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Regular paper

Vitamin D and disease activity in rheumatoid arthritis patients: a retrospective study in a Romanian cohort

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Background. The relationship between the serum levels of Vitamin D and the severity of RA is a subject of great interest for the future therapeutic strategies. Although the evidence on the relationship between hypovitaminosis D and early RA is contradictory, preliminary data suggest that the serum levels of vitamin D are inversely associated with the disease activity. Aim: the main objectives of this study include: (1) to analyze the serum levels of vitamin D in patients with RA in comparison to healthy controls; (2) to investigatea possible correlation with disease activity. Materials and Methods. This was a retrospective, comparative study conducted on 37 subjects suffering from RA and a group of 21 healthy matched controls. The following were determined in all studied subjects: erythrocyte sedimentation rate (ESR), white blood cells (WBC), hemoglobin (Hb), platelets (PLT), serum calcium (Ca), serum phosphorus (Phos), and serum 25 hydroxy-vitamin D. Moreover, in the RA group the IgM-Rhematoid Factor (RF) and anti-citrullinated protein antibodies (ACPA) (immune-enzymatic method) were assessed. The Disease Activity Score of 28 joints (DAS28) was calculated for the RA patients. Results. We observed that vitamin D deficiency is more common in RA patients than in healthy controls. No significant correlation between 25OHvitD and DAS28-ESR was found in our study cohort. Conclusions. There is no significant association of serum 25(OH)D with disease severity in a Western Romanian cohort with RA. However, this result could have implications for the disease management, as patients with RA could be supplemented with vitamin D even in the absence of disease activity.

Key words: Disease activity, rheumatoid arthritis, 25(OH) vitamin D, DAS 28

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Abbreviations: RA, rheumatoid arthritis; ESR, erythrocyte sedimentation; WBC, white blood cells; Hb, hemoglobin; PLT, platelets; Ca, serum calcium; Phos, serum phosphorus; ACPA, anti-citrullinated protein antibodies; 25(OH)D, serum 25 hydroxy-vitamin D; 25(OH) D3, calcifediol; 1,25(OH)2 D, calcitriol; DMARDS, disease modifying antirheumatic drugs; DAS28, disease activity score 28

INTRODUCTION

Vitamin D refers to a group of fat-soluble secosteroids with an important role in phospho-calcic metabolism. In humans, vitamin D circulates in two forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol).

Vitamin D3 is metabolized in the liver to 25-hydroxyvitamin D3 [25(OH)D3] (calcifediol), and it represents the major circulating form of vitamin D. In the kidneys, 25(OH)D3 is additionally hydroxylated to 1,25 dihydroxyvitamin D ($1,25(OH)_2$ D) or calcitriol, being the active form of vitamin D. Because of its long half-life and higher concentration, 25-hydroxyvitamin D [25(OH) D3] is commonly measured to assess and monitor the vitamin D status in individuals (Feldmann *et al.*, 2017).

Lately, there is a growing interest in the assessment of vitamin D status in several autoimmune diseases.

Rheumatoid arthritis (RA), an autoimmune progressive systemic disease of the connective tissue, is particularly manifested by destructive alterations in the joints and synovitis (Bumbea *et al.*, 2017).

It is known that RA affects 0.72% of the worldwide population and occurs more frequently in women over 40 years of age (Myasoedova *et al.,* 2020). Studies on the incidence and prevalence of RA suggest variations between different populations even within the same country. For instance, in Romania, 200 000 people suffer from arthritis, and women are three times more likely to develop RA (Șuța *et al.,* 2014).

The etiology and pathogenesis of RA is still obscure and many factors can be involved. The etiology of the disease could be attributed to genetic and non-genetic factors, such as hormonal, environmental, and infectious factors. Moreover, it has been shown that vitamin D is an environmental risk factor involved in the pathogenesis of this disease (Cutulo *et al.*, 2009).

Vitamin D receptors have been identified in the areas of cartilage erosion, in the chondrocytes and synoviocytes of patients with RA. Thus, detection of vitamin D receptors in the immune system cells may elucidate associations between this vitamin and RA (Tetlow *et al.*, 1999).

