tumour necrosis factor alpha.

18-year FU. As shown in table 5, the strongest predictor, S100A9, made a unique statistically significant contribution (p=0.019) to the model, when controlling for the other variables. S100A9 levels >453 pg/mL at the baseline visit predicted having active disease at the 18-year FU with an OR of 2.88 (95% CI 1.19 to 7.00). None of the predictors for non-achievement of remission at the 18-year FU made a unique contribution to the model (table 5).

We computed ROC curves to determine the optimal cut-off point (in terms of accuracy) and assessed various predictive measures for the variable as a predictor. We investigated the contribution of the serum biomarkers as baseline predictors of both inactive disease and remission off medication 18 years after disease onset. Doing this, we fitted a 'small, conventional model' and 'large, combined clinical and biomarker model' logistic regression model for each outcome and constructed ROC curves based on the estimated probability of the outcome in question. The two models were compared using ANOVA. The small, conventional model included commonly obtained clinical variables at baseline (ie, gender, age at onset, active and cumulative joints, ESR and CRP). The extended, combined model included the same clinical variables as above plus the biomarkers (IL-1ß, IL-4, IL-6, Il-10, IL-12, IL-13, IL-17A, IL-18, TNFα, MMP-3, CCL-2, sCD25, GM-CSF, MPO, S100A9 and S100A12) (figure 3). We compared the two regression models and tested if the biomarkers in the combined model with an AUC of 0.80, *as a whole*, provided additional significant explanatory value (figure 3). The extended model including the serum biomarkers was significantly better (p=0.024) at predicting inactive disease as an outcome after 18 years than was the conventional model, leading to an improvement in the AUC from 0.59 to 0.80. The equivalent comparison for remission off medication 18 years after disease onset did not yield significant results (p=0.4458) though the AUC was increased from 0.60 to 0.71 (figure 3). The prediction model selectively including the non-systemic patients (n=226) was also analysed (figure 3). The extended prediction model including the serum biomarkers was significantly better both at predicting inactive disease and remission off medication as an outcome after 18 years than was the conventional clinical model, leading to an improvement in the AUC from 0.64 to 0.85, p=0.0004, and from 0.61 to 0.80, p=0.01, respectively (figure 3). The prediction model selectively including the DMARD-naïve patients (n=180) was analysed. The extended prediction model including the serum biomarkers was significantly better at predicting inactive disease as an outcome after 18 years than was the conventional clinical model, leading to an improvement in the AUC from 0.61 to 0.81, p=0.038

