Arthritis Care & Research
Vol. 76, No. 12, December 2024, pp 1723–1732
DOI 10.1002/acr.25417

American College of Rheumatology
Empowering Rheumatology Professionals

Biomarker Changes in Response to Tofacitinib Treatment in Patients With Polyarticular-Course Juvenile Idiopathic Arthritis

Ekemini A. Ogbu,[1] Hermine I. Brunner,[2] Esraa Eloseily,[3] Yonatan Butbul Aviel,[4] Kabita Nanda,[5] Heinrike Schmeling,[6] Heather Tory,[7] Yosef Uziel,[8] Diego Oscar Viola,[9] Dawn M. Wahezi,[10] Stacey E. Tarvin,[11] Alyssa Sproles,[12] Chen Chen,[13] Nicolino Ruperto,[14] Bin Huang,[13] Alexei Grom,[15] and Sherry Thornton,[15] for the Investigators of the PRINTO and PRCSG Networks

Objective. We examine levels of candidate blood-based biomarkers (CBBs) in patients with juvenile idiopathic arthritis (JIA) treated with tofacitinib.

Methods. Patients with JIA who participated in clinical trial NCT02592434 received tofacitinib from baseline to week 18. Serial serum samples were assayed for CBBs (S100A8/9, S100A12, interleukin-18 [IL-18], serum amyloid A, resistin, vascular endothelial growth factor, angiopoietin-1, angiopoietin-2, matrix metalloproteinase 8 [MMP8], MMP2, tissue inhibitor of metalloproteinases 1, leptin, chemokine [C-X-C motif] ligand 9, soluble IL-2 receptor, intercellular adhesion molecule 1, soluble tumor necrosis factor receptor, IL-6, IL-23, monocyte chemotactic protein 1, chemokine [C-C motif] ligand 18 [CCL18], and CCL20). Association of CBBs with JIA response to treatment from baseline to week 18 were assessed.

Results. This study included 166 patients with polyarticular-course JIA. Paired serum samples from 143 patients were available at both baseline and week 18. Thirty-five percent (50 of 143) of patients had a JIA-American College of Rheumatology 90 (JIA-ACR90) level improvement, whereas 90, 121, and 137 (63%, 85%, and 96%) achieved JIA-ACR70, 50, and 30 improvement at week 18. Despite small numerical differences by JIA category, there were no baseline CBB values that independently predicted a decrease in Juvenile Arthritis Disease Activity Score (JADAS-27) or JIA-ACR90 response by week 18. Decrease in resistin level (baseline to week 18) was significantly associated with week 18 improvement in JADAS-27 and JIA-ACR90 response after adjusting for age, sex, JIA disease duration, and baseline resistin (r^2 0.79, SE 0.070, $P < 0.01$, and odds ratio [95% confidence interval] 1.134 [1.018–1.264]). HLA-B27 positivity was significantly associated with not achieving a JIA-ACR90 response at week 18 ($P = 0.0097$).

Conclusion. Among the CBBs included, only resistin was significantly associated with treatment response, and no CBB was identified that forecasts JIA improvement after initiation of tofacitinib. The association of HLA-B27 positivity with lower response to tofacitinib in JIA is intriguing and merits further study.

Serum samples were donated by Pfizer. Pfizer was not involved in the study design, biomarker assays, data analysis, or data interpretation.

Supported by the Ralph & Marian Falk Medical Trust Catalyst Award awarded to Drs Thornton, Brunner, and Grom and the Cincinnati Children's Hospital Medical Center Pediatric Musculoskeletal & Rheumatology Innovation Core center (PORTICO). The study was also supported by the National Institutes of Health (grants 5P30-AR-076316 and P30-AR-070549).

[1]Ekemini A. Ogbu, MD, MSc: Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Ohio, and Johns Hopkins University, Baltimore, Maryland; [2]Hermine I. Brunner, MD, MSc, MBA: Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Ohio; [3]Esraa Eloseily, MD: Cincinnati Children's Hospital Medical Center, Ohio, and Assiut University Faculty of Medicine, Assiut, Egypt; [4]Yonatan Butbul Aviel, MD: Rambam Health Care Campus, Haifa, Israel; [5]Kabita Nanda, MD: Seattle Children's Hospital and University of Washington School of Medicine; [6]Heinrike Schmeling, MD: Alberta Children's Hospital, University of Calgary, Calgary, Alberta, Canada; [7]Heather Tory, MD, MPH: University of Connecticut School of Medicine, Farmington, and Connecticut Children's Medical Center, Hartford; [8]Yosef Uziel, MD: Meir Medical Center, Kfar-Saba, Tel Aviv School of Medicine, Tel Aviv University, Israel; [9]Diego Oscar Viola, MD: Instituto CAICI SRL, Rosario, Argentina; [10]Dawn M. Wahezi, MD, MS: Children's Hospital at Montefiore, Bronx, New York; [11]Stacey E. Tarvin, MD, MS: Riley Hospital for Children at Indiana University, Indianapolis; [12]Alyssa Sproles, BS: Cincinnati Children's Hospital Research Foundation, Ohio; [13]Chen Chen, PhD, Bin Huang, PhD: Cincinnati Children's Hospital Medical Center, Ohio; [14]Nicolino Ruperto, MD, MPH: IRCCS Istituto Giannina Gaslini, Servizio Sperimentazioni Cliniche Pediatriche/Gaslini Trial Centre, PRINTO, Genoa, Italy; [15]Alexei Grom, MD, Sherry Thornton, PhD: Cincinnati Children's Hospital Medical Center and Cincinnati Children's Hospital Research Foundation, Ohio.

Additional supplementary information cited in this article can be found online in the Supporting Information section (https://acrjournals.onlinelibrary.wiley.com/doi/10.1002/acr.25417).

Author disclosures are available at https://onlinelibrary.wiley.com/doi/10.1002/acr.25417.

Address correspondence via email to Hermine I. Brunner, MD, MSc, MBA, at hermine.brunner@cchmc.org.

Submitted for publication March 19, 2024; accepted in revised form August 6, 2024.

