Vitamin D has multiple immunosuppressant properties. In vitro, 1,25-dihydroxyvitamin D \([1,25(\text{OH})_2\text{D}]\) inhibits the activation of monocytes to dendritic cells, decreasing interleukin 12 (IL-12) production; it inhibits B cell proliferation, decreasing production of antibodies; and it also inhibits T cell proliferation and activation. Many studies have observed that decreasing production of antibodies; and it also inhibits T cell leukemia-12 (IL-12) production; it inhibits B cell proliferation, activation of monocytes to dendritic cells, decreasing interleukin-2, interferon-γ, IL-1, IL-6, and tumor necrosis factor-α synthesis and promoting Th2 dominance, increasing IL-4, IL-5, and IL-10 synthesis. Considerably less is known about serum levels of 25-hydroxyvitamin D in patients with autoimmune diseases in humans, including IBD, type 1 diabetes, multiple sclerosis, RA, and SLE. Most studies in humans have documented lower serum levels of 25-hydroxyvitamin D in patients with autoimmune diseases. Serum 25(OH)D levels in adults with SLE are significantly lower than in controls.

Vitamin D deficiency has been linked to several autoimmune diseases in humans, including IBD, type 1 diabetes, multiple sclerosis, RA, and SLE. As defined as serum 25(OH)D < 20 ng/ml, as well as factors associated with serum 25(OH)D levels, children and adolescents attending a pediatric rheumatology clinic. A second aim was to ascertain whether there was a difference in serum levels of 25(OH)D and vitamin D deficiency rates among children with autoimmune disorders as compared to those with nonautoimmune conditions.

**MATERIALS AND METHODS**

We performed a cross-sectional analysis of serum 25(OH)D levels of patients attending the pediatric rheumatology clinic in the Floating Hospital for Children at Tufts Medical Center between November 2008 and October 2009. All patients who had routine phlebotomy performed were included. Our study was approved by the Institutional Review Board of Tufts Medical Center.
circulating 25(OH)D$_3$ and 25(OH)D$_2$. The laboratory participates in the College of American Pathologists proficiency testing and the Vitamin D External Quality Assessment Scheme (www.deqas.org; Charing Cross Hospital, London, UK), an international consortium of laboratories set up to ensure the analytical reliability of 25(OH)D. The laboratory also validated the assay using the Standard Reference Material 972 (National Institute of Standards and Technology, Gaithersburg, MD, USA). Interassay coefficients of variation are 6.5%–11% for 25(OH)D$_2$ over the range of 5–80 ng/ml and 9%–13% for 25(OH)D$_3$ over the range of 2.5–60 ng/ml. Patients were classified into 2 mutually exclusive categories according to their vitamin D status: deficient [serum 25(OH)D < 20 ng/ml] or nondeficient [serum 25(OH)D level ≥ 20 ng/ml].

Information concerning patients’ demographics (sex, age, and ethnicity), body mass index (BMI), diagnosis, disease activity, use of medications and supplements, and season of serum 25(OH)D measurement were identified based on the review of medical records.

Patients were classified into 2 distinct groups based on their clinical diagnoses. The first group included patients with definite autoimmune disorders, including juvenile idiopathic arthritis (JIA), juvenile SLE, juvenile dermatomyositis, scleroderma mixed connective tissue disease (MCTD), and vasculitis, while the second included patients who had a nonautoimmune condition (e.g., noninflammatory disorders, infectious diseases, pain amplification syndrome).

Disease activity was evaluated only for patients with autoimmune disorders. This was defined as active or inactive based on the attending physician’s assessment at the time of the index clinic visit.

BMI was divided into 2 categories: obese and nonobese. Children were classified as obese if their BMI was ≥ the 95th percentile, and nonobese if BMI was below the 95th percentile.

