

Early reduction of serum interleukin-6 levels as a predictor of clinical remission in systemic juvenile idiopathic arthritis

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Abstract

Background: Interleukin (IL)-6 is the main proinflammatory cytokine in systemic juvenile idiopathic arthritis (SJIA).

Objective: To determine if serial changes in serum IL-6 levels can predict outcomes of SJIA patients.

Methods: This was a retrospective cohort study. Medical records of patients aged 2–19 years with active SJIA between January 2012 and February 2014 were reviewed. Baseline characteristics were recorded at enrollment. Serum IL-6 levels were measured at enrollment and at 2–4 weeks, 6–8 weeks, 3 months, and 6 months thereafter. Treatment response and clinical remission were assessed after 2 years of follow-up.

Results: Of the 35 patients with active SJIA, 16 were in remission at the end of the study. IL-6 levels in the remission group returned to normal within 6 months, whereas they remained persistently high in the non-remission group. At the 3-month follow-up, patients were assigned to groups A and B based on reductions in serum IL-6 levels of >50% and ≤50%, respectively. At the end of the study, more patients in group A (72.2%) than in group B (17.6%) achieved clinical remission ($p < 0.05$). After multivariate analysis, a >50% reduction in serum IL-6 levels at the 3-month follow-up visit was a predictor of clinical remission at 2 years (odds ratio 22.74, 95% confidence intervals 2.16–239.85, $p < 0.01$).

Conclusions: An early reduction in serum IL-6 levels is significantly associated with clinical remission at 2 years in SJIA patients. Monitoring of serial changes in serum IL-6 levels is beneficial for predicting clinical remission.

Keywords: systemic juvenile idiopathic arthritis, juvenile idiopathic arthritis, systemic arthritis, interleukin-6 levels, Stills disease, chronic arthritis

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Background

Systemic juvenile idiopathic arthritis (SJIA) is distinct from other JIA subtypes and is characterized by fever, lymphadenopathy, evanescent rash, arthritis, and serositis.¹ The severity and course of SJIA are highly variable. About 40% of patients experience monophasic SJIA, whereas about 50% have disease activity that persists throughout life. Patients with severe disease activity often develop complications and side effects from treatment.^{2–4} Many studies have sought to identify predictors of SJIA outcomes to reduce the possibility of unfavorable complications.^{5,6} Schneider et al. showed that persistent systemic symptoms and thrombocytosis (platelet count $\geq 600 \times 10^9/L$) during the first 6 months of treatment were predictors for joint

destruction at later times.⁷ Additionally, polyarticular patterns and hip involvement in the first 6 months after disease onset were shown to be predictors for poor articular outcome in SJIA patients.⁸

The pathophysiology of SJIA mainly centers on innate immunity and proinflammatory cytokine production.^{9–13} In particular, interleukin (IL)-6 is a major proinflammatory cytokine in SJIA. Previous studies have shown that serum IL-6 levels are higher in SJIA patients with active disease than in patients with inactive disease or in healthy controls. Furthermore, IL-6 levels have been shown to correlate with the severity of inflammation and the number of inflamed joints and

fever spikes.¹⁴⁻¹⁷ Several biomarkers have been identified that are useful for diagnosis of SJIA and/or correlate with disease activity, for example, IL-1, IL-18, IL-6, and S100.¹⁸⁻²⁰ Vastert et al. demonstrated that persistently elevated IL-18, S100A12, and S100A8/9 levels might be predictors of unsuccessful tapering of anakinra when used as first-line therapy in new-onset SJIA patients.²¹ However, most of these studies were cross-sectional and may not reflect true disease activity due to individual differences in circadian rhythms and cytokine production. Therefore, we considered that longitudinal monitoring of cytokine levels may be a more reliable and accurate indicator of outcomes. Although serum IL-6 levels correlate well with SJIA disease status, there have been few studies on the utility of longitudinal serial assessment of this cytokine. Therefore, the purpose of this study was to determine whether changes in serum IL-6 levels over a 6-month period can predict the long-term outcomes of SJIA patients.

