Bone Mineral Density and Vitamin D Status in Patients with Autoimmune Polyglandular Syndromes: A Single Tertiary Care Center Experience

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Introduction
Autoimmune polyglandular syndromes (APS) are defined by the coexistence of at least two autoimmune-mediated endocrinopathies [1]. Specific clustering of monoglandular autoimmune diseases depends on genetic and non-genetic environmental factors. It differs considerably at the time of presentation, distinguishing between two major subtypes of APS. The juvenile APS-1 shows a monogenetic inheritance. In contrast, multiple genes contribute to the etiopathogenesis of the adult APS [1]. Based on the various combinations of autoimmune endocrinopathies, the adult APS is subdivided into types 2-4 [2]. Patients with APS are at high risk for developing non-glandular autoimmune diseases [3]. APS-2 is a rare condition defined by the presence of Addison’s disease (AD) along with autoimmune thyroid disease (ATD) either by hypothyroidism [Hashimoto's thyroiditis, (HT)], or hyperthyroidism [Graves' disease, (GD)] and/or type 1 diabetes (T1D). With a relative prevalence of approximately 40%, APS type 3 is the most frequent subtype, encompassing T1D and an AD [2].

Several epidemiological studies and meta-analyses demonstrated a significant association between impaired serum 25-hydroxyvitamin D (25-OHD) levels and increased incidence of several autoimmune diseases [4, 5]. Among the endocrine disorders, low 25-OHD levels have been described in ATD [6], T1D [7], and AD [8]. In particular, a polymorphism of the gene coding for 1-alphahydroxylase (CYP27B1), the enzyme that catalyzes the conversion of 25-OHD to 1,25(OH)2D3, is associated with AD, ATD, and T1D [9].

ABSTRACT
Immunological abnormalities, the resulting endocrinopathies, and their treatments may impact bone health and 25-hydroxyvitamin D (25-OHD) in patients with autoimmune polyglandular syndromes (APS). Several etiologies contribute to increased risk for low bone mineral density (BMD), including vitamin D deficiency. This study evaluated the vitamin D level and BMD of patients with APS. We performed a cross-sectional study on 44 patients with APS and 55 age and gender-matched control subjects. Among patients with APS, 14 were classified as APS-2 [Addison's disease (AD) + autoimmune thyroid disease (ATD) and/or type 1 diabetes (T1D)]. In contrast, the other 30 were APS-3 (ATD + T1D + other autoimmune diseases). Serum samples were analyzed for vitamin D levels. The lumbar spine and femoral neck BMD were measured by dual X-ray absorptiometry. Z-scores were obtained by comparison with age- and gender-matched average values (both patients and controls). The accepted normal levels were Z-score > –1 and 25-OHD > 30 ng/ml. Patients with APS showed 25-OHD levels and BMD significantly lower than healthy controls (p < 0.001 and p < 0.05, respectively). The highest prevalence of abnormal BMD was observed in the APS-2 subgroup (13 out of 14 patients, 92.6%). Identifying and treating vitamin D deficiency and low BMD is critical in APS patients. The fact that the significant endocrine component of APS-2 is AD, and these patients receive chronic long-term glucocorticoid therapy can be shown as the reason for this result. However, more extensive prospective controlled studies are needed to confirm these findings.
Furthermore, Bellastella et al. highlighted that patients with APS present reduced vitamin D circulating levels, but the vitamin D status is not different between patients with single or multiple autoimmune diseases [4]. The resulting endocrinopathies and their treatment may impact bone health. Vitamin D deficiency and secondary hyperparathyroidism are generally associated with decreased bone mineral density (BMD) and lead to increased bone turnover, which is catabolic for both cortical and trabecular bone.

