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Eva Zold (zold_eva@yahoo.com)
Peter Szodoray (szodoray@gmail.com)
Janos Gaal (gaaljan@gmail.com)
Janos Kappelmayer (kappel@gmail.com)
Laszlo Csathy (csatyl@gmail.com)
Edit Gyimesi (gyimed@yahoo.com)
Margit Zeher (zehmargit@yahoo.com)
Gyula Szegedi (szegedigyul@gmail.com)
Edit Bodolay (bodolai@iiibel.dote.hu)

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Vitamin D deficiency in Undifferentiated Connective Tissue Disease

Eva Zold[1], Peter Szodoray[1], Janos Gaal[2], János Kappelmayer[3], Laszlo Csathy [3], Edit Gyimesi[1]

Margit Zeher [1],Gyula Szegedi [1], Edit Bodolay [1]

[1] Division of Clinical Immunology, 3rd Department of Medicine, Medical and Health Science Center, University of Debrecen, Moricz Zs. Str. 22., Debrecen, 4032, Hungary

[2] Department of Rheumatology, Kenézy County Hospital, Bartok Bela Str. 4., 4043, Debrecen, Hungary

[3] Department of Clinical Biochemistry and Molecular Pathology, Medical and Health Science Center, University of Debrecen, Moricz Zs. Str. 22., Debrecen, 4032, Hungary

Corresponding author:

Eva Zold, MD

3rd Department of Internal Medicine

University of Debrecen

Moricz Zs. Str. 22

Debrecen, 4032

Hungary

T/F: (36-52)-414-969

Email: zold_eva@yahoo.com

Abstract

Introduction: Both experimental and clinical data provide evidence that vitamin D is one of those important environmental factors that can increase the prevalence of certain autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, insulin-dependent diabetes mellitus, and inflammatory bowel disease. The aim of the present study was to investigate the prevalence of vitamin D insufficiency in patients with undifferentiated connective tissue disease (UCTD).

Methods: Plasma 25(OH)D3 levels in 161 UCTD patients were measured both in summer and winter periods. Autoantibody profiles (ANA, anti-U1 RNP, anti-SSA, anti-SSB, anti-Jo1, anti-Scl70, anti-dsDNA, anti-centromere, anti-cardiolipin, RF, anti-CCP) and the patients' clinical symptoms were assessed.

Results: Plasma levels of 25(OH)D3 in UCTD patients were significantly lower compared to controls both in summer and winter periods (UCTD-summer: 33±13.4 ng/ml vs. control: 39.9±11.7 ng/ml; p=0.01; UCTD-winter: 27.8±12.48 vs. control 37.8±12.3 ng/ml; p=0.0001). The presence of dermatological symptoms (photosensitivity, erythema, chronic discoid rash) and pleuritis was associated with low levels of vitamin D. During the average 2.3 years follow-up period 35/161 patients (21.7 %) with UCTD further developed into well established connective tissue disease (CTD). Patients who progressed into CTDs had the lower vitamin D levels than those who remained in UCTD stage (Vitamin D levels: CTD: 14.7± 6.45 ng/ml vs. UCTD: 33.0± 13.4 ng/ml, p=0.0001).

Conclusions: In patients with UCTD a seasonal variance in levels of 25(OH)D3 was identified and showed that these levels were significantly lower than in controls during the corresponding seasons. Our results suggest that vitamin D deficiency in UCTD patients may play a role in the subsequent progress into well-defined CTDs.

Introduction

Environmental factors play an important role in the development and progression of systemic autoimmune diseases, along with susceptible genetic and hormonal background. It has been recently suggested that vitamin D is an environmental factor, which by modulating the immune system affects the prevalence of autoimmune syndromes; respectively vitamin D deficiency may have a role in the pathogenesis of systemic autoimmune diseases.

The classic and well-known function of vitamin D is to regulate mineral homeostasis and thus bone formation and resorption. On the other hand, less-traditional function of vitamin D has been demonstrated, including substantial effects on the regulation of cell proliferation and differentiation, also the vitamin D has been described to modulate immune responses[1-6].