Already known to have an important role in osteoporosis and fractures, which are frequent complications in RA (Bellan *et al.*, 2019; Tan *et al.*, 2018), and also in the modulation activities of the immune cells, including lymphocytes and macrophages (Marques *et al.*, 2010), vitamin D can be the key factor leading to the development or worsening of the disease.

Some epidemiological studies showed that vitamin D deficiency is common in RA patients when compared to the healthy population (Haque *et al.*, 2010; Kerr *et al.*, 2011), whereas other investigations did not confirm these findings (Cutolo *et al.*, 2006; Turhanoğlu *et al.*, 2010).

Due to these controversies that have not yet demonstrated whether vitamin D deficiency is a result or primary phenomenon of RA, new findings are needed to help understand the association between the RA disease activity and levels of 25 (OH) D.

Therefore, the main objectives of this retrospective study include: (1) to analyze serum levels of 25 (OH) D in patients with RA in comparison to healthy matched controls; (2) to investigate a possible correlation with disease activity.

METHODS

Study population. The study was performed on a group of 37 patients with RA and 21 healthy subjects matched for age and gender, as controls.

Patients were included in this study only if they met all of the following criteria: 1. Adults aged >30 years, 2. Definite diagnosis of RA according to the 1987 ACR criteria, 3. Disease duration for more than 1 year, 4. Treatment with at least one or more disease modifying antirheumatic drugs (DMARDS) or biologic agents, but not cortisone, 5. Data were collected during winter.

Exclusion criteria were as follows: cardiopulmonary diseases, diabetes mellitus treated by insulin, uncontrolled arterial hypertension, dyslipidemia, chronic kidney disease, thyroid dysfunction, Cushing's syndrome, osteoporosis, current smokers, diseases associated with hypercalcaemia (lymphoma, sarcoidosis, tuberculosis infection, primary hyperparathyroidism), treatment with vitamin D and/or calcium supplementation or drugs affecting the bone and mineral metabolism.

The control group had no rheumatic diseases or other skeletal symptoms based on history and clinical examination.

Clinical measurements and laboratory tests. In all subjects, the following data were collected: age, gender, disease duration, body weight, height, and body mass index (BMI: Kg/m^2).

Blood samples were taken from all patients in order to assess: erythrocyte sedimentation rate (ESR), white blood cells (WBC), hemoglobin (Hb), platelets (PLT), serum calcium (Ca), serum phosphorus (Phos), using standard laboratory methods. The IgM-Rhematoid Factor (RF) (nephelometric method) and anti-citrullinated protein antibodies (ACPA) (immune-enzymatic method) were also assessed. In order to evaluate vitamin D status in our study population, serum levels of 25(OH) vitamin D (ng/ml), the most stable circulating form of this molecule, were also measured using the chemiluminescent immunoassay technique.

In controls, the following were determined: erythrocyte sedimentation rate (ESR), white blood cells (WBC), hemoglobin (Hb), platelets (PLT), serum calcium (Ca), serum phosphorus (Phos), and vitamin D levels, using the same methods.

The rheumatoid arthritis activity was assessed using the Disease Activity Score 28 (DAS28). DAS28 was calculated based on ESR, tender joint count (28 joints), swollen joint count (28 joints), and the patient's assessment of global well-being (100 mm visual analogue scale).

High disease activity was defined as DAS28>5.1, moderate disease activity was defined as 3.2<DAS28≤5.1, and low disease activity was defined as 2.6≤DAS28≤3.2, while remission was set as DAS28≤2.60 (Feser *et al.*, 2009).

Values of vitamin D≥30 ng/ml were considered as normal, while vitamin D insufficiency was defined as a level between 15 and 29 ng/ml, and vitamin deficiency at levels<15 ng/ml (Watson, 2013).

All subjects gave their informed consent for inclusion before they participated in the study. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Ethics Committee.

