Winter lupus flares are associated with low vitamin D levels in a retrospective longitudinal study of Italian adult patients

F. Dall'Ara[1,2], L. Andreoli[1], N. Piva[1], S. Piantoni[1,2], F. Franceschini[1], A. Tincani[1]

[1]Rheumatology and Clinical Immunology, Department of Clinical and Experimental Sciences, Spedali Civili and University of Brescia, Brescia, Italy;
[2]Rheumatology Chair, University of Pavia, Pavia, Italy.

Abstract

Objective

Patients with systemic lupus erythematosus (SLE) are prone to hypo-vitaminosis D because of their photosensitivity. Vitamin D (vit.D) has beneficial effects not only on bone metabolism but also on the function of the immune system. The relationship between SLE disease activity and vit.D status is controversial and little is known on the effects of current supplementation strategies given for osteoporosis in raising vit.D levels.

Methods

Vit.D levels were measured longitudinally in 50 SLE patients from Northern Italy at two time-points (winter and summer) during disease remission. Thirty patients were also evaluated during a flare. As controls, 170 healthy donors were enrolled. All the samples were analysed for 25-OH vit.D levels by a chemiluminescence assay (DiaSorin SpA, Italy).

Results

SLE patients had lower vit.D levels than controls in the summer (median 29.4 vs. 39.2 ng/ml, p=0.005) but not in the winter (26.4 vs. 21.6 ng/ml). During wintertime, 36 SLE patients were supplemented with vit.D drops (n=24; 48%), vit.D+calcium tablets (n=12; 24%), while 14 (28%) received no supplementation. Patients on oral drops had significantly higher vit.D levels than patients on tablets. The median weekly dosage was higher for oral drops than for tablets (6250 vs. 4560 UI, p=0.009). Winter flares were associated with lower vit.D levels in comparison with remission during the same season for each patient (21.1 vs. 30 ng/ml).

Conclusion

Current strategies of vit.D supplementation seem to be not sufficient for reaching an optimal vit.D status in Italian SLE patients. Vit.D and calcium tablets were less effective, probably because of lower vit.D content and poorer compliance. Vit.D insufficiency detected in the wintertime can be either a predisposing factor for flare or the consequence of the flare itself in SLE patients.

Key words

vitamin D, 25-OH vit.D, calcidiol, cholecalciferol, systemic lupus erythematosus, flare, seasonality, supplementation

Francesca Dall'Ara, MD
Laura Andreoli, MD
Nicole Piva, MD
Silvia Piantoni, MD
Franco Franceschini, MD
Angela Tincani, MD

Please address correspondence to:
Dr Angela Tincani,
Rheumatology and Clinical Immunology,
Department of Clinical and Experimental Sciences,
Spedali Civili and University of Brescia,
Piazzale Spedali Civili 1,
25123 Brescia, Italy.
E-mail: angela.tincani@unibs.it

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Introduction

Vitamin D (vit.D) is a hormone with pleiotropic effects. It is not only important in the regulation of bone metabolism, but has also potent immunomodulatory effects (1). The discovery that the immune cells (antigen presenting cells, natural killers B cells, B and T lymphocytes) possess the vit.D receptor suggested a key role in mediating the communication between the innate and adaptive pathways of the immune system (2). These findings posed the question whether a poor vit.D status may contribute to the onset and perpetuation of autoimmune diseases, although the receptor genetic variability and the consequent functional status may result in significant inter-individual difference (3).

Vit.D deficiency is highly prevalent worldwide (4) and it particularly affects patients with rheumatic diseases (5-7). Among these patients, those with SLE are particularly at risk for vit.D insufficiency because of their well-known photosensitivity (4, 8, 9). SLE patients are strongly recommended to avoid direct sun exposure and to use sunscreens. These measures should help to prevent SLE flares triggered by increased cell apoptosis due to ultraviolet radiation-induced injury (10).