Methods

This study was approved by the Ethics Committee of Ramathibodi Hospital and conformed to the provision of the World Medical Association's Declaration of Helsinki. Written informed consent was provided by the legal guardian of the study participant prior to study enrollment.

Patients

This was a retrospective cohort study. We enrolled patients with active SJIA aged between 2–19 years who were seen in the Pediatric Department of Ramathibodi Hospital between January 2012 and February 2014. Patients were classified according to the International League of Association for Rheumatology criteria. Active disease was defined as the presence of systemic symptoms (high-grade fever plus at least one of hepatosplenomegaly, salmon rash, lymphadenopathy, and serositis) with or without arthritis, and with an erythrocyte sedimentation rate (ESR) >20 mm/h. Baseline characteristics, clinical manifestations, and serial measurements of serum IL-6 levels were reviewed from the medical records at enrollment and after 2–4 weeks, 6–8 weeks, 3 months, and 6 months after enrollment. Patients were followed up every 3 months for 2 years. The study was terminated at 2 years after enrollment, at which time the treatment response and clinical remission were assessed.

Data collection

Clinical manifestations and laboratory data included age, sex, duration of disease, number of active and limited joints, Thai version of Childhood Health Assessment Questionnaire (CHAQ),²² Physician Global Assessment (PGA), Parent/patient Global Assessment (PtGA), Juvenile Arthritis Disease Activities Score-27 (JADAS-27), medications, complete blood count (CBC), ESR, hematocrit (Hct), and serum IL-6 levels.

Outcome measures

The American College of Rheumatology Pediatric (ACR Pedi) 30, 50, 70, and 90 criteria were used to determine treatment response.²³ Disease outcomes were classified as (1) remission, for patients with no evidence of active arthritis or

systemic features, including fever, rash, serositis, hepatosplenomegaly, and lymphadenopathy; no active uveitis; normal ESR; and physician assessment showing no disease activity, with or without medication, for at least 6 consecutive months, and (2) non-remission, for patients with active arthritis and/or systemic features.

Serum IL-6 measurement

Samples of 5 mL of venous blood were collected into sterile tubes, incubated for 30 min at 37°C to allow clotting, and centrifuged at 2000×g for 10 min at room temperature. Serum was transferred to sterile tubes. Serum IL-6 was quantified using an electrochemiluminescence immunoassay kit (Roche Diagnostics, Mannheim, Germany). The lower level for detection was 1.5 pg/mL of purified human IL-6, and the normal level in this study was ≤7 pg/mL.

Statistical analysis

Simple descriptive statistics were used for baseline characteristics. Differences between group medians and means were analyzed using quantile regression and Student's t-test, respectively. Differences in categorical data were analyzed using Fischer's exact test or Chi-square test. The time to reach clinical remission was assessed using the Kaplan–Meier method with log-rank test. Logistic regression analysis was used to determine potential predictors of clinical remission. All analyses were performed using STATA version 14.1 (StataCorp, College Station, TX, USA). A p value of ≤0.05 was considered significant.

Results

Of the 35 active SJIA patients enrolled, 16 were in remission at the end of the study and the remaining 19 were assigned to the non-remission group. Baseline characteristics and laboratory data at enrollment for the remission and non-remission groups are shown in **Table 1**. At enrollment, patient characteristics (age, sex, JADAS-27, CHAQ, number of active joints, number of limited joints, PGA, and PtGA) and laboratory data (CBC, ESR, Hct, and IL-6 concentrations) were not significantly different between the two groups. However, the disease duration prior to study enrollment was longer for the non-remission group than the remission group (median 41.4 vs 4.0 months, respectively) and more patients in the non-remission group had received disease-modifying antirheumatic drugs (DMARDs, 74% vs 25%; **Table 1**). Serum IL-6 levels in the remission and non-remission groups during the first 6 months of follow-up are shown in **Figure 1**. The median serum IL-6 levels at baseline were not significantly different between the two groups. However, levels in the remission group decreased rapidly and returned to normal within 6 months of active disease onset, whereas levels in the non-remission group remained high throughout the 2-year follow-up period.