SIGNIFICANCE AND INNOVATIONS

- Biomarkers measured in the serum before tofacitinib initiation have limited utility in anticipating response to JAK inhibition in polyarticular-course juvenile idiopathic arthritis (JIA).
- HLA-B27 positivity may be a biomarker for children with JIA with lower likelihood of improving with tofacitinib treatment. However, similar findings from larger cohorts of adults with arthritis are lacking.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is among the most common chronic rheumatic diseases of childhood. Adopted worldwide, the International League against Rheumatism classification recognizes seven distinct categories of JIA, informed by the number of joints involved, existence of extra-articular features such as fever or psoriasis, and the presence of certain laboratory markers such as HLA-B27 and rheumatoid factor (RF), respectively.[1] Diverse immunologic pathways are currently implicated in the development of the various JIA categories.[2] Despite this advancement in the understanding of JIA immunopathology and the common treatment with biologic therapy that target certain cytokines, B cells, T cells, or signaling pathways involved in inflammation, much-desired personalized treatment regimens for JIA remain elusive. Indeed, current treatment guidelines for JIA recommend treatments that are predominantly based on JIA category, inflammation of certain joints, and the occurrence of some extra-articular features such as uveitis.[3]

Tofacitinib, a JAK inhibitor (JAKi), is among the newer approved advanced medications for the treatment of JIA.[3,4] Tofacitinib acts by inhibiting a large number of cytokines, chemokines, and growth factors via mostly targeting the JAK1 and JAK3 signaling pathways. In a double-masked, placebo-controlled random withdrawal clinical trial, tofacitinib was found to be beneficial for the treatment of JIA.[5] In brief, among 184 patients with polyarticular forms of JIA receiving tofacitinib at now-approved dosages, there was improvement at JIA-American College of Rheumatology (ACR) 30 and 70 levels[6] in 77% and 51% of patients, respectively, during the 18-week open-label part 1 of the study. At week 18, 142 patients who had improved on open-label tofacitinib entered the placebo-controlled double-masked 26-week withdrawal part 2 of the study, where 51% continued tofacitinib and 49% newly received placebo. The JIA flare rate was statistically significantly lower in patients who continued tofacitinib (29%) than those newly receiving placebo (53%) after week 18.[5]

Despite advances in the understanding of JIA pathophysiology, and the growing use of biologic or small molecule therapies for the management of JIA, personalized treatments remain elusive. Tofacitinib inhibits a large number of markers of inflammation.[7] Previously proposed candidate biomarkers of JIA probe pathways involving peripheral inflammatory cytokines including interleukin-6 (IL-6), IL-1, IL-18, and the IL-12/IL-23 axis.[8–10]

Therefore, the objectives of our study were to investigate whether these candidate blood-based biomarkers (CBBs) when measured before starting tofacitinib can anticipate response to treatment and whether there are differences in changes in CBB levels over time between tofacitinib responders compared with nonresponders in patients with JIA.

PATIENTS AND METHODS

Patients. For this exploratory study, we used longitudinal data and available serum samples from the phase 3 placebo-controlled randomized withdrawal clinical trial in JIA (NCT02592434).[5] In brief, all patients received tofacitinib twice daily from baseline to week 18 with samples collected at baseline, ie, just before the start of tofacitinib, and at week 18. The patients continued their stable background immunotherapies, which included methotrexate (≤25 mg per week or ≤20 mg/m^2 per week) and oral glucocorticoids (≤0.2 mg/kg per day of prednisone equivalent or ≤10 mg per day). In this study, we only included the 166 patients aged 2 to <18 years with JIA who had serum samples available for additional analysis. Our six JIA categories of interest included polyarticular RF-positive JIA (PJIA-RF+), polyarticular RF-negative JIA (PJIA-RF–), extended oligoarticular JIA (exo-JIA), juvenile psoriatic arthritis (JPsA), enthesitis-related arthritis (ERA), and systemic JIA without active systemic features (SJIA). Only 143 patients had paired serum samples available from baseline and week 18. All included serum samples were consented for use in additional research. This study was approved by the Institutional Review Board of Cincinnati Children's Hospital Medical Center (CCHMC) (IRB# 2021-0465). The data that support the findings of this study are available from the corresponding author upon reasonable request.

Outcome measures of interest. Treatment responses were defined by the change in the Juvenile Arthritis Disease Activity Score 27 (JADAS-27) and as per the JIA-ACR improvement criteria between the start of tofacitinib at baseline and week 18. Both the JADAS-27 and JIA-ACR response criteria are widely accepted measures for assessing JIA activity and treatment effects in JIA.[11,12]

The JADAS-27 is a composite measure of disease activity that measures disease activity based on a 27-joint count and C-reactive protein (CRP) level.[12,13] The JADAS is composed of 1) the physician global assessment (PGA) of JIA disease activity measured on a visual analogue scale (VAS) (range: 0–10; 0, no activity); 2) a VAS of parent/patient global assessment of overall well-being (Pat-GA; range: 0–10; 0, very well; 10, very poor); 3) the number of active joints, ie, with swelling or, in absence of swelling, limited range of motion plus pain on motion or palpation;

and 4) a measure of inflammation—here, the CRP (in mg/L, normalized to range 0–10). The JADAS-27 score ranges from 0 to 57 with higher scores indicating more active disease (high, >8.5; moderate, 3.9–8.5; low, 1.1–3.8; inactive disease, ≤1).[12,13]

Using the criteria for improvement of JIA, a JIA-ACR90 response reflects profound improvement of disease. It is defined as ≥90% improvement in 3 out of 6 JIA core set variables (CSVs) with no more than one of the remaining CSVs worsening by ≥30%. The CSVs are 1) active joint count; 2) number of joints with limited range of motion; 3) PGA; 4) Pat-GA; 5) measure of functional ability, often using the Childhood Health Assessment Questionnaire Disability Index (CHAQ-DI)[14]; and 6) measure of inflammation (CRP) or erythrocyte sedimentation rate (ESR).[12]