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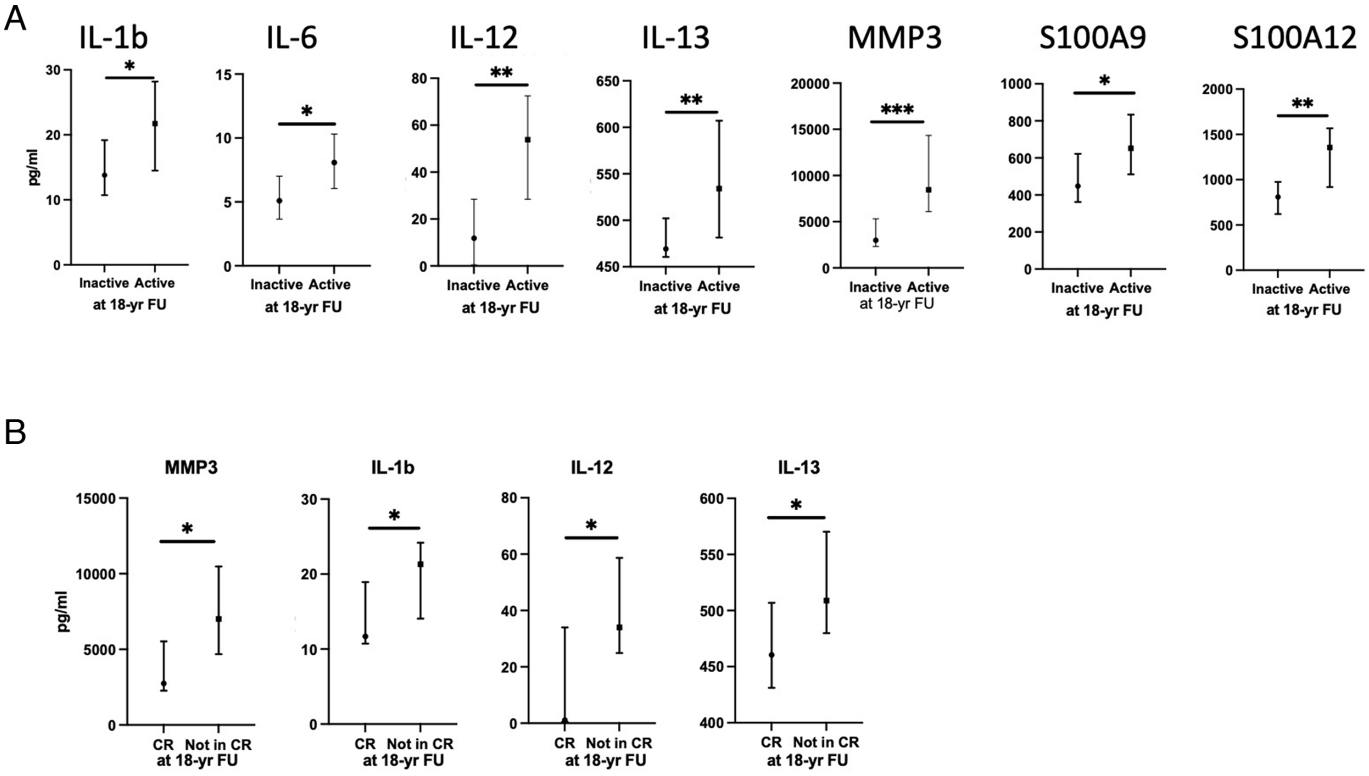

Figure 2 (A) Serum biomarker levels at baseline visit comparing patients with inactive disease (as defined by Wallace *et al*[25 26] (n=112) and active disease (n=82) at 18-year follow-up (FU). (B) Serum biomarker levels at baseline visit comparing patients in complete remission off medication (CR; as defined by Wallace *et al*[25 26] (n=76) and non-achievement of CR (n=117) at 18-year follow-up. Values are in picograms/millilitre (pg/mL) expressed as median with 95% CI. *P<0.05; **p<0.01; ***p<0.001. IL, interleukin; MMP-3, matrix metalloproteinase 3; S100A9, S100 calcium-binding protein A9; S100A12, S100 calcium-binding protein A12.

(figure 3). The equivalent comparison for remission off medication 18 years after disease onset gave a small but insignificant increase in AUC from 0.61 to 0.74, p=0.258 (figure 3).

DISCUSSION

From this unique population-based Nordic JIA cohort, we have previously substantiated the characterisation of JIA as a chronic disease, showing that 46% of patients still exhibited active disease even after 18 years of FU.[1] In the present study, we combined clinical data with data from analysis of a large panel of inflammatory biomarkers, both at baseline and after 18 years of FU. Patients who exhibited inactive disease[25] 18 years later demonstrated significantly lower levels of a large panel of proinflammatory biomarkers (IL-1β, IL-6, IL-12, IL-13, MMP-3, S100A9 and S100A12) at baseline. By comparing logistic regressions between a traditional clinical model and an integrated model combining clinical and biomarker data (encompassing a panel of inflammatory biomarkers from baseline), our analysis revealed a significant enhancement in predictive capacity. This augmentation was particularly evident in forecasting the outcome of inactive/active disease status 18 years later.

To our knowledge, this is the first study of inflammatory biomarkers obtained early in the disease course in a population-based JIA cohort evaluating the prediction of inactive disease and remission at long-term FU. Previous biomarker investigations in JIA have been performed in smaller cohorts or over shorter FU durations.[20 27–30] In this Nordic JIA cohort, we have previously studied complement lectin pathway protein levels, showing increased serum M-ficolin levels at baseline and a decrease at FU, reflecting the course of clinical disease activity.[31] However, neither M-ficolin nor other lectin pathway proteins showed predictive abilities for long-term remission status.[31]