Active vitamin D has been shown to inhibit the differentiation and maturation of myeloid dendritic cells to reduce the expression of MHC II, co-stimulatory molecules (CD80, CD86, CD40) and the maturation proteins (CD1a, CD83)[7]. In addition, antigen-presenting capacity of macrophages and dendritic cells is suppressed and the immune stimulatory IL-12 is inhibited by active vitamin D[8]. Th1 and Th2 cells are direct targets of active vitamin D. Vitamin 1,25(OH2)D3 decreased the proliferation of Th1 cells moreover inhibited the production of IL-2, IFN-γ and TNF-α of Th1 cells and had an anti-proliferative effect [3;9]. Furthermore, vitamin D silences the Th17 response, moreover repairs the number and function of the CD4+/CD25+ regulatory T cells, which may prevent the development of autoimmune diseases [9;10]. These findings suggest that the effect of vitamin D is predominantly tolerogenic.

Cantorna et al. have showed that vitamin D status as an environmental factor affecting autoimmune disease prevalence. The determination of exact connection is difficult, because of

the complexity of the vitamin D regulatory system, moreover complicated interactions could occur between genes that may affect autoimmune disease susceptibility [11]. Serum levels of vitamin D were significantly lower in systemic lupus erythematosus (SLE) and type-1 diabetes mellitus (IDDM) than in the healthy population [12-15]. Recently, it was also found that lower levels of vitamin D were associated with higher disease activity in rheumatoid arthritis (RA) [6]. An inverse correlation has been described between the supplementation of vitamin D and the development of IDDM and multiple sclerosis (MS)[1;12].

The evolution of diseases with immune pathogenetic background is usually slow and progressive. The term undifferentiated connective tissue disease (UCTD) has been used since 1980 to describe a group of connective tissue disorders (CTDs) that lack the characteristics of any distinctive disease. There is great deal of information available regarding the clinical and serological profile of UCTD and the rate of evolution into well–defined CTD [16-18]. About 30-40% of patients with UCTD will evolve to defined CTD during years of follow-up. The higher rate of disease evolution, mostly can be seen between the 1st and 2nd year. [18;19]. UCTD has specific signs and/or autoantibodies, which are characteristics for autoimmune disease. Mosca et al. and our previous studies described that the most frequent clinical manifestations of UCTD were polyarthralgy/polyarthritis, Raynaud's phenomenon, serositis (pleuritis, pericarditis), photosensitive rash, xerostomia and xerophthalmia, as well as central nervous system involvement. During the follow-up period new organ manifestations can appear and the existing clinical and immunological abnormalities can increase in the intensity or even become permanent. Evolution to systemic lupus erythematosus (SLE) and to other systemic autoimmune diseases (Mixed Connective Tissue Disease-MCTD, Systemic Sclerosis, Sjögren's syndrome, Polymyositis/Dermatomyositis, Rheumatoid Arthritis, Systemic Vasculitis) has also been described.

Until now, there has been no data on the 25(OH)D3 levels in UCTD patients. The aim of our study was to assess the vitamin D deficiency in UCTD patients and compare it to controls. We examined the seasonal variance of the vitamin D levels during the summer and winter months. We also determined a possible connection between low levels of vitamin D and the clinical and serological manifestations of the disease.

In addition, we determined the prevalence of the 25(OH)D3 levels in patients with UCTD and assessed its probable pathogenic role in the progression towards a well-defined CTD.

Materials and Methods

The study population involved 161 patients (154 women and 7 men) with UCTD followed-up and treated at the Division of Clinical Immunology, 3rd Department of Internal Medicine, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary. All patients with UCTD were enrolled based on the following criteria: 1. symptoms and signs suggestive of a CTD not fitting the accepted classification criteria for any of the defined CTDs, 2. disease duration of at least 1 year and 3. the presence of at least one non-organ specific autoantibody. No patients had received corticosteroids, immune-suppressive or cytotoxic drugs.

Patients with a defined CTD were diagnosed according to the corresponding ACR (American College of Rheumatology) classification criteria [17;18;20-25]. Informed consent was signed by all patients approved by the Ethical Committee of the University.

Age and sex matched healthy individuals served as controls with no autoimmune/endocrine or malignant neoplastic diseases (residents and health-care workers).

Clinical data were obtained by a questionnaire or from patients' charts, including age, body mass index, age at the diagnosis and therapy.

All patients were followed-up in every 4 months. Diagnostic procedures for all patients included chest X-ray, spirometry/diffusion capacity, Doppler echocardiography and high resolution computed tomography (HRCT), Schirmer's test, sialometry, and radionuclide esophageal transit scingtigraphy.

