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U. Müller-Ladner, Bad Nauheim
U. Lange, Bad Nauheim

Yang Liu · Hongyan Wen

Department of Rheumatology, the Second Hospital of Shanxi Medical University, Shanxi Medical University, Taiyuan, China

Impact of vitamin D deficiency on clinical parameters in treatment-naïve rheumatoid arthritis patients

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease which has a prevalence of approximately 0.5–1% of the population of the industrialized world [1]. It is characterized by cartilage destruction and bone erosion, eventually leading to a high risk of morbidity and mortality. The etiopathogenesis of RA remains unknown, with the involvement of more than one mechanism in disease development. Emerging evidence suggests that vitamin D plays an important role in immune regulation, including RA. Beyond its endocrine role in bone metabolism, vitamin D is endowed with remarkable immunomodulatory properties, acting as a hormone precursor [2]. The importance of the immune cell as a target of vitamin D in RA was illustrated by Wei et al. [3]. Vitamin D could modulate innate immunity by promoting monocytes' transformation into macrophages and affecting the release of related chemokines and cytokines [2]. In the adaptive immune system, vitamin D could inhibit B cells' and T cells' proliferation and differentiation, and downregulates the expression of immunoglobulins and autoantibodies to regulate humoral and cellular immunity [4, 5]. Despite evidence supporting the inverse association between the levels of vitamin D and disease activity [6, 7], results of previous studies are contradictory [8] regarding the level of vitamin D and affliction with disease. Besides, there are few studies targeting the association between the absolute numbers of immunocytes and vitamin D in RA. Therefore, there still needs to be a comprehensive study to assess the issue. Given this gap in the literature, this study was performed to determine if patients with RA are at higher risk of vitamin D deficiency and to explore a potential association between vitamin D and clinical/experimental characteristics of RA.

Methods

Patients and enrolment criteria

In a cross-sectional case–control study, we evaluated the charts of over 6000 RA patients. A total of 280 consecutive treatment-naïve RA patients from rheumatologic clinic of the Second Hospital of Shanxi Medical University were enrolled from September 2015 to November 2016. All patients fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for RA [9]. The 140 age- and sex-matched healthy volunteers were mostly recruited from patients' acquaintances that lived with them (patients' relatives), to minimize the influence of lifestyles such as dressing and nutritional habits on vitamin D status. Patients receiving or who had ever received vitamin D, corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs), or tumor necrosis factor antagonists, and those who had hepatic or renal insufficiency were excluded. Additional exclusion criteria were as follows: older than 85 years, malabsorption, pregnancy, lactation, systemic lupus erythematosus (sunlight exposure limited), malignancy, diabetes mellitus, hyperthyroidism, celiac disease, inflammatory bowel diseases, current and/or long-term usage of tuberculosis or fungal medications.

Study design

Demographic data like gender, age, weight, height, body mass index (BMI = kg/m^2), and disease duration (time elapsed since symptom onset) were included. Stratified by BMI, RA individuals were classified into four groups: underweight (BMI < 18.5 kg/m^2), normal (BMI 18.5–23.9 kg/m^2), overweight (BMI ≥ 24 kg/m^2), and obese (BMI ≥ 28 kg/m^2) [10]. DAS28-CRP scores were calculated using following equations for patients with complete data [11]:

DAS28-CRP = [0.56 * sqrt (tender joint count) + 0.28 * sqrt (swollen joint count) + 0.36 *ln (CRP +)] * 1.10 + 1.15.

Patients were categorized into three groups by DAS28: high disease activity (DAS28: >5.1), moderate disease activity (DAS28: 3.2–5.1), and low disease activity (DAS28: <3.2) [12, 13].

Vitamin D deficiency was defined as 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), the active form of vitamin D, levels <25 ng/ml, as measured by enzyme-linked immunosorbent assay (ELISA) in accordance with expert consensus [14]. Erythrocyte sedimentation rate (ESR) was analyzed by the Westergren method. Immunophenotypes were determined by a FACSCalibur flow cy-

Table 1 The RA individuals were compared with the healthy volunteers in general ($\bar{x} \pm s$)

Group	Gender	*N*	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)
RA individuals	Male	72	55.83 ± 12.06	66.73 ± 11.53	1.69 ± 0.07	23.10 ± 3.39
	Female	208	52.29 ± 14.71	58.18 ± 9.30	1.60 ± 0.06	22.77 ± 3.25
	Total	280	53.55 ± 13.87	61.23 ± 10.90	1.63 ± 0.08	22.89 ± 3.28
Healthy volunteers	Male	42	52.21 ± 11.22	63.59 ± 7.08	1.64 ± 0.06	23.55 ± 2.40
	Female	98	50.45 ± 12.16	60.18 ± 6.44	1.60 ± 0.03	23.29 ± 2.57
	Total	140	51.32 ± 11.87	61.43 ± 6.84	1.62 ± 0.04	23.40 ± 2.48

Results are given as mean ± standard deviation
RA rheumatoid arthritis, *N* numbers of cases, *BMI* body mass index

Table 2 Main characteristics of RA patients and healthy volunteers, and the association between RA patients with vitamin D insufficiency or with vitamin D sufficiency

Variables	Healthy volunteers	All RA patients *N* = 280	RA patients with vitamin D insufficiency *N* = 265	RA patients with vitamin D sufficiency *N* = 15	*P*-value (RA with vitamin D insufficiency versus sufficiency)
Women	98 (70%)	208 (74.3%)	201 (75.8%)	7 (46.7%)	**0.012**
Man	42 (30%)	72 (25.7%)	64 (24.2%)	8 (53.3%)	**0.001**
Age (years)	51.32 ± 11.87	53.55 ± 13.87	56.1 ± 12.20	51.9 ± 13.20	0.075
$1,25(OH)_2D_3$ (ng/ml)	21.08 ± 7.14	12.24 ± 6.68	11.15 ± 4.74	31.62 ± 6.46	**0.001**
Disease duration (years)	–	9.00 ± 9.34	9.02 ± 9.374	8.78 ± 8.94	0.925
ESR (mm/h)	–	59.06 ± 36.48	59.33 ± 36.73	54.47 ± 32.54	0.617
DAS28	–	4.75 ± 1.43	4.77 ± 1.43	4.51 ± 1.53	0.494
T cell (/μl)	1338.15 ± 358.27	1242.47 ± 585.53	1249.32 ± 586.75	1122.93 ± 569.67	0.417
B cell (/μl)	210.30 ± 84.83	204.40 ± 182.28	208.21 ± 185.97	137.87 ± 72.57	0.146
NK cell (/μl)	289.58 ± 128.33	237.42 ± 187.45	233.78 ± 188.78	300.93 ± 153.99	0.178
Th1 cell (/μl)	87.65 ± 76.95	88.29 ± 116.11	87.58 ± 116.66	103.38 ± 108.91	0.691
Th2 cell (/μl)	10.95 ± 6.43	12.73 ± 10.82	12.85 ± 10.99	10.12 ± 5.79	0.461
Th17 cell (/μl)	7.54 ± 4.07	9.27 ± 9.02	9.33 ± 9.15	8.01 ± 6.06	0.669
Treg cell (/μl)	33.77 ± 13.67	33.46 ± 23.46	33.00 ± 22.44	41.4.0 ± 37.44	0.438
Th17/Treg	0.25 ± 0.15	0.42 ± 0.64	0.42 ± 0.65	0.41 ± 0.45	0.964

