# Changes in Markers of Bone Turnover and Inflammatory Variables During Alendronate Therapy in Pediatric Patients with Rheumatic Diseases

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***ABSTRACT.*** ***Objective.*** Alendronate treatment for 12 months in pediatric patients with rheumatic diseases and secondary low bone mass was reported to result in a substantial increase in bone mineral density (BMD). In this study, we evaluated the changes in bone metabolism and disease activity markers in 45 patients ages 5 to 18 years (31 female, 14 male) with rheumatic diseases treated with alendronate for 12 months.

***Methods.*** Variables analyzed included demographic and anthropometric data, biochemical markers of bone metabolism, disease activity indexes, and BMD values. For all variables, the differences between levels at baseline and at 12 months were calculated; correlations between the variables and between the BMD variation over 12 months and baseline levels of the different variables were also evaluated.

***Results.*** There was a statistically significant decrease of both bone resorption and bone formation markers over the 12 month treatment period. By contrast, no disease activity index changed significantly over one year. BMD Z score change over one year did not correlate with erythrocyte sedimentation rate, matrix metalloproteinase-3, interleukin 6, or C-reactive protein variations over the same period.

***Conclusion.*** These results support the conclusion that alendronate treatment is accompanied by a reduction of bone turnover in pediatric patients and that the observed BMD increase is not secondary to a reduction of inflammatory activity. (J Rheumatol 2002;29:1786–92)

*Key Indexing Terms:*
ALENDRONATE BISPHOSPHONATES JUVENILE IDIOPATHIC ARTHRITIS
BONE TURNOVER BONE MARKERS RHEUMATIC DISEASES

Rheumatic diseases are frequently complicated by osteopenia and/or osteoporosis. These complications are particularly worrisome in the growing child, since the attainment of satisfactory peak bone mass may be compromised. Indeed, recent data have shown that young adults with a history of juvenile chronic arthritis may be at risk of developing premature osteoporosis and associated fractures[1,2]. Osteoporosis in pediatric rheumatic diseases is multifactorial, but systemic inflammation is likely to play a major role[3-5], as several cytokines are known to have an effect on bone remodeling.

In a recent multicenter study, we demonstrated that alendronate treatment can improve bone mass in children and adolescents with rheumatic diseases, and that at least in the short term the drug is well tolerated and free of significant side effects[6]. However, in that study we did not investigate the mechanisms of bone mass increase in these patients. Moreover, several studies have shown the usefulness of biochemical markers of bone metabolism in the followup of adult patients treated with bisphosphonates[7-13], but these aspects have not been clearly addressed in pediatric patients.

We therefore performed serial measurements of different biochemical indicators of inflammation and bone metabolism in a group of 45 children and adolescents with chronic rheumatic diseases treated with alendronate, and evaluated their changes over one year.

## MATERIALS AND METHODS

*Patients.* This study involved a group of children and adolescents followed

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*Submitted July 30, 2001; revision accepted February 28, 2002.*

in 5 pediatric rheumatology centers of northern Italy. These patients had low bone mass and were therefore treated with alendronate for one year in an open trial. The majority of the patients (38 out of 45) are described in our previous report[6]; 7 additional patients received alendronate for one year and were followed with the same protocol, but were not enrolled in the original study because they began their treatment after the onset of the open trial. The main characteristics of the 45 patients (31 female, 14 male) at baseline are shown in Table 1. Juvenile idiopathic arthritis (JIA) was classified according to the International League of Associations for Rheumatology criteria[14], systemic lupus erythematosus (SLE) according to the 1982 American Rheumatism Association criteria[15], and juvenile dermatomyositis according to the criteria of Bohan and Peter[16]. As shown in Table 1, 37 out of 45 patients were treated with corticosteroids, and this treatment was continued throughout the study. Physical activity was very low (i.e., walking with difficulty for a short time, unable to participate in gymnasium or outdoor activities) in 18 patients, moderate (walking normally, but unable to practice sports) in 21 patients, and high (participating in sports) in 6.

*Methods*. The study protocol is described in detail[6]. Procedures of recruitment and followup were approved by local ethics committees. Informed consent was obtained from patients or parents. Alendronate was administered at a dosage of 5 mg daily for body weight < 20 kg, and 10 mg for body weight ≥ 20 kg. The children and their parents were instructed to take the alendronate pill after an overnight fast, at least 60 min before breakfast, with at least 100 ml of water, and to stay seated or upright for at least 30 min. Throughout the study patients were allowed to continue their prescribed drug treatment, with all required adjustments on the basis of clinical needs. All postpubertal girls and/or their parents were informed about the need to avoid a pregnancy during the study and for at least 6 months after discontinuation of the drug. At each visit (at baseline and at 3, 6, 9, and 12 mo) a detailed history was taken and physical examination was performed.