Laboratory measurements included erythrocyte sedimentation rate (ESR), full blood count, urine analysis, serum creatine phosphokinase, serum calcium, kidney and liver function tests, thyroid stimulating hormone (TSH), and PTH. PTH was measured using a Siemens Advia Centaur autoanalyser (Deerfiled, IL, USA) using reagents and protocols provided by the manufacturer.

At the time of study, all patients with UCTD underwent bone densitometry using a Lunar-DPX-L DEXA instrument. BMD of lumbar 2-4 vertebrae and femoral neck was assessed and T scores determined. Osteoporosis or least osteopenia was according to the WHO classification criteria (T-score < -1) [26]. All patients and control subjects had normal mean BMD values.

Patients had no signs of renal insufficiency and they did not take any vitamin D supplements previously and in parallel with the investigations.

At diagnosis of UCTD the plasma 25(OH)D3 levels were measured. We assessed the plasma 25(OH)D3 levels of 161 UCTD patients and 59 control subjects during the summer (from June to October) and during the winter (from January to May) period.

Immuneserological analyses

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on HEp-2 cells. Anti-U1-RNP, anti-Sm, anti-SSA, anti-SSB, anti-Jo1, anti-Scl70, anti-cardiolipin (anti-CL) were analyzed in all patients by enzyme-linked immune-absorbent assay (ELISA) according to the manufacturer's instructions (Pharmacia & Upjohn, Diagnostic GmbH, Freiburg, Germany and Cogent Diagnostics, Edinburgh, UK).

IgM RF was assessed by nephelometry, values above 50 U/l were considered positive.

Anti-CCP levels were measured using a second generation ELISA (Quanta Lite^{TM}, CCP ELISA; Inova Diagnostics Inc., San Diego, CA, USA), utilizing synthetic citrullinated peptides bound to surface of a microtiter plate as antigen. The test was performed according to the manufacturer's instructions.

Serum samples, collected immediately after the initial diagnosis of patients, were separated and stored at -70 ^{o}C.

Determination of vitamin D levels

Plasma levels of 25(OH)D3 vitamin of patients and controls were assessed at the Department of Clinical Biochemistry and Molecular Pathology Laboratory, University of Debrecen Medical and Health Science Center. Samples were analyzed by a high performance liquid chromatograph (HPLC) method using Jasco HPLC system (Jasco Inc., Easton, MD, USA) and Bio-Rad reagents kit (Bio-Rad Laboratories Hercules, CA, USA). The sample (500µL plasma from EDTA anticoagulated blood) was purified from proteins and 50µL of the cleaned supernatant was injected into the instrument. Separation was achieved with a reverse phase C18 Bio-Rad column (90*3.2mm) (Bio-Rad Laboratories Hercules, CA, USA). The mobile phase (methanol-water mixture) had a flow rate of 1.1 mL/min. For quantitative determination of the separated compound a diode array detector (set at 265nm) was used.
According to current recommendations, plasma 25(OH)D3 levels <30 and 10 ng/ml were defined as vitamin D insufficiency and vitamin D deficiency, respectively. [27-29].

Statistical analysis:

Data were presented as percentages or mean values ± standard deviation (SD). GraphPad Software (San Diego, California, USA) was used in data interpretation (two-tailed t-test, chi-squared test and Fisher's exact test, logistic regression). Pierce regression coefficient assay was also performed when required. Multiple linear regression models was used to examine relationship between vitamin D level clinical signs, smoking, and seasonality. P-values below 0.05 were considered to be statistically significant.

Results

Clinical and serological data of 161 patients with UCTD

The mean age at the diagnosis of 161 UCTD was 44.91±12.7 years (woman/man: 22/1). The mean duration of symptoms at the time of the enrollment into the study was 4.09±2.36 years. The rate of woman and men was very similar in the two groups (control group number: 59; mean age:43.9±15.1; woman/man: 18/1). There was no difference between the body weight, height, BMI, and BMD values in patients and the controls.

The most frequent clinical manifestation of UCTD was polyarthritis (28.5%). Skin lesions (photosensitivity, erythema and lymphocytic vasculitis) were 22.9%, Raynaud's phenomenon 17.3%. Xerophthalmia was observed in 15.5% of patients. Pleuritis (5.59%), neuropathy (4.96%), deep vein thrombosis (2.48%) myositis (1.2%) and pulmonal manifestations (1.2%) were less frequent among the patients' first clinical symptoms.