Results are given as mean ± standard deviation
RA rheumatoid arthritis, *ESR* erythrocyte sedimentation rate, *DAS28* 3-variable Disease Activity Score, *NK cell* natural killer cell, *Th1cell* T helper 1cell, Th2 cell T helper 2 cell, *Th17 cell* T helper 17 cell, *Treg cell* $CD4^+$ regulatory T cell, *Th17/Treg* the radio of Th17 cell versus Treg cell
The levels of vitamin D insufficiency were defined as <25 ng/ml; the levels of vitamin D sufficiency were defined as >25 ng/ml

tometer (Becton Dickinson Bio, USA). The cells were stained with following antibodies: total T cell ($CD3^+CD19^-$; normal range [NR]: 955 ~ 2860/μl), total B cell ($CD3^-CD19^+$; NR: 90 ~ 560/μl), natural killer (NK) cell ($CD3^-/CD16^+CD56^+$; NR: 150 ~ 1100/μl), T helper 1 (Th1) cell (IFN-γ; NR: 5.52 ~ 182/μl), T helper 2 (Th2) cell (IL-4; NR: 4.04 ~ 21/μl), T helper 17 (Th17) cell (IL-17; NR: 3.07 ~ 14/μl), and regulatory T (Treg) cell ($CD4^+CD25^+Foxp3$; NR:17.7 ~ 54.2/μl). The ratio of Th17 cell versus Treg cell (Th17/Tregs) was calculated (NR: 0.09 ~ 0.47). Serum autoantibodies including rheumatoid factor (RF), antiperinuclear factor (APF), anti-keratin antibody (AKA), anti-mutated citrullinated vimentin (anti-MCV), and anti-cyclic-citrullinated peptides (anti-CCP) antibodies were examined by the chemiluminescence microparticle immunoassay. Quality control protocols were included, running known standards each day before testing samples. In addition, the laboratory was enrolled in external quality assurance testing programs with the College of American Pathologists and the United Kingdom National External Quality Assurance Service.

Statistical analysis

Nominal variables results were expressed as mean ± standard deviation (SD). Categorical variables were compared by chi-square test, and continuous variables were compared by one-way analysis of variance. Linear regression was performed to examine associations and estimate the contributing factors affecting serum $1,25(OH)_2D_3$ levels. *P* value of less than 0.05 was considered statistically significant. Statistical analyses were performed by SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

Results

Clinical information and serum $1,25(OH)_2D_3$ levels of RA individuals and healthy volunteers

There was no significant difference in age, gender, and BMI between RA individuals and healthy volunteers ($p > 0.05$; **Table 1**). The average age of RA patients was 53.55 ± 13.87 years, ranging from 22 years to 85 years. Among all the subjects, 208 (72.7%) were female. The mean serum $1,25(OH)_2D_3$ levels of the RA patients were significantly lower than those of healthy controls (12.24 ± 6.68 ng/ml versus 21.08 ± 7.14 ng/ml; $p < 0.05$; **Table 2**). The main RA-related findings with vitamin D insufficiency or with vitamin D sufficiency are shown in **Table 2**, which also includes the main characteristics of the healthy controls. Using recommended cut-off points shown in **Table 3**, 132 (46.2%) RA patients had severe vitamin D deficiency

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Y. Liu · H. Wen

Impact of vitamin D deficiency on clinical parameters in treatment-naïve rheumatoid arthritis patients

Abstract

Objective. To determine if patients with rheumatoid arthritis (RA) are at risk of vitamin D deficiency and whether the levels of vitamin D are correlated with clinical parameters in RA.

Methods. A total of 280 treatment-naïve RA patients, and 140 age- and sex-matched healthy volunteers were enrolled. Serum levels of 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), the active form of vitamin D, were measured by enzyme-linked immunosorbent assay (ELISA). Concentrations of $1,25(OH)_2D_3$ less than 25 ng/mL were defined as insufficient. Linear regression was performed to evaluate correlations as (modifying and) confounding factors were controlled.

Results. The levels of serum $1,25(OH)_2D_3$ in RA individuals (12.24 ± 6.68 ng/ml) were significantly lower than in healthy controls (21.08 ± 7.14 ng/ml; $p < 0.05$). An inverse association was found between the levels of $1,25(OH)_2D_3$ and ESR in obese and overweight individuals with RA ($\beta_{obese} = -0.385$, $\beta_{overweight} = -0.395$, both $p < 0.05$), but not in normal and underweight subjects. A significant negative association between levels of $1,25(OH)_2D_3$ and DAS28 score ($\beta = -0.164$, $p = 0.018$) was observed. The levels of $1,25(OH)_2D_3$ were associated moderately and inversely with the absolute numbers of Th-17 ($\beta = -0.158$, $p = 0.019$) and positively with those of $CD4^+$ regulatory T (Treg) cell ($\beta = 0.146$, $p = 0.025$). The levels of $1,25(OH)_2D_3$ in anti-cyclic citrullinated peptide (anti-CCP)-positive patients with RA were lower than in the anti-CCP-negative RA patients (10.86 ng/ml versus 15.98 ng/ml; $t = -3.08$, $p < 0.01$).

Conclusions. A significant association was observed between levels of vitamin D and parameters of disease, including body mass index (BMI), DAS28, Th17 cell counts, Treg cell counts, and presence of anti-CCP antibody in RA patients.