To focus on the relationship between the rate of serum IL-6 reduction and clinical outcomes, patients were classified into two groups based on the percentage reduction in serum IL-6 levels between baseline and the 3-month follow-up: patients with reductions >50% and ≤50% were assigned to group A (n = 18) and B (n = 17), respectively. The median (interquartile range [IQR]) serum IL-6 levels at baseline between group A (105.4

Table 1. Baseline characteristics and laboratory data of SJIA patients in the non-remission and remission groups.

Characteristic	Non-remission group (n = 19)	Remission group (n = 16)	P value
Age, years [†]	8.7 ± 4.3	8.3 ± 3.6	0.90
Female, n (%)	12 (63)	10 (63)	1.00
Disease duration prior to study enrollment, months	41.4 (41.3)	4.0 (20.8)	<0.01*
Duration of follow-up, months [†]	26.8 ± 3.1	26.5 ± 2.8	0.37
JADAS-27	14.1 (22.6)	20.1 (11.4)	0.37
Number of active joints	4.0 (7.0)	2.0 (1.0)	0.20
Number of limited joints	3.0 (8.0)	1.0 (1.0)	0.28
CHAQ	0.2 (0.9)	0.2 (0.6)	1.00

Characteristic	Non-remission group (n = 19)	Remission group (n = 16)	P value
PGA	5.0 (5.5)	6.0 (5.3)	0.53
PtGA	3.0 (6.0)	5.5 (4.2)	0.23
ESR, mm/h	66.0 (55.0)	61.0 (49.0)	0.66
WBC (× 10 ⁶ /L)	11,810 (6,840)	13,605 (9,595)	0.49
Hct (%) [†]	33.2 ± 5.3	32.0 ± 4.4	0.46
Serum IL-6, pg/mL	80.1 (107.2)	65.5 (94.6)	0.91
Medications, n (%)			
- Prednisolone	13 (68)	7 (45)	0.18
- DMARDs	14 (74)	4 (25)	0.01
- Tocilizumab	9 (47)	5 (31)	0.49

* Statistically significant, [†] mean (standard deviation). All other data are presented as the median (interquartile range). n, number of individuals; CHAQ, Childhood Health Assessment Questionnaire (range 0–3); DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; Hct, hematocrit; IL-6, interleukin-6; JADAS-27, Juvenile Arthritis Disease Activity Score (range 0–57); PGA, Physician Global Assessment (range 0–10); PtGA, Patient/parent Global Assessment (range 0–10); SJIA, systemic juvenile idiopathic arthritis; WBC, white blood cell count.