CBB selection and measurement. CBBs were selected based on their known involvement in pathogenic pathways in inflammatory arthritis (Table 1). Our study used stored peripheral venous blood samples collected at baseline (before starting tofacitinib treatment) and at week 18. Whole blood was collected per participant per time point, and samples were then stored at room temperature until clotted (~30–45 minutes). After clotting, samples were centrifuged at 1,500*g* to 2,000*g* for 10 minutes in a refrigerated centrifuge (2–8°C). Serum samples were frozen at −70°C to −80°C in a nondefrosting freezer within 30 minutes of centrifugation. **Serum samples remained frozen until their use in this study.** The CBBs were quantitated using custom-made Luminex Human Magnetic Assay (24-PLEX) and enzyme-linked immunosorbent assay. Measurements were performed by the Rheumatology Research Flow Cytometry Core using previously described methodology.[33–35] Briefly, duplicate samples were incubated with antibody coated beads overnight at 4°C with shaking. Plates were washed twice, and the secondary antibody was added. Then, the samples were incubated at room temperature for 1 hour with shaking. Streptavidin-R- Phycoerythrin was then added directly to the secondary antibody and incubated for 30 minutes at room temperature with shaking. The plates were again washed, and 150 µL of sheath fluid was added with shaking for 5 minutes before detection with the Multiplex Analyzer (Millipore Sigma, Darmstadt, Germany). Concentrations were calculated from standard curves using recombinant proteins. CBBs were measured in pg/mL, except for serum amyloid A (SAA), which was measured in ng/mL.

Table 1. CBBs of interest implicated in JIA, rheumatoid arthritis, and other immune-mediated arthritides*

Pathway involved in immune-mediated arthritides	Selected CBBs
Innate immunity activation	S100A8/9, S100A12, IL-18, SAA, resistin[15–18]
Endothelial dysfunction	VEGF[19]
Angiogenic activity in synovial tissue	Ang-1, Ang-2[20]
Tissue and extracellular matrix degeneration	MMP2, MMP8, TIMP-1, leptin[21–23]
Interferon induced pathways	CXCL9[24]
T and B cell activation	sIL2r-α, ICAM-1[25,26]
Cytokine activity	sTNFr, IL-6, IL-23[10,27–29]
Trafficking of immune cells to sites of inflammation	MCP-1, CCL18, CCL2, CCL20[30–32]

* Ang, angiopoietin; CBB, candidate blood-based biomarkers; CCL, chemokine (C-C motif) ligand; CXCL9, chemokine (C-X-C motif) ligand 9; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; JIA, juvenile idiopathic arthritis; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; SAA, serum amyloid A; sIL2r, soluble interleukin-2 receptor; sTNFr, soluble tumor necrosis factor receptor; TIMP-1, tissue inhibitor matrix metalloproteinase-1; VEGF, vascular endothelial growth factor.

Statistical analysis. Summary statistics were performed for demographics (sex, race, ethnicity, and age at diagnosis), clinical parameters, and CBBs at baseline and at week 18. Sex, race, and ethnicity were self-reported. Clinical parameters included concurrent JIA medication treatment, disease duration, baseline JADAS-27, and HLA-B27 positivity status. Demographics, clinical parameters, and CBBs were subanalyzed by JIA category, treatment response (change in JADAS-27 and JIA-ACR90 level improvement yes/no), and CBB level change from baseline to week 18. Changes in JADAS-27 and CBB levels were defined as the absolute difference in values at baseline and week 18.

Categorical and noncategorical measures were summarized as frequencies (with percentages), and as means or medians (with SD or interquartile ranges [IQRs]), respectively. For univariate and two-group comparisons, chi-square or Fisher's exact test were used for categorical variables, while Wilcoxon's rank sum or Kruskal–Wallis tests were used for continuous variables, as appropriate.

CBB values below lower limit to quantification (LLOQ) were imputed using the LLOQ value. CBB values above the upper limit of quantification (ULOQ) were imputed using the ULOQ values. Before use in statistical analyses, CBB quantities were log10 transformed to minimize outlier related statistical complication. Linearity and collinearity of the CBB were also assessed. The Spearman correlation between treatment responses and CBB values (at baseline, and the changes from baseline to week 18) were evaluated as appropriate.

To determine the association between baseline JADAS-27 and CBBs (at baseline and the changes from baseline to week 18), simple linear regression was used. Multiple linear regression using a stepwise selection procedure was used to determine the strength of the association between baseline JADAS-27 and log10-transformed CBBs (at baseline, and the changes from baseline to week 18) adjusting for JIA category, demographics (age, sex, race), and disease duration.

Comparisons were also made between the JIA-ACR90 responders and nonresponders at week 18 by demographics, clinical parameters, and CBB levels. The association between

JIA-ACR90 response at week 18 and CBBs (at baseline and the changes from baseline to week 18) were determined by logistic regression analyses. Using stepwise logistic regression procedures, important CBBs associated with JIA-ACR90 response were first identified within each inflammatory pathway (Table 1) and then included with important CBBs identified from the other probed inflammatory pathways, both after adjusting for JIA category, baseline JADAS-27, demographics (age, sex, race), and disease duration. All stepwise selection procedures employed an entry criterion of 0.25 and a stay criterion of 0.15 to select CBB variables for inclusion in the final model. All logistic regression involving JIA categories used Firth correction to mitigate data sparsity. Sensitivity analyses were performed excluding patients with SJIA at baseline and at week 18. Since this was an exploratory investigation, no multiple comparison procedures were applied. Statistical analyses were performed using SAS/STAT version 15.1, and two-sided *P* values <0.05 were deemed statistically significant.

RESULTS

Patient baseline characteristics. The 166 patients included in this study constituted a subset of patients participating in the clinical trial.[5] As shown in Table 2, our patients were predominantly White (89%), not Hispanic (77%), and female (75%). The median age was 13 years (IQR 9–15). Median duration of disease and baseline JADAS-27 were both higher in patients with SJIA compared with other JIA categories. However, all 166 patients had high baseline disease activity by JADAS-27 exceeding a value of 8.5. Furthermore, there were no differences between JIA categories by baseline ESR and CRP. The median baseline CHAQ-DI score was 0.8 (IQR 0.3–1.4). Among patients also taking methotrexate (113 of 166), treatment was highest in those with PJIA-RF– (66 of 83, 80%) and PJIA-RF+ (19 of 27, 70%) compared with those in other JIA categories (chi-square; *P* = 0.006). Fifty-one patients were treated with glucocorticoids, most frequently patients with SJIA (5 of 7, 71%) than those in other JIA categories (chi-square; *P* = 0.0010).