Osteoporosis and decreased BMD are frequently observed in several autoimmune endocrine disorders, including T1D, AD, growth hormone deficiency, and premature ovarian failure (POF), all diseases with increased risk of hip and vertebral fractures and associated morbidity, mortality, and health care costs. Many reports suggest altered bone and mineral metabolism in T1D and AD, which are the components of APS [10, 11]. The relationship between T1D and decreased BMD is well known [12]. The onset of T1D typically occurs at a young age when bone mass is still increasing. People with T1D may achieve lower peak bone mass (PBM), the maximum strength and density that bones reach. People usually reach their peak bone mass in their 20s. Low PBM can increase one’s risk of developing osteoporosis later in life. AD requires lifelong glucocorticoid replacement therapy, which, in excessive doses, may result in impaired bone quality and reduced bone mass [11]. Although thyroid dysfunction has been known to represent a risk factor for bone disease, the role of thyroid hormone excess or deficiency in the pathogenesis of osteoporosis and risk factors of fractures has been underestimated, and the underlying mechanisms are still uncertain [13].

However, no study evaluated whether the association of APS further affects BMD. Therefore, our study aimed to examine the possible adverse effects on vitamin D levels and BMD in patients with APS and investigate its relation with age, body mass index (BMI), and disease duration.

Materials and Methods

Study design and participants

In a cross-sectional study, we evaluated patients with APS (n = 44) who were 18–45 years of age and receiving outpatient care at Uludag University Medical School Endocrinology and Metabolism Clinic in Bursa. Age-, gender- and ethnicity-matched healthy controls (n = 55). They were volunteer blood donors without personal or a family history of autoimmune diseases and were randomly recruited from staff personnel or medical students from the University Hospital. The respective type of APS was included according to the accepted clinical, endocrinological, and immunological criteria. APS-2 is characterized by the presence of AD along with ATD and/or T1D [2]. In APS-3, ATD can co-occur with any autoimmune disorder but not AD [1]. APS-2 was found in 31.8% (n = 14) and APS-3 in 68.1% (n = 30). In APS-2, AD coexisted with ATD, T1D, or the combination of these conditions in 64% (n = 9), 18% (n = 3), and 13% (n = 2) of cases, respectively. In addition, three non-endocrine organ-specific autoimmune disorders (vitiligo, autoimmune hepatitis, and myasthenia gravis) were detected. In APS-3, only one autoimmune endocrinopathy (T1D), five non-endocrine organ-specific (vitiligo, autoimmune hepatitis, pernicious anemia, myasthenia gravis, and alopecia) autoimmune endocrinopathies were detected in combination with ATD. All patients with APS are treated according to standard guidelines. Ages at the diagnosis of disease manifestations were collected from hospital records.

Exclusion criteria for both groups were represented by calcium and vitamin D supplementation, bone active therapy (antiresorptive/bone-forming therapy), liver disease, moderate and severe chronic kidney disease, history of parathyroid or rheumatological disease, inflammatory bowel disease, gastric surgery, isolated malabsorption syndrome, congenital adrenal hyperplasia, surgical adrenalectomy, severe obesity (BMI > 35 kg/m²) or underweight (BMI ≤ 19 kg/m²). Postmenopausal women, smokers, and alcohol users were also excluded from the study.

The study was approved by the Uludag University Clinical Research ethical committee (decision no: 2013–1/21, dated January 15, 2013) and was conducted according to the principles of the Declaration of Helsinki. All participants provided written consent.

Data collection

Anthropometric data, including their height (cm), body weight (kg), and BMI, were recorded. The BMI was calculated as the ratio of weight and the square of the height.

Blood serum samples were collected following 10–12 hours of fasting and obtained to measure serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), 25-OHD, and parathormone (PTH). The laboratory parameters (Ca, P, ALP) were analyzed photometrically in a Roche c-702 autoanalyzer (Roche Diagnostics, Ankara, Turkey). Serum concentrations of PTH and 25-OHD were measured by electroluminescence with a Roche Cobas e-601 (Roche, USA). According to the Endocrine Society Clinical Guidelines, vitamin D level was classified as normal (≥ 30 ng/ml), insufficient (> 20 to < 30 ng/ml), or deficient (< 20 ng/ml) [14]. To avoid the interference of season variations of values, we measured 25-OHD levels on plasma samples of patients and controls drawn from June through early September, considering that these months usually express the peak of secretion of vitamin D.