Characteristics of RA patients and controls. The two groups were similar in terms of age, height, weight and BMI. Thus, mean age in the RA patients and control group was 57±12.58 and 50.19±16.41, respectively. Also, the mean BMI of the RA patients was 26.48±4.44 kg/m^2 versus 26.76±3.6 kg/m^2 of the controls, indicating an overweight sample.

A higher level of serum ESR was observed in RA patients, when compared *to healthy* controls.

RA patients were positive for the rheumatoid factor and anticitrullinated peptide antibodies. All patients enrolled in the study received a DMARD treatment without concomitant corticosteroids or vitamin D supplementation. Thus, 56.8% of the patients received methotrexate, 32.4% leflunomide, and 5.45% sulfasalazine.

Statistical analysis. Data analysis was performed using SPSS v.17. Continuous variables were presented as mean and standard deviations, and nominal data were presented as counts (percentages). We performed a descriptive and inferential statistics analysis to summarize the characteristics of the study population. In order to evaluate the proportion of categorical data in the groups, we applied the chi-squared test ($\chi 2$). The results of the Shapiro-Wilk normality test showed a non-Gaussian distribution, which is why we continued to use nonparametric tests. To compare patients with/without RA or vitamin D deficiency, we applied the Mann-Whitney U test. In order to highlight the relationship between group characteristics and levels of vit D we performed Spearman's correlation. A multivariate linear regression analysis was used to evaluate the independent factors associated with the DAS28 variable and risk of vit D deficiency. A p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

The demographic data of patients and controls are presented in Table 1.

We registered significant differences between the groups, with lower mean values of 25(OH) vitD in the RA patients than in the controls (p=0.003).

25(OH) vitD deficiency, insufficiency, and sufficiency were found in 30% (n=11), 54% (n=20) and 16% (n=6) of the RA patients, while 25(OH)D deficiency, insufficiency and sufficiency were found in 9% (n=2), 43% (n=9) and 48% (n=10) of the controls.

In our study group, the mean score of DAS28-ESR was 3.59±1.05, providing a moderate disease activity group. Moreover, the 25-OH vitamin D levels of the patients from the high disease activity group were found to be the lowest, but they were not significantly different between the groups.

The Spearman nonparametric correlation test revealed that there was a significantly weak positive correlation between DAS28, WBC (p=0.013) and Phos (p=0.049). In contrast, correlation was strong in the case of ESR (p<0.001) (Table 2).

We found no significant correlation between 25OH-vitD and DAS28 or other studied parameters.

Following the linear regression analysis, only ESR was established as a significant predictive factor for DAS28 (p=0.003) (Table 3).

The baseline characteristics of RA patients according to the disease activity are presented in Table 4. About

Table 1. Characteristics of RA patients (n=37) and healthy controls (*n*=21)

Variable	RA patients	Healthy controls	P
Age (years)[a]	57±12.58	50.19±16.41	0.083
Female (%)[b]	28 (75.7%)	21(100%)	0.012*
Disease duration (years)[a]	2.73±2.22	–	–
Height (cm)[a]	164.97±8.49	163.81±6.19	0.820
Weight (kg)[a]	72.19±14.29	71.78±10.43	0.428
BMI (kg/m2)[a]	26.48±4.44	26.76±3.6	0.588
WBC (×1000/μL)[a]	7.01±2.1	7.63±1.99	0.234
Hb (g/dl)[a]	12.9±1.68	13.76±0.96	0.031*
PLT(×1000/μL)[a]	269.68±99.06	261.86±75.5	0.871
25OHVit D (ng/ml)[a]	20.34±8.36	27.87±9.81	0.003*
Ca (mmol/l)[a]	9.48±0.41	9.27±0.41	0.099
Phos (mmol/l)[a]	3.27±0.67	4.28±0.94	<0.001*
ESR (mm/h)[a]	25.54±20.18	12.33±10.14	0.002*
DAS28-ESR[a]	3.59±1.05	–	–
Treatment: Leflunomide (%)[b] Methotrexate (%)[b] Sulfasalazine (%)[b]	 12 (32.4%) 21 (56.8%) 2 (5.4%)	–	–