We found significantly higher levels of IL-18, S100A9 and S100A12 in systemic JIA than in non-systemic disease categories, which is in accordance with previous findings.[20 27] These three proinflammatory biomarkers have been suggested as valuable predictors for discriminating between children with systemic JIA and children with other diseases that can be easily misdiagnosed as systemic JIA.[14 32 33] Ganeva *et al*[20] investigated whether baseline biomarker levels gave information about disease extension and analysed their potential in predicting the development of extended versus persistent oligoarticular JIA after 1 year. Contrary to their expectations, they observed that extended oligoarthritis did not necessarily correlate with increased biomarker activity; rather, higher baseline cytokine levels were discovered in patients with

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Table 4 Area under the curve (AUC) model for each biomarker measured at baseline on prediction of 'Inactive disease' and 'remission off medication' according to Wallace definition,[25 26] number of active joints and JADAS71≤1[35] at 18-year follow-up

Baseline predictor candidates	Active disease versus inactive disease* (n=81/112)		Non-achievement of remission versus remission off medication* (n=117/76)		Active joints at 18-year FU versus no active joints (n=46/153)		JADAS71>1 versus JADAS71≤1 at 18-year FU (n=109/75)	
	Missing=43		Missing=43		Missing=37		Missing=52	
	AUC	P value	AUC	P value	AUC	P value	AUC	P value
IL-1β	0.60 (0.52–0.68)	0.020*	0.603 (0.52–0.68)	0.016*	0.63 (0.53–0.72)	0.011*	0.66 (0.58–0.74)	<0.001***
IL-4	0.55 (0.47–0.63)	0.245	0.532 (0.45–0.62)	0.455	0.53 (0.43–0.62)	0.572	0.59 (0.51–0.67)	0.031*
IL-6	0.61 (0.53–0.69)	0.011*	0.572 (0.49–0.66)	0.093	0.59 (0.50–0.68)	0.044*	0.61 (0.53–0.69)	0.009**
IL-10	0.54 (0.46–0.63)	0.326	0.563 (0.48–0.65)	0.143	0.53 (0.43–0.62)	0.582	0.55 (0.47–0.64)	0.243
IL-12p70	0.63 (0.55–0.71)	0.003**	0.588 (0.51–0.67)	0.038*	0.62 (0.53–0.71)	0.009**	0.59 (0.51–0.67)	0.033*
IL-13	0.61 (0.53–0.69)	0.010*	0.593 (0.51–0.67)	0.029*	0.66 (0.57–0.75)	<0.001***	0.68 (0.60–0.76)	<0.001***
IL-17	0.55 (0.46–0.63)	0.280	0.550 (0.46–0.64)	0.244	0.57 (0.48–0.66)	0.134	0.62 (0.53–0.70)	0.006**
IL-18	0.54 (0.46–0.62)	0.333	0.542 (0.46–0.63)	0.321	0.54 (45–0.64)	0.394	0.56 (0.48–0.65)	0.150
TNFα	0.56 (0.48–0.64)	0.147	0.571 (0.49–0.66)	0.096	0.54 (0.45–0.63)	0.400	0.60 (0.51–0.68)	0.023*
MMP-3	0.64 (0.56–0.72)	<0.001**	0.599 (0.51–0.68)	0.020*	0.66 (0.58–0.75)	<0.001***	0.64 (0.55–0.72)	0.001**
CCL-2	0.52 (0.44–0.60)	0.615	0.548 (0.46–0.63)	0.265	0.45 (0.36–0.54)	0.287	0.51 (0.43–0.60)	0.758
sCD25	0.51 (0.42–0.61)	0.817	0.513 (0.42–0.60)	0.788	0.52 (0.39–0.64)	0.786	0.52 (0.43–0.62)	0.638
GM-CSF	0.58 (0.50–0.67)	0.056	0.518 (0.43–0.60)	0.678	0.59 (49–0.70)	0.072	0.54 (0.46–0.63)	0.339
MPO	0.54 (0.46–0.62)	0.370	0.517 (0.44–0.60)	0.688	0.60 (0.51–0.70)	0.38	0.57 0.48–0.65)	0.121
S100A9	0.60 (0.52–0.68)	0.020*	0.557 (0.47–0.64)	0.181	0.64 (0.56–0.72)	<0.001***	0.62 (0.53–0.70)	0.006**
S100A12	0.63 (0.55–0.71)	0.002**	0.576 (0.49–0.66)	0.074	0.69 (0.60–0.77)	<0.001***	0.63 (0.54–0.71)	0.003**
ESR	0.59 (0.50–0.68)	0.048*	0.579 (0.49–0.67)	0.093	0.59 (0.49–0.70)	0.083	0.55 (0.46–0.64)	0.281
CRP	0.58 (0.49–0.67)	0.090	0.544 (0.45–0.64)	0.359	0.59 (0.49–0.70)	0.088	0.57 (0.48–0.66)	0.127
CHAQ	0.51 (0.42–0.61)	0.813	0.555 (0.47–0.64	0.240	0.48 (0.37–0.59)	0.678	0.52 (0.43–0.62)	0.642
Age at onset	0.51 (0.41–0.61)	0.834	0.518 (0.44–0.60)	0.667	0.45 (0.35–0.54)	0.278	0.57 (0.48–0.65)	0.135
Active joints	0.63 (0.53–0.72)	0.012*	0.578 (0.497–0.66)	0.069	0.55 (0.46–0.65)	0.276	0.55 (0.47–0.64)	0.229
Cumulative joints	0.64 (0.54–0.73)	0.006**	0.584 (0.501–0.67)	0.050	0.61 (0.52–0.71)	0.022*	0.55 (0.47–0.64)	0.204
JADAS71	0.58 (0.48–0.68)	0.121	0.578 (0.48–0.68)	0.141	0.54 (0.43–0.66)	0.441	0.54 (0.44–0.65)	0.442