Keywords

1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) · Body mass index (BMI) · DAS28 · Immunocyte · Autoantibodies

Einfluss eines Vitamin-D-Mangels auf klinische Parameter bei therapienaiven Patienten mit rheumatoider Arthritis

Zusammenfassung

Ziel. Ziel war es zu untersuchen, ob Patienten mit rheumatoider Arthritis (RA) von Vitamin-D-Mangel bedroht sind und ob der Vitamin-D-Spiegel mit klinischen Parametern bei RA korreliert ist.

Methoden. In die Studie wurden 280 therapienaive RA-Patienten sowie 140 nach Alter und Geschlecht passende gesunde Probanden aufgenommen. Die Serumspiegel von 1,25-Dihydroxycholecalciferol ($1,25(OH)_2D_3$), der aktiven Form von Vitamin D, wurden mittels ELISA („enzyme-linked immunosorbent assay") gemessen. $1,25(OH)_2D_3$-Konzentrationen unter 25 ng/ml waren als unzureichend definiert. Eine lineare Regressionsanalyse wurde durchgeführt und Störfaktoren berücksichtigt.

Ergebnisse. Bei RA-Patienten waren die $1,25(OH)_2D_3$-Serumspiegel (12,24 ± 6,68 ng/ml) signifikant niedriger als bei den gesunden Kontrollen (21,08 ± 7,14 ng/ml; $p < 0,05$). Ein inverser Zusammenhang fand sich zwischen den Werten für $1,25(OH)_2D_3$ und Blutsenkungsgeschwindigkeit (BSG) bei adipösen und übergewichtigen Personen mit RA ($\beta_{adipös} = -0,385$; $\beta_{übergewichtig} = -0,395$; beide $p < 0,05$), nicht jedoch bei normal- und untergewichtigen Personen. Es wurde eine signifikante negative Assoziation zwischen den $1,25(OH)_2D_3$-Werten und dem Disease Activity Score (DAS28) festgestellt ($\beta = -0,164$; $p = 0,018$). Der $1,25(OH)_2D_3$-Wert war mittelgradig und invers mit der absoluten Zahl an Th-17-Zellen ($\beta = -0,158$; $p = 0,019$) und positiv mit der Zahl der CD4-positiven regulatorischen T-Zellen (Treg-Zellen; $\beta = 0,146$; $p = 0,025$) assoziiert. Die Werte für $1,25(OH)_2D_3$ bei für Anti-CCP (antizyklisches zitrulliniertes Peptid) positiven RA-Patienten waren niedriger als bei den für Anti-CCP negativen RA-Patienten (10,86 ng/ml vs. 15,98 ng/ml; $t = -3,08$; $p < 0,01$).

Schlussfolgerung. Zwischen den Vitamin-D-Spiegeln und Krankheitsparametern einschließlich Body-Mass-Index (BMI), DAS28, Zahl der Th17-Zellen, Zahl der Treg-Zellen und Vorliegen von Anti-CCP-Antikörpern bei RA-Patienten wurde ein signifikanter Zusammenhang beobachtet. Diese Feststellungen stehen möglicherweise in Übereinstimmung mit der Hypothese, dass ein Vitamin-D-Mangel ein prädisponierender Faktor für den Beginn und das Fortschreiten einer RA sein könnte. Vitamin D könnte sich ggf. als alternative Substitutionsbehandlung bei RA eignen.

Schlüsselwörter

1,25-Dihydroxycholecalciferol ($1,25(OH)_2D_3$) · Body-Mass-Index (BMI) · DAS28 · Immunozyt · Autoantikörper

with $1,25(OH)_2D_3$ levels <10 ng/ml, 118 (41.3%) of the subjects had below 20 ng/ml, and only 15 (5.2%) had over 25 ng/ml. Using the suggested threshold (≤25 ng/ml) [14], the overall prevalence of vitamin D insufficiency for RA was 94.8% (265 out of 280) compared with 52.1% (73 out of 140) for healthy controls (**Table 3**).

$1,25(OH)_2D_3$ and BMI in RA individuals

When stratified using subtypes of BMI, a significant difference of serum $1,25(OH)_2D_3$ levels was found among obese (BMI ≥ 28 kg/m²; 5.21 ± 2.08 ng/ml), overweight (BMI ≥ 24 kg/m²; 11.6 ± 6.29 ng/ml), normal-weight (BMI: 18.5–23.9 kg/m²; 16.80 ± 4.12 ng/ml), and underweight (BMI < 18.5 kg/m²; 27.22 ± 3.18 ng/ml) in RA individuals ($F = 31.41$, $p = 0.001$; **Fig. 1a**). An inverse association was found between $1,25(OH)_2D_3$ and ESR in obese and overweight RA individuals ($\beta_{obese} = -0.385$, $\beta_{overweight} = -0.395$, both $p < 0.05$), but not in normal- and underweight (**Table 4**).

Table 3 Distribution of serum $1,25(OH)_2D_3$ levels in the studied cohort

$1,25(OH)_2D_3$ (ng/ml)	RA individuals (%)	Healthy volunteers (%)
>25	15 (5.2)	67 (47.9)
20.1–25	15 (5.2)	58 (41.4)
10.1–20	118 (41.3)	15 (10.7)
<10	132 (46.2)	0 (0)
≤25	265 (94.8)	73 (52.1)

Table 4 The associations of serum $1,25(OH)_2D_3$ concentrations with ESR or DAS28 in RA individuals stratified on subtypes of BMI, disease activity, and anti-CCP

Group		ESR		DAS28 scores	
		N	β-coefficient (*P*)	*N*	β-coefficient (*P*)
BMI	Obese	14	−0.385 (**<0.05**)	14	−0.118 (0.101)
	Overweight	92	−0.395 (**<0.05**)	92	−0.123 (0.401)
	Normal weight	152	−0.001 (0.988)	152	−0.010 (0.843)
	Underweight	22	0.046 (0.531)	22	0.020 (0.675)
Disease activity	High	110	−0.387 (**<0.01**)	110	−0.567 (**<0.01**)
	Moderate	126	−0.072 (0.421)	126	−0.060 (0.505)
	Low	44	−0.132 (0.392)	44	−0.051 (0.741)
Anti-CCP	Positive	230	−0.116 (0.104)	230	−0.464 (**<0.01**)
	Negative	50	−0.039 (0.552)	50	−0.030 (0.644)