BMD values in the lumbar spine (L1–L4), femur total, and femur neck regions were measured by the dual X-ray absorptiometry (DXA) method using a Hologic Delthi-w serial no: 70232 bone densitometer. BMD results were evaluated according to the Z-score criteria the World Health Organization (WHO) recommended. The Z-score is the number of standard deviations (SDs) a given BMD measurement differs from the mean for age- and gender-matched reference populations. A Z-score of ≤ –2.0 was defined as low BMD or “below the expected range for age”; a Z-score between −1.0 and −2.0 was defined as the low range of normality [15]. An abnormal BMD was determined as low or normal BMD (Z-score < –1.0) [16].

Statistical analysis

All data obtained in the study were recorded in the SPSS 21.0 database (SPSS, Chicago, IL, USA). Statistical analyses were applied. The analysis used mean ± standard deviation, median, minimum, and maximum values for continuous variables and frequency and percentage values for categorical variables as descriptive statistics. The chi-square test of independence or Fisher’s exact was used to test categorical variables between groups. The Shapiro–Wilk test was used to examine whether the data showed normal distribution.

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Results

For the study, 44 patients with APS and compatible criteria defined for the study and 55 individuals for the healthy control group were included. Of the total subjects, 83 (83.8%) were females, and 16 (16.2%) were males, with a mean age of 36.4 ± 12.0 years. The demographic and laboratory findings of the study and control groups are presented in Table 1. Demographic variables such as age and gender were similar in both groups (p > 0.05). Only the BMI in the APS patients was lower than in the control subjects (p = 0.01).

The differences between APS and controls regarding several laboratory findings, such as Ca, P, and ALP, were insignificant (p > 0.05). In contrast, the differences between the two groups regarding the PTH and 25-OH vitamin D values were significant (p < 0.05) (Table 1). Compared with controls, APS patients showed significantly lower 25-OH vitamin D levels (25.4 ± 7.1, 17.2 ± 7.2 ng/ml, respectively; p < 0.001). Moreover, there was no significant difference between APS subpopulations (16.4 ± 2.80 in APS-2 and 18.7 ± 8.9 ng/ml in APS-3; p = 0.62). Among the 55 controls, 9 showed vitamin D deficiency (16.3%), and 33 showed vitamin D insufficiency (60%). The prevalence of vitamin D deficiency was significantly higher in patients with APS (52.2%) than in controls (p < 0.001) (▶ table 1). There was no significant correlation between serum Ca and vitamin D levels (p > 0.05). In contrast, a statistically significant negative correlation existed between serum vitamin D levels and PTH values (r = −0.23, p = 0.01).

The BMD of the patients was evaluated in terms of osteoporosis risk. The BMD measurement results are summarized in ▶ Table 2. Lumbar BMD (LBMD), femur neck BMD (FnBMD), femur total BMD (FTBMD), and Z-scores in patients with APS patients were significantly lower than those of controls.

The prevalence rates of low BMD, defined by the lowest Z-score ≤ −2.0 in one or more sites (lumbar spine, total femur, and femoral neck), were significantly lower in the APS patients compared with the control group (25% vs. 3.6%; p = 0.04). In total, 24 (54.5%) APS patients and 4 (7.2%) controls had abnormal BMD (Z-score ≤ −1.0). Also, these were found in patients with APS type 2 and type 3 at 13/14 (92.6%) and (11/30) 36.6%, respectively. The low BMD rate in type 2 and type 3 APS and the control group was (8/14) 57.1, (3/30) 10, and (2/55) 3.6%, respectively. Among the 24 (54.5%) APS patients with abnormal BMD Z-scores, eight patients (33.3%) had deficient, and 5 (20.8%) patients had insufficient vitamin D values. Serum vitamin D levels and abnormal BMD values were not found to be related.

