Growth Velocity and Interleukin 6 Concentrations in Juvenile Idiopathic Arthritis

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ABSTRACT. Objective. To evaluate associations of growth velocity with inflammatory markers and cumulative dose of glucocorticoid in a cohort of patients with juvenile idiopathic arthritis (JIA) followed during 1 year.

Methods. Seventy-nine patients were evaluated. Disease activity was evaluated by a pediatric rheumatologist. Anthropometric data were classified according to the World Health Organization standards. Tanner growth velocity curves were used; values below the Z-score ≤ –2 were considered low growth velocity. Serum concentrations of interleukin 6 (IL-6) were measured by ELISA, and values > 1 pg/ml were considered elevated.

Results. The prevalence of low growth velocity was 25.3%, and it was associated with active disease on followup visit, elevated IL-6, erythrocyte sedimentation rate and C-reactive protein, and higher cumulative glucocorticoid doses. In the multiple linear regression with growth velocity as the dependent variable, only elevated IL-6 level was independently and negatively associated with growth velocity.

Conclusion. Low growth velocity is highly prevalent in children with JIA. Elevated IL-6 levels seem to have an important negative influence on growth in these children, while total glucocorticoid exposure appears to be a secondary factor. (First Release Oct 1 2008; J Rheumatol 2008;35:2265–71; doi:10.3899/jrheum.080199)

Key Indexing Terms: JUVENILE IDIOPATHIC ARTHRITIS GROWTH VELOCITY INTERLEUKIN 6

Growth is often affected in pediatric diseases characterized by chronic inflammation, such as juvenile idiopathic arthritis (JIA), cystic fibrosis, and Crohn’s disease. In JIA the prevalence of low stature ranges from 10.4% to 41.0%. Evidence has shown that proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) are important mediators of chronic inflammation in JIA. IL-6 is a pleiotropic cytokine that regulates immune response, hematopoiesis, acute-phase response, and inflammation. In animal models of chronic inflammatory disease, as well as in human IL-6 transgenic murine lines, IL-6 appears to be the main proinflammatory cytokine involved in growth retardation. Experiments in transgenic mice suggested that growth retardation might be due to IL-6-induced perturbations in growth hormone (GH) signal transduction pathways, which include a reduction of GH receptor mRNA synthesis.

The influence of high levels of IL-6 on growth has been reported in children with perinatal human immunodeficiency virus infection. In these patients, increased spontaneous and phytohemagglutinin-stimulated IL-6 release from peripheral blood mononuclear cells was associated with low growth velocity and low serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3).

Circulating and synovial fluid levels of IL-6 are markedly elevated in patients with JIA, and they are associated with laboratory and clinical variables of disease activity. An association has also been observed between inflammatory clinical activity and growth retardation. However, no study has specifically evaluated elevated IL-6 levels as a compromising factor for growth in JIA. Considering the therapeutic approaches under investigation involving inhibition of actions of IL-6 by receptor blockade, a better comprehension of their potentially beneficial effects on the growth of children with JIA would be important.

Another factor that may intensify growth retardation is the use of glucocorticoids. Few observational studies have associated the use, duration, or cumulative dose of glucocorticoid with low stature in JIA. On the other hand, we recently reported that, on multivariable analysis, glucocorticoids were found not to be independently associated with...
low stature, in contrast with disease duration and inflammatory activity. There are few studies evaluating growth velocity in JIA, and the results have indicated that reduced growth velocity was associated with inflammatory activity, drug therapy with glucocorticoid, and reduced serum levels of IGF-I.

Our objective was to study growth velocity in a group of patients with JIA, including children in the pubertal stages, and the factors associated with its compromise, with special attention to glucocorticoid use and serum levels of IL-6 and other inflammatory markers.

MATERIALS AND METHODS

Patients. The study was performed at the Rheumatology Outpatient Clinic, Hospital de Clínicas de Porto Alegre. Patients were recruited consecutively and evaluated prospectively during 1 year. They came from 3 reference centers for treatment of children with rheumatic diseases in the city of Porto Alegre, Brazil. Seventy-nine patients (53 female, 21 male, aged 2 to under 20 yrs) fulfilled the diagnostic criteria for JIA according to the International League of Associations for Rheumatology. Exclusion criteria were presence of other diseases associated with JIA and/or the impossibility of performing the anthropometric measures.