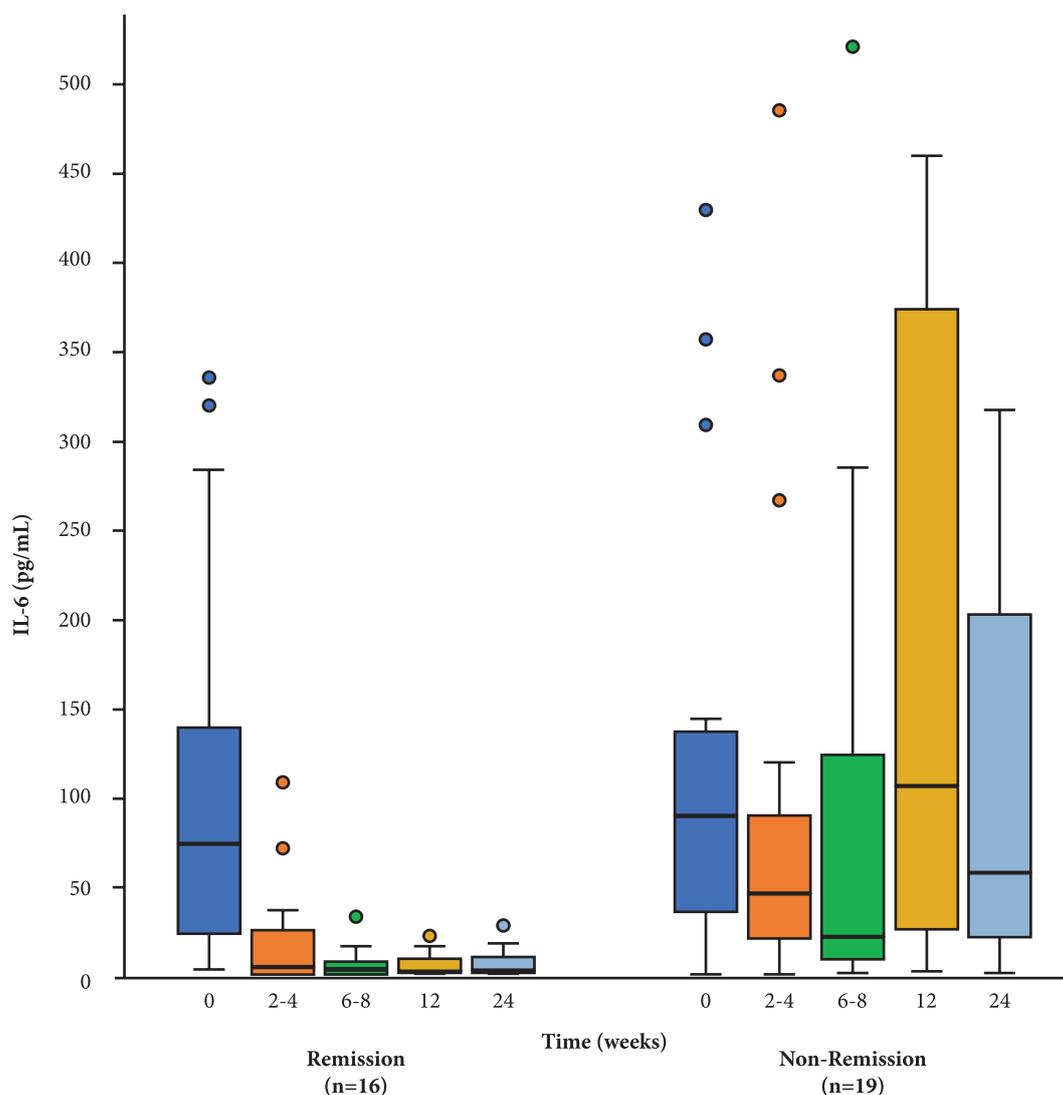


Figure 1. Serum IL-6 levels in patients in the non-remission and remission groups during the first 6 months of follow-up. Box and whisker plots show the median, interquartile range, maximum/minimum values, and outliers.

[265.8] pg/mL and group B (62.0 [83.6] pg/mL) were not significantly different ($p = 0.11$). There were 7/18 (38.9%) patients and 7/17 (41.2%) patients receiving tocilizumab in group A and B respectively. The changes in serum IL-6 levels between baseline and at the 3-month follow-up for each patient in the two groups are shown in **Figure 2**.

At the end of the 2-year study, more patients in group A reached ACR Pedi 30, 50, 70, and 90 and achieved clinical remission than did patients in group B (**Figure 3**). With the exception of the ACR Pedi 50 score ($p = 0.06$), these differences

were all significant ($p < 0.05$). The number of patients in clinical remission at the end of the study was 13 (72.2%) in group A and 3 (17.6%) in group B ($p < 0.05$). A Kaplan–Meier analysis revealed that the median time to clinical remission was shorter for group A (21.6 months) than group B (32.4 months) ($p = 0.006$, **Figure 4**).

The potential predictors of clinical remission were analyzed by logistic regression, and variables that showed significant associations by univariate analysis were included in the multivariate analysis. As shown in **Table 2**, we found that a $\geq 50\%$