Of the 166 patients, 143 (86%) had paired serum samples available from baseline and week 18. There were no differences in age, sex, race, and ethnicity between the initial study cohort (n = 166) and these 143 patients (Supplementary Table 1).

CBB levels at baseline by JIA category and in relation to disease activity. At baseline, median levels of only a few CBBs differed among JIA categories (Table 3 and Supplementary Table 2). For CBBs associated with innate immunity activation, patients with SJIA showed the highest median levels of S100A12 and IL-18, whereas resistin was highest in patients with JPsA. Intercellular adhesion molecule-1 and CCL20 were also higher in patients with SJIA compared with those in the other JIA categories. Vascular endothelial growth factor, a marker of endothelial dysfunction, was highest in JPsA. The cytokines, soluble tumor necrosis factor receptor (sTNFr) and IL6, were highest in patients in the PJIA-RF– and PJIA-RF+ categories compared with those in other JIA categories. There were no baseline differences by JIA categories in the other CBBs, including those probing for synovial tissue angiogenic activity, tissue and extracellular matrix degeneration, and interferon induced pathways.

At baseline, there were no more than weak associations between CBB levels and the JADAS-27. The strongest correlations were observed for IL-6 (Spearman r = 0.26, *P* = 0.002), CXCL9 (Spearman r = 0.20, *P* = 0.015), IL-18 (Spearman r = 0.18, *P* = 0.028), and SAA (Spearman r = 0.18, *P* = 0.03), respectively (Supplementary Table 3). All other tested CBBs did not show a significant correlation with baseline JADAS-27. Specifically, there was no correlation of baseline JADAS-27 with baseline levels of the markers of endothelial dysfunction, synovial tissue angiogenic activity, tissue and extracellular matrix degeneration, T and B cell activation, and inflammatory cell chemotaxis (Supplementary Table 3).

Association of CBB levels with treatment response at week 18. For the 143 patients with available samples at baseline and week 18, we compared treatment response to tofacitinib considering reduction (improvement) of JADAS-27 and JIA-ACR90 responses from baseline to week 18. There was no significant difference between groups in concomitant glucocorticoid or methotrexate treatment by JIA-ACR90 response status (Supplementary Table 4).

Multiple linear regression analyses showed that reduction in resistin levels from baseline to week 18 was associated with improvement in JADAS-27 after adjusting for differences in baseline resistin and other covariates (r^2 = 0.79, SE 0.070, *P* <0.01) (Supplementary Table 5). Partial regression plot of the change in resistin and change in JADAS-27 from baseline to week 18 showed that a greater reduction in resistin level was associated with a greater improvement in JADAS-27 (Figure 1). Partial regression plot of the change in S100A12 levels also showed positive association with greater change in JADAS-27 from baseline to week 18 (Figure 2 and Supplementary Table 6). Change in the levels of other CBBs did not show significant associations with change in JADAS-27 from baseline to week 18.

Baseline characteristics and CBB levels of the patients who achieved a JIA-ACR90 response compared with JIA-ACR nonresponders are shown in Supplementary Table 4. At week 18, 50 (50 of 143, 35%) patients achieved JIA-ACR90 level improvement, and 90, 121, and 137 patients (63%, 85%, and 96%) achieved JIA-ACR70, 50, and 30 level improvement, respectively. JIA-ACR90 level response was more common among girls (42 of 106, 39.6%) compared with boys (8 of 37, 8.1%). There were no significant differences in CBB levels

Table 2. Baseline demographic and clinical characteristics of the 166 patients with JIA*

Descriptors	All JIA	PJIA-RF+	PJIA-RF−	Exo-JIA	JPsA	ERA	SJIA	*P* value[a]
No. of patients	166 (100)	27 (16)	83 (50)	19 (11)	14 (8)	16 (10)	7 (4)	
Sex, female	124 (75)	25 (93)	64 (77)	12 (63)	12 (86)	10 (63)	1 (14)	<0.006[b]
Age, y	13 (10,15)	14 (12–16)	13 (8–15)	12 (6–15)	15 (12–16)	13 (11–16)	14 (11–16)	0.120
Race, White	147 (89)	21 (78)	76 (92)	18 (95)	11 (79)	15 (94)	6 (86)	0.268
Ethnicity, Hispanic	39 (23)	10 (37)	19 (23)	4 (21)	3 (21)	2 (13)	1 (14)	0.511
Disease duration, y	2.6 (1–5.6)	2 (1–3.6)	3.4 (1–6.8)	2.7 (1–5.2)	1.6 (1–4.1)	1.7 (1–3.5)	8.2 (3–11)	0.019
ESR, mm/h	14 (9–28)	22 (7–40)	14 (10–26)	14 (10–28)	14 (9–24)	11.5 (8–45)	8 (5–25)	0.709
CRP, mg/dL	0.19 (0.04–0.75)	0.54 (0.10–1.08)	0.16 (0.03–0.63)	0.16 (0.03–0.69)	0.32 (0.14–0.66)	0.09 (0.03–0.89)	0.19 (0.04–1.84)	0.546
HLA-B27+	24 (15)	0 (0)	10 (13)	3 (16)	1 (8)	9 (56)	1 (14)	<0.001[b]
Methotrexate treatment, yes	113 (68)	19 (70)	66 (80)	14 (74)	4 (29)	7 (44)	3 (43)	<0.001[b]
Glucocorticoid treatment, yes	51 (31)	13 (48)	28 (34)	3 (16)	2 (14)	0	5 (71)	0.001[b]
JADAS-27	19.71 (15.61–25.29)	21.21 (17.21–26.93)	20.55 (15.21–28.21)	19.71 (15.71–23.21)	17.71 (13.51–20.06)	16.21 (11.71–20.21)	23.71 (17.21–29.26)	0.026[b]
Active joint count	10 (6–14)	10 (8–20)	10 (7–14)	7 (5–10)	12.5 (6–18)	7 (6–11.5)	11 (7–15)	0.026[b]

* Values are n (%) of 166 total patients with JIA or median (interquartile range) unless indicated otherwise. ANOVA, analysis of variance; CRP, C-reactive protein; ERA, enthesitis-related arthritis; ESR, erythrocyte sedimentation rate; exo-JIA, extended oligoarticular JIA; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; JPsA, juvenile psoriatic arthritis; PJIA-RF+, polyarticular rheumatoid factor–positive JIA; PJIA-RF−, polyarticular rheumatoid factor–negative JIA; SJIA, systemic JIA.
[a] Kruskal–Wallis ANOVA test or chi-square test; statistically significant with *P* value < 0.05.
[b] Statistically significant with *P* value <0.05.