Informed consent was obtained from each patient, parents, or legal guardian. The study was approved by the Ethics in Research Committee at Hospital de Clínicas de Porto Alegre.

Clinical assessment. After recruitment, the patients submitted to a standardized interview assessing disease characteristics, use of medication, anthropometric measures, and family income. The clinical assessment was repeated during the followup period, 1 year after the baseline assessment.

Cumulative dose of glucocorticoids. The cumulative dose of glucocorticoids was calculated by reviewing patient records and electronic prescriptions.

Disease activity. Disease activity was determined at baseline assessment and during the followup by a pediatric rheumatologist, according to Gäre, et al.

The disease was considered (1) active when the number of joints affected increased despite the use of drug therapy; (2) stable when the number of joints affected was stable using drug therapy; (3) inactive — no evidence of synovitis and/or extraarticular involvement without drug therapy for < 2 years; or (4) in remission — no evidence of synovitis and/or extraarticular involvement without drug therapy for ≥ 2 years.

Assessment of puberty. Puberty was assessed according to the Tanner classification during the baseline and followup periods. Children in the growth-spurt phase were stratified, characterized as Tanner 2 (T2) for girls and Tanner 3 (T3) for boys.

Anthropometric variables. Anthropometric data were measured according to the World Health Organization (WHO) standards and always by the same researcher (LSS). Weight was measured on a Filizola scale, with 10-g graduation; patients were barefoot and wearing a standard gown. Stature was measured with a stadiometer; the patients were barefoot, standing with their feet together, heels against the wall, and the height reading was rounded to the closest 0.5 cm. According to the WHO, the body mass index (BMI) was calculated using the weight/square of height formula and was compared with reference values of the curves of BMI percentiles for children developed by the National Center of Health Statistics in collaboration with the US Centers for Disease Control and Prevention growth charts. Percentiles obtained in this manner were classified according to the WHO

Growth velocity (cm/yr) was defined by the difference between followup and baseline measures, divided by followup time. Values were normalized according to chronological age, expressed as standard deviation (Z-score). According to the WHO, the calculation of the Z-score uses the following formula: Z-score = (value - mean) / SD (for age, sex, and sample), where the means of growth values at the 50th percentile and standard deviation were as defined by Tanner, et al.

Biochemical variables. Blood was collected the same day as the baseline clinical evaluation to measure erythrocyte sedimentation rate (ESR); reference value 0 to 10 mm/h and C-reactive protein (CRP); reference value < 3.0 mg/l. Serum aliquots obtained after centrifugation (3000 rpm for 10 min) were frozen at −80°C until testing for IGF-I and IL-6, which was performed at the same time.

IGF-I levels were measured in 73 patients, using a 2-site immunoradiometric assay (IRMA Active IGF-1 DSL-5600); Diagnostic Systems Laboratories, Webster, TX, USA). IGF-I levels are expressed in ng/ml, and their reference values vary according to age and sex, as suggested by the manufacturer.

IL-6 levels were measured in 78 patients by ELISA (Cell Science, Diaclone, Canton, MA, USA). The plates, previously sensitized with human anti-IL-6 monoclonal antibody, were incubated simultaneously with the serum samples and with anti-IL-6 monoclonal antibody conjugated with biotin for 1 h at room temperature. After washing, the plates were incubated with streptavidin-peroxidase for 30 min at room temperature. The plates were washed and incubated again with tetramethylbenzidine for 10 min. The enzymatic reaction was interrupted by sulfuric acid, and optical density was read with a 450 nm filter. IL-6 levels were considered to be elevated when > 1 pg/ml and low when ≤ 1 pg/ml.

Statistical analysis. Sample size was calculated based on the correlation between growth velocity and the values of IL-6 in JIA. Considering a 5% level of significance, a power of 95%, and a moderate correlation coefficient (r ≥ 0.4) between variables, a minimum of 75 individuals was estimated.

Variables with a symmetrical distribution were described by mean and standard deviation, whereas those with asymmetrical distribution were described by median and 25th and 75th percentiles. The Kolmogorov-Smirnov test was applied to assess the distribution of variables.

Absolute and relative frequencies were used to describe qualitative variables. Due to the asymmetry of the growth velocity variable, nonparametric tests were applied to evaluate its association with the variables studied. Spearman correlation was applied for quantitative variables. Mann-Whitney test was used for dichotomous qualitative variables, and the Kruskal-Wallis test for the polytomic variables. A ranked transformation in growth velocity was applied to use Tukey’s test as post-hoc of the Kruskal-Wallis test.