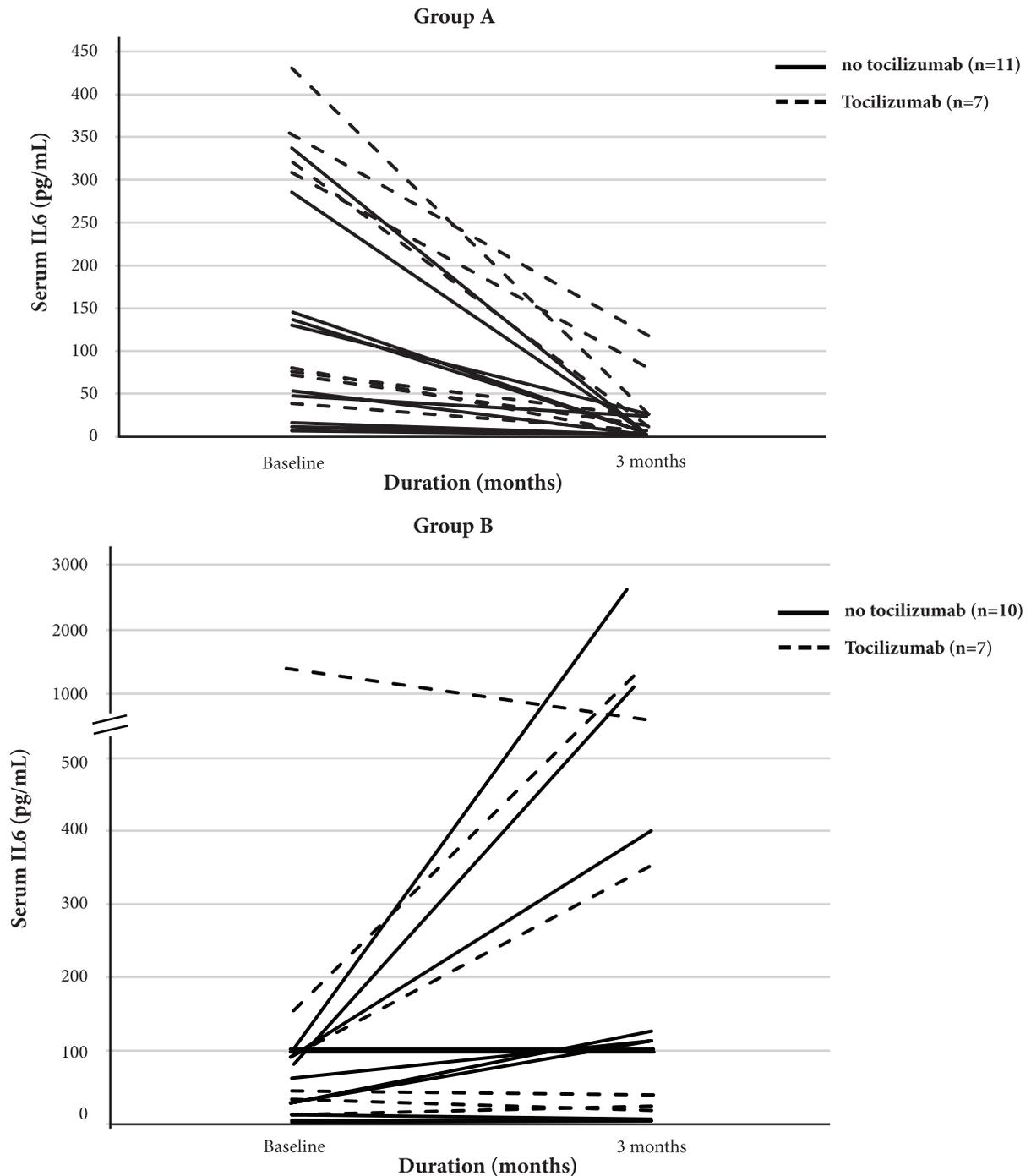


Figure 2. Serum IL-6 levels in SJIA patients between baseline and the 3-month follow-up in groups A ($n = 18$) and B ($n = 17$); group A and B, patients with $>50\%$ reduction (A) or $\leq 50\%$ reduction (B) in IL-6 at the 3-month follow-up visit. Dashed line and solid line are patients with and without tocilizumab treatment, respectively.

