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Bone involvement in clusters of autoimmune diseases: Just a complication?

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ABSTRACT

Bone loss, described in individual groups of patients with Type 1 diabetes (T1D), autoimmune thyroid disease (ATD) or celiac disease (CD) is usually viewed as a complication of these diseases. There is increasing evidence that alterations in the immune system may directly affect bone mass. Clustering of autoimmune diseases in the same individual might predispose to higher risk of osteopenia due to imbalance in immune regulation. The aim of this study was to evaluate bone involvement in clusters of the most common autoimmune diseases (T1D, ATD and CD) in children.

The study was performed at a tertiary care center for the care of pediatric diabetes. One-hundred-two patients with T1D alone or associated with ATD and/or CD were studied; 13 patients had cluster of three autoimmune diseases. Amplitude-dependent speed of sound (AD-SoS) was measured by phalangeal quantitative ultrasound and expressed as standard deviation score (SDS). AD-SoS SDS < -2 was considered indicative of osteopenia.

Osteopenia was equally distributed among children with T1D alone (8.1%), T1D associated with ATD (7.7%) or CD (10.3%), while it was 53.8% in patients presenting with three autoimmune diseases. Poor compliance to gluten-free diet increased osteopenia to 18.8% in patients with T1D and CD and 80% in patients with three autoimmune disorders. No difference among groups was found with regard to gluco-metabolic control, calcium metabolism, thyroid function.

In conclusion bone impairment in multiple autoimmune diseases might be considered not only a complication due to endocrine or nutritional mechanisms, but also a consequence of an immunoregulatory imbalance. Alterations of homeostatic mechanisms might explain an imbalance of osteoclast activity leading to osteopenia.

Introduction

Type 1 diabetes (T1D) may be frequently associated with other immune mediated diseases, such as autoimmune thyroid disease (ATD) or celiac disease (CD) [1]. Clustering of these diseases in the same individual is not unusual, suggesting that common immune mechanisms may facilitate immune mediated processes. A shared genetic background might explain this association [2,3]. Routine screening for ATD or CD, that often occur asymptomatic or oligosymptomatic, allows an early diagnosis and a possible prevention of their potential negative effects, by replacing normal thyroid function, if altered, or adopting a strict gluten-free regimen [4]. Impairment of bone mineral density has been a matter of investigation in patients suffering from T1D, ATD or CD [5,6]. The exact mechanisms accounting for bone loss in these diseases have been variably explained by metabolic derangements due to the impaired hormonal function in T1D [7] or ATD [8], or calcium malabsorption and secondary hyperparathyroidism in untreated CD patients [9]. Therefore osteopenia has been usually considered as a complication of these diseases. However, there is increasing evidence that alterations in the immune system may be responsible of mechanisms leading to osteoporosis [10,11]. By using phalangeal bone ultrasonography we recently demonstrated that the risk to develop osteopenia was not increased in patients with T1D and CD versus patients with only T1D, provided good compliance to gluten-free diet (GFD) [12].

The aim of the present investigation was to extend our observation on bone involvement to patients affected by three immune mediated diseases, T1D, ATD and CD, that represent the most common autoimmune diseases found in pediatric age. The additional role of compliance to GFD was also examined.

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Materials and methods

Patients

Data from 102 children, adolescents and young patients (49 boys, 53 girls) with type 1 diabetes, who had participated in previous research studies of bone health [12] were used for the present study. They were in regular follow up at the two regional centers for the care of Pediatric diabetes in Campania region (Departments of Pediatrics at "Federico II" University and "Second University" of Naples). The following inclusion criteria were considered: age <22 years, absence of microalbuminuria (albumin excretion rate <20 μg/min in two of three timed overnight urine collections or an albumin-to-creatinine ratio <2.5 mg/mmol), normal thyroid function when the study was performed, no previous intake of medications known to affect bone and mineral metabolism. Mean age was 12.8 ± 4.5 years; mean duration of diabetes was 5.9 ± 3.9 years. Forty-eight patients (47%) had completed puberty (Tanner stage V). Patients were treated with two to four daily injections of insulin.

Diagnosis of ATD and CD

In our population 26 patients (25.5%) had ATD; 52 (50.9%) had CD. In particular 13 patients had T1D associated with ATD, 39 had T1D associated with CD and 13 had T1D associated with both ATD and CD.