Statistical analyses. Descriptive statistics were used to analyze the data. We determined the prevalence of vitamin D deficiency in the study sample. Univariate analyses were conducted using ANOVA and t-tests to determine the association of categorical and binary variables with serum 25(OH)D levels. A multivariate linear regression was used to analyze variables identified as a priori associated with serum 25(OH)D levels, including age, supplement use, autoimmune disorder, BMI, ethnicity, and season. A multivariate logistic regression was used to determine the OR of vitamin D deficiency in patients with autoimmune disorders, after adjusting for confounders (supplement use, BMI, ethnicity, and season), identified a priori. JMP version 8 (SAS Institute Inc., Cary, NC, USA) was used for data analyses. All tests were 2-sided, and statistical significance was established with an α < 0.05.

RESULTS
Sample. A total of 254 patients had serum 25(OH)D measurements performed during the study period; of these, 169 patients (67%) had autoimmune disorders and 85 had nonautoimmune conditions. The average age of all children was 12.3 (± 4.5) years, 67% were female, and 80% were white. There were no differences in various demographic characteristics according to disease group (Table 1).

Vitamin D deficiency. Mean serum level of 25(OH)D in the complete sample was 28.6 (± 11) ng/ml. The range was 2 to 59 ng/ml. Average serum levels of 25(OH)D in patients with autoimmune disorders were 28.1 (± 11.4) ng/ml, compared to 29.7 (± 10.3) ng/ml in patients with nonautoimmune disorders (p = 0.25).

In patients with autoimmune disorders, 23% had vitamin D deficiency, while in patients with nonautoimmune conditions this rate was 14%. The OR of patients with autoimmune disorders being vitamin D deficient, after adjusting for supplement use, BMI, ethnicity, and season, was 2.3, in relation to patients with nonautoimmune disorders (p = 0.04; Table 2).

Variables associated with serum 25(OH)D levels. Age, ethnicity, BMI, season, and use of supplements containing vitamin D were significantly associated with serum 25(OH)D levels, as expected (Table 3).

Serum 25(OH)D and medications. Among patients with autoimmune disorders, 60 (36%) were not taking any medications. Of the remainder, medication use included nonsteroidal antiinflammatory drugs (NSAID; 39, 23%), prednisone (24, 14%), biologic drugs (adalimumab, etanercept, infliximab, anakinra, and rituximab; 24, 14%), disease-modifying antirheumatic drugs (DMARD; methotrexate, cyclosporine, cyclophosphamide, azathioprine, and hydroxychloroquine; 20, 12%), and intravenous immunoglobulin infusions (2, 1%).

Mean serum 25(OH)D levels by each medication used were 29 ng/ml for NSAID, 22 ng/ml for prednisone, 28 ng/ml for biologic drugs, 29 ng/ml for DMARD, 32 ng/ml for intravenous immunoglobulin, and 30 ng/ml for patients taking no medications. Mean serum 25(OH)D levels did not differ in relation to the use of particular medications (p = 0.12).

No patients with nonautoimmune conditions were taking long-term medications (> 1 month), other than vitamins and oral contraceptives.

Serum 25(OH)D and rheumatologic disorders. Mean serum 25(OH)D levels were 29 ng/ml in JIA (n = 124), 21 ng/ml in SLE (n = 18), 29 ng/ml in vasculitis (n = 9), 32 ng/ml in dermatomyositis (n = 8), 21 ng/ml in MCTD (n = 3), 26 ng/ml in scleroderma (n = 3), and 32 ng/ml in Sjögren’s syndrome (n = 2). Serum 25(OH)D levels did not vary by diagnosis in patients with autoimmune disorders (p = 0.05).

There were 117 patients (69%) with autoimmune disorders who had active disease. Mean serum 25(OH)D levels did not differ in patients with active (27.9 ± 11.7 ng/ml) or inactive (28.5 ± 10.8 ng/ml) disease (p = 0.78).

In patients with nonautoimmune conditions, mean serum 25(OH)D levels did not differ among diagnoses (p = 0.96).

In the multivariate linear regression, autoimmune disorder was not associated with serum 25(OH)D levels, after adjustment for age, supplement use, BMI, ethnicity, and season (p = 0.06; Table 3).