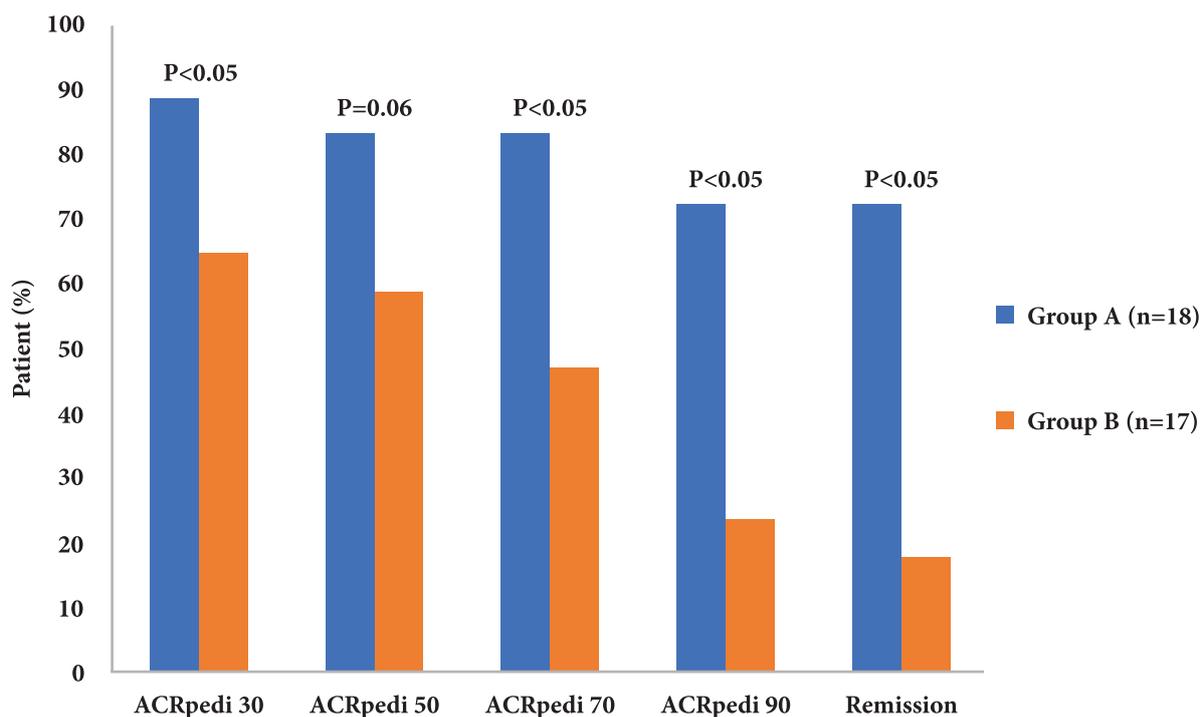


Figure 3. ACR Pedi 30, 50, 70, 90 and clinical remission rates at the end of the study.

ACR Pedi, American College of Rheumatology Pediatric Criteria; group A and B, patients with >50% reduction (A) or ≤50% reduction (B) in IL-6 at the 3-month follow-up visit.

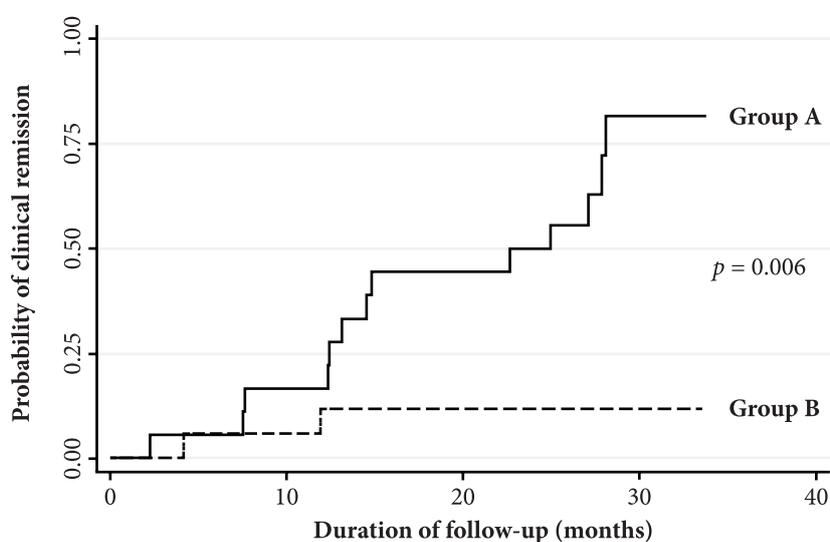


Figure 4. Kaplan–Meier analysis of clinical remission in systemic juvenile idiopathic arthritis patients. Groups A (n = 18) and B (n = 17) are patients with >50% and ≤50% reductions, respectively, in serum IL-6 levels at the 3-month follow-up visit.