Table 3. CBB parameters with significant differences in baseline values for the 166 patients with JIA*

CBB[a,b]	All JIA (N = 166)	PJIA-RF+ (n = 27)	PJIA-RF− (n = 83)	Exo-JIA (n = 19)	JPsA (n = 14)	ERA (n = 16)	SJIA (n = 7)	*P* value[c]
Innate immunity activation								
S100A12	122.3 (77.12–315.56)	227.4 (89.03–340.23)	152.9 (82.46–383.74)	89.6 (52.75–219.7)	93.1 (54.19–225.79)	83.6 (63.15–176.17)	242.3 (135.37–1,920[d])	0.009
IL-18	0.25 (0.19–0.34)	0.21 (0.17–0.27)	0.27 (0.21–0.38)	0.23 (0.17–0.43)	0.24 (0.21–0.29)	0.22 (0.18–0.28)	0.30 (0.18–9.96)	0.041
Resistin	11.46 (9.09–16.43)	9.84 (8.59–12.92)	11.46 (8.56–15.99)	10.79 (9.53–12.68)	17.98 (11.29–23.32)	14.75 (10.84–18.09)	12.53 (9.2–21.61)	0.028
Endothelial dysfunction								
VEGF	0.06 (0.03–0.1)	0.05 (0.03–0.07)	0.06 (0.03–0.1)	0.08 (0.05–0.12)	0.09 (0.04–0.13)	0.04 (0.03–0.08)	0.08 (0.05–0.24)	0.026
T and B cell activation								
ICAM-1	282.8 (208.83–374.26)	256.7 (171.95–326.35)	304.5 (216.88–390.66)	265.2 (184.86–399.29)	229.2 (170.21–242.83)	263.0 (216.46–315.86)	342.8 (299.39–456)	0.033
Cytokine activity								
sTNFr	1.31 (1.06–1.64)	0.99 (0.69–1.3)	1.37 (1.11–1.72)	1.13 (0.99–1.61)	1.46 (1.32–2.16)	1.43 (1.19–1.8)	1.25 (0.83–2.68)	<0.001
IL-6	2.17 (1.36–6.82)	12.69 (4.24–45.42)	1.58 (1.36–6.29)	1.36 (0.94–4.33)	1.35 (0.52–5.04)	1.77 (1.36–4.03)	5.66 (1.36–40.77)	<0.001
Trafficking of immune cells to sites of inflammation								
CCL20	0.03 (0.00–0.06)	0.00 (0.00–0.03)	0.04 (0.01–0.07)	0.04 (0.01–0.06)	0.01 (0.00–0.03)	0.02 (0.00–0.06)	0.07 (0.06–0.10)	<0.001

* Values are the median (interquartile range) unless indicated otherwise. ANOVA, analysis of variance. CBB, candidate blood-based biomarker; CCL20, chemokine (C-C motif) ligand 20; ERA, enthesitis-related arthritis; exo-JIA, extended oligoarticular JIA; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; JIA, juvenile idiopathic arthritis; JPsA, juvenile psoriatic arthritis; PJIA-RF−, polyarticular rheumatoid factor–negative JIA; PJIA-RF+, polyarticular rheumatoid factor–positive JIA; SAA, serum amyloid A; SJIA, systemic JIA; sTNFR, soluble tumor necrosis factor receptor; VEGF, vascular endothelial growth factor.

[a] Raw values are presented for all CBBs.

[b] Median (interquartile range) in ng/mL, except for IL6, which is in pg/mL.

[c] *P* values <0.05 by Kruskal–Wallis ANOVA test are statistically significant.

[d] At upper limit of detection of the assay.

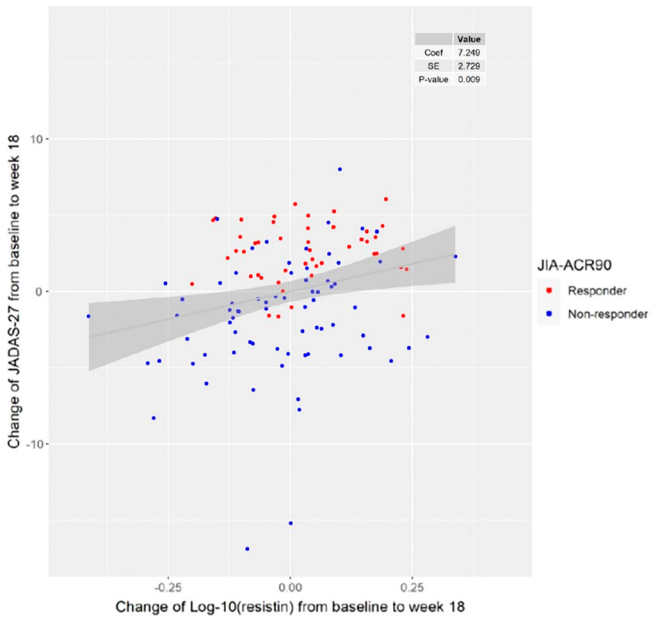

Figure 1. Partial regression plot of the change in resistin compared to change in JADAS-27 from baseline to week 18. ACR, American College of Rheumatology; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis.