DISCUSSION
In this sample of children and adolescents with autoimmune and nonautoimmune conditions, we found a high prevalence of vitamin D deficiency. Serum levels of 25(OH)D were associated with age, ethnicity, BMI, season, and use of supplements. After adjustment for confounding variables, patients with autoimmune disorders were 2.3 times more likely to be vitamin D deficient than patients with nonautoimmune conditions; however, there was no difference in mean serum 25(OH)D levels between patients with autoimmune and nonautoimmune disorders.
Our findings are in part consistent with a large number of cross-sectional studies on vitamin D status and autoimmune rheumatologic diseases in adults, which show lower serum levels of 25(OH)D, or higher rates of vitamin D deficiency, in patients with autoimmune disorders. Interventional studies also show an interesting effect of vitamin D in autoimmunity. For example, high-dose oral α-calcidiol therapy reduced disease activity in 89% of 19 patients with RA. In another uncontrolled study of 11 adults with systemic sclerosis, 1,25(OH)₂D₃ administered for a period of 6 months to 3 years was associated with significant improvement in clinical measures. There are few studies in children comparing serum levels of 25(OH)D in patients with autoimmune disorders and nonautoimmune disorders, or healthy controls. One study assessed serum 25(OH)D levels in children with rheumatologic disorders and did not find decreased levels in patients with active or inactive disease. However, in that study serum levels of 25(OH)D ≥ 15 ng/ml were considered normal and no healthy controls were used for comparison.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
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<td>Body mass index (nonobese)</td>
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<td>Supplementation (none)</td>
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<td>0.33</td>
<td>0.02</td>
<td>4.9</td>
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</table>

NS: nonsignificant; NA: not applicable.
levels have been compared between JIA and healthy controls in 3 different studies; however, results were inconsistent, since 2 of them, from the same group of authors, found a normal range of serum 25(OH)D that did not differ from controls38,39, in the other study, children with JIA had serum 25(OH)D levels significantly lower than those in controls40.

Although there was no statistically significant difference between serum 25(OH)D levels of patients with different autoimmune disorders, the differences found might be clinically meaningful. Patients with MCTD and SLE had lower serum 25(OH)D levels in comparison to other autoimmune disorders. Lower serum levels of 25(OH)D in adult patients with SLE have been reported2,11,12,15,16,25. These findings may therefore be recommended15,22.

Unlike other studies, we did not find an association between disease activity and serum levels of 25(OH)D in patients with autoimmune disorders. Others have found lower levels of serum 25(OH)D in adult patients with active SLE, in comparison to patients with inactive disease15,17,19,25, and negative correlations between serum 25(OH)D levels and disease activity in adults with RA25,33,34. However, other studies failed to show any correlation between disease activity and serum 25(OH)D levels in adult patients with SLE12,13,16. The lack of association between disease activity and serum 25(OH)D levels in our study might be explained by the tool used to measure disease activity, which was based only on the attending physician’s assessment, and no structured or composite instrument was used.

Although prednisone use is known to cause reduced serum levels of 25(OH)D22,40,42, we did not find a significant association between this use and serum 25(OH)D levels. Similar findings were reported by others in adults with SLE11 or Behçet’s disease43.

Our study had several limitations, including being retrospective, and a lack of information available about diet, sun exposure, and use of sunscreen [all of which influence serum 25(OH)D levels]. One possible explanation for why we did not find a significant difference in serum 25(OH)D levels between patients with autoimmune and nonautoimmune disorders was inadequate statistical power, due to our sample size. Another possibility is that serum 25(OH)D levels actually differ by autoimmune disorder, and this difference was missed when different diagnoses were combined. However, this is the first report comparing serum 25(OH)D levels between children with several autoimmune rheumatologic disorders and controls.

Twenty percent of children and adolescents attending a pediatric rheumatology clinic were vitamin D deficient. Serum 25(OH)D levels were associated with age, ethnicity, BMI, season, and use of supplements. Patients with autoimmune disorders were 2.3 times more likely to be vitamin D deficient than patients with nonautoimmune conditions. Hence, screening of serum 25(OH)D levels should be performed for patients with autoimmune disorders. Future studies are needed evaluating serum 25(OH)D levels and disease activity in childhood rheumatologic disorders.

**REFERENCES**