The prevalence of dysmotility was 13.6% in UCTD patients. The most frequent immune serological abnormality in the serum of patients was the presence of ANA, which was found in 64.59% of patients. The earliest antibody at the onset of UCTD was anti-SSA, presented in 43 patients (26.7%). Anti-CL autoantibodies could be detected in 40 patients (24.8 %).

Anti-U1-RNP antibodies were found in the sera of 29 patients (18.0%), anti-Sm antibody in 8 (4.9%), anti-CCP in 14 (8.6%), anti-dsDNA in 12 (7.4%), anti-SSB in 9 (5.59%), ANCA in 4 (2.48%), IgM RF in 2 patients (1.2%) at the initial diagnosis of UCTD.

The levels of vitamin D in patients with UCTD

The summer and winter levels of 25(OH)D3 in patients with UCTD were significantly lower compared to healthy individuals (UCTD-summer: 33.0±13.4 ng/ml vs. control:

39.9±11.7 ng/ml; p= 0,010; UCTD-winter: 27.8±12.48 vs control: 37.8± 12.3 ng/ml; p=0.0001) (**Figure 1**.). In UCTD patients the winter levels of vitamin D were considerably lower than the summer levels (UCTD summer: 33.0±13.4 ng/ml, UCTD winter: 27.8 ±12.48 ng/ml, p=0.001). In the control group, vitamin D levels were lower in winter than in the summer, but the difference was not significant (controls-summer: 39.9±11.7 ng/ml, controls-winter: 37.8± 12.3; ns.). Hereafter, the summer levels of 25(OH)D3 in controls were used for comparison.

There was vitamin D insufficiency (below 30ng/ml vitamin D level) in 41.6% of UCTD patients (67 patients) during the summer months, in 54.3% of patients (88 patients) during the winter, and in 18.64% of controls (11 subjects) (**Table 1**.). In UCTD patients with vitamin D insufficiency, the winter levels of vitamin D were significantly lower than the summer levels (UCTD (<30 ng/ml) summer: 21.9±4.7 and winter: 18.1±5.9; p=0.03). Vitamin D deficiency (below <10 ng/ml vitamin D level) were found in 5 of the UCTD cases.

Correlation of clinical and laboratory parameters with plasma levels of vitamin D

Dermatological symptoms (photosensitivity, erythema, discoid skin lesions) (p=0.0046) and pleuritis (p=0.0346) were also more frequent in UCTD patients with low levels of vitamin D (<30 ng/ml) (**Table 2**).

Interestingly, patients with high serum levels of anti-U1-RNP, anti-SSA and anti-CCP antibodies were found to have the lowest vitamin D levels (anti-U1-RNP: p=0.024, anti-SSA: p=0.029, anti-CCP: p=0.0001).

During the follow-up period, there were 35 out of 161 UCTD patients (21.7%) who developed an established CTD (**Figure 2**.). The evolution to defined CTD was an average of 2.3±1.2 years. Among these patients, 12 developed RA, 6 SLE, 6 MCTD, 6 Sjögren's syndrome, 2 systemic vasculitis and 3 antiphospholipid syndrome.

Surprisingly, we found significantly the lowest levels of vitamin D in those patients who eventually developed CTD, compared to patients who remained in the UCTD stage (established CTD patients**:** 14.7±6.45 ng/ml, remained in the UCTD stage: 33.0±13.4 ng/ml; p=0.0001) (**Table 3.**).

Discussion

Epidemiological studies suggest that the development of systemic autoimmune disease is affected by geographical areas and lifestyle. Presumably, in these processes vitamin D is a significant environmental factor**.** Vitamin D deficiency has been linked to several different diseases, including malignant and immune-pathogenetical disorders.

Age, sex, lifestyle, geographical areas, sunlight and vitamin D supplementation are important determinants of vitamin D levels. In countries with temperate climates, such as Hungary, serum vitamin D concentrations rise and fall throughout each year, in parallel with the sun exposure [30-33]. The prevalence of vitamin D deficiency is much higher in Europe than in Asia, Australia or USA. In Hungary, high prevalence of D hypovitaminosis in healthy postmenopausal women has been described [34].

In the present study we first analyzed the circulating levels and seasonal variance in the levels of 25(OH)D3 in a large cohort of patients with UCTD. According to our studies, in UCTD patient vitamin D levels were significantly lower than in the control group both during the summer and winter months. Circulating levels of vitamin D fluctuate seasonally in UCTD patients, with low levels of 25(OH)D3 in the winter months and high levels during the summer months.