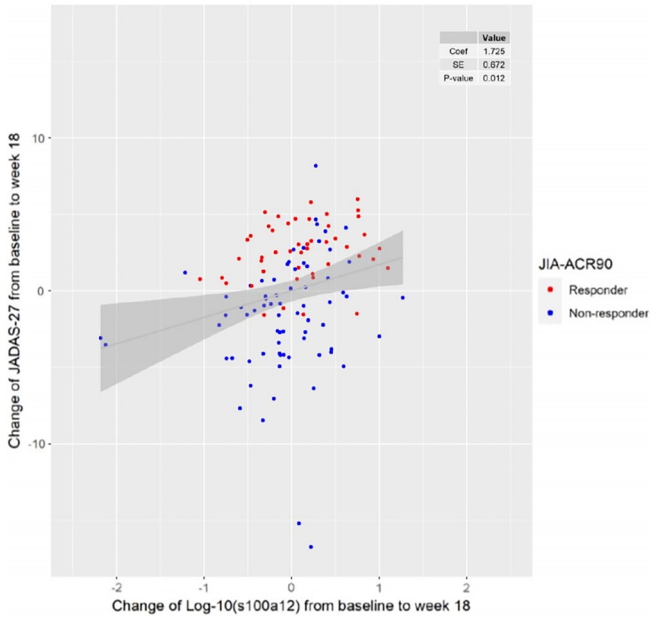

Figure 2. Partial regression plot of the change in S100A12 levels shows a positive association with greater change in JADAS-27 from baseline to week 18. ACR, American College of Rheumatology; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis.

at baseline or at week 18 by JIA-ACR90 response status. (see Supplementary Figure 1).

Among the 21 patients testing positive for HLA-B27, only 2 patients achieved a JIA-ACR90 level response (2 of 21, 10%) compared with 46 HLA-B27–negative patients (46 of 115, 40%). The JIA categories of these 21 patients with positive HLA-B27 status were as follows: 10 patients with PJIA-RF–, 2 patients with exo-JIA, 7 patients with ERA, 1 patient with JPsA, and 1 patient with SJIA.

Multiple logistic regression analyses showed that reduction of resistin levels from baseline to week 18 was associated with higher odds of achieving a JIA-ACR90 response at week 18 after adjusting for baseline resistin (odds ratio 1.134; 95% confidence interval 1.018–1.264). Change in the other CBB levels did not show significant associations with achieving JIA-ACR90 response by week 18. We excluded consideration of HLA-B27 status from the multivariable analyses because it caused a zero-cell issue (Table 2).

Sensitivity analyses. Sensitivity analyses conducted excluded patients with SJIA (baseline: n = 7, week 18: n = 4 of 143; see Supplementary Table 7) and yielded similar findings in CBB associations with treatment response. The effect of reduction in resistin remained significant in the sensitivity analyses when applying the same multiple regression. Our sensitivity analyses also found that HLA-B27 status was significantly associated with JIA-ACR90 nonresponse at week 18 (Supplementary Table 4 and Supplementary Table 8).

DISCUSSION

To promote precision treatment in children with polyarticular-course JIA, we measured CBBs in the serum before starting tofacitinib at approved dosages and investigated whether these CBBs can anticipate response to treatment and how CBB levels change in tofacitinib responders compared with nonresponders over time in patients with JIA. Our results show that CBB levels do not provide strong actionable information as to whether patients with JIA will respond to tofacitinib. Furthermore, our analyses suggest that HLA-B27 positivity is associated with not achieving a JIA-ACR90 response to tofacitinib treatment.

The reported positive associations of JADAS-27 with IL-6, IL-18, and SAA are in accordance with prior research showing marginal correlations. Serum IL-6 levels have been shown to be positively correlated with the extent and severity of joint involvement, ESR, and CRP in patients with RF+/RF– PJIA and exo-JIA.[36] Likewise, serum IL-18 levels are positively correlated with disease activity measures in patients with SJIA, PJIA-RF– or RF+, and oligoarticular JIA.[15] Similarly, SAA concentrations were reported to positively correlate with JIA disease activity in the past.[16] In our study, we found associations between disease activity and the aforementioned CBBs to be weaker than previously reported. This might be explained by the heterogeneity of our population with JIA and the difference in the size of the prior studies compared to ours.

Although considered an adipokine, resistin is also expressed in macrophages and plays important roles in systemic inflammation; resistin appears to be a predominant proinflammatory protein associated with both acute and chronic inflammation[37] and signals through the MAP-kinase pathway, promotes angiogenesis and synovial inflammation, and induces cartilage destruction.[38] Compared with healthy controls, patients with rheumatoid arthritis and JIA have higher serum and synovial fluid levels of resistin, especially with high disease activity.[17,39] Notably, our study of JIA biomarkers using whole-blood gene-expression profiles from the same clinical trial participants also reported that patients with JIA with strongly up-regulated MAPK signaling pathways are expected to be poor responders to tofacitinib.[40]

Similar to resistin, S100 proteins are major monocyte/macrophage proteins that bind calcium, constituting ~40% of the cytosolic protein in neutrophil granulocytes. Prior research supports the relevance of S100A8/9 and S100A12 as CBBs of JIA disease control.[18,41] In our study, serum levels of both of these CBBs were confirmed to correlate with disease course in JIA.

In this study, HLA-B27 positivity was associated with lower response to tofacitinib. This contrasts with the adult phase III randomized, double-masked, placebo-controlled clinical trial that reported superiority of tofacitinib over placebo in patients with active axial spondyloarthropathy.[42] The difference may be explained by the small number of patients in this study or could be attributed to a difference in disease pathophysiology between children and adults. Notably, HLA-B27 positivity was not found to be predictive of treatment response in adult patients with psoriatic arthritis,[43] but HLA-B27 status influences the effectiveness of TNF inhibitors in the treatment of axial spondylarthritis, in which higher risk of drug discontinuation is encountered by HLA-B27–negative patients.[44]

In this study, the association of CBBs and clinical responses were only measured at baseline and week 18 due to availability of samples for testing at those time points. In future studies, additional measurements of CBBs earlier in the disease course, such as after the first two to four weeks on tofacitinib, may be more useful in assessing the predictive properties of the CBBs. This is because clinical response from tofacitinib can occur as early as two weeks and inactive disease as early as four weeks.[5] The observation that HLA-B27 positivity is associated with lower response of JIA to tofacitinib should be interpreted with much caution given the small number of HLA-B27–positive pediatric patients in this cohort. In addition, the total number of patients is also quite small and includes patients from several JIA categories. Larger numbers of patients might be helpful to establish relationships with disease activity for the various biomarkers and include correction for multiple comparisons in the analyses, which we were unable to do with our exploratory study. The panel of CBBs used for our study was limited, and the individual CBBs may not fully represent the inflammatory pathway understudy. Therefore, broader panels to better interrogate required pathways may be considered in future studies. Finally, our study population was composed of clinical trial participants, which potentially limits the generalizability of our results to other populations with JIA.