Controversial results have been reported on the relationship between 25-OH vitamin D (25-OH vit.D) and SLE disease activity (11). Recently, Birmingham *et al*. (12) showed that in non-African American SLE patients unusually large declines in 25-OH vit.D during low daylight months may be mechanistically related to SLE flare, whereas relatively high 25-OH vit.D levels during high daylight months may be protective. Abou-Raya *et al*. described how vit.D supplementation in SLE patients seemed to ameliorate inflammatory and haemostatic markers, with a tendency towards subsequent clinical improvement (13). Other studies confirmed a profound hypovitaminosis D in SLE patients, but failed to find a relationship with SLE flare, disease duration, activity or damage (14-15). To note, many studies had a cross-sectional design and did not take into consideration important factors that may potentially influence vit.D status, such as seasonality and the concomitant supplementation with vit.D.

The aim of this study was to assess the vit.D status in a cohort of Italian SLE patients. These patients were longitudinally evaluated with regard to the seasonality, the disease activity (remission *vs*. flare) and the ongoing supplementation with vit.D given as primary prophylaxis of glucocorticoid-induced osteoporosis.

Patients and methods

This is a retrospective study of a 50 SLE patients (45 females, 5 males; median age 34 years, range 17–68), classified according to the revised American College of Rheumatology (ACR) criteria. All the patients were regularly attending every 3 to 6 months the Rheumatology and Clinical Immunology Unit in Brescia, located in northern Italy (latitude 45° N). In our centre, SLE patients were asked at the beginning of follow-up to give their consent for serum storage for medical research purposes. All the samples were stored at -80°C and used for biomarkers testing upon specific consent. This serum bank was functional to identify serum samples at different time points during the follow-up of the patients. Specifically, we identified three time points for 30 patients (group A) and two time points for 20 patients (group B). The aim of multiple time points was to assess the temporal relationship between 25-OH vit.D levels, disease activity (flare *vs*. remission) and season (winter: October to March *vs*. summer: April to September). The 3 time points in group A included a sample during a disease flare and 2 samples during remission state (1 in the same season as the flare, 1 in the opposite season). In group B, 2 samples during remission state were analysed (1 during the winter, 1 during the summer). Disease flare was defined as an increase in SLEDAI-2K score of ≥4 from the previous visit and as an alteration of clinical and/or serological parameters that required a treatment change such as a double prednisone dose and/or a change or new introduction of an immunosuppressive drug. Remission state was defined as stable if unchanged for at least 6 months. Clinical and laboratory

features of the 50 patients were derived from clinical charts (summarised in Table I). In Table II, characteristics of the 30 disease flares are presented.

As controls, 170 healthy blood donors (106 females, 64 males, median age 35 years, range 18–65) from the same geographical area were studied (117 samples were collected during winter and 53 samples during summer).

Vit.D levels were determined by Liason® 25-OH Vitamin D Assay (DiaSorin S.p.A., Saluggia, Italy) with the kind support of the company. Vit.D levels above 30 ng/ml were considered as sufficient, according to international guidelines (16). Insufficiency was defined as values between 10 and 30 ng/ml and deficiency as values below 10 ng/ml.

The study was approved by the Local Institutional Ethics Committee in Brescia and conducted in accordance with the Declaration of Helsinki.

Vit.D levels of patients and controls were not normally distributed (by the Shapiro-Wilk test), consequently the non-parametric Wilcoxon test was applied to paired data, while the Mann-Whitney U-test was used for unpaired data. Categorial variables were analysed with the Chi-squared test. For the analysis of longitudinal samples collected at 3 different times (group A) the Friedman test was applied. Results were considered as statistically significant for *p*-values (two-tailed) below 0.05.

The statistical analysis was performed with the software "R", an online available environment for statistical computing and graphics.

Table I. Demographic and clinical characteristics of 50 systemic lupus erythematosus (SLE) patients.

50 SLE patients	n. patients (n, %)
Age in years (median age, range)	34 (17-68)
Sex (male, female)	5, 10% (45, 90%)
Race (Caucasian, non-Caucasian)	48, (96%) 2 (4%)
Cigarette smoke	15 (30%)
Indoor working	50, (100%)
Criteria at disease onset (median number at the onset, range)	5 (4-7)
Criteria during follow-up (median number during follow-up, range)	6 (4-7)
1) Malar rash	25 (50%)
2) Discoid rash	2 (4%)
3) Photosensivity	25 (50%)
4) Oral Ulcer	15 (30%)
5) Arthritis	35 (70%)
6) Serositis (pulmonary/pericardic effusion)	11 (22%)
7) Renal involvement	33 (66%)
8) NPSLE*	0 (0%)
9) Haematological involvement	23 (46%)
10) Immunologic involvement	50 (50%)
10a) Anti-dsDNA	50 (50%)
10b) Anti-Sm	7 (14%)
10c) aPL	18 (36%)
11) ANA	48 (96%)
Other related autoimmune disease: Antiphospholipid syndrome	8 (16%)