Routine laboratory tests [erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood count, creatinine, total protein, albumin, transaminases, calcium, phosphate, total alkaline phosphatase] were performed every 3 months at the 5 participating centers' laboratories, while measurements of bone-specific markers and cytokines were performed at baseline and after 6 and 12 months in 2 specialized laboratories (Istituto Auxologico Italiano for bone markers and Istituto Giannina Gaslini for cytokines). For these measurements, the patients' serum and urine samples were collected, frozen, and sent to the reference laboratories, where the following tests were performed: parathyroid hormone (PTH), markers of bone formation, i.e., osteocalcin (OC) and bone-specific alkaline phosphatase (BSAP), markers of bone resorption, i.e., pyridinoline (PYR) and N-terminal telopeptide of collagen type I (NTx), and inflammatory markers, i.e., interleukin 6 (IL-6) and matrix metalloproteinase-3 (MMP-3). All tests were performed on serum samples except for NTx levels, which were determined on urine samples from 24 h collection. To reduce the sources of variability all blood samples were drawn in the morning (9:00 AM) after an overnight fast; the samples were adequately stored and measured together with the same lot of assay kits in the same laboratory.

Serum concentrations of IL-6 and MMP-3 were tested by commercial ELISA kits (Amersham, UK) according to the manufacturers' instructions. Assays for IL-6 and MMP-3 detect bound and free IL-6 and MMP-3, respectively. PTH was measured by an immunoradiometric assay (DiaSorin Inc., Stillwater, MN, USA). OC (RIA; TechnoGenetics, Milano, Italy) and BSAP (RIA; Metra Biosystems Inc., Mountain View, CA, USA) were evaluated as bone formation markers. Urinary NTx (ELISA; Ostex Intern Inc., Seattle, WA, USA) and serum PYR (competitive enzyme immunoassay; Metra Biosystems) were evaluated as bone resorption markers.

Bone mineral density (BMD) was measured every 6 months at the lumbar spine (L2–L4, posterior–anterior) using dual energy x-ray absorptiometry (DEXA) devices (4 Hologic and one Lunar) under a strictly standardized protocol[6]. As software-calculated BMD for area (mg/cm$^2$) does not account for bone volume, which is important in evaluating a growing skeleton and is strictly related to body size (weight and height), BMD for area was subsequently adjusted for body surface. Z scores were calculated on the basis of the BMD (similarly adjusted for body surface) of local reference groups of healthy children matched for sex and age. For each patient all the DEXA scans were performed with the same machine.

*Statistics*. Relevant variables were entered into a customized database and included demographic and anthropometric data, biochemical markers of bone metabolism (serum levels of calcium, phosphate, BSAP, PYR, OC, and PTH and urinary NTX), disease activity measures (ESR, CRP, hemoglobin, platelet count, IL-6, MMP-3, and SLE Disease Activity Index where applicable), and BMD values. For all variables differences between levels at baseline and at 12 months were evaluated by the Student t test for paired data, and a linear trend between baseline and 6 and 12 months was assessed by an ANOVA model for repeated measures. Relationships between the variables were evaluated using the Pearson correlation coefficient. Relationships between the BMD changes over 12 months and baseline levels of the different variables were also evaluated to identify possible predictors of a response to treatment. A Bonferroni correction was performed when applicable. BMD changes over 12 months were also correlated to variations of the disease activity indexes over the same period, in the total study group and in the subgroup of patients with JIA.

*Table 1*. Demographics of the study population.

| | |
|---|---|
| No. of patients | 45 |
| Male | 14 |
| Female | 31 |
| Diagnosis | |
| Systemic lupus erythematosus | 14 |
| Juvenile dermatomyositis | 7 |
| Systemic juvenile idiopathic arthritis | 8 |
| Polyarticular juvenile idiopathic arthritis | 10 |
| Other* | 6* |
| Age at study onset, yrs | |
| Mean ± SD | 12.9 ± 3.8 |
| Range | 5–18 |
| Disease duration, mo | |
| Mean (range) | 67 (13–169) |
| Steroid treatment, n = 37 patients | |
| Mean duration, mo ± SD | 41.7 ± 4.8 |
| Cumulative dose prednisone, mg ± SD | 14,498 ± 1512 |
| Current dose prednisone, mg/day ± SD | 9.3 ± 1.2 |
| Pubertal status, Tanner stage | |
| T1, T2 | 20 |
| T3 | 6 |
| T4 | 4 |
| T5 | 15 (menarche 14) |

* Other: Behçet's disease 2, undifferentiated connective tissue disease 2, Wegener's granulomatosis 1, inflammatory bowel disease related arthritis 1.