ATD was revealed by positivity of thyroglobulin (TG) and/or thyroperoxidase (TPO) antibodies, and confirmed by diffuse reduction in thyroid echogenicity on thyroid ultrasound. No patient exhibited hyperthyroidism, while patients with subclinical or clinical hypothyroidism were on L-thyroxin treatment; thyroid function was tested by serology every 6 months in order to keep TSH within the reference interval.

Eight patients received CD diagnosis before diabetes onset for the presence of classical symptoms, 25 were diagnosed by screening within 1 year from diabetes onset and 19 during the follow-up. Screening of CD was performed by measuring serum total IgA values and subsequently serum IgA endomysial antibodies (EmA) and anti-tissue transglutaminase antibodies (IgA-tTg) on a single random blood sample. Serum from patients with total IgA deficiency (<5 g/l) was alternatively analyzed for IgG anti-gliadin antibodies (AGA). All patients who were positive for antibodies underwent a diagnostic small bowel biopsy procedure and morphometric/immunohistochemical studies were carried out to confirm CD diagnosis, according to the revised European Society for Pediatric Gastroenterology and Nutritional criteria [13]. CD patients were advised to start GFD under the supervision of a trained pediatric dietician and were tested yearly by serology. Compliance to diet was assessed by a dietician-administered inquiry to assess gluten transgressions over the previous 3 months and search for IgA-tTg and/or -EmA antibodies. Patients were distinguished into compliant (no reported transgressions and persistent negativity for IgA-tTG Ab and/or -EmA) and not compliant (occasional or habitual transgressions and positivity for IgA-tTG Ab and/or –EmA).

Physical and biochemical measurements

Height and weight were measured and the Body Mass Index (BMI) was calculated [weight (kg)/height (m^2)]. Since height and BMI are variables age and gender related, they were also expressed as standard deviation score (SDS), based upon the established Center for Disease Control normative curves [14]. The total daily calcium intake was calculated using a food-frequency questionnaire, specifically compiled for a pediatric population [15]. Concentrations of serum total calcium, phosphorus and alkaline phosphatase were determined in the morning after 12-h fast, and were analyzed using a standard auto analyzer. TSH, free T_3 (FT_3) and free T_4 (FT_4), anti-thyroid peroxidase and anti-thyroglobulin antibodies were measured by immunoenzymatic method (normal values: TSH 0.3–4.2 mU/l, FT_3 3.0–6.7 pmol/l, FT_4 11.5–21.8 pmol/l). EmA were detected by means of indirect immunofluorescence assay while IgA-tTG and IgG- were measured by ELISA method. The mean of four HbA_{1c} determinations during the previous year (HbA_{1c}-last year) was considered as representative of long term metabolic control for each patient; HbA_{1c} measurements were done by the Ames DCA 2000 tm Analyzer (immunoassay) (normal values: 4.2–6.5%).

Assessment of phalangeal quantitative ultrasound parameters

Phalangeal quantitative ultrasound (DBM Sonic, IGEA, Carpi, Modena, Italy) is based on the transmission of ultrasound through the distal end of the proximal phalangeal diaphysis, in the proximity of the condyles of the last four fingers of the hand. The condyles at the distal diaphysis provide a convenient point for placing the probes, which is an essential feature for reproducibility of measurements. The distal end of the diaphysis of the proximal phalanges contains both cortical and trabecular bone, as well as a small medullary canal; the anatomic region is mostly cortical. This region does not have a growth plate, so that it is not a confounding factor in ultrasound measurement. Two transducers of twelve millimeter diameter on a high precision caliper (±0.02 mm), which measures the distance between the two probes, were positioned on the lateral and medial surface of each finger; the coupling of the probes with the skin was mediated by standard ultrasound gel. The amplitude-dependent speed of sound (AD-SoS, m/s) through the phalanx was measured in the last four fingers and calculated by measuring the width of the finger divided by the time of flight (defined as the time from emitted pulse to received signal, considering the signal which reaches a predetermined minimum amplitude value (2 mV) for the first time). The average over the four measurements was calculated and then expressed as SDS, on the basis of the Italian standards provided by the manufacturer [16]. AD-SoS SDS < −2 was considered indicative of osteopenia. All measurements were performed by the same skilled operator; the coefficient of variation was 0.8% as previously described [17].