NPSLE: Neuropsychiatric Systemic Lupus Erythematosus; Anti-dsDNA: anti-double-stranded DNA autoantibody; Anti-Sm: anti Smith antibody; ANA: antinuclear antibodies; aPL antiphospholipid antibodies: Lupus Anticoagulant, and/or anticardiolipin antibodies, and/or anti-β2-glycoprotein I antibodies.

Results

The frequency of insufficient 25-OH vit.D levels (<30 ng/ml) among SLE patients and healthy subjects was respectively 67% and 82% during the winter and 55% and 24% during the summer. Vit.D seasonal variations were observed in both groups, with vit.D levels higher in summer than in winter. During the summer, SLE patients had significantly lower median values than healthy controls (29.4 ng/ml *vs*. 39.2 ng/ml; p=0.005). Conversely, no difference was found between the two groups during the winter (Fig. 1).

The fact that during the winter SLE patients do not have a higher frequency of hypovitaminosis D as compared to healthy subjects could be explained by the possible beneficial effect of vit.D supplementation. In fact, most of the patients were taking vit.D supplements (none of the patients was taking bisphosphonates). Irrespective of the season, 30 out 50 patients (60%) were supplemented with oral vit.D, while the other 20 were not. Eighteen were supplemented with oral vit.D drops (14 with calcidiol; 4 with cholecalcipherol), while the other 12 patients were supplemented with calcium and cholecalchipherol in tablets (Fig. 2). No vit.D supplementation was taken by 19 patients (38%) during the summer and by 14 (28%) during the winter. These patients had significantly higher vit.D values in summer than in winter, although insufficient in both seasons (27.5 ng/ml *vs*. 15.6 ng/ml; p=0.049).

Those patients who were taking vit.D oral drops had higher vit.D levels than those patients who were taking vit.D-calcium tablets (32 *vs*. 21 ng/ml, p=0.008) (Fig. 2). A possible confounding factor of this observation comes from the different cumulative dose between the two compounds: those taking vit.D drops received 6250 IU per week on average, while those treated with vit.D-calcium tablets about 4560 IU per week (p=0.001).

No significant associations were found between vit.D insufficiency and SLE therapy (corticosteroids, other immunesuppressants, hydroxychloroquine). No significant associations were found with either cigarette smoking habits, working environment or body mass index. No cases of chronic renal insufficiency were observed. Among the 30 patients with a disease flare, 23 (77%) had a renal flare. Since proteinuria may account for vit.D levels, we verified that no significant difference was present between vit.D levels of patients with renal flare and non-renal flare.

Vit.D levels during a disease flare were not significantly different between supplemented and non-supplemented patients. By considering winter and summer flares separately and comparing them with samples from periods of remission in the same and in the opposite season, we found that winter flares were associated with significantly low-

Table II. Type of flare and treatment at the time of blood sampling in 30 SLE patients with disease flare.

30 SLE flare	number of patients (%)
Type of flare	
Renal flare	23 (77%)
Articular and cutaneous flare	6 (20%)
Pericardial and pulmonary effusion	1 (3%)
SLEDAI-2K score (median, range)	
During flares	12 (5–20)
During remission in summertime	4 (0–4)
During remission in wintertime	4 (0–6)
Therapy at the time of blood sample (dosage and kind of compound)	
Steroids (prednisone):	
Low dose (<7.5 mg/day)	7 (23.3%)
Moderate dose (7.5–25 mg/day)	18 (60.0%)
High dose (>25 mg/day)	2 (6.6%)
Hydroxychloroquine (200–300 mg/day)	23 (76,6%)
Immunosuppressants	
Azathioprine	9 (30%)
Methotrexate	2 (6.6%)
Cyclosporine-A	3 (10%)
Mycophenolate mofetil	3 (10%)