Associations of age, disease duration, BMI, PTH, and 25-OH vitamin D levels were analyzed with the subjects’ lumbar total, femur neck, and femur total BMD (▶ Table 3). This study did not find any significant correlation between the 25-OH vitamin D, PTH values, and the BMD values of the lumbar total, femur total, and femur neck (p > 0.05) (▶ Table 3). It was determined that there was a significant positive correlation between the age and BMI values and the patient’s lumbar total BMD values and that the duration of the disease was negatively correlated with the lumbar total, femur neck, and femur total BMD values (p < 0.05) (▶ Table 3).
Discussion

The first aim of our study was to evaluate whether there was a change in BMD and vitamin D levels in patients with adult APS. To the best of our knowledge, this is the first study examining BMD exclusively in these patients. As a second aim, we also investigated the correlations between age, BMI, disease duration, PTH, 25-OHD, and BMD in patients with adult APS.

We found only one study in the literature that evaluated vitamin D status in patients with adult APS [4]. This study demonstrated a high prevalence of vitamin D deficiency in adult APS compared to the control subjects; among the APS subgroups, all patients with vitamin D deficiency consisted of APS-3. This finding suggested that it is likely linked to an impairment of this vitamin’s absorption or metabolic steps at the skin, liver, or kidney level [4].

Our study found that circulating 25-OHD levels were significantly reduced in both groups of APS patients compared to control subjects, but there was no significant difference in 25-OHD levels between APS subgroups. However, it was observed that the prevalence of vitamin D deficiency was higher in those with APS-2. This can be explained by the fact that AD only exists in patients with APS-2 who use long-term high-dose glucocorticoids. Only a few observational studies investigated the link between vitamin D plasma levels and AD [17]. A record-linkage study by Ramagopalan et al. showed that a large cohort of patients admitted to a UK hospital for vitamin D deficiency significantly elevated rates of AD (rate ratio = 7.0 % CI: 3.6–12.3) and other autoimmune diseases [8]. Another recent study by Zawadzka et al. retrospectively analyzed medical records of 31 adult patients diagnosed with AD, in whom

<table>
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<th>Table 2</th>
<th>Absolute BMD levels and Z-scores of lumbar and femur areas of the study groups.</th>
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<tr>
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<td>APS-2</td>
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<td></td>
<td>(n=14)</td>
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<tr>
<td>BMD (g/cm²)</td>
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<tr>
<td>Lumbar L1–L4</td>
<td>1.01 ± 0.55</td>
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<tr>
<td>P-value</td>
<td>0.29</td>
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<tr>
<td>Femur neck</td>
<td>0.57 ± 0.01</td>
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<tr>
<td>P-value</td>
<td>0.11</td>
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<tr>
<td>Femur total</td>
<td>0.91 ± 0.04</td>
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<tr>
<td>P-value</td>
<td>0.31</td>
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| Z-score   |        |        |           |          |
| Lumbar L1–L4 | −0.54 ± 1.27 | −0.59 ± 1.78 | −0.58 ± 1.42 | −0.03 ± 1.13 |
| P-value     | 0.81   | 0.04   | 0.60  | <0.001|
| Femur neck  | −1.56 ± 1.18 | −1.13 ± 1.09 | −1.23 ± 1.20 | 0.11 ± 0.90 |
| P-value     | 0.60   | <0.001 | 0.77  | <0.001|
| Femur total | −1.33 ± 1.41 | −1.15 ± 1.87 | −1.21 ± 1.12 | 0.23 ± 0.81 |
| P-value     | 0.04   | 0.03   | 0.77  | 0.03  |

Low BMD, n (%) | 8 (57.1) | 3 (10) | 11 (25) | 2 (3.6) |

Data are expressed as mean ± SD; BMD: Bone mineral density. *Low BMD was defined as a Z-score of ≤ –2 in at least one of three sites. p<0.05: Comparisons between patients with APS-2 and patients with APS-3. p<0.01: Comparisons between controls and patients with adult APS.