## RESULTS

Median changes in the different variables studied are shown in Table 2. There was a statistically significant decrease of both bone resorption and bone formation markers after 6 months of alendronate treatment, which continued steadily throughout the 12 months of treatment with a linear trend (NTX, $p = 0.001$; PYR, $p < 0.0001$; BSAP, $p < 0.0001$; OC, $p = 0.006$). PTH increased slightly at the end of 12 months. Figure 1 shows the mean values of BSAP, OC, NTX, and PYR during the study period. By contrast, none of the

*Table 2*. Percentage and absolute changes of variables before and after one year treatment with alendronate and t test for paired data (2 tailed) with p values.

| | Median % Change | Min, Max % Change | Mean Absolute Change ± SD | p |
|---|---|---|---|---|
| BMD Z score | 34.08 | –18.5, 167 | 0.87 ± 0.57 | < 0.001 |
| Calcium, mg/dl | –1.35 | –19.4, 13.6 | –0.09 ± 0.71 | 0.440 |
| Phosphate, (mg/dl) | 0.75 | –3.7, 7.9 | 0.04 ± 0.8 | 0.897 |
| PTH, pg/ml | 47.17 | –71.3, 975.2 | 11.66 ± 26 | 0.011 |
| BSAP, µg/l | –40.77 | –64.1, 62.3 | –17.78 ± 18.82 | < 0.001 |
| OC, µg/l | –38.51 | –82.1, 660 | –9.59 ± 20.15 | 0.006 |
| NTx, nM/mM Cr | –40.27 | –87.5, 183 | –184.87 ± 301.62 | 0.001 |
| Pyr, nM/l | –29 | –68.6, –1.7 | –0.91 ± 0.55 | < 0.001 |
| ESR, mm/h | 0 | –93.5, 220 | –5.4 ± 21.4 | 0.114 |
| CRP, mg/l | 2.19 | –95.2, 663.3 | –2.4 ± 13.4 | 0.329 |
| Hb, g/dl | 0.77 | –32.5, 47.5 | 0.15 ± 1.41 | 0.490 |
| IL-6, pg/l | –13.84 | –97.6, 7818 | 15.75 ± 531.44 | 0.872 |
| MMP-3, ug/l | –20.83 | –86.8, 133.3 | –81.30 ± 211.70 | 0.094 |
| Platelets | –6.89 | –49.9, 211.8 | –10,119 ± 136,083 | 0.632 |
| SLEDAI | –28.57 | –100, 500 | –3.2 ± 7.9 | 0.261 |

PTH: parathyroid hormone, BSAP: bone-specific alkaline phosphatase, OC: osteocalcin, NTX: urinary N-terminal telopeptide of procollagen type 1, Pyr: pyridinoline, IL-6: interleukin 6, MMP-3: matrix metalloproteinase-3, SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