*P<0.05; **p<0.01; ***p<0.001.
*Definitions of inactive/active disease and remission off medication as per Wallace *et al*.[25 26]
CCL-2, chemokine ligand 2; CHAQ, Childhood Health Assessment Questionnaire; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; FU, follow-up; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; JADAS71, Juvenile Arthritis Disease Activity Score for 71 joints; MMP-3, matrix metalloproteinase 3; MPO, myeloperoxidase; S100A9, S100 calcium-binding protein A9; S100A12, S100 calcium-binding protein A12; sCD25, soluble CD25; TNFα, tumour necrosis factor alpha.

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Table 5 Logistic regression on active disease and remission off medication as dependent variables at 18-year follow-up (FU)

	B	SE	Wald	df	Sig	Exp(B)	95% CI
Active disease							
IL-1β	0.903	0.746	1.464	1	0.226	2.467	0.57 to 10.65
IL-6	−0.098	0.642	0.023	1	0.879	0.907	0.26 to 3.19
MMP-3	0.51	0.612	0.695	1	0.404	1.665	0.50 to 5.52
IL-12	0.015	0.510	0.001	1	0.976	1.015	0.37 to 2.76
IL-13	0.211	0.798	0.070	1	0.791	1.235	0.26 to 5.91
S100A9	1.106	0.472	5.480	1	**0.019**	**3.022**	**1.20 to 7.63**
S100A12	−0.165	0.560	0.087	1	0.768	0.848	0.28 to 2.54
ESR log	0.537	0.624	0.741	1	0.389	1.711	0.50 to 5.82
Active joints log	0.566	1.157	0.239	1	0.625	1.761	0.18 to 17.00
Cumulative joints log	0.186	0.984	0.036	1	0.850	1.205	0.18 to 8.29
Remission off medication							
IL-1β	−0.515	0.497	1.071	1	0.301	0.598	0.23 to 1.58
MMP-3	−0.531	0.372	2.042	1	0.153	0.588	0.28 to 1.22
IL-12	−0.151	0.377	0.161	1	0.689	0.860	0.41 to 1.80
S100A9	−0.313	0.330	0.901	1	0.342	0.731	0.38 to 1.40
Cumulative joints log	−0.582	0.378	2.369	1	0.124	0.559	0.27 to 1.17

For the goodness of fit, the 'omnibus tests of model coefficients' ('Active disease': χ^2=27.22, p=0.002; 'Remission off medication': χ^2=14.029, p=0.015) and the Hosmer and Lemeshow test ('Active disease': χ^2=5.597, p=0.692; 'Remission off medication': χ^2=6.332, p=0.610) both indicated support for the model.
Bold values indicate a unique statistically contribution to the model.
ESR, erythrocyte sedimentation rate; IL, interleukin; log, logarithm; MMP-3, matrix metalloproteinase 3.

subsequent persistent oligoarticular JIA.[20] We could, however, not confirm these results in our study but found that baseline MMP-3 was significantly lower in patients with persistent oligoarticular JIA at 18-year FU compared with those with a non-persistent subtype. We found no disparities in other baseline biomarker levels between patients with persistent oligoarticular JIA and patients with non-persistent oligoarticular subtype at the 18-year FU.