[a]Mean ±S.D.; Mann-Whitney U Test; [b]number (percentage); Chi-square test; significance threshold value reached

Table 2. Correlation between DAS28 and other studied parameters (Spearman nonparametric correlation test)

DAS28	Age	Disease duration	BMI	WBC	Hb	PLT	Ca	Phos mmol/l	ESR
Spearman Coefficient	–0.038	–0.273	–0.071	0.405*	–0.189	–0.019	0.079	0.326*	0.693*
p – value	0.825	0.102	0.676	0.013	0.263	0.912	0.644	0.049	<0.001

*Significance threshold value reached

Table 3. Predicted variables of the linear regression model (by the Enter method) considering DAS28 as the dependent variable

Predictors	Unstandardized coefficients		Standardized coefficients	T	Sig.
	B	Std. Error	Beta		
(Constant)	–19.624	33.354		–0.588	0.562
Age	–0.008	0.013	–0.095	–0.602	0.553
Disease duration	–0.112	0.073	-0.237	-1.543	0.136
Height	0.124	0.188	0.998	0.656	0.518
Weight	–0.134	0.201	–1.828	–0.667	0.511
BMI	0.346	0.551	1.460	0.628	0.536
WBC	0.127	0.082	0.254	1.561	0.132
Hb	0.072	0.124	0.116	0.582	0.566
PLT	–0.003	0.002	–0.241	–1.609	0.121
25OHVitD	–0.026	0.021	–0.204	–1.199	0.242
Ca	0.238	0.487	0.093	0.489	0.629
Phos (mmol/l)	0.144	0.292	0.092	0.492	0.627
ESR	0.031	0.009	0.600	3.356	0.003*

*Significance threshold value reached

Table 4. Characteristics of rheumatoid arthritis patients based on the disease activity status.

Variable	Remission (DAS 28 < 2.6)	Low disease activity (2.6≤DAS 28≤3.2)	Moderate disease activity (3.2<DAS 28≤5.1)	High disease activity (DAS 28 > 5.1)	*p*
	(N=8, 22%)	(N=7, 19%)	(N=15, 40%)	(N=7, 19%)	
Age[a]	66.13±5.69	48.29±13.34	53.13±11.75	63.57±11.13	0.009*
Sex[b]	5 (62.5%)	5 (71.4%)	12 (80.0%)	6 (85.7%)	0.715
Disease duration[a]	2.44±1.24	2.79±1.29	3.2±2.91	2±2.24	0.292
Height[a]	165.88±6.92	164.57±12.03	165.93±6.63	162.29±10.83	0.500
Weight[a]	73.75±15.54	72.14±16.21	71.8±14.22	71.29±14.28	0.979
BMI[a]	26.63±3.66	26.49±4.43	26.19±5.65	26.93±2.83	0.814
WBC[a]	6.58±2.45	6.94±2.06	6.35±1.35	9.01±2.2	0.025*
Hb[a]	13.49±2.32	12.99±1.43	12.86±1.26	12.21±1.98	0.775
PLT[a]	271.75±106.59	287.14±122.16	269.4±75.57	250.43±128.76	0.857
25OHvitD[a]	19.95±6.75	22.55±9.44	22.37±9.07	14.24±5.06	0.212
Ca[a]	9.52±0.5	9.45±0.14	9.33±0.4	9.79±0.41	0.152
Phos[a]	3.17±0.87	2.98±0.79	3.3±0.62	3.61±0.26	0.181
ESR[a]	12.38±4.14	17.86±21.66	27.53±17.3	44±23.42	0.003*

[a]Mean ±S.D.; Kruskal-Wallis Test; [b]number (percentage); Chi-square test; *significance threshold value reached

19% (*n*=7) of the participants were classified as showing high disease activity, 40% (*n*=15), 19% (*n*=7), and 22% (*n*=8) were classified as having moderate, low disease activity, and in remission, respectively.

Although, age was significantly higher in the remission group and in the high disease activity group (Kruskal-Wallis Test, *p*=0.009), no differences between the remission and the high disease activity groups were observed (Mann-Whitney U Test, *p*=0.861).