RA rheumatoid arthritis, *ESR* erythrocyte sedimentation rate, *DAS28* 3-variable Disease Activity Score, *N* numbers of patients for each group, *BMI* body mass index, *anti-CCP* anti-cyclic-citrullinated peptides

Levels of $1,25(OH)_2D_3$ and disease activity in RA individuals

A considerable proportion of RA patients (39.3%) had a mean DAS28 score of 6.17 ± 0.77, representing high disease activity (DAS28 scores >5.1). In addition, 126 (45.0%) patients (4.30 ± 0.52) had moderate disease activity (DAS28 scores 3.2–5.1), and 44 (15.7%) patients (2.56 ± 0.60) had low disease activity (DAS28 scores <3.2). A significant difference in serum $1,25(OH)_2D_3$ levels was found among those three groups of different disease activity ($F = 554.64$, $p < 0.05$; **Fig. 1b**). Significantly lower values of $1,25(OH)_2D_3$ (8.10 ± 3.64 ng/ml) were found in the high disease activity group compared to those of moderate and low disease activity (27.00 ± 3.54 ng/ml and 30.84 ± 2.67 ng/ml; $p < 0.05$ and $p < 0.05$, respectively; **Fig. 1c**). A significantly negative association between $1,25(OH)_2D_3$ levels and DAS28 scores ($\beta = -0.164$, $p = 0.018$) was observed. In addition, serum $1,25(OH)_2D_3$ levels were inversely associated with ESR and DAS28 scores ($\beta = -0.387$, -0.567, $p < 0.01$) in RA patients with high disease activity (**Table 4**).

$1,25(OH)_2D_3$ and anti-CCP in RA individuals

The serum levels of $1,25(OH)_2D_3$ for RA patients in the presence of anti-CCP (10.86 ng/ml) were lower than those in the absence of anti-CCP (15.98 ng/ml; $t = -3.08$, $p < 0.01$; **Fig. 1d**). Serum $1,25(OH)_2D_3$ levels in the anti-CCP-positive group were negatively associated with DAS28 scores ($\beta = -0.464$, $p < 0.01$). There was no obvious association between the $1,25(OH)_2D_3$ and RF, APF, AKA, or anti-MCV (**Table 4**).

Levels of $1,25(OH)_2D_3$ and Th 17 and Treg cell counts in RA individuals

The relationship between $1,25(OH)_2D_3$ and immune cells is summarized in **Fig. 2**. No statistically substantial associations were observed between $1,25(OH)_2D_3$ and certain variables (T cell, B cell, NK cell, Th1 cell, Th2 cell, Th17/Treg) in RA patients (**Fig. 2a–f**). However, the levels of $1,25(OH)_2D_3$ were inversely associated with Th 17 cell counts ($\beta = -0.158$, $p = 0.019$) and positively associated with Treg cell counts ($\beta = 0.146$, $p = 0.025$; **Fig. 2g, h**).

Discussion

In this study, concentrations of $1,25(OH)_2D_3$ in the patients with RA were significantly lower than those in healthy controls, consistent with previous cross-sectional studies in white [15], Chinese [16], Indian [17], and Iranian [6] RA patients. With an increasing incidence of vitamin D deficiency in patients with established RA, nearly 95% of subjects had $1,25(OH)_2D_3$ levels below the currently accepted thresholds, indicating that vitamin D deficiency is a general problem. Vitamin D insufficiency was more common in women, with a prevalence of 72.7%, which was in good agreement with our previous studies [18]. The finding in men as an adverse risk factor in low serum $1,25(OH)_2D_3$ levels is intriguing, and possibly due to their higher levels of androgens [19, 20]. We also found an inverse correlation between $1,25(OH)_2D_3$ and ESR in obese and overweight RA individuals (high BMI), consistent with Nikiphorou's study [21]. A recent cross-sectional analysis of 120 men and women reported that a decrease in $1,25(OH)_2D_3$ serum value was observed in parallel with an increase in BMI level ($r = -0.266$, $p = 0.037$) [22]. BMI is a well-established risk factors for vitamin D deficiency and these associations were confirmed in the recent study [23].

We found that the worse the indices of disease activity, the lower the $1,25(OH)_2D_3$ levels or the higher the proportion of patients with vitamin D deficiency. Wen H et al. [18], Cutolo et al. [24], and Patel et al. [25] all shared a similar outcome with our current analysis, and reported an inverse association between DAS28 and $1,25(OH)_2D_3$ levels in their entire samples ($n = 132$, 118, and 206, respectively). However, it does have contradictory results to previous studies [26] in which $1,25(OH)_2D_3$ failed to yield a significant association with