Our data suggested that patients with UCTD have vitamin D insufficiency in 41% of cases in the summer months and even more became vitamin D deficient during the wintertime. In UCTD patient, the winter levels of vitamin D were considerably lower than the summer levels. Plasma levels of 25(OH)D3 in UCTD patients were significantly lower compared to controls both in summer and winter periods. Vitamin D deficiency was found in 5 of the UCTD cases (3.1%), compared to none in the control group.

A clear correlation between the frequency of IDDM, MS, RA, SLE, inflammatory bowel disease (IBD) and the North-South latitude, sunshine exposure and vitamin D levels

has been shown [12;35-37]. MS and IBD are diseases prevalent in Canada, the northern parts of the USA and Europe. The severity of MS has been shown to fluctuate seasonally, with exacerbations occurring mostly during the spring time [38;39]. Munger at al. found that the risk of MS was 40% lower in women taking more vitamin D [40]. This condition is explained by the fact that the northern hemisphere receives less sunlight, especially during the winter. MS, IDDM and RA are more prevalent in temperate high latitudes, than at the equatorial latitude. It seems that high vitamin D intake, regardless of sunlight exposure are associated with reduced risk of developing IDDM, RA and MS.

A study on 29.000 women showed that vitamin D intake reduced the risk of developing RA [41].

2000 IU/day vitamin D supplementation during infancy also significantly reduced the subsequent development of IDDM, evaluated 30 years later [12]. Vitamin D deficiency is common in patients with Crohn disease even when the disease is in remission [42].

25(OH)D3, 1,25$(OH)_2$D3 and PTH levels in 25 Caucasian SLE (disease duration 1-8 years) and in 25 female patients with fibromyalgia were studied and found no significant difference between the two groups [43] .

Müller at al assessed the levels of 25(OH)D3 and 1,25$(OH)_2$D3 in 21 SLE patients, 29 RA patients, 12 osteoarthritis patients and they found that vitamin D levels in SLE patients were significantly lower compared to patients with osteoarthritis and controls [44].

Significantly lower serum 25(OH)D3 levels were found among recently diagnosed 123 SLE patients compared to 240 age- and sex-matched controls from the Carolina Lupus Study [13]. Levels of 25(OH)D3 were significantly lower among African-Americans compared to Caucasians. Levels of vitamin D were the highest in the summer and lowest in the winter. Vitamin D deficiency was found in 18% of the SLE patients with the presence of severe renal disease and photosensitivity [13].

In our results, the probability to develop dermatologic symptoms (photosensitivity, vasculitis, erythema) and pleuritis correlated with vitamin D insufficiency. The presence of anti-U1-RNP, anti-SSA and anti-CCP were more frequently occurred in these particular patients.

In our study as well as data reported by others, suggest that UCTD may develop into any well-defined CTDs [16-18]. Evolution into a specific established CTD was found in 21.7% of patients with UCTD during the follow-up period. UCTD most frequently progressed into RA, while SLE, Sjögren's syndrome and MCTD had about the same prevalence.

Interestingly, the lowest levels of vitamin D (below 30ng/ml) were measured in UCTD patients, who subsequently evolved to defined CTDs. In our previous study, we found the shift toward Th1 with increased IFNγ production in patients with UCTD combined with the degree of immunoregulatory disturbances characterized by the progressive divergent shifts in natural and induced T-regulatory cell populations [45]. Therefore, immunoregulatory abnormality signify the transition from undifferentiated to definitive CTD [45]. Since vitamin D is an important regulator of the immune system, it raises the possibility that vitamin D deficiency may contribute to the progression into well-defined CTD-s.

Several factors can lead to low levels of vitamin D in our patients with UCTD. Although, the physical activity of most patients was not limited, nonetheless, patients with photosensitive rashes do seem to have a reduced exposure to sunlight and they generally use very high UV protection. As another vitamin D-reducing factor, anti-vitamin D antibodies have been described in patients with SLE, antiphospholipid syndrome, and pemphigus vulgaris, and these autoantibodies were associated with anti-dsDNA antibodies in SLE [46].

The observed low vitamin D levels underline the importance of an intensified routine vitamin D supplementation as opposed to the current administration practice. This is further

supported by a few prospective studies showing that the intake of vitamin D significantly reduces the incidence and/or progression of autoimmune diseases [40;47;48].