Association between categorical variables was assessed using the chi-square test.

In order to compare variables with symmetrical and asymmetrical distribution in relation to disease subtype, the one-way analysis of variance (ANOVA) and Kruskal-Wallis test were used, respectively. Student t and Mann-Whitney tests were used, respectively, to compare the quantitative variables with symmetrical and asymmetrical distribution in relation to the dichotomized IL-6 (≤ 1 pg/ml or > 1 pg/ml).

The multiple linear regression model was applied to control confounding factors and to evaluate predictors of growth velocity. Logarithmic transformation was used for this analysis. Variables included in the regression model were those that presented p ≤ 0.20 in the bivariate analysis.
The level of significance adopted was 5% and the tests were performed using SPSS version 11.034.

RESULTS

Patients’ demographic and clinical characteristics are described in Table 1. Females (67.1%) and the polyarticular subtype of the disease (75.6%) predominated in the sample. The mean growth velocity of all patients was 4.23 ± 2.89 cm/yr in the total sample, 5.24 ± 2.69 cm/yr in boys and 3.73 ± 2.88 cm/yr in girls (p = 0.029). In the overall sample the mean of the Z-score of growth velocity was –0.59 ± 2.56. During the growth-spurt phase, characterized as Tanner stage T2 for girls and T3 for boys, the mean growth velocity in Z-score for girls was 1.56 ± 1.34, with a mean age of 11.49 ± 1.86 years (n = 6), and for boys was –1.07 ± 6.70, mean age 12.97 ± 1.27 years (n = 3) (data not shown). The prevalence of low growth velocity (Z-score ≤ –2) was 25.3% (n = 20). Twelve (60.0%) of these 20 patients presented levels of IL-6 > 1 pg/ml, while of the 58 patients with normal growth velocity, only 16 (27.6%) presented levels of IL-6 > 1 pg/ml (p = 0.020). No statistically significant association was found between the Z-score of the growth velocity and sociodemographic and anthropometric measures (data not shown).

No significant association was found between growth velocity and disease subtypes or disease activity during the baseline period. All the children who were experiencing their growth spurt during the baseline period had stable disease activity. There was a tendency for patients with active disease during the followup period to present lower growth velocities. No significant associations were found between growth velocity and IGF-I (Table 2).

There was a significant inverse association between growth velocity (Z-score) and cumulative glucocorticoid dose in the bivariate analysis, i.e., lower growth velocities were found in patients with higher cumulative doses of medication (r = –0.263, p = 0.044; data not shown). An inverse statistically significant association was observed between growth velocity and laboratory measures of ESR (r = –0.269; p = 0.022), CRP (r = –0.386, p = 0.001), and IL-6 (r = –0.337, p = 0.003) (Figure 1). Patients with IL-6 levels > 1 pg/ml (n = 28, 35.9%) presented a mean growth velocity significantly lower than those with IL-6 levels ≤ 1 pg/ml (mean Z-scores of –1.66 ± 2.44 vs –0.07 ± 2.48, respectively; p = 0.006).

Elevated IL-6 levels (> 1 pg/ml) were significantly associated with the polyarticular and systemic subtypes, lower age at onset of disease, longer duration of disease, active and stable forms of disease, and low weight (Table 3).

Applying a multiple linear regression model using growth velocity (Z-score) as the dependent variable, it was
observed that growth velocity was negatively associated with elevated IL-6 levels (> 1 pg/ml) and positively associated with the growth spurt (T2 for girls, T3 for boys), independently of the other variables in the model. Despite a significant association in the bivariate analysis, there was no independent association of the cumulative dose of glucocorticoids with growth velocity (Table 4).

DISCUSSION

We assessed growth velocity in a cohort of patients with JIA, and attempted to identify the factors that affect the growth of this population, with special attention to disease activity, acute-phase proteins, IL-6 concentrations, and cumulative dose of glucocorticoids.

Only 4 studies have previously evaluated growth velocity in patients with JIA not exposed to growth hormone therapy; 3 were retrospective and one prospective. Different methodologies were used to assess and classify growth velocity in these studies, rendering it difficult to compare them. None of the studies evaluated growth velocity during the growth spurt, as in the present study.