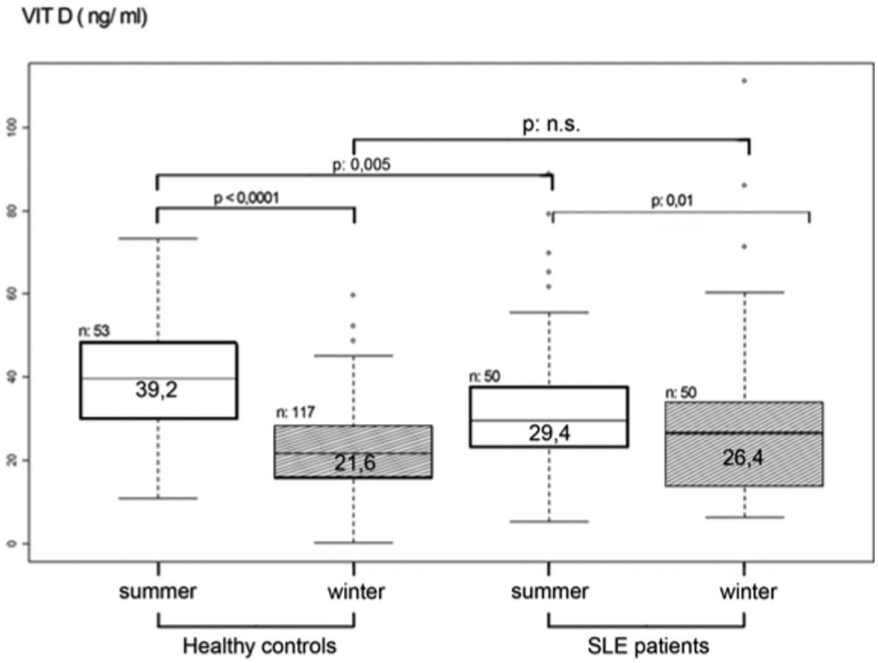

Fig. 1. Vitamin D levels in SLE patients (right-hand boxes) and healthy controls (left-hand boxes) in different seasons, summer (April–September; white boxes) and winter (October–March; striped boxes). The bold line in the plots shows the median value, the whiskers represent values within two standard deviations.
Vit.D: vitamin D; SLE: systemic lupus erythematosus.

er levels of vit.D (21.1 ng/ml) as compared to remission (30 ng/ml during winter and 33.5 ng/ml during summer Friedman test, p=0.0131) (Fig. 3). No difference was observed for summer flares.

Discussion

This is to our knowledge the first study assessing the relationships between vit.D status and SLE disease activity in an Italian cohort of SLE adult patients. Differently to previous cross-sectional studies performed in other geographical areas, we tried to include in the analysis as many as possible relevant factors potentially affecting vit.D levels.

Firstly, we selected both SLE patients and healthy controls living in the same geographical area (latitude), assuring a similar exposure to daylight for all the patients and controls. Secondly, we verified that cigarette smoking, working environment (outdoor *vs*. indoor), and body mass index were not significantly influencing vit.D levels in our cohort. Thirdly, we performed a detailed analysis of two major factors affecting vit.D levels, namely season and oral supplementation with vit.D, which has been traditionally given to our patients as part of the prophylaxis against glucocorticoid-induced osteoporosis. The most relevant peculiarity of this study is probably the longitudinal design, which allowed us to evaluate each single patient during a disease flare and during remission, in order to rule out individual variations of vit.D levels upon disease activity.

A study on Spanish SLE patients (17) found that increasing 25-OH vit.D levels upon supplementation had a beneficial on fatigue. However, seasonality was not considered in this study and it must be underlined that median 25-OH vit.D values upon supplementation did not reach sufficient levels (>30 ng/ml), questioning the efficacy of the supplementation itself. A multi-ethnic study from the USA used a longitudinal design to show that abnormally large seasonal declines in vit.D may trigger SLE flare, but the contribution of vit.D supplementation cannot be ruled out, since only 3 out of 82 patients were taking vit.D (12).