In contrast to previous studies,[19 20 34] we did not relate the baseline biomarker levels to a specific medical treatment response in the present study. Medical responses to treatment with corticosteroids, synthetic DMARDs and biologics might interfere with the baseline biomarker levels, and sera from patients on DMARDs may not truly reflect baseline values. However, at the time of the baseline serum sampling, a high proportion of the patients were DMARD naïve in our cohort (76%), which was not the case in the previous study by Ganeva *et al* where only 20% were DMARD naïve.[20] None of the patients in our study had started biologics when blood was taken at baseline. Levels of IL-1β, IL-4, IL-6, IL-10, IL-13, MMP-3, S100A9 and S100A12 were significantly higher in patients treated with DMARD at baseline than in DMARD-naïve patients, which may seem surprising. However, since the baseline measures were obtained no later than 8 months after disease onset, they indicated that the DMARDs had been administered shortly before blood samples were taken. In fact, the group exposed to DMARD represented individuals with increased disease activity compared with the DMARD-naïve group.

By using the Wallace provisional criteria for defining CID[25] and the preliminary criteria for remission off medication,[26] we used even stricter definitions of 'inactive disease' than were used in the paper by Ganeva *et al*[20] who applied the 'inactive disease' definition of clinical JADAS≤1.[35] Even so, many of our results align with their previously published findings, despite the substantially extended FU in our study compared with the 1-year FU in Ganeva *et al*'s study.[20] The ROC curve for each of the variables showed that AUCs for seven biomarkers (IL-1β, IL-6, IL-12p70, IL-13, MMP-3, S100A9 and S100A12) and three conventional variables (ESR, number of active joints and number of cumulative joints) at baseline could significantly predict the outcome of inactive disease at the 18-year FU. Including all these variables in the same equation, we found that only S100A9 could significantly predict the outcome of active disease at the 18-year FU. Using the stricter 'remission off medication' as the dependent variable, we found that only the biomarkers IL-1β, IL-12p70, IL-13 and MMP-3