However, the mean value of vitamin D level was 14.24±5.06 ng/mL in the high disease activity group, 22.37±9.07 ng/mL in the moderate disease activity group, and 22.55±9.44 ng/mL in the low disease activity group.

Also, ESR values are significantly increased in the high disease activity group (Kruskal-Wallis test, *p*=0.003). However, the ESR values in the high disease activity group are not significantly different when compared to the moderate disease activity group (Mann-Whitney U test, *p*=0.067).

DISCUSSION

Our study showed that vitamin D deficiency and insufficiency are more prevalent in patients with RA than in healthy controls. The mean value of vitamin D was significantly lower in the RA patients than in controls (*p*=0.003).

Although many studies have shown that vitamin D deficiency is common in RA, other studies did not find significant differences between RA patients and healthy controls (Craig *et al.*, 2010; Cutolo *et al.*, 2006; Hitchon *et al.,* 2012; Turhanoğlu *et al.*, 2010).

A large study from 15 countries, the COMORA study, explored the status of vitamin D in RA patients. The authors concluded that vitamin D deficiency, in patients with RA, varies between different countries. This variability may be explained by the association of many factors such as: racial or ethnic differences, climates, lack of sun exposure, skin color, clothing style, or latitude. Until recently, vitamin D insufficiency and deficiency were common in countries with less sun, particularly in North versus South Europe, with a circannual rhythm (Cutolo *et al.*, 2006). In fact, current data indicates that tropical or subtropical areas, such as Central America and the Middle East, are also widely affected. This finding could be explained by several factors: avoidance of sun exposure, colored skin, wearing protective clothing, country latitude, vitamin D receptor (VDR) gene polymorphism and its expression (Vojinovic *et al.*, 2017)

We believe that this variability is also determined by characteristics of the RA patients included in different studies, by inclusion and exclusion criteria taken into account or by methodology used in these studies.

Another large multicentric study collected data on vitamin D serum levels in 625 RA patients from 13 different European countries. Similarly to our study, the mean serum concentration of 25(OH)D in RA patients was significantly lower when compared to controls. 25(OH) D deficiency was found in 66% of the RA patients, and insufficiency was found in 27% of them (Merlino *et al.*, 2004). On the contrary, in our study 54% of the patients had insufficiency, and only 30% had deficiency, while 16% of the RA patients had normal serum levels of vitamin D.

The study found a statistically significant difference in vitamin D levels between some European countries, with significantly higher 25(OH)D serum concentrations in RA patients from Spain, Latvia, and Serbia when compared to Romania (Merlino *et al.*, 2004).

Although several studies have shown that vitamin D levels are inversely related to the RA disease activity, we found no correlation between the 25(OH)D levels and disease activity in our cohort. However, despite the lack of a significant correlation, our patient population had a well-controlled disease. This weak correlation may be explained by the absence of other assessed factors that could be involved in the functional deficiency of vitamin D (seasonal differences, sun exposure, nutritional status, skin color, clothing style, etc).

So far, evidence for the relationship between hypovitaminosis D and RA disease activity is contradictory (Ga-

Table 5. Summary of the studies conducted on correlation between DAS and serum 25(OH)D values in RA patients