Statistical analysis

The results were reported as mean ± SD, unless otherwise indicated. All data were normally distributed, apart from calcium intake. Univariate unadjusted analyses were performed with One-way ANOVA and Bonferroni correction, to compare variables normally distributed, and Kruskal–Wallis test for the variable not normally distributed. Fisher exact test or chi square test were used for categorical variables. A p-value less than 0.05 was considered significant. All statistical analyses were carried out using SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) for Windows software program release 15.0.

The study was approved by the local ethics committee; informed consent was obtained from all the patients and/or parents or the legal guardians.

Results

Patients were analyzed according to the presence of T1D alone ($n = 37$) or T1D associated with either ATD ($n = 13$) or CD ($n = 39$) or both ($n = 13$). A significant difference among these groups was found in the AD-SoS-SDS values (ANOVA $p = 0.005$) (Fig. 1, panel A) with the lowest values in patients with three autoimmune diseases. CD patients were further distinguished according to good (GFD^+) or poor (GFD^-) compliance to gluten-free diet and the following four

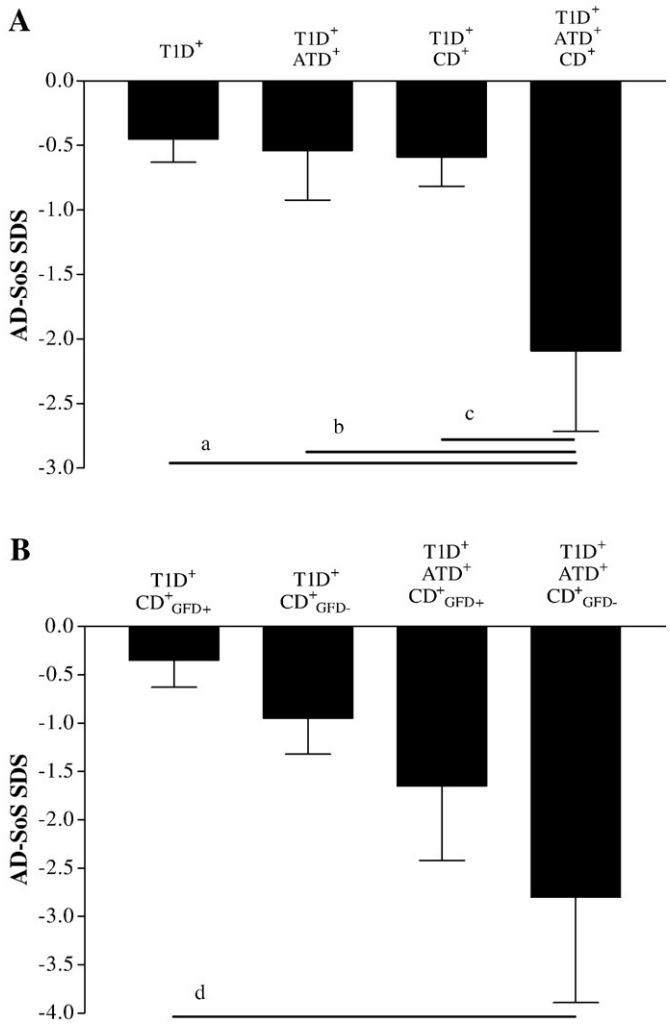

Fig. 1. Comparison of AD-SoS SDS values among T1D patients stratified according to the presence or less of ATD and/or CD (Panel A) and compliance to GFD (Panel B) (GFD^+: good compliance; GDF-: poor compliance). Data are expressed as mean ± SEM. $^aP<0.004$, $^bP<0.04$, $^cP<0.01$, $^dP<0.02$.

groups were identified: $T1D^+CD^+_{GFD+}$ ($n=23$), $T1D^+CD^+_{GFD-}$ ($n=16$), $T1D^+ATD^+CD^+_{GFD+}$ ($n=8$) and $T1D^+ATD^+CD^+_{GFD-}$ ($n=5$). A significant trend towards lower AD-SoS-SDS values was also found among these groups, with the lowest values in patients with three autoimmune diseases and poor compliance to GFD (ANOVA $p=0.02$) (Fig. 1, panel B).