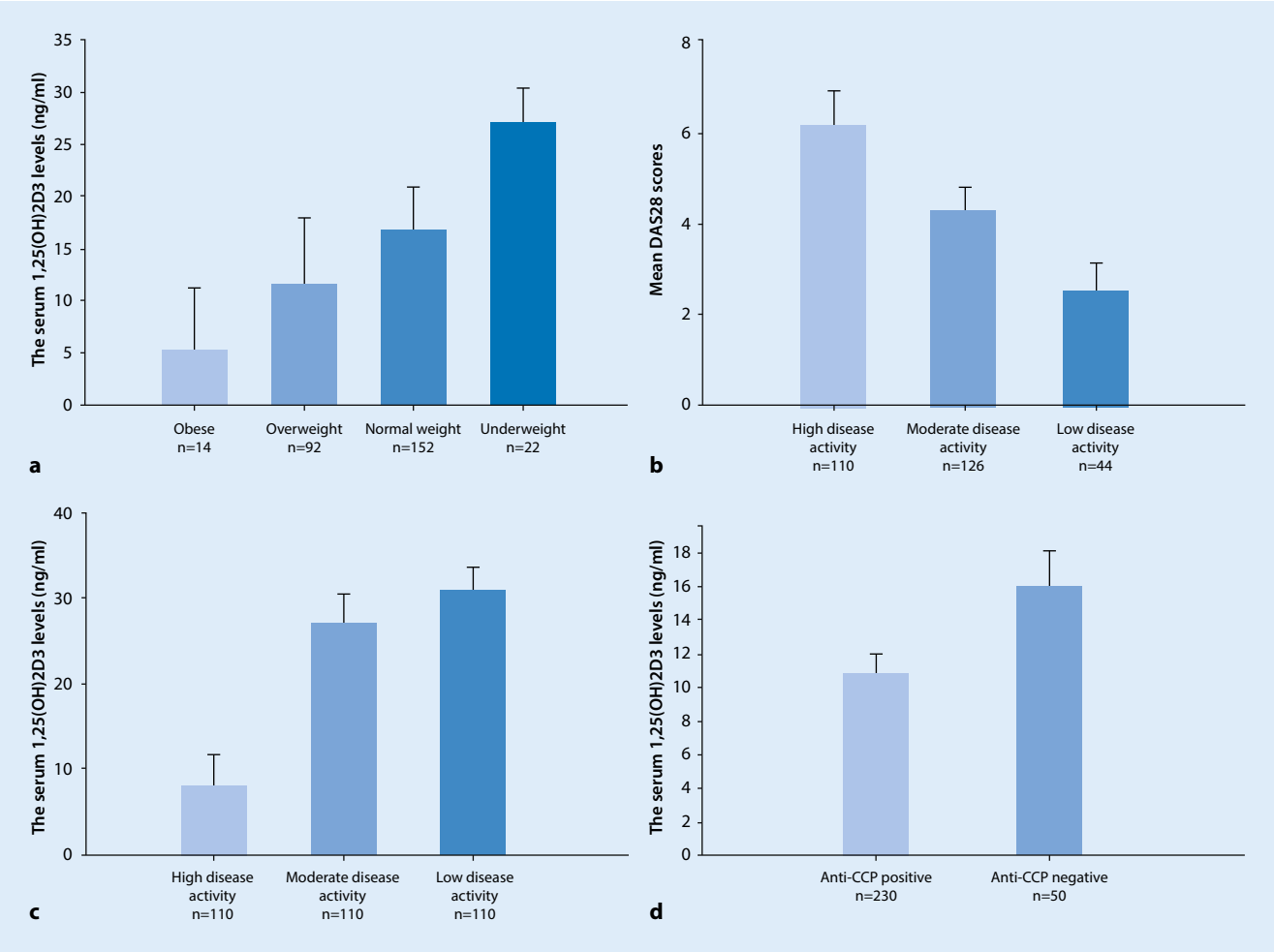

Fig. 1 ▲ The association between 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and body mass index (BMI) score, disease activity, and anti-cyclic-citrullinated peptides (anti-CCP) in rheumatoid arthritis (RA) individuals. **a** A significant difference of serum $1,25(OH)_2D_3$ levels was found among obese (BMI ≥ 28 kg/m^2), overweight (BMI ≥ 24 kg/m^2), normal-weight (BMI:18.5–23.9 kg/m^2), and underweight (BMI < 18.5 kg/m^2) RA individuals ($F = 31.41$, $p = 0.001$). **b** The scores of DAS28 for high, moderate, and low disease activity. **c** Significantly lower $1,25(OH)_2D_3$ values were found in high disease activity group compared to those having moderate and low disease activity ($p < 0.05$). **d** The serum $1,25(OH)_2D_3$ levels for RA patients in the presence of anti-CCP were lower than that in the absence of anti-CCP ($t = -3.08$, $p < 0.01$)

disease activity, mainly regarding differences in exposure to the number of cases, measurement methods, environments, illumination time or geographical locations.

Interestingly, our study reported a relationship between low $1,25(OH)_2D_3$ body supplies and positive anti-CCP in RA patients. Our study is in agreement with Kerr et al. [27]. Sahebari et al. [28] also suggested that in the early diagnosed RA patients, $1,25(OH)_2D_3$ and anti-CCP serum values were negatively correlated ($r = -0.5$, $p = 0.04$). These findings may be in line with the hypothesis of $1,25(OH)_2D_3$ deficiency as a predisposing factor for the initiation of autoimmune process. As noted, the documents in agreement with the bone-preserving role of $1,25(OH)_2D_3$ on the specific facets of human immunity have been shown to be on the rise in recent years, but it seems that the associations between $1,25(OH)_2D_3$ and specific autoantibodies are not clear yet. A large number of studies need to be investigated further.

Interestingly, we have shown that $1,25(OH)_2D_3$ may act by restoring Th17 and Treg balance, thereby restoring immune homeostasis, which is interesting in light of the correlations between these cells and vitamin D in RA. Colin [5] also suggested that $1,25(OH)_2D_3$ may contribute its bone-sparing effects in RA patients by modulating levels of Th17 and inhibiting Th17 cytokines, which is in good agreement with our data. Besides $1,25(OH)_2D_3$ stimulating Treg activity, polarized Tregs express a higher level of Treg-associated markers such as CTLA4, PD1, and CD25, and their suppressive capacity is enhanced by $1,25(OH)_2D_3$ [2]. Also, the important role of Th17 cells and the suppressive capacity of Treg cells were highly related to the beneficial effect of $1,25(OH)_2D_3$ in multiple sclerosis [29], systemic lupus erythematosus [30], asthma [31], and renal transplant [32] patients. Therefore, it is hypothesized that $1,25(OH)_2D_3$ suppresses autoimmu-