We confirmed a high prevalence of hypovitaminosis D in SLE patients as already described in several studies (2, 18, 19). However, we found that also healthy subjects had vit.D levels below 30 ng/ml in 24% of the cases during summer and 82% during winter, confirming what is called "epidemic hypovitaminosis D" in the general population (8). Both SLE patients and healthy people displayed seasonal variability in vit.D levels, with significantly higher levels in the summertime. This finding may suggest that even SLE patients, despite a reduced sun exposure and the use of sunscreen, may benefit from the summer season in terms of vit.D production. As a matter of fact, there are data in the literature suggesting that the use of full-screen protective cream does

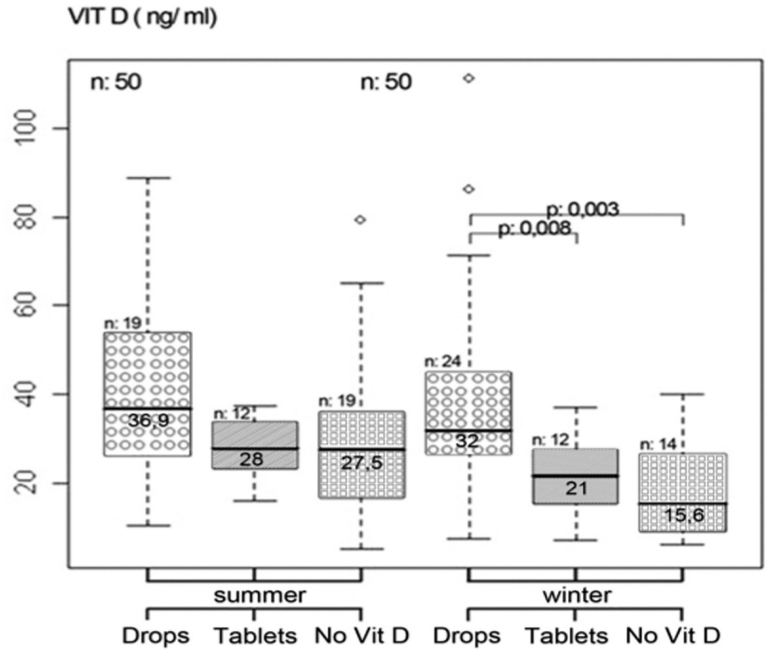

Fig. 2. Vitamin D levels in SLE patients supplemented with different compounds: patients supplemented with oral vitamin D drops (circled boxes), patients supplemented with vitamin D and calcium tablets (striped boxes) and patients without any supplementation (squared boxes) in different seasons, summer (April–September) and winter (October–March). The bold line in the plots shows the median value, the whiskers represent values within two standard deviations.

not reduce the metabolism of vit.D so much to induce a hypovitaminosis (20). However, this benefit may somehow be irrelevant for SLE patients, since their median values were significantly lower than healthy controls (29.4 *vs*. 39.2 ng/ml, p=0.0059) and still below the threshold for sufficiency (30 ng/ml).

Another major point of our study was the investigation of the effects related to the type of supplementation (vit.D drops and vit.D-calcium tablets). In fact, it is accepted that the oral absorption of vit.D, a fat-soluble molecule, is supported by the presence of food. So we may speculate that chewable tablets containing calcium and vit.D, possibly taken on an empty stomach, may have a reduced efficacy in the absorption of the vitamin. In summer, there was no difference between patients supplemented with different compounds, while during the winter drops were more efficacious than tablets (32 *vs*. 21.7 ng/ml, p=0.008). A possible confounding factor of this observation comes from the different cumulative dose between the two compounds, since those patients taking drops received a significantly higher amount of vit.D. In any case, we can say that tablets containing calcium and vit.D did not allow in achieving sufficient vit.D values in our patients (median values of 28 ng/ml in the summer, 21.7 ng/ml in the winter). This may be probably due to the relatively low content of vit.D (usually 400–600 UI per tablet), but also to poor compliance generally associated with these products because of dyspeptic symptoms (related to the digestion of calcium). Surprisingly, we found out that more than a quarter of patients were not taking any vit.D supplementation (38% in summer, 28% in winter), despite a strong promotion of osteoporosis prophylaxis made by physicians. These subjects showed vit.D levels significantly higher in summer than in winter (27.5 *vs*. 15.6 ng/