<table>
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<th>Table 3</th>
<th>The correlation between demographic and some laboratory values with bone mineral density values.</th>
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<tr>
<td></td>
<td>Lumbar L1–L4 (g/cm²)</td>
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<td></td>
<td>p</td>
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<tr>
<td>Adult APS (n=44)</td>
<td></td>
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<tr>
<td>Age</td>
<td>0.037</td>
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<tr>
<td>BMI</td>
<td>0.047</td>
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<tr>
<td>Disease duration</td>
<td>0.042</td>
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<tr>
<td>PTH</td>
<td>0.228</td>
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<tr>
<td>25-OH D</td>
<td>0.322</td>
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| Controls (n=55) |        |        |        |        |        |        |
| Age       | 0.541 | −0.08  | 0.496 | −0.264 | 0.844 | 0.023 |
| BMI       | 0.181 | 0.243  | 0.258 | 0.369  | 0.142 | 0.19  |
| PTH       | 0.234 | −0.283 | 0.062 | −0.268 | 0.235 | −0.39 |
| 25-OH D   | 0.895 | −0.001 | 0.622 | −0.058 | 0.836 | −0.006 |

serum vitamin D was measured. A total of 90.3 % of AD patients had inadequate vitamin D concentrations (< 30 ng/ml), and 19.3 % of them were found to be severely vitamin D deficient (< 10 ng/ml). They also found only serum calcium concentrations significantly correlated with VD status (r = 0.53, p = 0.006) [17]. This and similar studies have shown that vitamin D could play a protective role in the pathogenesis of AD [5]. Also, some studies have shown that glucocorticoid replacement therapy may frequently be administered in doses more than necessary in patients with AD [18]. Glucocorticoids decrease the production of active vitamin D by inhibiting vitamin D hydroxylation in the liver and Ca absorption in intestinal mucosal cells when glucocorticoids are administered more than necessary. In addition, glucocorticoids have been known to increase Ca and P excretion by inhibiting renal tubular reabsorption. PTH secretion rises to compensate for the decreased intestinal absorption and renal Ca excretion. The developed secondary hyperparathyroidism condition provides serum Ca to be in balance [19]. In our study, depending on this condition, the patient’s serum Ca level is in the normal range. PTH levels are significantly higher in patients with all types of APS than in healthy controls.

In this study, we also evaluated the BMD of the patients in terms of the risk of osteoporosis that may develop due to secondary hyperparathyroidism arising from vitamin D and Ca absorption problems. Regarding BMD, patients were compared with their peers using Z scores. It was observed that the BMD of those with APS was significantly lower statistically than the healthy control group. BMD reduction was detected in both the femur and lumbar vertebrae measurements. Among the APS subgroups, the low BMD rate was approximately six times higher in APS-2 than in APS-3 (57.1 % vs. 10 %).

In the literature, there is no data about BMD in APS. It has been widely analyzed with monoglandular autoimmune endocrine diseases, but most of these studies were performed on patients with T1D, AD, and ATD [11, 13].

We found a low BMD rate in the patients with APS-2, in line with previous studies on patients with AD [19–22]. This can be explained by the fact that AD is only present in patients with APS-2. The most extensive cross-sectional study comprising 292 Addison patients from Norway, Britain, and New Zealand demonstrated a reduced BMD at the femoral neck and lumbar spine [22]. The decreased BMD in patients with AD is believed to be reduced because glucocorticoid therapy used for replacement purposes is generally given more than physiological needs. Steroids directly inhibit bone formation. Since they shorten the active formation period in the bone remodeling cycle, they also reduce the number and activity of osteoblasts. Thus, bone matrix formation is reduced. The serum osteocalcin and bone ALP levels decrease within the first 24 hours, depending on the dose. Systemic steroids also accelerate bone resorption by reducing androgen and estrogen and increasing PTH secretion. As a result, the bone resorbs. These effects are dose and duration-dependent, and the effect increases in high doses and long-term use [20–22]. Although it has been suggested in some studies that the osteopenia detected in Addison’s patients is not related to the dose and duration of glucocorticoid replacement therapy, many studies are showing that BMD in Addison’s patients who use very low doses close to physiological needs are not different from healthy controls [23, 24]. A recent prospective two-year study on adult patients with AD demonstrated a significant increase in femoral neck and lumbar spine BMD Z-scores in the patients with a cautious reduction in hydrocortisone equivalent dose [25]. In our study, the leading cause of the low BMD rate of patients with APS-2 could be glucocorticoid therapy, likely to be long-term and supra-physiological doses. In addition, the sudden decrease in BMD might be related to the lack of protective role played by adrenal gland androgens in the body.