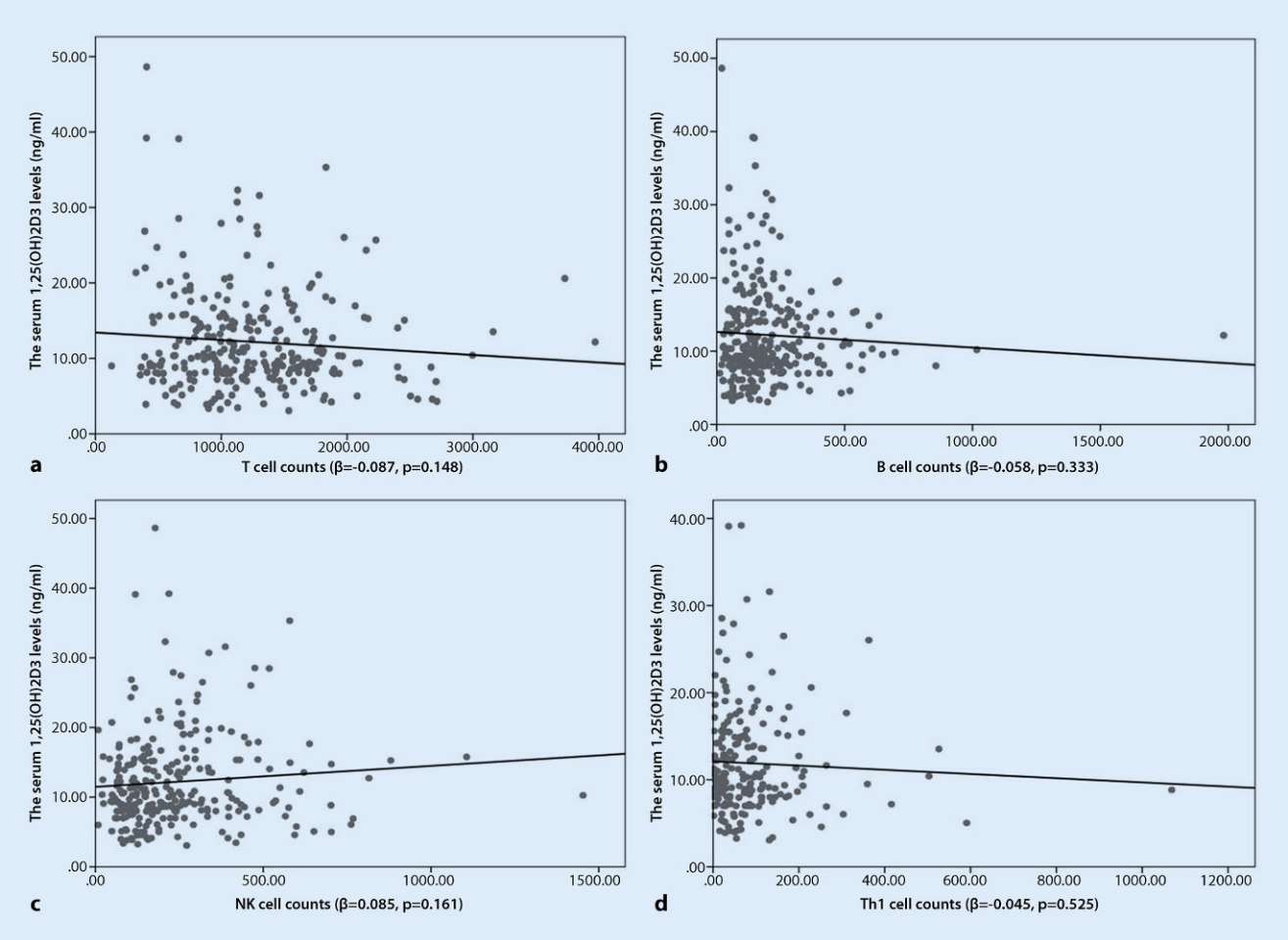

Fig. 2 ▲ The association between 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and immune cells in rheumatoid arthritis (RA) patients. **a**–**f** No significant association was found between $1,25(OH)_2D_3$ and T cell, B cell, natural killer (NK) cell, T helper 1(Th1)-cell, T helper 2(Th2)-cell, or T helper 17 (Th17)/regulatory T (Treg) (β = –0.087, –0.058, 0.085, –0.045, –0.104, –0.105, respectively, all $p > 0.05$). The levels of $1,25(OH)_2D_3$ were inversely associated with Th 17 cell counts (β = –7 cell $p = 0.019$; **g**) and positively associated with Treg cell counts (β = 0.146, $p = 0.025$; **h**)

nity at least partially via inhibition of Th17 activity and optimization of Treg function [33].

The existence of a worse response to treatment in patients with hypovitaminosis D compared to those with normal $1,25(OH)_2D_3$ levels at RA onset was demonstrated by Di Franco [34]. Furthermore, Hajjaj-Hassouni found out that absence of supplementation of vitamin D is related to higher prevalence of vitamin D deficiency in a COMORA study from 15 countries [35]. Despite some controversies [2], the majority of reports reinforce the idea of the added value for the treatment of autoimmunity [36]. A possible explanation for this observation could be precisely the $1,25(OH)_2D_3$ screening and supplementation strategies employed both at the clinic but also at the national level, minimizing the risk of $1,25(OH)_2D_3$ deficiency, even though there is no consensus concerning the amount that should be indicated.

Several limitations of this study should be noted. First, the study population was selected from the Second Hospital of Shanxi Medical University. Thus, these findings may not be representative of patients in other areas. Second, since intake of $1,25(OH)_2D_3$ was able to be affected by different seasons, geographical limits, outdoor exercise or not, sun exposure, and other factors, we did not strictly balance the factors upon selection of experimental patients. Therefore, a longer follow-up period of our cohort is required to validate the role of vitamin D (or analog) supplementation, beyond correction of deficiency, as a treatment modality, and to determine if higher vitamin D levels lead to standard clinical improvement in disease activity and immune regulation.

Conclusions

Our preliminary data showed a mutual influence of vitamin D on disease activity, BMI index, Th17 cell, Treg cell, and anti-CCP antibody, especially in female RA patients. These findings may be in line with the hypothesis that vitamin D deficiency might be a predisposing factor for the initiation and progression of RA. However, we cannot rule out the possible impact of the cross-sectional design on