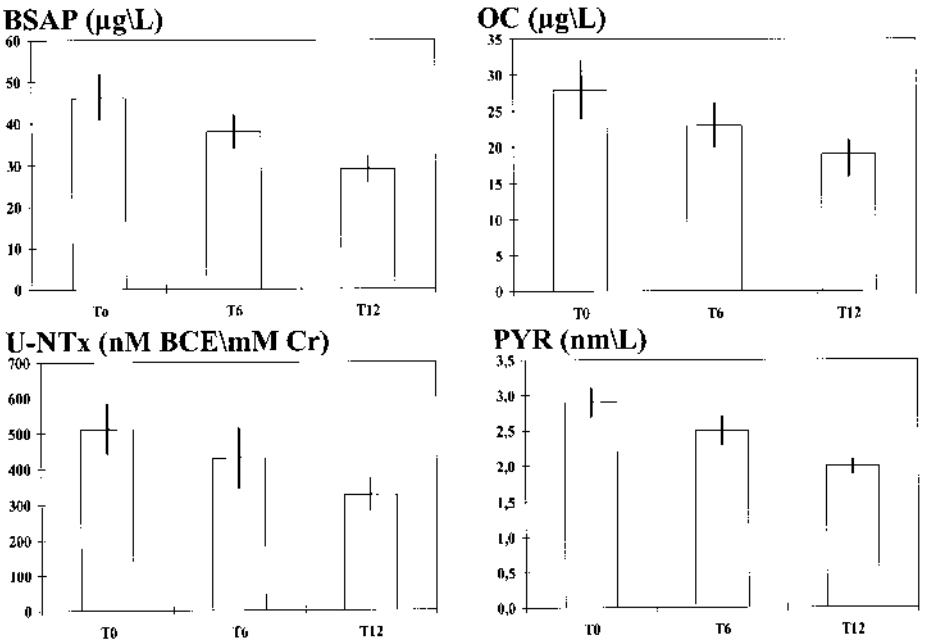



*Figure 1*. Mean levels of serum bone-specific alkaline phosphatase (BSAP), osteocalcin (OC), pyridinoline (PYR), and urinary N-terminal telopeptide of type I collagen (NTx) at baseline and after 6 and 12 months of alendronate treatment. Bars represent standard deviations. BCE: bone collagen equivalent, Cr: creatinine. Units of measurements are on vertical axes, time of study period on horizontal axes (T0: baseline, T6: after 6 mo, T12: after 1 year).

disease activity indexes, including a physician global assessment, changed significantly over the same period. The great majority (41/45) of patients were judged by the treating physician as clinically stable at 12 months compared to baseline.

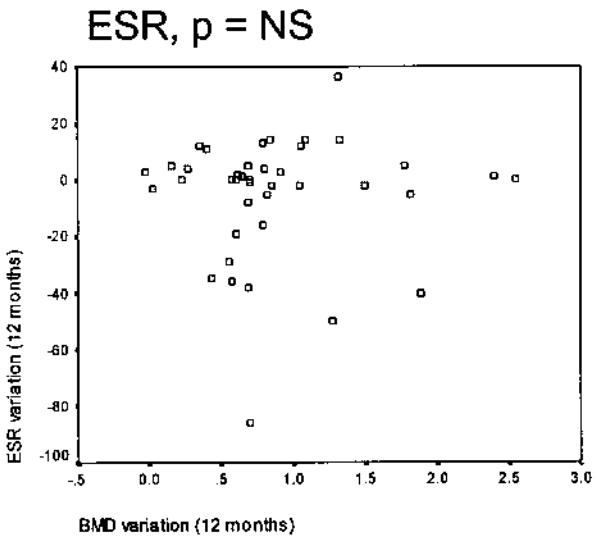
Univariate analysis between change in BMD expressed as Z score and baseline variables showed a significant correlation for BSAP (p = 0.005), OC (p = 0.005), and NTX (p = 0.009). Baseline BMD Z score was not correlated with the subsequent BMD increase (p = 0.22). We also analyzed possible correlations between baseline BMD Z score versus all recorded baseline variables, but no significant correla-

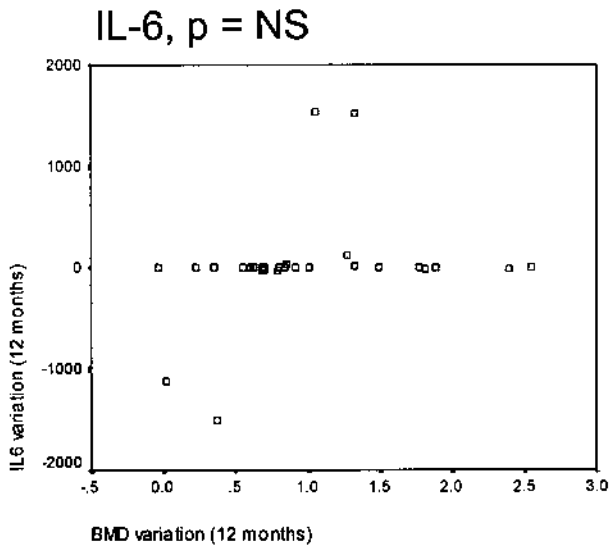
tions were found. Also, correlations between the changes in BMD Z score and the changes of all other variables were not statistically significant. In particular, the BMD Z score changes over one year did not correlate with ESR, MMP-3, IL-6, or CRP changes (Figure 2); this would indicate that the increase in bone density is not likely to be secondary to a change in disease activity or inflammation. An interesting finding, however, is shown in Figure 3, where the inverse correlation between changes in serum MMP-3 levels and changes in BMD only in the subgroup of patients with JIA is shown. As can be seen, this correlation is highly significant ($r = –0.88$, $p = 0.001$).