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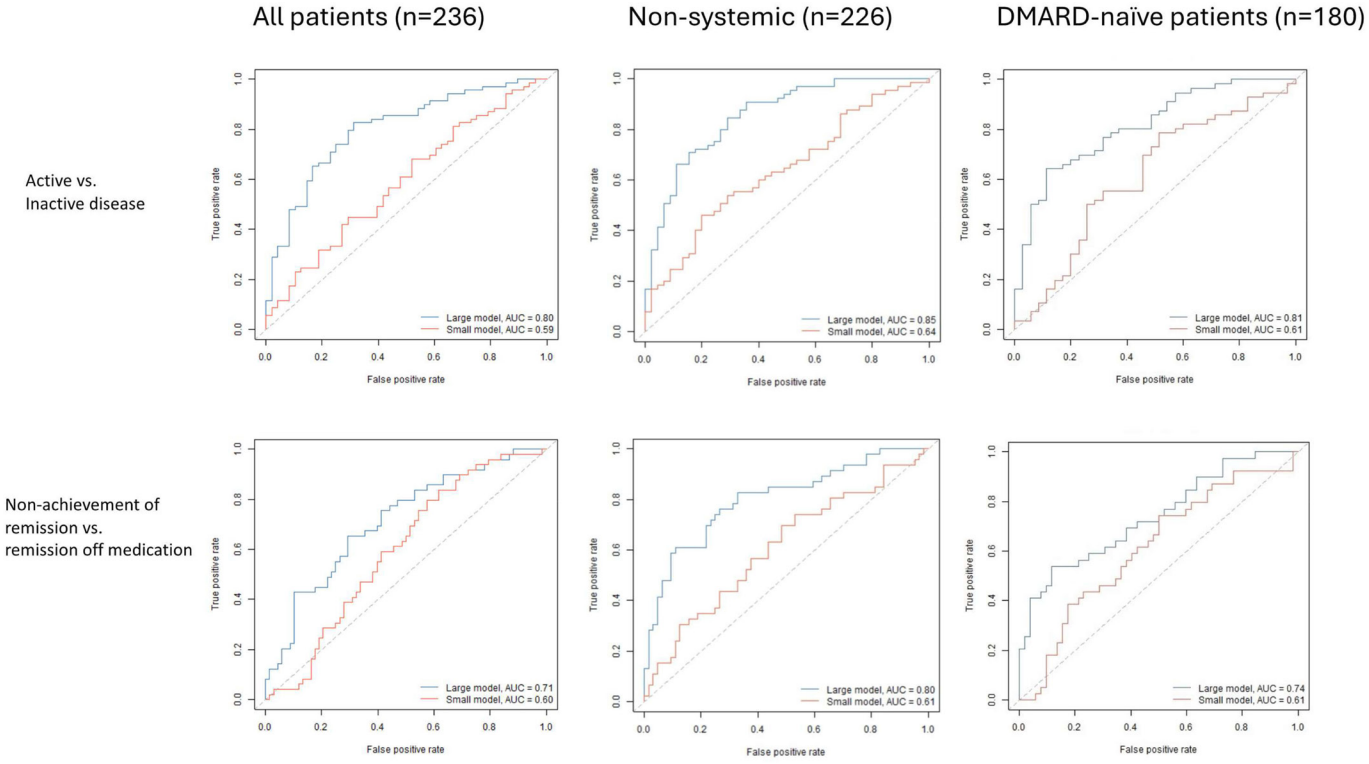

Figure 3 Comparison of a small prediction model including only clinical variables and a large model including the same clinical variables along with all biomarkers for all patients (n=236), patients with non-systemic juvenile idiopathic arthritis (JIA) (n=226) and disease-modifying antirheumatic drug (DMARD)-naïve patients (n=180). Receiver operating characteristic (ROC) curves illustrating area under the curve (AUC) model for prediction of active disease or non-achievement of remission at 18-year follow-up (FU). Small (conventional) model included common clinical variables at baseline visit: gender, age at onset, active and cumulative joints, erythrocyte sedimentation rate and C reactive protein. Large, combined model included same clinical variables as above plus biomarkers (interleukin 1β (IL-1β), IL-4, IL-6, IL-10, IL-12, IL-13, IL-17A, IL-18, tumour necrosis factor alpha, matrix metalloproteinase 3, chemokine ligand 2, soluble CD25, granulocyte-macrophage colony-stimulating factor, myeloperoxidase, S100A9, S100A12).

significantly predicted the outcome. Including all these variables in the same equation, we found that none of the variables predicted the outcome of remission off medication at the 18-year FU. Comparing the clinical model (only clinical variables included) with the combined clinical/biomarker model (clinical data and biomarkers included), our analysis demonstrated a noteworthy enhancement in predictive capacity. The inclusion of biomarkers significantly augmented the accuracy of predicting the attainment of inactive disease status or its absence after 18 years of disease duration.

The findings from this study, which highlight S100A9 as the strongest predictor of long-term outcomes, underscore the substantial importance of S100 proteins as reliable biomarkers. The S100 proteins, a family of Ca^{2+}-binding proteins, have a broad range of cellular functions, including cell migration, differentiation, tissue repair and inflammation.[36] The S100 proteins, S100A8/S100A9 and S100A12, are released from cells of the myeloid lineage during activation of the innate immune system,[37] acting as ligands for Toll-like receptor 4 and receptors for advanced glycation end-products, thus activating phagocytes and promoting further recruitment of leucocytes to sites of damage.[38] In a multicentre randomised controlled trial including 364 children with clinically inactive JIA, Foell *et al*[39] found that serum levels of S100A8/S100A9 prior to stopping methotrexate (MTX) were significantly higher in patients who later had flares than in patients with stable remission. Moreover, they found that the serum levels of S100A8/S100A9 were predictive of disease flare within 12 months of observation. The results could support the clinical decision on when to withdraw MTX therapy.[39] MMP-3 plays an important role in the pathogenesis of inflammatory arthritis as it facilitates accumulation of inflammatory cells, promotes vascular invasion in the synovium, degrades cartilage matrix and promotes osteoclast differentiation.[40] We found that MMP-3 performed well as a predictor of long-term outcome at the 18-year FU, which was also recently reported by Ziegler *et al* in a non-population-based JIA cohort.[41]