Based on our findings we conclude that measurement of serum vitamin D is crucial in UCTD patients and the effective supplementation of vitamin D may be important in these patients. Future prospective studies are needed to determine the efficacy of supplementation of vitamin D in the prevention of subsequent evolution of UCTD to well-defined CTDs and to establish the role of vitamin D in the treatment of autoimmune diseases.

Conclusions

Vitamin D has pivotal role in the maintenance of immune-homeostasis. In various systemic autoimmune diseases low levels of vitamin D have been described previously. We showed that in patients with UCTD, serum levels of vitamin D was significantly lower compared to healthy individuals, moreover critically low levels of the vitamin clearly correlated with the progression to well-established connective tissue diseases. Our findings support the idea that vitamin D may be a key regulator of autoimmune processes in patients with UCTD.

Abbreviations

UCTD	undifferentiated connective tissue disease
CTD	connective tissue disease
SLE	systemic lupus erythematosus
IDDM	type-1 diabetes mellitus
RA	rheumatoid arthritis
MS	multiple sclerosis
MCTD	Mixed Connective Tissue Disease
anti-CL	anti-cardiolipin
anti-U1-RNP	anti-U1 ribonucleoprotein antibody

ANA	Anti-nuclear antibody
anti-CCP	anti-cyclic citrullinated peptide antibody
anti-dsDNA	anti-double-stranded deoxyribonucleic acid antibody
anti-CL	anti-cardiolipin
ANCA	anti-neutrophil cytoplasmic antibody
IBD	inflammatory bowel disease

Competing interests

The authors declare that they have to no competing interests. The submission fee has partially been sponsored by TEVA Hungary Ltd.

Authors' contributions

EZ: acquisition of data and analysis; PS: interpretation of data and manuscript preparation; JG: interpretation of data, drafting the manuscript, JK: analysis and interpretation; LC: analysis and interpretation; EG: analysis and interpretation; MZ: interpretation of data, drafting the manuscript; GS: interpretation of data, drafting the manuscript; EB: given fine approval of the version to be published

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Figure Legends

Figure 1. Comparison of vitamin D of UCTD patients with healthy controls during the summer and winter months.

Figure 2. Evolution of undifferentiated connective disease to defined connective tissue diseases (Abbreviation: **CTD:** connective tissue disease, **UCTD:** undifferentiated connective tissue disease)

Tables

Table 1. Seasonal fluctuation of the levels of vitamin D in patients with UCTD

	UCTD patients Summer N=161	**UCTD patients Winter n=161**	**Control Summer n=59**
Vitamin D insufficiency (<30 ng/ml)	67 patients (41.6%) 21.0 ± 5.79[a]	88 patients (54.3%) 18.4 ± 6.7[b]	11 (18.64 %) 25.0 ± 4.65[c]
Vitamin D deficiency (<10 ng/ml)	5 (3.1 %) (1 RA, 4 UCTD)	5 (3.1%) (2RA, 3 UCTD)	

Significance

a-c: summer-control: p= 0.14

b-c: winter-control : p=0.016

a-b: summer-winter : p=0.03

Table 2. Relationship between low serum levels of vitamin D and clinical/ serological manifestations of UCTD patients

Correlation between clinical symptoms and vitamin D insufficiency	**P**	**OD**
Xerostomia/xerophtalmia	0.8276	0.8909 0.3768-2.107
Polyarthritis	0.4834	0.7621 0.3772-1.540
Skin lesion	0.0046	2.987 1.397-6.385
Pleuritis	0.0346	5.367 1.078-26.719
Central and peripheral nervous system manifestations	1	0.8344 0.1924-3.619
Raynaud's phenomenon	0.5331	0.7407 0.3178-1.726
Oesophageal involvement	0.3448	1.650 0.6569-4.144
Correlation between antibodies and vitamin D insufficiency		
Anti-U1-RNP	0.0240	5.083 1.286-20.094
Anti-SSA	0.029	3.474 1.147-10.520
Anti-CCP	0.0001	12.0 3.541-42.036
Anti-dsDNA	0.2551	0.4896 0.1751-1.369
ANCA	0.2978	0.4024 0.064-2.51
Anti-CL	0.3728	1.715 0.6535-4.501
Anti-SSB	0.2945	0.5254 0.1484-1.860