Author/year of publication/Country	Mean age (years)	Number of subjects	Vitamin D deficiency (%)	Vitamin D insufficiency (%)	Findings
Craig *et al.*, 2010/ USA	51	266 RA patients	49.6%	14.7	Significant inverse correlation between serum 25(OHD) levels and DAS28 (by univariate analysis)
Rossini *et al.*, 2010/ Italy	58	1191 RA patients 1019 controls	52%	–	Inverse association of serum 25OHvitD levels with disease activity
Braun-Moscovici *et al.*, 2011/USA	56	85 RA patients	42.1%	–	No correlation between vitamin D levels and DAS28, ESR, CRP
Higgins *et al.*, 2013/ UK	64	176 RA patients	18%	50%	No significant correlation between vitamin D and DAS28 scores with or without the inclusion of VAS
Sahebari *et al.*, 2014/ Iran	44	99 RA patients 68 controls	–	75.7%	No correlation between 25(OH)D serum values and DAS28, duration of disease, anti CCP, ESR, VAS, and the number of tender and swollen joints.
Azzeh *et al.*, 2015/ Saudi Arabia	50	102 RA patients	57.8%	31.4%	Significant inverse correlation between serum 25(OHD) levels and DAS28
Akkar *et al.*, 2016/ Morocco	52	73 RA patients	17.8%	70%	Vitamin D deficiency is associated with higher disease activity
Gamal *et al.*, 2016 Egypt	43.8	55 RA patients and 25 healthy controls	–	21.8%	Significant negative correlation between 25(OH)D and ESR No correlation between 25(OH)D and other parameters
Hajjaj-Hassouni *et al.*, 2017/ COMORA Study	57.9	1413 RA patients	8.5%	54.6%	Vitamin D levels were inversely correlated with disease activity DAS28
Vojinovic *et al.*, 2017/ European multicentre pilot study	55	625 RA patients 276 controls	66%	27%	Negative correlations were found between 25(OH)D serum levels and DAS28-CRP, and HAQ scores
Polasik *et al.*, 2017/ Poland	48	35 RA patients 38 controls	71.43%	17.14%	No significant correlation between vitamin D and DAS28 scores or joint damage (Steinbrocker criteria) A positive correlation between 25(OH)D and IL-6 was observed

mal *et al.*, 2016; Higgins *et al.*, 2013; Polasik *et al.*, 2017; Rossino *et al.*, 2010).

In a prospective cohort study on 29,368 women without a history of RA at baseline, it was demonstrated that low or deficient levels of 25(OH)D were inversely associated with the risk for development of RA (n=152) (Akkar *et al.*, 2016). However, correlation between the disease activity and vitamin D deficiency has been demonstrated in many other studies (Azzeh *et al.*, 2015; Braun-Moscovici *et al.*, 2011; Hajjaj-Hassouni *et al.*, 2017; Sahebari *et al.*, 2014).

The findings of several studies included in our literature review and the characteristics of the study participants (mean age, number of subjects, vitamin D deficiency and insufficiency) are summarized in Table 5.

Limitations of the study

The study presented here has some limitations. The first limitation is the small sample size, being a single-center study. Study participants were enrolled from the Western part of Romania where sun exposure rates (and resulting vitamin D concentrations) may differ from other populations living in other geographical areas. Moreover, we did not assess other factors that could be involved in the functional deficiency of vitamin D (seasonal differences, sun exposure, nutritional status, skin color, clothing style, etc).

Secondly, only the serum 25(OH)D was determined, but not 1.25-hydroxyvitamin D. We did not collect data on parathyroid hormone levels or other parameters involved in vitamin D deficiency.

Finally, another limitation is the lack of a follow-up study in our population, given that all parameters were assessed only at enrollment.

CONCLUSIONS

We observed that vitamin D deficiency is more common in the RA patients than in the controls. There is no significant association of serum 25(OH)D with disease severity in a Western Romanian cohort with RA.

We found that the serum values of 25 (OH)D are not a predictive factor for the disease activity in the RA patients. The hypothesis of vitamin D deficiency as a predisposing factor in triggering an increased activity of the disease needs to be tested on a larger group of RA patients. However, our results could have implications for disease management, as patients with RA could be supplemented with vitamin D even in the absence of disease activity.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Akkar O, Ichchou L (2016) Vitamin D status and its association with disease activity, severity and physical disability in rheumatoid arthritis patients. *Ann Rheum Dis* **75** (Suppl 2): 711. https://doi.org/10.1136/annrheumdis-2016-eular.5083

Azzeh FS, Kensara OA (2015) Vitamin D is a good marker for disease activity of rheumatoid arthritis disease. *Dis Markers* 260725. https://doi.org/10.1155/2015/260725

Bellan M, Pirisi M, Sainaghi PP (2015) Osteoporosis in rheumatoid arthritis: role of the vitamin D/parathyroid hormone system. *Rev Bras de Reumatol* **55**: 256–263. https://doi.org/10.1016/j.rbr.2014.10.007