Our study showed important factors associated with nonresponse to tofacitinib. However, accurate serum biomarkers that independently predict tofacitinib response in JIA are still needed. Our analyses suggest that HLA-B27 positivity may be associated with lower response to tofacitinib in patients with JIA. Confirmation of this finding in an independent validation cohort is recommended.

ACKNOWLEDGMENTS

We thank the Pediatric Rheumatology Collaborative Study Group (PRCSG) and Pediatric Rheumatology International Trials Organization (PRINTO) Coordinating Center for the assistance with the measurement of patient response to therapy for the clinical trial of tofacitinib in polyarticular-course JIA (NCT02592434). The authors thank Pfizer for providing blood samples, PRCSG and PRINTO investigators for the collection of serum samples and data used, and the University of Cincinnati Center for Clinical and Translational Science and Training. We thank Sanjeev Dhakal, MS, Angela Merritt, BA, and Megan Quinlan-Waters, BA, for their regulatory and data management support.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr Brunner had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ogbu, Brunner, Grom, Thornton.

Acquisition of data. Brunner, Aviel, Nanda, Schmeling, Tory, Uziel, Viola, Wahezi, Tarvin, Sproles, Ruperto, Grom, Thornton.

Analysis and interpretation of data. Huang, Ogbu, Thornton, Aviel, Nanda, Uziel, Viola, Wahezi, Tarvin, Sproles, Ruperto, Eloseily, Chen, Grom.

REFERENCES

1. Petty RE, Southwood TR, Manners P, et al; International League of Associations for Rheumatology. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31(2):390–392.
2. Lin YT, Wang CT, Gershwin ME, et al. The pathogenesis of oligoarticular/polyarticular vs systemic juvenile idiopathic arthritis. Autoimmun Rev 2011;10(8):482–489.
3. Ringold S, Angeles-Han ST, Beukelman T, et al. 2019 American College of Rheumatology/Arthritis Foundation guideline for the treatment of juvenile idiopathic arthritis: therapeutic approaches for non-systemic polyarthritis, sacroiliitis, and enthesitis. Arthritis Care Res (Hoboken) 2019;71(6):717–734.
4. Brunner HI, Foeldvari I, Alexeeva E, et al; Paediatric Rheumatology INternational Trials Organization (PRINTO) and Pediatric Rheumatology Collaborative Study Group (PRCSG). Secukinumab in enthesitis-related arthritis and juvenile psoriatic arthritis: a randomised, double-blind, placebo-controlled, treatment withdrawal, phase 3 trial. Ann Rheum Dis 2023;82(1):154–160.
5. Ruperto N, Brunner HI, Synoverska O, et al; Paediatric Rheumatology International Trials Organization (PRINTO) and Pediatric

Rheumatology Collaborative Study Group (PRCSG). Tofacitinib in juvenile idiopathic arthritis: a double-blind, placebo-controlled, withdrawal phase 3 randomised trial. Lancet 2021;398(10315):1984–1996.