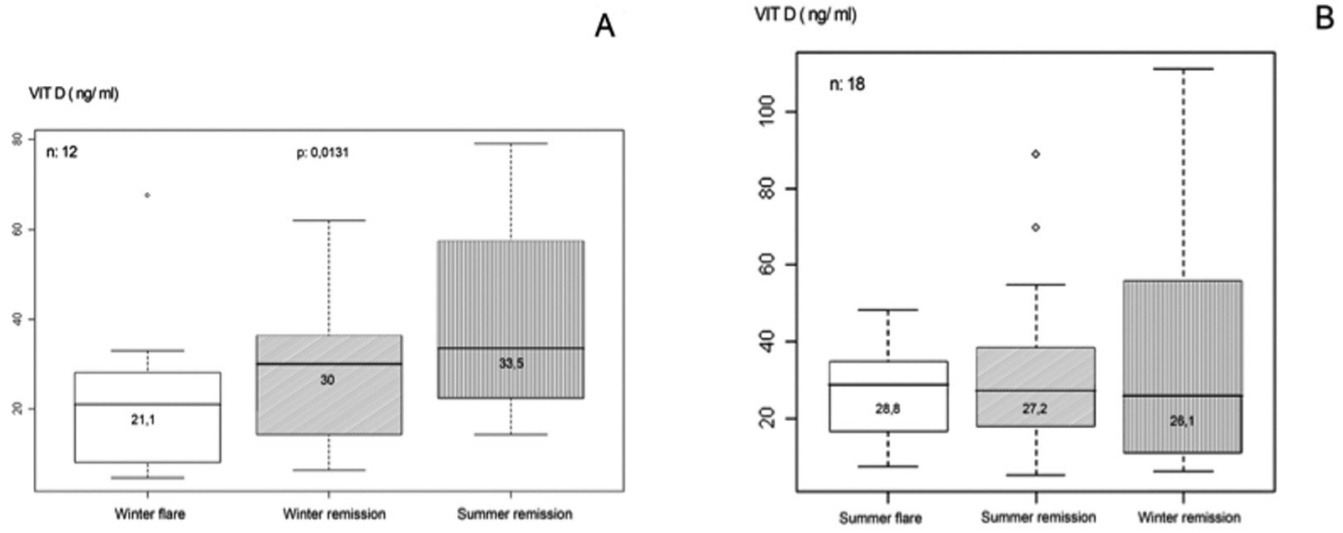

Fig. 3 A. Vitamin D levels in 30 SLE patients tested during a winter disease flare and during a remission period both in the same (winter) and in the opposite season (summer). Friedman test: p=0.0131.
B. Vitamin D levels in 30 SLE patients tested during a summer disease flare and during a remission period both in the same (summer) and in the opposite season (winter). Friedman test: p=not significant.

ml, p=0.049), confirming the fact that SLE patients are able to produce vit.D through sun exposure, but still remaining in the insufficiency range.

A main focus of our study was also on the relationship between vit.D status and disease activity. Many cross-sectional and prospective studies reported discordant data on this topic, probably because of methodological issues. Some studies reported an inverse relationship between 25-OH vit.D levels and disease activity (12), while other studies failed to find such a correlation (13, 14). In the present study, we found a higher frequency of hypovitaminosis D during winter flares than during remission state, both in the same and in the opposite season (Fig. 3). No difference was observed for summer flares. Given that the majority of the flares were renal, we checked whether proteinuria could be a confounding factor due to the loss of vit.D binding protein. This was not the case, since no significant difference was found in vit.D levels of renal and non-renal flares. Therefore, our data may suggest that hypovitaminosis D, especially if profound during the winter, might be a contributing factor to the onset of a disease flare confirming the findings of the previously cited American study that reported that large seasonal declines in vit.D status may trigger SLE flare in non-African Americans (12).

We acknowledge that this study has some limitations in the generalisation of the findings, due to the fact that no analysis could be performed upon ethnicity (mostly Caucasian patients were included) and type of disease flare (84% of flares were renal).

In conclusion, our study provides a complete overview of the relationships between vit.D status and SLE in adult Italian patients. Our findings, primarily the high frequency of hypovitaminosis D among SLE patients, are in line with recently published data on a group of Italian childhood-onset SLE patients (21).

Oral supplementation with vit.D, at the dosage traditionally recommended for osteoporosis prophylaxis, was shown not to be sufficient to determine an optimal vitamin status. In order to optimise vit.D supplementation, we suggest that each SLE patient undergoes periodically testing for 25-OH vit.D levels. This would allow pursuing optimal values throughout the course of the year, and reversing the profound deficiency observed especially during the winter, when the lack of vit.D is more likely to become a predisposing factor for the onset of a disease flare.

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