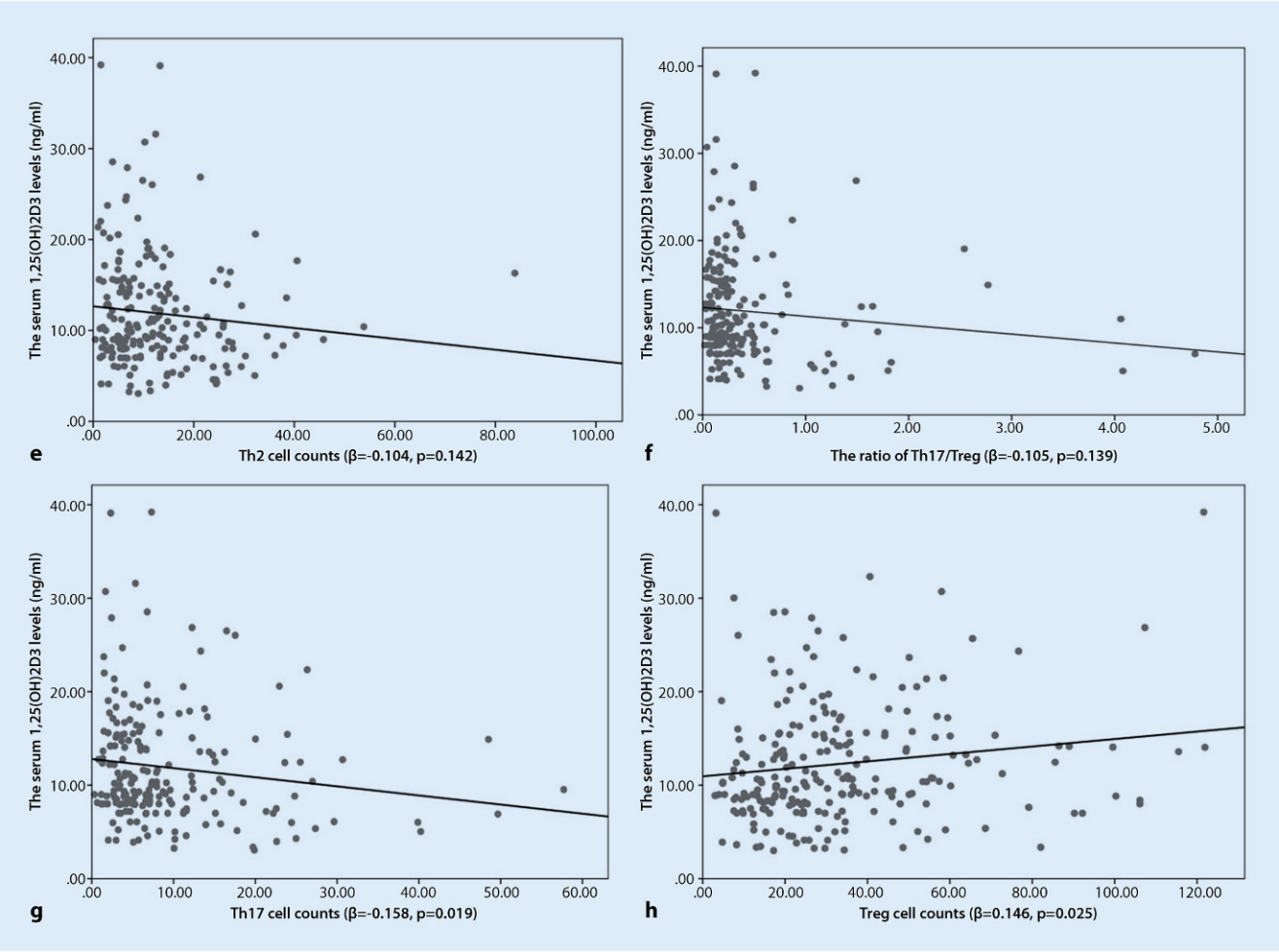

Fig. 2 ▲ (Continued)

the study results and we also are not able to determine whether vitamin D deficiency directly impacts RA disease activity and immune regulation, or whether the reverse may be true. In spite of this, serum $1,25(OH)_2D_3$ levels could be a marker to monitor disease activity in RA patients and vitamin D may be an alternative supplementary treatment for RA.

Corresponding address

H. Wen, MD, PhD
Department of Rheumatology, the Second Hospital of Shanxi Medical University, Shanxi Medical University
No. 382 Wu Yi Road, 030001 Taiyuan, Shanxi province, China
wenhongyan0509@aliyun.com

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Compliance with ethical guidelines

Conflict of interest. Y. Liu and H.-y. Wen declare that they have no competing interests.

Informed consent was obtained from all patients. The experimental protocol was approved by the Medical Ethics Committee of the Second Hospital of Shanxi Medical University and complied with the Declaration of Helsinki (approval number: 2013ky007).

References

1. McFarlane IM, Ozeri DJ, Pathiparampil J et al (2017) Prevalence and clinical characteristics of rheumatoid arthritis in an inner city population with sickle cell disease. Rheumatology (Sunnyvale) 7(2):218. https://doi.org/10.4172/2329-8731.1000218
2. Dankers W, Colin EM, van Hamburg JP, Lubberts E (2016) Vitamin D in autoimmunity: molecular mechanisms and therapeutic potential. Front Immunol 7:697
3. Wei R, Christakos S (2015) Mechanisms underlying the regulation of innate and adaptive immunity by vitamin D. Nutrients 7:8251–8260
4. Hewison M (2012) An update on vitamin D and human immunity. Clin Endocrinol (Oxf) 76:315–325
5. Colin EM, Asmawidjaja PS, van Hamburg JP et al (2010) 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. Arthritis Rheum 62:132–142
6. Rajaee E, Ghorbani A, Mowla K, Zakerkish M, Mohebi M, Dargahi-Malamir M (2017) The relationship between serum level of vitamin D3 and the severity of new onset rheumatoid arthritis activity. J Clin Diagn Res 11:OC28–OC30
7. Lin J, Liu J, Davies ML, Chen W (2016) Serum vitamin D level and rheumatoid arthritis disease activity: review and meta-analysis. PLoS ONE 11:e146351
8. Cote J, Berger A, Kirchner LH, Bili A (2014) Low vitamin D level is not associated with increased incidence of rheumatoid arthritis. Rheumatol Int 34:1475–1479
9. Arnett FC, Edworthy SM, Bloch DA et al (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324