Osteopenia (AD-SoS values < − 2 SD) was found in 15 out of 102 (14.7%) patients, with a slightly higher prevalence in girls (60%) than males (not significant). The frequency of osteopenia did not significantly differ among patients with diabetes alone (8.1%), diabetes associated with ATD (7.7%) or diabetes associated with CD (10.3%), while it was significantly higher (53.8%) in patients with three autoimmune diseases ($p=0.004$, Fig. 2, panel A). Poor compliance to GFD further increased the frequency of osteopenia to 18.8% in patients with T1D and CD and 80% in patients with T1D, ATD and CD ($p=0.001$, Fig. 2, panel B).

No differences among groups were found with regard to the following variables: gender distribution, age, diabetes duration, HbA_{1c}-last year, daily insulin dose, daily calcium intake, height-SDS, BMI-SDS (Table 1). Serum total calcium, phosphorus and alkaline phosphatase were also similar among groups. Thyroid function was normal in all patients.

Discussion

Metabolic or nutritional imbalance in endocrine or intestinal autoimmune diseases may negatively influence bone health. A new body of evidence suggests the possibility that bone can be also involved in autoimmune disorders [10,11]. Bone remodeling involves complex interactions between osteoclasts, the primary bone-resorption cells and other cells in their microenvironment (marrow stromal cells, osteoblasts, macrophages, T-lymphocytes and marrow cells) [18,19]. Besides their role in calcium mobilization from bone and initiation of bone remodeling, osteoclasts are now considered as the innate immune cells in the bone, since they are able to produce and respond to cytokines and chemokines [11,20,21]. Some of these cytokines can promote osteoclast formation and activity, namely the cytokine receptor activator of NFkB ligand (RANKL) and the macrophage colony stimulating factor (M-CSF) [22]. In vitro and in vivo models have demonstrated that also interleukin-1, M-CSF, tumor necrosis factor and interleukin-6 can stimulate the formation and bone-resorption capacity of osteoclasts. The relative proportion of these cytokines in the marrow microenvironment may play a critical role in regulating osteoclast activity.