Table 2. Predictors of clinical remission in SJIA patients.

Predictive factor	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Reduction in serum IL-6 levels of >50% at 3 months of follow-up	12.13 (2.41–61.20)	0.003*	22.74 (2.16–239.85)	0.009*
DMARDs	0.12 (0.03–0.55)	0.006*	0.04 (0.16–3.02)	0.332
Disease duration prior to study enrollment of <2 years	8.40 (1.83–38.57)	0.006*	9.71 (0.78–120.90)	0.077

*Statistically significant. CI, confidence intervals; DMARDs, disease-modifying antirheumatic drugs; IL-6, interleukin-6; OR, odds ratio; SJIA, systemic juvenile idiopathic arthritis

reduction in serum IL-6 levels at the 3-month follow-up was a predictor of clinical remission, with an odds ratio of 22.74 (95% confidence interval 2.16–239.85, $p < 0.01$).

Discussion

The clinical presentation of SJIA differs from that of other JIA subtypes and includes prolonged fever, salmon rash, hepatosplenomegaly, serositis, and/or lymphadenopathy accompanied by arthritis. Moreover, the disease course varies considerably among individual patients. Earlier studies investigated whether clinical features such as fever, arthritis, and ESR could be used to predict SJIA disease course.⁵ However, because many factors can influence these events,²⁴ in this study, we sought to identify a more specific predictive biomarker. Three proinflammatory cytokines, IL-6, IL-1, and IL-18, have been investigated for their involvement in SJIA.^{25,26} IL-6 is elevated in the peripheral blood and synovial joint fluid of active SJIA patients and its levels correlate with disease activity, suggesting that this cytokine may play a major role in SJIA pathogenesis.¹⁴ Therefore, monitoring of IL-6 levels should be useful in clinical practice. Although other studies have sought to identify predictive biomarkers for SJIA, serial changes in serum IL-6 levels have not been studied. Here, we demonstrated that the rate of change in serum IL-6 levels during the first 3 months of active disease could be a predictor of clinical remission in SJIA patients.

At study enrollment, the disease severity and laboratory data were not significantly different between the patients who did and did not achieve remission at 2 years follow-up. However, a much higher proportion of the non-remission group had received DMARDs, and the duration of disease prior to enrollment was much longer for the non-remission group than the remission group. This is not surprising, since patients with chronic conditions and/or more severe arthritis may well require more extensive DMARD treatment. The longer disease duration may have been due to patients with poor prognosis resulting from excessive production of proinflammatory cytokines, especially IL-6. There was no significant difference between the number of patients in the non-remission and remission groups who received other immunosuppressive medications, including prednisolone and tocilizumab. Since the two patient groups had comparable clinical and laboratory data, including IL-6, at baseline, these results indicate that one-time measurements in patients with active disease cannot predict the long-term response. In previous cross-sectional studies, serum IL-6 levels correlated well with various aspects of disease activity, including fever peak, severity of arthritis, and thrombocytosis during active and inactive disease status.^{14,16} These findings imply that SJIA is an IL-6-mediated disease. Our study showed a striking difference in serum IL-6 levels between patients who did and did not achieve remission. Those who did enter remission showed significant decreases in serum IL-6 levels during the 6-month follow-up period, whereas patients who failed to achieve remission had persistently high serum IL-6 levels. Therefore, longitudinal assessment of serum IL-6 over the disease course appears to provide valuable information that cannot be gained with one-time measurements.