We found a prevalence of low growth velocity (Z-score ≤ –2) of 25.3%, the same percentage observed in the study of Garcia-Consuegra, et al. Patients with low growth velocity tended to present the active form of the disease on the followup visit. This is also in agreement with the study of Garcia-Consuegra, et al, where low growth velocity was associated with disease activity due to the increased number of inflamed joints, as well as with the study of Saha, et al, where diminished growth velocity was more prevalent in the polyarticular subtype and in patients with more severe clinical disease activity. We also observed that the acute-phase reactants ESR and CRP were correlated with lower growth velocity. Therefore, there is strong evidence correlating disease activity and low growth velocity.

To better understand the role of inflammation in stunted growth, we investigated whether serum levels of the proinflammatory cytokine IL-6 were associated with low growth velocity. The choice of IL-6 was due to its well recognized central role in JIA, especially in the systemic-onset subtype, and because a number of studies with animal models have demonstrated its negative effects on growth. We observed an inverse association between growth velocity and serum levels of IL-6. No previous study on growth velocity assessed IL-6 serum levels.

Figure 1. Associations between Z-score of the growth velocity and (A) ESR, (B) CRP, and (C) interleukin 6 by Spearman test.

Although patients with the polyarticular and systemic subtypes presented lower growth Z-scores compared with the oligoarticular subtype, this difference did not reach statistical significance, probably due to the low number of patients in each group. However, significantly more patients with the polyarticular and systemic subtypes presented high IL-6 levels (> 1 pg/ml), and we and others have previously reported that children with the polyarticular and systemic subtypes have shorter stature. Moreover, there was
an association between high levels of IL-6 and disease activity, in agreement with other studies in JIA. Since the polyarticular and systemic subtypes usually progress with higher inflammatory activity levels, and considering that elevated IL-6 levels were associated with lower Z-score values for growth velocity, we believe that IL-6 could be an essential link between inflammation and growth deficit.

In our patients with JIA we observed that the longer the duration of disease, the higher the proportion of elevated IL-6 levels. In our previous study, we observed that the activity and longer duration of disease were independently associated with lower stature. Garcia-Consuegra, et al. have observed that the longer duration of disease and the higher degree of functional involvement appeared to be risk factors for height growth impairment. These observations further stress the importance of immediate action in controlling the inflammatory activity to prevent stunted growth.

We also observed an association between high IL-6 levels and low weight. The association between high IL-6 levels and reduction of stature and weight measures has been shown in experimental studies. It has been observed that the inflammatory clinical activity of JIA disease was associated with reduction of BMI and percentage of body fat. Weight loss and reduced appetite have been associated with elevated circulating levels of IL-6 in other disorders. Therefore, one could argue that the mechanism by which IL-6 might be involved in retarded growth is by decreased caloric intake. However, it was reported that human-IL-6-transgenic mice that presented markedly reduced growth without detectable tissue inflammatory activity had normal food intake and serum glucose, indicating that their growth defect was not caused by a nutritional disorder.

Multivariable analysis confirmed the negative correlation of high IL-6 levels (> 1 pg/ml) and growth velocity. However, even if an inverse association had been observed between growth velocity and cumulative dose of glucocorticoids in bivariate analysis, when the multiple linear regression model was applied, it was seen that it was not inde-

Table 3. Association between interleukin 6 (IL-6) and clinical and anthropometric characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IL-6, n = 78</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 1 pg/ml,</td>
</tr>
<tr>
<td></td>
<td>n = 50</td>
</tr>
<tr>
<td>Baseline BMI, n (%)</td>
<td></td>
</tr>
<tr>
<td>Low weight</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td>Eutrophy</td>
<td>35 (70.0)</td>
</tr>
<tr>
<td>Overweight</td>
<td>12 (24.0)</td>
</tr>
<tr>
<td>Disease activity at baseline, n (%)</td>
<td></td>
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<tr>
<td>Active</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Stable</td>
<td>38 (76.0)</td>
</tr>
<tr>
<td>Inactive or remission</td>
<td>12 (24.0)</td>
</tr>
<tr>
<td>Disease activity at followup, n (%)</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Stable</td>
<td>34 (68.0)</td>
</tr>
<tr>
<td>Inactive or remission</td>
<td>14 (28.0)</td>
</tr>
<tr>
<td>Disease subtype, n (%)</td>
<td></td>
</tr>
<tr>
<td>Oligoarticular</td>
<td>25 (50.0)</td>
</tr>
<tr>
<td>Polyarticular</td>
<td>22 (44.0)</td>
</tr>
<tr>
<td>Systemic</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Psoriatic</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Age at disease onset, median (25th–75th)</td>
<td>4.8 (2.8–8.1)</td>
</tr>
<tr>
<td>Duration of disease, median (25th–75th)</td>
<td>4.5 (2.7–6.6)</td>
</tr>
<tr>
<td>Total cumulative dose of glucocorticoids, mg, median (25th–75th)</td>
<td>2150 (1162–4293)</td>
</tr>
</tbody>
</table>

* Pearson chi-square test; ** Mann-Whitney test. BMI: body mass index.