We also performed statistical comparisons dividing patients by diagnoses, but there was no significant difference in the results (changes of different variables) among the different disease groups. As well, we divided patients in 2 groups, prepubertal (Tanner stage 1 to 3) and pubertal (Tanner 4 and 5). The only variable that was significantly different between these 2 groups was osteocalcin, which increased by 24.2% in the former and decreased by 51.8% in the latter group, who had already achieved pubertal maturity.

## DISCUSSION

Therapeutic use of bisphosphonates is now more than 30 years old. Their use in pediatrics, however, is relatively new, and only recent clinical studies have been published[17-31]. The main indication for the use of these agents in children is the treatment of osteogenesis imperfecta[19,22,24,26], but to our knowledge our recent study dealing with chronic rheumatic diseases[6] included the largest patient sample. In that study patients' BMD increased by an average of 14.9% (± 9.8) ($p < 0.002$ versus baseline). The bone mass increase was much higher than in nontreated matched controls. Moreover, there



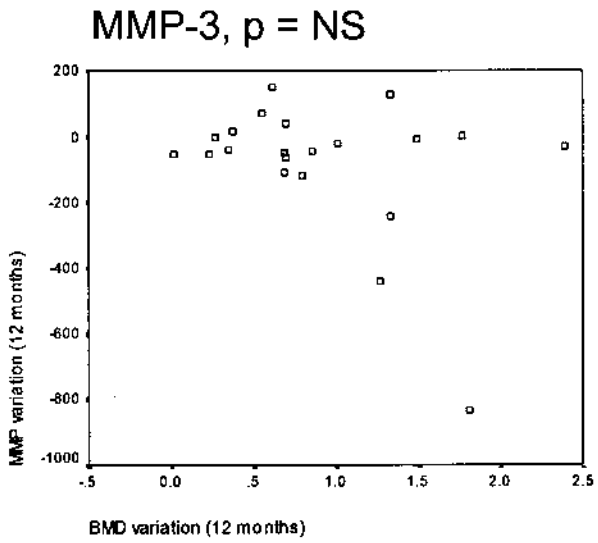




*Figure 2.* Relationships between bone mineral density (BMD) variation over 12 months and variations of ESR, IL-6, and MMP-3 serum levels. NS: not significant.

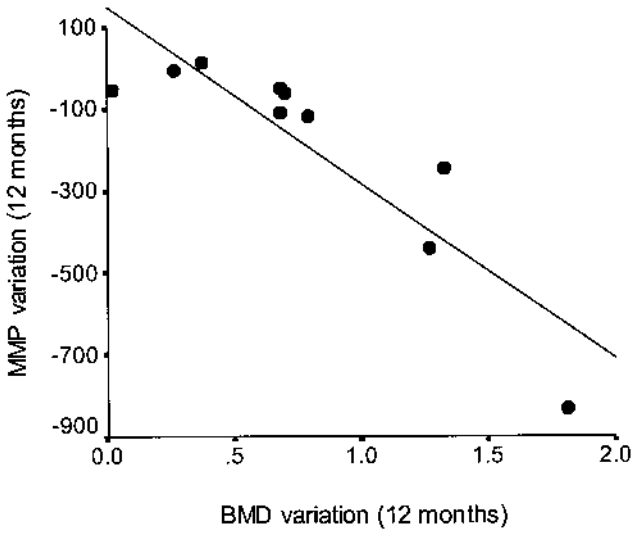



*Figure 3.* Significant correlation (p = 0.001) between bone mineral density (BMD) variation over 12 months and variations of MMP-3 serum levels only in patients with juvenile idiopathic arthritis.

was a large increase in BMD (15.3 ± 9.9%) in 16 patients who had been followed with DEXA scans in the year immediately preceding alendronate therapy, during which there had been almost no increase, and frequently a decrease of BMD.