10. Zhou B, Coorperative Meta-Analysis Group Of China Obesity Task Force (2002) Predictive values of body mass index and waist circumference to risk factors of related diseases in Chinese adult population. Zhonghua Liu Xing Bing Xue Za Zhi 23:5–10
11. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL (1995) Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 38:44–48
12. Fransen J, Stucki G, Riel PLCMV (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 (Hoboken) 49:S214–S224
13. Sabbagh Z, Markland J, Vatanparast H (2013) Vitamin D status is associated with disease activity among rheumatology outpatients. Nutrients 5:2268–2275
14. Spiro A, Buttriss JL (2014) Vitamin D: an overview of vitamin D status and intake in Europe. Nutr Bull 39:322–350
15. Haque UJ, Bartlett SJ (2010) Relationships among vitamin D, disease activity, pain and disability in rheumatoid arthritis. Clin Exp Rheumatol 28:745–747
16. Hong Q, Xu J, Xu S, Lian L, Zhang M, Ding C (2014) Associations between serum 25-hydroxyvitamin D and disease activity, inflammatory cytokines and bone loss in patients with rheumatoid arthritis. Rheumatology (Oxford) 53:1994–2001
17. Sharma R, Saigal R, Goyal L et al (2014) Estimation of vitamin D levels in rheumatoid arthritis patients and its correlation with the disease activity. J Assoc Physicians India 62:678–681
18. Wen HY, Luo J, Li XF (2015) Vitamin D levels and associations with disease activity in Chinese Han patients with early rheumatoid arthritis. J Clin Rheumatol 21:276–277
19. Tedeschi SK, Bermas B, Costenbader KH (2013) Sexual disparities in the incidence and course of SLE and RA. Clin Immunol 149:211–218
20. Tobon GJ, Youinou P, Saraux A (2010) The environment, geo-epidemiology, and autoimmune disease: rheumatoid arthritis. J Autoimmun 35:10–14
21. Nikiphorou E, Uksila J, Sokka T (2017) A cross-sectional study of vitamin D levels in a large cohort of patients with rheumatic diseases. Clin Rheumatol. https://doi.org/10.1007/s10067-017-3870-8
22. Goshayeshi L, Saber H, Sahebari M et al (2012) Association between metabolic syndrome, BMI, and serum vitamin D concentrations in rheumatoid arthritis. Clin Rheumatol 31:1197–1203
23. Rossini M, Maddali Bongi S, La Montagna G et al (2010) Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability. Arthritis Res Ther 12:R216
24. Cutolo M, Otsa K, Laas K et al (2006) Circannual vitamin d serum levels and disease activity in rheumatoid arthritis: northern versus southern Europe. Clin Exp Rheumatol 24:702–704
25. Patel S, Farragher T, Berry J, Bunn D, Silman A, Symmons D (2007) Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis Rheum 56:2143–2149
26. Pakchotanon R, Chaiamnuay S, Narongroeknawin P, Asavatanabodee P (2016) The association between serum vitamin D Level and disease activity in Thai rheumatoid arthritis patients. Int J Rheum Dis 19:355–361
27. Kerr GS, Sabahi I, Richards JS et al (2011) Prevalence of vitamin D insufficiency/deficiency in rheumatoid arthritis and associations with disease severity and activity. J Rheumatol 38:53–59
28. 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 Intern Med 5:148–155
29. Smolders J, Thewissen M, Peelen E et al (2009) Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. PLoS ONE 4:e6635
30. Marinho A, Carvalho C, Boleixa D et al (2017) Vitamin D supplementation effects on FoxP3 expression in T cells and FoxP3(+)/IL-17A ratio and clinical course in systemic lupus erythematosus patients: a study in a Portuguese cohort. Immunol Res 65:197–206
31. Chary AV, Hemalatha R, Murali MV, Jayaprakash D, Kumar BD (2016) Association of T-regulatory cells and CD23/CD21 expression with vitamin D in children with asthma. Ann Allergy Asthma Immunol 116:447–454.e2
32. Aly MG, Zhu L, Weimer R et al (2017) Low utility of serum 25-hydroxyvitamin D3 and 1, 25-dihydroxyvitamin D3 in predicting peripheral Treg and Th17 cell counts in ESRD and renal transplant patients. Transpl Immunol 43–44:3–10
33. Xie Z, Chen J, Zheng C et al (2017) 1,25-dihydroxyvitamin D3 induced dendritic cells suppress experimental autoimmune encephalomyelitis by increasing proportions of the regulatory lymphocytes and reducing Th1, Th17 cells. Immunology 152(3):414–424. https://doi.org/10.1111/imm.12776
34. Di Franco M, Barchetta I, Iannuccelli C et al (2015) Hypovitaminosis D in recent onset rheumatoid arthritis is predictive of reduced response to treatment and increased disease activity: a 12 month follow-up study. BMC Musculoskelet Disord 16:53
35. Hajjaj-Hassouni N, Mawani N, Allali F et al (2017) Evaluation of vitamin D status in rheumatoid arthritis and its association with disease activity across 15 countries: "the COMORA Study". Int J Rheumatol. https://doi.org/10.1155/2017/5491676
36. Franco AS, Freitas TQ, Bernardo WM, Pereira RMR (2017) Vitamin D supplementation and disease activity in patients with immune-mediated rheumatic diseases: a systematic review and meta-analysis. Medicine (Baltimore) 96:e7024

Fachnachrichten

Neue Erkenntnisse über die Autoimmunkrankheit Morbus Bechterew

Wissenschaftler an der Jacobs University in Bremen haben die molekularen Mechanismen hinter der Krankheit näher entschlüsselt.

Morbus Bechterew führt zu langwierigen und schmerzhaften Entzündungen der Gelenke und letztlich zu einer Verformung der Wirbelsäule. Forscher vermuten als Ursache für die Krankheit ein bestimmtes Protein, welches die meisten Patienten in ihren Zellen aufweisen: HLA-B27. Es wird vermutet, dass dieses Protein durch seine besonders langsame und komplizierte Faltungsweise die Krankheit auslöst.

Forscher der Jacobs University haben gemeinsam mit Kollegen der Freien Universität Berlin herausgefunden, wie die Faltung und die Qualitätskontrolle des HLA-B27-Proteins ablaufen. Dr. Zeynep Hein, Postdoc in der Forschungsgruppe von Prof. Dr. Sebastian Springer an der Jacobs University, hat dazu den Transport des Proteins genau untersucht. Dazu wird eine gentechnisch stabilisierte Form des HLA-B27-Proteins hergestellt und mit dem in unserem Körper vorkommenden Protein verglichen.

Unter anderem konnten Hein und ihr Team herausfinden, dass das Protein sich nur sehr schwer in seine spezielle Struktur falten kann. Und selbst wenn es sich falten kann, neigt es dazu, gleich wieder zu zerfallen und dadurch seine Funktion zu verlieren. "Natürlich können wir zu diesem Zeitpunkt noch nicht sagen, wie genau diese Grundlagenkenntnisse später zu einer möglichen Therapie der Bechterew-Krankheit beitragen", sagt Springer. "Das Erforschen fundamenteller Mechanismen ist unabdingbar, um letztendlich Therapien und Medikamente zu entwickeln."

*Z. Hein, et al, Distinct mechanisms survey the structural integrity of HLA-B*27:05 intracellularly and at the surface. PLOS ONE 2018; doi:10.1371/journal.pone.0200811.*

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