6. Giannini EH, Ruperto N, Ravelli A, Lovell DJ, Felson DT, Martini A. Preliminary definition of improvement in juvenile arthritis. Arthritis Rheum 1997;40(7):1202–1209.
7. Maeshima K, Yamaoka K, Kubo S, et al. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon-γ and interleukin-17 production by human CD4+ T cells. Arthritis Rheum 2012;64(6):1790–1798.
8. Cargill M, Schrodi SJ, Chang M, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am J Hum Genet 2007;80(2):273–290.
9. Pascual V, Allantaz F, Arce E, et al. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005;201(9):1479–1486.
10. de Benedetti F, Massa M, Robbioni P, et al. Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. Arthritis Rheum 1991;34(9):1158–1163.
11. Brunner HI, Lovell DJ, Finck BK, et al. Preliminary definition of disease flare in juvenile rheumatoid arthritis. J Rheumatol 2002;29(5):1058–1064.
12. Consolaro A, Giancane G, Schiappapietra B, et al. Clinical outcome measures in juvenile idiopathic arthritis. Pediatr Rheumatol Online J 2016;14(1):23.
13. Consolaro A, Bracciolini G, Ruperto N, et al; Paediatric Rheumatology International Trials Organization. Remission, minimal disease activity, and acceptable symptom state in juvenile idiopathic arthritis: defining criteria based on the juvenile arthritis disease activity score. Arthritis Rheum 2012;64(7):2366–2374.
14. Miyamae T, Tani Y, Kishi T, et al. Updated version of Japanese Childhood Health Assessment Questionnaire (CHAQ). Mod Rheumatol 2020;30(5):905–909.
15. Lotito APN, Campa A, Silva CAA, et al. Interleukin 18 as a marker of disease activity and severity in patients with juvenile idiopathic arthritis. J Rheumatol 2007;34(4):823–830.
16. Cantarini L, Giani T, Fioravanti A, et al. Serum amyloid A circulating levels and disease activity in patients with juvenile idiopathic arthritis. Yonsei Med J 2012;53(5):1045–1048.
17. Gheita TA, El-Gazzar II, El Shazly RI, et al. Elevated serum resistin in juvenile idiopathicarthritis: relation to categories and disease activity. J Clin Immunol 2013;33(1):297–301.
18. Hinze CH, Foell D, Johnson AL, et al. Serum S100A8/A9 and S100A12 levels in children with polyarticular forms of juvenile idiopathic arthritis: relationship to maintenance of clinically inactive disease during anti–tumor necrosis factor therapy and occurrence of disease flare after discontinuation of therapy. Arthritis Rheumatol 2019;71(3):451–459.
19. Vignola S, Picco P, Falcini F, et al. Serum and synovial fluid concentration of vascular endothelial growth factor in juvenile idiopathic arthritides. Rheumatology (Oxford) 2002;41(6):691–696.
20. Scola MP, Imagawa T, Boivin GP, et al. Expression of angiogenic factors in juvenile rheumatoid arthritis: correlation with revascularization of human synovium engrafted into SCID mice. Arthritis Rheum 2001;44(4):794–801.
21. Parackova Z, Zentsova I, Horvath R, et al. Immunomodulation of neutrophils and platelets by TNF blockage in patients with juvenile idiopathic arthritis. Clin Immunol 2022;245:109170.
22. Gattorno M, Gerloni V, Morando A, et al. Synovial membrane expression of matrix metalloproteinases and tissue inhibitor 1 in juvenile idiopathic arthritides. J Rheumatol 2002;29(8):1774–1779.
23. Markula-Patjas KP, Ivaska KK, Pekkinen M, et al. High adiposity and serum leptin accompanied by altered bone turnover markers in severe juvenile idiopathic arthritis. J Rheumatol 2014;41(12):2474–2481.
24. Al-Jaberi L, Simonds MM, Brescia AMC. CCL24, CXCL9 and CXCL10 are increased in synovial fluid in patients with juvenile idiopathic arthritis requiring advanced treatment. Rheumatology (Oxford) 2023;62(7):2594–2600.
25. Ziegler L, Fuehner S, Kessel C, et al. Soluble interleukin-2 receptor serum levels facilitate prediction of relapses in subgroups of patients with juvenile idiopathic arthritis. Rheumatology (Oxford) 2022;61(12):4975–4984.
26. Grieshaber-Bouyer R, Exner T, Hackert NS, et al. Ageing and interferon gamma response drive the phenotype of neutrophils in the inflamed joint. Ann Rheum Dis 2022;81(6):805–814.
27. Eberhard BA, Laxer RM, Andersson U, et al. Local synthesis of both macrophage and T cell cytokines by synovial fluid cells from children with juvenile rheumatoid arthritis. Clin Exp Immunol 1994;96(2):260–266.
28. Nistala K, Moncrieffe H, Newton KR, et al. Interleukin-17-producing T cells are enriched in the joints of children with arthritis, but have a reciprocal relationship to regulatory T cell numbers. Arthritis Rheum 2008;58(3):875–887.
29. Gaur P, Myles A, Misra R, et al. Intermediate monocytes are increased in enthesitis-related arthritis, a category of juvenile idiopathic arthritis. Clin Exp Immunol 2017;187(2):234–241.
30. El-Behi M, Ciric B, Dai H, et al. The encephalitogenicity of TH17 cells is dependent on IL-1-and IL-23-induced production of the cytokine GM-CSF. Nat Immunol 2011;12(6):568–575.
31. Yao TC, Kuo ML, See LC, et al. RANTES and monocyte chemoattractant protein 1 as sensitive markers of disease activity in patients with juvenile rheumatoid arthritis: a six-year longitudinal study. Arthritis Rheum 2006;54(8):2585–2593.
32. Bosco MC, Delfino S, Ferlito F, et al. Hypoxic synovial environment and expression of macrophage inflammatory protein 3γ/CCL20 in juvenile idiopathic arthritis. Arthritis Rheum 2008;58(6):1833–1838.
33. Kessel C, Holzinger D, Foell D. Phagocyte-derived S100 proteins in autoinflammation: putative role in pathogenesis and usefulness as biomarkers. Clin Immunol. 2013;147(3):229–241.
34. Holzinger D, Frosch M, Kastrup A, et al. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. Ann Rheum Dis 2012;71(6):974–980.
35. Yasin S, Fall N, Brown RA, et al. IL-18 as a biomarker linking systemic juvenile idiopathic arthritis and macrophage activation syndrome. Rheumatology (Oxford) 2020;59(2):361–366.
36. De Benedetti F, Robbioni P, Massa M, et al. Serum interleukin-6 levels and joint involvement in polyarticular and pauciarticular juvenile chronic arthritis. Clin Exp Rheumatol 1992;10(5):493–498.
37. Qi Y, Gong F, Zhang Q, et al. Reverse regulation of soluble receptor for advanced glycation end products and proinflammatory factor resistin and S100A12 in Kawasaki disease. Arthritis Res Ther 2012;14:R251.
38. Tripathi D, Kant S, Pandey S, et al. Resistin in metabolism, inflammation, and disease. FEBS J 2020;287(15):3141–3149.
39. Senolt L, Housa D, Vernerová Z, et al. Resistin in rheumatoid arthritis synovial tissue, synovial fluid and serum. Ann Rheum Dis 2007;66(4):458–463.
40. Eloseily E, Pickering A, Dhakal S, et al. OP0030 Differential gene expression in response to tofacitnib treatment in polyarticular juvenile idiopathic arthritis. Ann Rheum Dis 2023;82:20. https://doi.org/10.1136/annrheumdis-2023-eular.680
41. Gerss J, Tedy M, Klein A, et al. Prevention of disease flares by risk-adapted stratification of therapy withdrawal in juvenile idiopathic

arthritis: results from the PREVENT-JIA trial. Ann Rheum Dis 2022; 81(7):990–997.

42. Deodhar A, Sliwinska-Stanczyk P, Xu H, et al. Tofacitinib for the treatment of ankylosing spondylitis: a phase III, randomised, double-blind, placebo-controlled study. Ann Rheum Dis 2021; 80(8):1004–1013.
43. Mease PJ, Chakravarty SD, McLean RR, et al. Treatment responses in patients with psoriatic arthritis axial disease according to human leukocyte antigen-B27 status: an analysis from the CorEvitas Psoriatic Arthritis/Spondyloarthritis Registry. ACR Open Rheumatol 2022;4(5): 447–456.
44. Fröhlich F, Micheroli R, Hebeisen M, et al. HLA-B27 as a predictor of effectiveness of treatment with TNF inhibitors in axial spondyloarthritis: data from the Swiss Clinical Quality Management Registry. Clin Rheumatol 2023;42(5):1267–1274.