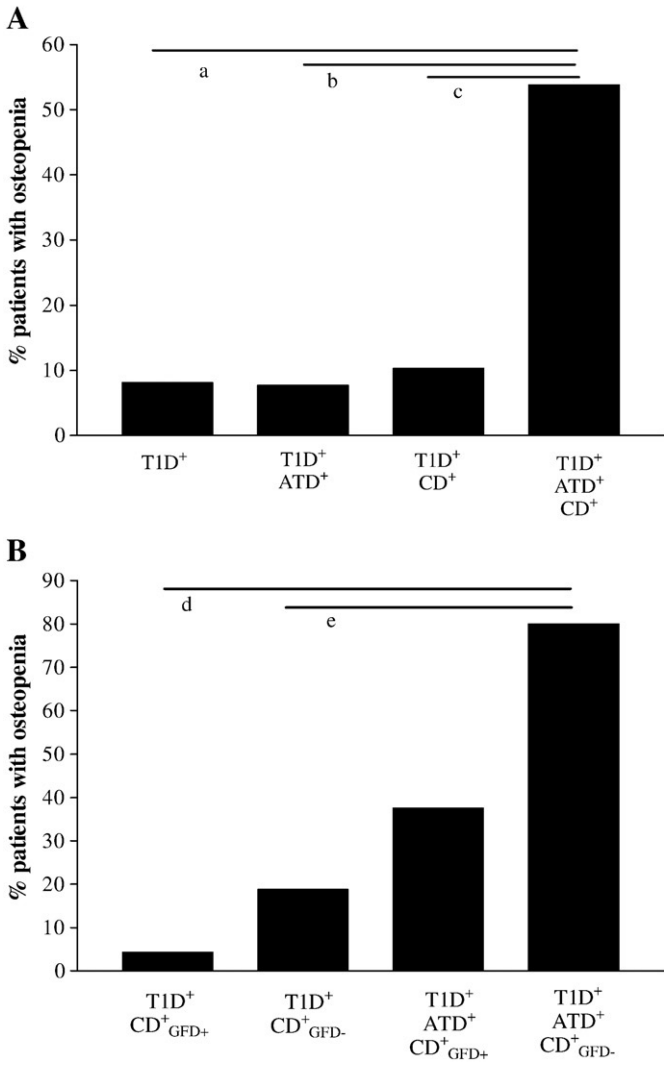

Fig. 2. Percentage of patients with osteopenia (AD-SoS-SDS below -2 SD) in T1D patients stratified according to the presence or less of ATD and/or CD (Panel A) and compliance to GFD (Panel B) (GFD^+: good compliance; GFD-: poor compliance). $^aP<0.001$, $^bP<0.03$, $^cP<0.002$, $^dP<0.01$, $^eP<0.025$.

Table 1
Clinical and laboratory data.

	$T1D^+$	$T1D^+ATD^+$	$T1D^+CD^+_{GFD+}$	$T1D^+CD^+_{GFD-}$	$T1D^+ATD^+CD^+_{GFD+}$	$T1D^+ATD^+CD^+_{GFD-}$
N	37	13	23	16	8	5
Male/female	23/14	6/7	10/13	6/10	1/7	3/2
Age (years)	11.6 ± 4.3	14.0 ± 2.4	13.0 ± 4.5	12.5 ± 5.8	15.6 ± 3.8	13.2 ± 6.3
Height (cm)	146.2 ± 21.3	159.9 ± 10.8	147.6 ± 18	143.2 ± 26	157.1 ± 10.2	150.7 ± 26.6
Height-SDS	0.33 ± 0.1	0.24 ± 0.7	−0.31 ± 0.8	−0.25 ± 0.7	−0.07 ± 0.9	0.58 ± 1.32
BMI (kg/m^2)	19.8 ± 3.8	21.9 ± 2.2	20.4 ± 2.6	20.6 ± 4.6	22.0 ± 2.8	20.8 ± 3.6
BMI-SDS	0.52 ± 0.8	0.74 ± 0.6	0.54 ± 0.9	0.71 ± 0.7	0.52 ± 0.9	0.6 ± 1.3
Time since DM diagnosis (years)	4.5 ± 3.3	6.3 ± 3.7	6.8 ± 4.5	6.9 ± 4.2	7.4 ± 2.7	6.0 ± 4.6
Time since CD diagnosis (years)	–	–	7.5 ± 5.2	5.5 ± 4.6	7.6 ± 2.2	5.2 ± 3.1
Insulin dose (U/kg/day)	0.9 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
HbA_{1c}-last year (%)	7.9 ± 1.3	8.2 ± 1.0	7.7 ± 0.9	8.1 ± 1.0	7.8 ± 0.9	7.8 ± 0.7
TSH (mU/l)	2.5 ± 0.8	2.4 ± 0.9	2.3 ± 0.9	2.3 ± 0.9	2.8 ± 1.4	2.4 ± 0.7
FT_4 (pmol/l)	12.9 ± 2.9	12.3 ± 11.7	13.1 ± 3.5	13.8 ± 11.8	12.2 ± 11.3	12.6 ± 14.4
FT_3 (pmol/l)	4.76 ± 1.0	5.08 ± 0.9	5.19 ± 1.2	5.03 ± 0.7	4.71 ± 1.0	5.27 ± 0.9
Calcium intake (mg/day)	1041 ± 527	910 ± 298	978 ± 330	905 ± 289	1267 ± 657	1111 ± 240