Table 4. Multiple linear regression analysis* to evaluate growth velocity predictors.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beta</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative dose of glucocorticoids</td>
<td>0.020</td>
<td>0.905</td>
</tr>
<tr>
<td>Growth-spurt phase (T2 female and T3 male)</td>
<td>0.428</td>
<td>0.003</td>
</tr>
<tr>
<td>ESR</td>
<td>0.212</td>
<td>0.273</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.169</td>
<td>0.377</td>
</tr>
<tr>
<td>IL-6 &gt; 1 pg/ml</td>
<td>-0.375</td>
<td>0.025</td>
</tr>
<tr>
<td>IGF-I (above normal)</td>
<td>-0.048</td>
<td>0.722</td>
</tr>
</tbody>
</table>

* ANOVA for the regression model: F (6,39) = 3.591 with p = 0.006; determination coefficient (R^2) = 35.6%. T: Tanner growth stage, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, IL-6: interleukin 6, IGF-I: insulin-like growth factor-I.
pendently associated with growth velocity. This confirms our previous findings\(^6\) that glucocorticoids by themselves do not seem to be the predominant negative factor affecting growth in these patients. There is controversy concerning the association between treatment with glucocorticoids and shorter stature. Some studies have observed this association\(^{44-46}\), while others did not\(^{4,6,35,47}\). Polito, et al\(^{36}\) reported growth impairment in patients with JIA who had never received steroids. Saha, et al\(^{23}\) reported that the cumulative total dose of glucocorticoids did not have a statistically significant influence on the velocity of growth. These observations support the notion that to preserve growth in children with JIA, aggressive control of inflammation might be a more relevant concern than sparing glucocorticoids, at least in the short-term perspective.

Some limitations in our study should be noted. The method we used to evaluate disease activity was developed by EULAR in 1983 and may not have been sensitive enough to identify smaller changes during the followup\(^25\). Recently, efforts have been made to validate methods for adequate measurement of JIA activity; however, there is still no consensus. The core set indices, such as the Disease Activity Score (DAS) and the American College of Rheumatology-30 pediatric response criteria, have the advantage of incorporating multiple aspects of disease activity and are widely used in clinical trials. Despite this, their implementation may demonstrate some pitfalls, since the ACR response criteria are less effective in describing an individual’s disease state at a specific moment in time, and the DAS may not be appropriately sensitive for children with oligoarticular disease\(^{48}\). In respect of the study population, a relatively small number of patients with the systemic JIA subtype was verified (n = 6), in which as we stated growth failure is seen more commonly and IL-6 has been shown to play a major pathogenetic role. We think these limitations do not severely compromise our main findings, because both of them would bias toward a negative association between growth failure and IL-6 levels. However, we acknowledge that the small number of patients with systemic JIA precludes more definitive conclusions on differences across JIA subtypes.

Recently, new therapeutic strategies utilizing cytokine inhibition, such as anti-TNF monoclonal antibodies and the TNF soluble receptor construct, have demonstrated significant efficacy in the control of joint inflammation\(^{48}\). The positive influence on growth velocity of treatment with anti-TNF in patients with severe JIA was recently shown in a prospective study performed during a 2-year followup period\(^{49}\). This study also demonstrated that inflammatory disease control was a significant predictor of increased growth velocity. Because of the correlation between high IL-6 levels and growth impairment we observed, we anticipate that therapies targeting this cytokine, such as the humanized anti-IL-6 receptor monoclonal antibody tocilizumab, will also be of value to preserve growth in these children\(^{19,20}\).

Low growth velocity was highly prevalent in our children with JIA. Elevated IL-6 levels seemed to have an important negative effect on growth in these children, while total glucocorticoid exposures were not independently associated with low growth. Aggressive control of inflammation, including inhibition of proinflammatory cytokines, rather than limiting glucocorticoid exposure, should be a priority to preserve growth in children with JIA.

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