Abbreviations: anti-U1-RNP: anti-U1 ribonucleoprotein antibody, **ANA**: Anti-nuclear antibody, **anti-CCP**: anti-cyclic citrullinated peptide antibody, **anti-dsDNA:** anti-double-stranded deoxyribonucleic acid antibody, **anti-CL:** anti-cardiolipin, **ANCA:** anti-neutrophil cytoplasmic antibody

Table 3. Comparison of 35 patients who developed to an established CTD with patients who remained in the UCTD stable stage

	Patients with evolution into defined CTD n=35	**Patients with "stable" UCTD n=126**	**p<**
Age (years) (mean±SD)	43.85±11.1 range:21-67	44.9 ±12.7 range:17-78	0.651
Duration of follow-up (years)	2.31±1.2 range:0.5-4	4.09±2.36 range:0.5-9	0.0006113
Vitamin D serum levels (ng/ml)	14.7±6.45 range: 4.7-25.2	33.0±13.4 range:6-88.9	0.0001

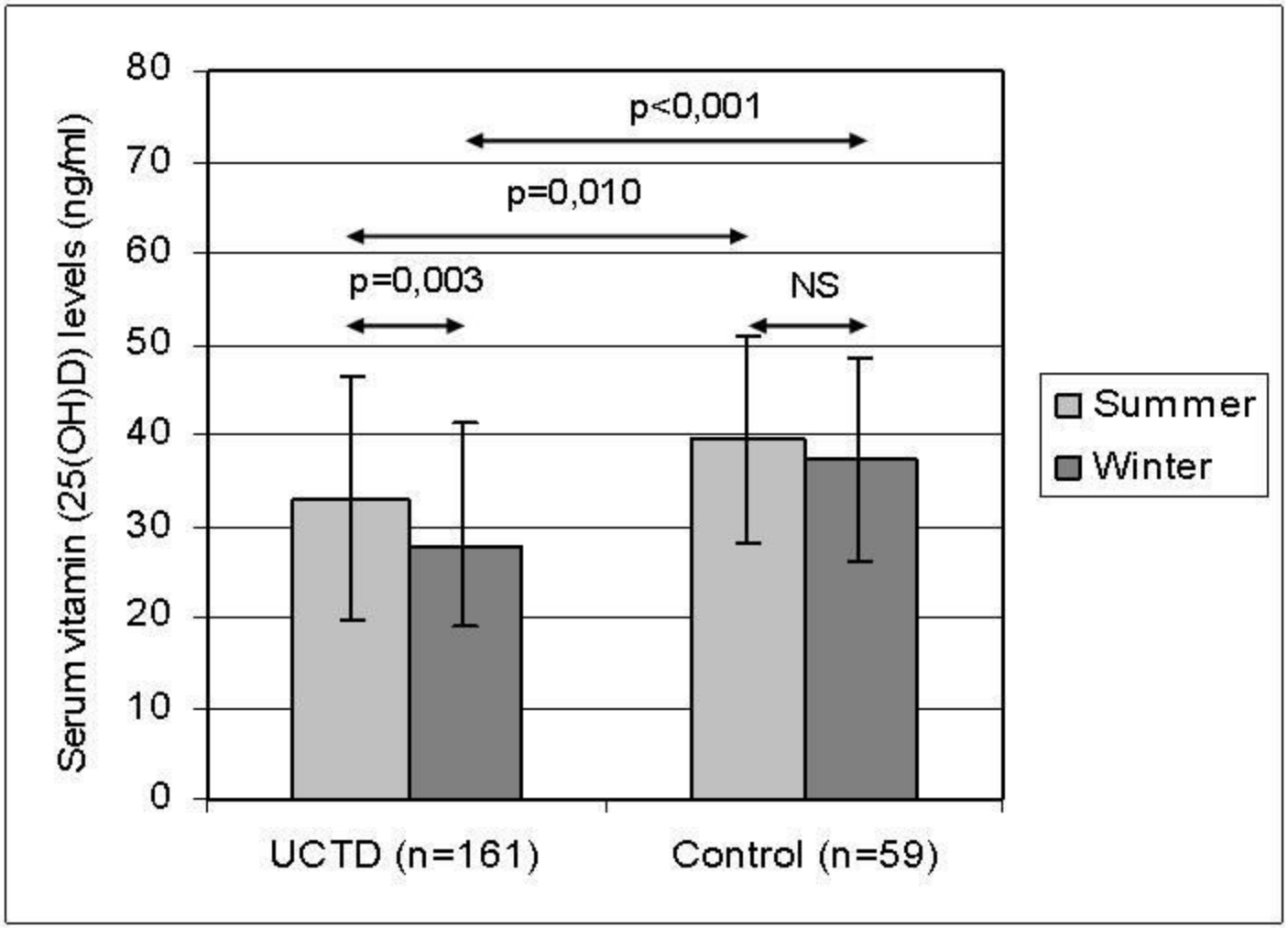
Serum vitamin (25(OH)D) levels (ng/ml)
80
70
60
50
40
30
20
10
0
p<0,001
p=0,010
p=0,003
NS
Summer
Winter
UCTD (n=161)
Control (n=59)