We found that SJIA patients with early decreases in serum IL-6 levels also showed higher ACR Pedi 30, 50, 70, 90 responses and had a shorter median time to remission compared with patients with persistently high serum IL-6 levels. These findings are consistent with a major role for IL-6 in SJIA and the correlation with clinical improvement. Thus, the rapid reduction in serum IL-6 levels likely reflects better treatment responses leading to remission, whereas treatment failure would explain the persistently high IL-6 levels in the non-remission group. Previous studies have demonstrated that IL-6 can promote the differentiation of naïve T cells to T helper (Th)17 cells, which can promote chronic inflammatory processes such as persistent arthritis.^{27,28} Indeed, increased levels of Th17 cells are found in the peripheral blood of SJIA patients.²⁹ Therefore, it is possible that the patients in our study with persistent IL-6 levels may also have had higher numbers of Th17 cells, leading to excessive cytokine production and chronic inflammation.

To identify possible predictors of clinical remission, we analyzed plausible factors using logistic regression models. In the univariate model, we found that a >50% reduction in serum IL-6 at the 3-month follow-up, no DMARD treatment, and disease duration prior to study enrollment of <2 years were significantly associated with remission. In the multivariate model, the reduction in serum IL-6 levels was the only predictive indicator of clinical remission. Therefore, although DMARD treatment and disease duration differed between the groups at the start of the study, they did not predict clinical remission. Approximately 80% of patients achieving clinical remission had lower serum IL-6 levels at 3 months, suggesting that patients with persistently high IL-6 levels during this period may require add-on therapy to improve their future response. This study has therefore demonstrated the utility of serial measurement of serum IL-6 levels for predicting disease outcome.

Since tocilizumab is an anti-human IL-6R monoclonal antibody, it has been reported to increase levels of free IL-6 after first administration due to a reduction in IL-6 clearance.³⁰ The peak of increased IL-6 levels was 2 weeks, following which the levels became steady, which represents the balance between IL-6 production and IL-6 clearance.³⁰ Therefore, the changes in serum IL-6 levels after a 2-week period should be able to reflect disease activity. Since the follow-up period in our study was 2 years, tocilizumab should not affect serum IL-6 levels in the long-term. In the remission group, serum IL-6 levels decreased over time after the disease control and they also decreased relative to disease activity in patients who had received tocilizumab treatment, as shown in **Figure 1**. This also explains the results of patients in group A and B, which was focused on IL-6 reduction rate at the 3-month follow-up in individuals more than one time measurement at baseline. Serum IL-6 levels in patients with tocilizumab treatment should already reach steady state by that time. Also, the number of patients receiving tocilizumab treatment in group A and B was similar, as shown in **Figure 2**. As described above, the changes of serum IL-6 levels in patients with tocilizumab treatment could be used in this study.

There are some limitations to this study. First, this was a retrospective cohort study and could potentially have been

limited by missing data. However, the routine approach to data collection in our clinic is rigorous and mitigates any weakness in the study design. Second, the study recruited both newly and previously diagnosed SJIA patients with active disease from a single tertiary care center. Third, the small sample size may also have biased the results. However, the confounding factors, including medications and disease duration prior to study enrollment, were adjusted by using multivariate analysis. The results indicate that these factors were not significantly associated with clinical remission. Moreover, we were interested in the change in serum IL-6 levels and its relationship to disease outcome, rather than to disease duration and/or medication. Therefore, we believe that the study results are meaningful. Further studies with larger sample sizes, multi-center analyses, and prospective cohort design are recommended to confirm our findings.

Conclusion

Longitudinal measurements of serum IL-6 levels better reflected disease activity than one-time measurements in SJIA patients with active disease. Patients with early reduction in serum IL-6 levels had better treatment outcomes and a higher chance of achieving clinical remission than did patients with persistently high serum IL-6 levels. Monitoring the serial change in serum IL-6 levels is thus beneficial in assessing the treatment response and predicting clinical remission in SJIA patients.

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Authors' contributions

BL and SV had equal contribution in conception and design, analysis and interpretation of data, and drafting of the manuscript. All authors read and approved the final manuscript.

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