Most APS patients with low BMD measurements may have secondary multifactorial causes such as malabsorption disorders, low BMI, smoking, and alterations in vitamin D metabolism or drug exposure that inhibits PBM gain during adolescence [26]. Considering these factors, patients with these characteristics were excluded from the study. One of the other primary etiology that comes to mind in the case of low BMD in patients with APS is celiac disease (CD), which can be seen with autoimmune comorbidities and malabsorption evolving because of it [27, 28]. It has been known for a long time that osteoporosis and bone deformities secondary to osteoporosis are more common in celiac patients. The reported prevalence of osteopenia or osteoporosis in CD is variable, ranging from 38–72 % of newly diagnosed patients [29]. However, in our study, no patient had CD and follow-up malabsorption findings.

Our results showed that age, BMI, and disease duration are important factors affecting BMD, and this has been widely recognized. However, we have demonstrated no correlation between BMD values and PTH and 25-OH vitamin D levels.

This study found a positive correlation between age and lumbar total BMD values. As known in the literature, advancing age is a risk factor for low BMD. It is important to note that bone mass is accrued throughout adolescence and early adulthood, and also stated that BMD could reach peak bone mass until the age of 35 at the latest [30]. In our study, an average age of 35 might correspond to PBM formation. Therefore, our positive correlation might be related to PBM.

One of the strongest predictors of BMD is body weight. Many studies have shown that obesity is important in maintaining BMD and quality [31]. Body weight depends on fat and lean mass, and a positive correlation was found between BMD and fat mass [31]. Our result in controls concord with this finding, indicating that controls with significantly higher BMI values tended to have higher BMD at all sites of the body than patients.

Some reports also indicate that disease duration is negatively associated with BMD at all sites in patients with AD treated with glucocorticoid, and bone loss occurs mainly in patients with longer disease duration [20, 21]. Therefore, we investigated the relationships between disease duration and BMD at all sites. In our study, in accordance with the literature, we found that the disease duration was negatively correlated with the femur total BMD; it was determined that with increased duration of the disease, a significant reduction of femur total BMD values might have occurred.

Limitations

The first limitation of our study is the cross-sectional design, which restricts the assessment of causal relationships. Although there was no significant difference between the groups concerning gender, the lack of subgroup analyses regarding gender is another limitation because we could not administer a subgroup analysis since...
the number of male patients was insufficient for statistical analysis. Second, we have excluded the patients with malabsorption as the study needs to include exact data regarding dietary habits or daily calcium intake. Finally, there needs to be more data on drugs used by patients in our study (as glucocorticoid replacement regimens have different effects on BMD). Despite these limitations, our study has contributed to the knowledge of vitamin D status and bone diseases in adult APS patients and the results of BMD as real-life data.

Conclusion

In this study, vitamin D and BMD values (lumbar total, femur neck, and femur total) are significantly lower in patients with all types of APS compared to healthy gender- and age-matched controls. These data suggest that osteoporosis screening and prevention among patients with APS need to be prioritized. It is also important for all of these patients to receive treatment to increase bone mass and reduce the risk of fractures. In addition, the effects of altered osteoimmunology on the function of bone cells and reduction of BMD need to be elucidated in future prospective studies on patients with APS.

Conflict of Interest

The authors declare that they have no conflict of interest.

References