The main strengths of our study are its population-based design and the extensive, uniform long-term observation for all JIA participants. These attributes enhance the generalisability of our findings. Furthermore, the paired FU of a large panel of inflammatory

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biomarker samples closely related to disease activity measures is another main strength of the present study. There are several limitations to the present study design and the analytical set-up. First, aliquots of sera for biomarker analysis obtained at baseline were available from only 67.6% of the initial JIA cohort, excluding the Finnish group which had no access to storage. Nevertheless, the missing samples of the Finnish patients did not interfere with the population-based setting. At the 18-year FU, the availability of the serum samples for biomarker analysis was acceptable (86%). However, when comparing the group of patients with available baseline serum samples with those without, no differences were observed in terms of onset age, gender and clinical disease activity. Another limitation is the lack of a control group; however, the same laboratory has previously demonstrated low serum levels of most biomarkers in healthy controls compared with all JIA categories.[20] Third, dilution with TBS was necessary due to small volumes of baseline samples. Low-abundance cytokines, like IL-1β and IL-4, behave non-linearly in Luminex when diluted[42 43] which may have resulted in unusually high absolute levels. However, this did not affect the relative differences. Fourth, the stability of the biomarkers may have suffered from the long-term storage even at −80°C. The S100 proteins have previously shown to be relatively stable after long-term storage.[12 44] However, we found that the median level of S100A9 was lower at the baseline visit compared with the 18-year time point. Since the disease activity at baseline was higher than at the 18-year FU we would have expected a high-baseline S100A9 level. The observed low level of S100A9 might be caused by a protein degradation during the many years of storage.

On the other hand, biomarkers like IL-1β, IL-6, IL-10, IL-13 and IL-17 may degrade up to 50% within 4 years.[45] However, it is noteworthy that despite the conceivable protein degradation over time, several of the biomarkers analysed from samples obtained at baseline ranged from 1.4 to 5.5 times higher than those obtained at the 18-year FU. Fifth, the baseline samples were obtained 6 months after the onset of the disease, and 19% of the patients were using DMARDs at the time of sample collection. However, the results showed that most inflammatory markers retained higher serum levels at the baseline visit than at the 18-year FU. None of the patients received biologics early on as the availability was very limited in 1997–2000. It can only be speculated whether early, more aggressive therapy could have altered the remission rates reported 18 years later. At the 18-year FU, almost 16% were on biologics and the disease activity was low in the total cohort suggesting that effective treatment had been initiated; however, compliance and immunosuppressive therapy may have affected the level of the biomarkers. These factors about medication at the two time points may have compromised the predictive accuracy.

CONCLUSION

The validated biomarker potential of specific S100 proteins along with the cytokines IL-1ß, IL-6, IL-12, IL-13 and MMP-3 may greatly improve the management of JIA. If these biomarkers together with conventional clinical variables could predict which children are unlikely to respond to first-line treatment with MTX, early second-line biologic therapy could be initiated instead. This could expedite remission and minimise adverse effect by reducing unnecessary drug exposure. Furthermore, prospectively using S100 proteins as biomarkers for detection of subclinical inflammation could possibly prevent inappropriate therapy interruption in patients likely to relapse. Our results suggest that these biomarkers could provide additional value in prediction models, possibly guiding decisions concerning JIA treatment and therapeutic withdrawal. Nevertheless, additional research is needed before definitive conclusions can be drawn.

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Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and the Nordic JIA cohort was approved by the Regional Committee for Medical Ethics and the Danish Data Protection Agency (Denmark 1-10-72-280-13, Norway 2012/2051, Sweden Dnr 2014/413-31, Finland 174/13/03/03/2014). The studies were approved by the Research Ethics Boards and data protection authorities at all participating institutions and performed following the Declaration of Helsinki, including informed written consent. Participants gave informed consent to participate in the study before taking part.

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