Several autoimmune disorders may be associated in the same individual, co-involving multiple organs and probably configuring distinct phenotypes. Bone could be an additional target of immune dysregulation. The immune mediators found to play a role in most autoimmune disorders (IL-1, TNF, IL-6), could be also involved in promoting bone loss. Imbalanced osteoclast activity relative to new bone formation might occur as a consequence of chronic inflammatory autoimmune diseases leading to osteopenia. Therefore we hypothesized that bone involvement could occur more frequently in individuals affected by several autoimmune diseases. To test this hypothesis we analyzed a population of patients with T1D, since this disease is frequently associated with other autoimmune diseases, such as ATD and/or CD and the simultaneous occurrence of these three diseases is not uncommon [23]. Our clinical observation indicates that while the presence of the second disease, either ATD or CD in patients with T1D did not increase the frequency of osteopenia, provided a good compliance to gluten-free diet in CD patients, clustering of three autoimmune diseases significantly increased the occurrence of osteopenia (37.5%). In these patients the impaired quality of the bone could be explained nor by thyreopathy or by the consequences of replacing therapy, since thyroid hormone levels and TSH were normal when the study was performed and kept in the normal range during the follow-up, nor by poor adherence to gluten-free diet since the specific antibodies were negative, nor by glycaemic control, since the HbA_{1c} values were similar to patients affected by diabetes alone or associated with the second autoimmune disease (ATD or CD). Furthermore, no difference in age or diabetes duration was found in patients suffering from three diseases with respect to the other groups. Therefore the observation that a longer duration of diabetes could have influenced the results was reasonably excluded.

Our findings prompted us to search in the literature a possible explanation of this phenomenon. The occurrence of poly-reactive autoimmune processes in the same individual is now emerging as a novel clinical entity within primary immunodeficiency. Along with environmental factors, genetic susceptibility represents a well established feature in the predisposition of individuals to certain autoimmune diseases. The co-occurrence of T1D and ATD in the same patient is considered one of the variants of the autoimmune polyglandular syndrome type 3, that, contrary to types 1 and 2, does not involve the adrenal cortex. Associations with non endocrine immune diseases (celiac disease, autoimmune gastritis or hepatitis) may also occur [24]. Cell-mediated immune processes play an important role in the immunopathogenesis of multiple autoimmune diseases. Multiple organs are involved when tolerance is lost to several self antigens and the onset of autoimmunity can be influenced by environmental factors leading to innate immune system activation. While epidemiological data point to a strong genetic influence on the shared susceptibility to T1D and ATD [25], T1DM and CD [26] or CD and ATD [27], very scarce data refer to the genetic susceptibility in patients with the three diseases. Among the proposed susceptibility genes, which may increase the risk for developing autoimmune disorders, Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is a strong candidate gene in patients with either isolated T1D, ATD or CD [28]. Interestingly, CTLA4 might represent the link between the immune system and bone that addresses the regulatory site of interaction between these two organ systems. In fact, it has been demonstrated in animal studies that CTLA4 expressed on T regulatory (Treg) cells impairs osteoclast formation by binding to osteoclast precursors and inhibiting their differentiation [29]. The strength of this suppressive effect on osteoclast formation is presumably accomplished by cytokines with anti-inflammatory properties (IL-4, IL-10, TGFβ). Therefore it can be hypothesized that failure of Treg cell function in clustering of multiple autoimmune diseases could represent a well-defined mechanism to explain both the occurrence of poly-reactive autoimmune processes and the increase of bone resorption in the same individual. Osteopenia in subjects with multiple autoimmune diseases would occur when the loss of anti-osteoclastogenic function of Treg cells adds to the the key role of inflammatory T cell subsets in stimulating osteoclast formation through RANKL expression.

As expected, poor compliance to GFD increased the occurrence of osteopenia, and the effect was stronger in patients with three autoimmune diseases (80%) than in those with two autoimmune diseases (18.8%). This result confirms our previous finding that osteopenia occurs more frequently in patients with diabetes and CD with poor compliance to gluten-free diet [12]. In our patient's population, time since CD diagnosis did not differ, as well. Recent observations indeed indicated an imbalance of cytokines relevant to bone metabolism in untreated celiac patients' sera and the direct effect of these sera on in vitro bone cell activity [30,31]. In particular the RANKL/osteoprotegerin (OPG) ratio was increased in patients not on gluten-free diet [32].

In conclusion, our clinical observation suggests that bone impairment in the context of multiple autoimmune diseases might be viewed as a consequence of an immunoregulatory imbalance. Assessment of quality of the bone through non-invasive methods, such as phalangeal ultrasonography, is advised in patients with clusters of autoimmune diseases to detect an involvement on skeletal, too. To our knowledge, this is the first study that explored bone involvement in clusters of autoimmune diseases. More studies in osteoimmunology, an interdisciplinary new research field combining the exciting fields of osteology and immunology, should be implemented in patients with multiple autoimmune diseases in order to understand the underlying link.

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