Braun-Moscovici Y, Toledano K, Markovits D, Rozin A, Nahir AM, Balbir-Gurman A (2011) Vitamin D level: is it related to disease activity in inflammatory joint disease? *Rheumatol Int* **31**: 493–499. https://doi.org/10.1007/s00296-009-1251-6

Bumbea AM, Mușetescu AE, Albulescu DM, Dumitrașcu RC, Bumbea BȘ, Popescu D, Albu VC (2017) The occurrence risk of demyelinating lesions in patients with rheumatoid arthritis and anti-TNF α biological therapy. *Farmacia* **65**: 917–922

Craig SM, Yu F, Curtis JR, Alarcón GS, Conn DL, Jonas B, Callahan LF, Smith EA, Moreland LW, Bridges Jr SL, Mikuls TR (2010) Vitamin D status and its associations with disease activity and severity in African Americans with recent-onset rheumatoid arthritis. *J Rheumatol* **37**: 275–281. https://doi.org/10.3899/jrheum.090705

Cutolo M (2009) Vitamin D and autoimmune rheumatic diseases. *Rheumatol* **48**: 210–212. https://doi.org/10.1093/rheumatology/ken394

Cutolo M, Otsa K, Laas K, Yprus M, Lehtme R, Secchi ME, et al (2006) Circannual vitamin D serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe. *Clin Exp Rheumatol* 24: 702–704

Feldmann D, Pike JW, Bouillon R, Giovannucci E, Goltzman D, Hewison M (2017) Vitamin D: Health, Disease and Therapeutic. In *Vitamin D*, 4th edn, pp 142–149. Academic Press.

Feser M, Derber LA, Deane KD, Lezotte DC, Weisman MH, Buckner JH, Mikuls T, O'Dell J, Gregersen PK, V Holers M, Norris JM (2009) Plasma 25,OH vitamin D concentrations are not associated with rheumatoid arthritis (RA)-related autoantibodies in individuals at elevated risk for RA. *J Rheumatol* **36**: 943–946. https://doi.org/10.3899/jrheum.080764

Fransen J, Stucki G, Van Riel P (2003) Rheumatoid arthritis measures: disease activity score (DAS), disease activity score-28 (DAS28), rapid assessment of disease activity in rheumatology (RADAR), and rheumatoid arthritis disease activity index (RADAI) *Arthritis Care Res* 49, Suppl, pp S214–S224. https://doi.org/10.1002/art.11407

Gamal RM, Gaber W, Sayed S, Mohey AM, Goma SH, Mohamed MSE (2016) Vitamin D status in Egyptian patients with rheumatoid arthritis. *Akt Rheumatol* **41**: 492–498. http://doi.org/10.1055/s-0035-1545308

Hajjaj-Hassouni N, Mawani N, Allali F, Rkain H, Hassouni K, Hmamouchi I, Dougados M (2017) Evaluation of vitamin D status in rheumatoid arthritis and its association with disease activity across 15 countries: "The COMORA Study". *Int J Rheumatol* 5491676. https://doi.org/10.1155/2017/5491676

Haque UJ, Bartlett SJ (2010) Relationships among vitamin D, disease activity, pain and disability in rheumatoid arthritis. *Clin Exp Rheumatol* **28**: 745–747.

Higgins MJ, Mackie SL, Thalayasingam N, Bingham SJ, Hamilton J, Kelly CA (2013) The effect of vitamin D levels on the assessment of disease activity in rheumatoid arthritis. *Clin Rheumatol* **32**: 863–867. https://doi.org/10.1007/s10067-013-2174-x

Hitchon CA, Sun Y, Robinson DB, Peschken CA, Bernstein CN, Siminovitch KA, El-Gabalawy HS (2012) Vitamin D receptor polymorphism rs2228570 (Fok1) is associated with rheumatoid arthritis in North American natives. *J Rheumatol* **39**: 1792–1797. https://doi.org/10.3899/jrheum.120387