The mechanism of action of alendronate on bone, according to many studies[32-37], is mainly the inhibition of bone resorption, and this characteristic makes it especially effective in the treatment of osteoporosis. Indeed, a high rate of bone turnover because of an increased activation frequency of new bone multicellular units (BMU) is considered the basis of the microarchitectural deterioration of bone tissue in osteoporosis. The activities of BMU are revealed in the serum and urine levels of different biochemical markers of bone turnover, and such markers have become an essential tool in the clinical management of osteoporosis. Accordingly, several studies on changes of bone turnover variables during alendronate treatment of adult patients have been published. Most of them, however, deal with postmenopausal osteoporosis, and only a few with osteoporosis secondary to chronic inflammatory conditions such as rheumatoid arthritis. As expected, we also observed in our young patients a marked decrease of bone turnover markers after only 6 months of treatment with alendronate, in accord with the results observed in adults; a further significant decrease of all markers was observed at the end of the 12 month period. Moreover, in children as well the link between bone resorption and formation markers was maintained. Indeed, bone formation and bone resorption processes are strictly coupled, and the decrease in bone resorption measures due to alendronate was quickly followed by a decrease in bone formation markers.

Of note, we used a newly available kit measuring serum pyridinoline. This is a useful marker of bone resorption, and our results indicate that there was a similar pattern of response to alendronate for this serum marker and the urinary NTX, which is considered specific for bone. This is particularly interesting, as a correct urine collection is often difficult in children.

One of the major goals of our study was to evaluate whether the observed bone mass increase was achieved through a direct action of alendronate on bone resorption or was simply a consequence of an independent reduction of the ongoing inflammatory process. The baseline levels of bone markers were correlated with the later changes in BMD, in accord with the results seen in adults[38,39]. On the other hand, no correlation was found between the changes in bone markers and changes in BMD, or between indexes of disease activity and BMD change. This latter finding suggests that the BMD increase observed in our patients was not secondary to a change in disease activity, but most likely represented an effect of the biological activity of alendronate treatment on bone resorption.

Diagnostic categories and pubertal status did not seem to have an influence on variations of the different variables studied, except for an increase in osteocalcin levels in prepubertal patients, presumably for a higher bone formation activity. Lack of statistically significant differences may, however, have been a result of the small number of patients in each group.

To further investigate the possible influence of inflammation on bone resorption, 2 biological variables were evaluated. IL-6 is known as a potent proinflammatory cytokine, usually found to be overexpressed in many autoimmune disorders. During systemic inflammation, IL-6 exerts a negative balance on bone mass, through its direct influence on osteoclast activation[40]. Notably, in many studies, IL-6 was found to correlate with disease activity measures, in childhood arthritis as well[41]. Matrix metalloproteinases (MMP) are a large family of proteolytic enzymes produced by fibroblasts, macrophages, chondrocytes, and osteoclasts upon stimulation by a number of growth factors, proinflammatory cytokines, and hormones[42]. The proteolytic activity of MMP is thought to be crucial for the removal of extracellular matrix during tissue resorption in both physiological and pathological processes. In particular, MMP act as selective proteolytic enzymes during bone remodeling for many macromolecules of the extracellular matrix[42], and it is noteworthy that MMP-3 could also play a role in bone resorption, as suggested by experimental data[43-45]. In chronic synovitis, MMP-3 has been found to be one of the most relevant MMP in the pathogenesis of tissue damage, and it has been shown to be overexpressed at the level of biological fluids and synovial membranes in arthritis in adults[46,47] and recently in children[48] as well. Moreover, a clear correlation between disease activity measures and MMP-3 serum concentration has been reported[49]. One of the most inter-

esting findings of our study was the strong negative correlation between the variation of serum MMP-3 and BMD Z score in the subgroup of patients with JIA. It is worth noting that in recent *in vitro* studies various bisphosphonates, including alendronate, have been shown to downregulate the production of MMP by osteoclasts[50]. It is therefore possible to hypothesize a local negative effect of alendronate on the MMP-3 production at the bone level, at least in the subgroup of patients where more information is available on the role of MMP on the pathogenesis of tissue damage.

In summary, we observed that alendronate treatment is accompanied by a reduction of bone turnover markers (resorption and formation) in pediatric patients. Moreover, the increase in BMD during treatment was not associated with a reduction of inflammatory activity. Further studies are needed to clarify the mechanisms of bone mass increase in children and adolescents treated with bisphosphonates. This class of drugs has been increasingly used in pediatric patients, and data on their mechanism of action in a growing skeleton will be useful. The longterm safety of these agents remains to be evaluated and, in light of their very long persistence in bone, there are concerns about their use, especially in young females who will eventually deliver children.

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