Figure 1

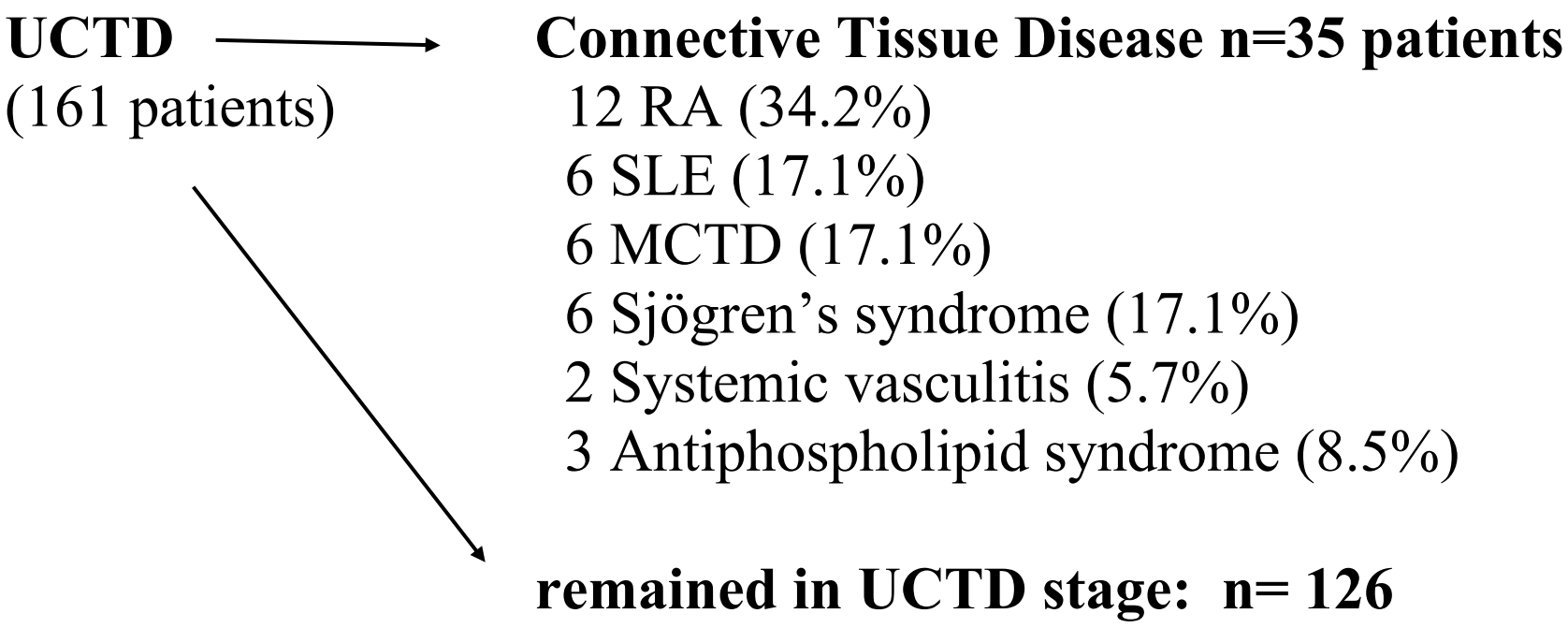
UCTD
(161 patients)
Connective Tissue Disease n=35 patients
12 RA (34.2%)
6 SLE (17.1%)
6 MCTD (17.1%)
6 Sjögren's syndrome (17.1%)
2 Systemic vasculitis (5.7%)
3 Antiphospholipid syndrome (8.5%)
remained in UCTD stage: n= 126

Figure 2