Kerr GS, Sabahi I, Richards JS, Caplan L, Cannon GW, Reimold A, Thiele GM, Johnson D, Mikuls TR (2011) Prevalence of vitamin D deficiency in rheumatoid arthritis and associations with disease severity and activity. *J Rheumatol* **38**: 53–59. https://doi.org/10.3899/jrheum.100516

Marques CD, Dantas AT, Fragoso TS, Duarte AL (2010) The importance of vitamin D levels in autoimmune diseases. *Rev Bras de Reumatol* **50**: 67–80. https://dx.doi.org/10.1590/S0482-50042010000100007

Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG (2004) Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. *Arthritis Rheum* **50**: 72–77. https://doi.org/10.1002/art.11434

Myasoedova E, Davis J, Matteson EL, Crowson CS (2020) Is the epidemiology of rheumatoid arthritis changing? Results from a population-based incidence study, 1985–2014. *Ann Rheum Dis* **79**: 440–444. http://dx.doi.org/10.1136/annrheumdis-2019-216694

Polasik K, Piotrowska E, Lipinska B, Witkowski JM, Bryl E, Tukaj S (2017) Vitamin D status in patients with rheumatoid arthritis: a correlation analysis with disease activity and progression, as well as serum IL-6 levels. *Acta Biochim Polon* **64**: 667–670. https://doi.org/10.18388/abp.2017_1636

Rossini M, Maddali Bongi S, La Montagna G, Minisola G, Malavolta N, Bernini L, Cacace E, Sinigaglia L, Di Munno O, Silvano Adami (2010) Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability. *Arthritis Res Ther* **12**: R216. https://doi.org/10.1186/ar3195

Sahebari M, Mirfeizi Z, Rezaieyazdi Z, Rafatpanah H, Goshyeshi L (2014) 25(OH) vitamin D serum values and rheumatoid arthritis disease activity (DAS28 ESR). *Caspian J Int Med* **5**: 148–155

Șuța C, Șuța M, Craiu E (2014) Prevalence of rheumatoid arthritis in Caucasian population across Europe. *Romanian J Rheumatol* **4**: 169–174

Tan LM, Long TT, Guan XL, Wu SF, Zheng W, Fu HY, Wang QH, Meng YM, Wu Y, Zeng TT, Tian YJ, Yu JL, Chen JJ, Li H, Cao LP (2018) Diagnostic value of vitamin D status and bone turnover markers in rheumatoid arthritis complicated by osteoporosis. *Ann Clin Lab Sci* **48**: 197–204

Tetlow LC, Smith SJ, Mawer EB, Woolley DE (1999) Vitamin D receptors in the rheumatoid lesion: expression by chondrocytes, macrophages, and synoviocytes. *Ann Rheum Dis* **58**: 118–121.

Turhanoğlu AD, Güler H, Yönden Z, Aslan F, Mansuroglu A, Ozer C (2010) The relationship between vitamin D and disease activity and functional health status in rheumatoid arthritis. *Rheumatol Int* **31**: 911–914. https://doi.org/10.1007/s00296-010-1393-6

Vojinovic J, Tincani A, Sulli A, Soldano S, Andreoli L, Dall'Ara F, Ionescu R, Simic Pasalic K, Balcune I, Ferraz-Amaro I, Tlustochowicz M, Butrimiene I, Punceviciene E, Toroptsova N, Grazio S, Morovic-Vergles J, Masaryk P, Otsa K, Bernardes M, Boyadzhieva V, Salaffi F, Cutolo M (2017) European multicentre pilot survey to assess vitamin D status in rheumatoid arthritis patients and early development of a new Patient Reported Outcome questionnaire (D-PRO). *Autoimmun Rev* **16**: 548–554. https://doi.org/10.1016/j.autrev.2017.03.002

Watson RR (2013) Handbook of vitamin D in human health: Prevention, treatment and toxicity. In *Defining vitamin D deficiency*. Wageningen Academic Publishers, pp 551–552. http://dx.doi.org/10.3920/978-90-8686-765-3