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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)

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ABSTRACT

Objective: To collect data on vitamin D (25(OH)D) serum levels in a large number of rheumatoid arthritis (RA) patients from different European countries, to investigate their relation with disease activity, disability, quality of life, and possibly to construct a new Patient Reported Outcome (PRO) questionnaire in order to self-estimate if they are at risk for vitamin D insufficiency/deficiency-related clinical implications (D-PRO).
Methods: This was a European League Against Rheumatism (EULAR) supported cross-sectional study (project No CLI064) which involved 625 RA patients (mean age 55 ± 11 years, mean disease duration 11 ± 9 years), 276 age and sex matched healthy subjects, and rheumatologists working in academic institutions or hospital centres, as well as PARE organizations (patient representatives) from 13 European countries. Serum samples for 25(OH)D level measurement were collected during winter time and analyzed in a central laboratory using chemiluminescence immunoassay (DiaSorin). Patient past medical history was recorded. RA patients were provided with three questionnaires: the Rheumatoid Arthritis Impact Diseases score (RAID), the Health Assessment Questionnaire (HAQ), and the new D-PRO questionnaire at the time of 25(OH)D serum sampling. D-PRO questionnaire consisted of three domains, Symptom Risk Score (SRS), Habitus Risk Score (HRS) and Global Risk Score (SRS + HRS = GRS), constructed with items possibly related to vitamin D deficiency. D-PRO was correlated with both clinical and PRO scores. DAS28-CRP was also evaluated. Statistical analysis was performed by non parametric tests.

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Results: Mean serum concentration of 25(OH)D in RA patients (17.62 ± 9.76 ng/ml) was found significantly lower if compared to the levels obtained in matched controls (18.95 ± 9.45 ng/ml) ($p = 0.01$), with statistically significant differences among several European countries. Negative correlations were found between 25(OH)D serum levels and DAS28-CRP ($p < 0.001$), RAID ($p = 0.05$) and HAQ ($p = 0.04$) scores in the RA patients group. Negative correlations were also found in the cohort of enrolled RA patients between 25(OH)D serum concentrations and SRS ($p = 0.04$), HRS ($p = 0.02$) and GRS ($p = 0.02$) domains of the D-PRO questionnaire.
Conclusions: This first multicentre European survey add new evidences that vitamin D insufficiency/deficiency is frequent in RA patients with statistically significant differences among several countries. Vitamin D serum concentrations seem to correlate negatively and significantly with the D-PRO Global Risk Score, clinimetric indexes for quality of life, disease activity and disability in present cohort of RA European patients.

1. Introduction

Experimental and clinical data provided evidence that vitamin D deficiency can be an important environmental risk factor influencing prevalence and severity of several autoimmune diseases especially in people of particular geographical, climate and ethnic background [1–4].

The term vitamin D refers to a precursor of the 1,25-hydroxy-vitamin D ($1,25(OH)_2D_3$) (calcitriol), the only natural form able to induce biological effects within the body, which is known to act at subnanomolar concentrations as an endocrine hormone. The D hormone ($1,25\text{-}(OH)_2D_3$) is a member of a group of steroid molecules generated from cholesterol (cholecalcipherol) with a plethora of biological actions and immune modulatory effetcs, orchestrated via the integrated operation of the D hormone endocrine system [5–6].

Individual vitamin D status is usually assessed by measurement of 25(OH)D (calcifediol) in serum, since calcitriol has a very short half-life that does not allow reliable measurements [7–8]. Accordingly, calcitriol deficiency may exist even when normal or even elevated calcifediol levels are present into circulation [9].

No real serum 25(OH)D threshold has been documented for rheumatoid arthritis (RA), but preliminary studies suggest that low serum concentrations of vitamin D may be common, and an inverse relationship between serum levels of vitamin D metabolites and disease activity or disability have been reported in RA patients [3,10–11]. Furthermore, it is not unusual for RA patients to have silent signs of vitamin D deficiency (i.e. amplified pain, muscle weakness, changes in hair and nail growth, mood changes etc.) influencing their perception of wellbeing but not attributable to disease itself [12–14]. Similarly, importance of vitamin D deficiency has been demonstrated in many other inflammatory conditions [15–20].

There is also a growing interest in the assessment of RA status from the patient's perspective, and patient reported outcomes (PRO) have been found to be very informative and sensitive. PROs are bringing additional information in the assessment of RA patients, especially in domains of health important from the patient's perspective [21].

The aim of this study was to collect data on vitamin D serum levels in a large number of RA patients from different European countries and to investigate its relation with disease activity, disability, quality of life, and possibly with a new Patient Reported Outcome (PRO) questionnaire constructed and cross-culturally validated for RA patients to self-estimate if they are at risk for vitamin D insufficiency/deficiency (D-PRO).

2. Patients and methods

This was an European League Against Rheumatism (EULAR) supported cross-sectional study (project No CLI064) which involved rheumatologists working in academic institutions or hospital centre and PARE organizations (patient representatives) from 13 European countries (Bulgaria, Croatia, Estonia, Italy, Latvia, Lithuania, Poland, Portugal, Romania, Russia, Serbia, Slovakia and Spain). The study was performed in accordance with GCP and Helsinki Declaration, and local Ethical Committee and Patient Informed Consent were obtained before patient enrolment at each national centre.

2.1. Study population and design

It was expected to enrol approximately a total number of 600–700 RA patients (50 from each participating centre). RA patients inclusion criteria were: age 25–65 years; RA diagnosis established by ACR/EULAR criteria [22] at least one year prior to study entry; patients on stable treatment with conventional synthetic DMARDs (i.e. Methotrexate, Leflunomide) during at least 3 months prior to enrolment; use of glucocorticoids ≤7.5 mg/day of prednisone or equivalent, for at least one month prior to enrolment.

Data from 625 RA patients were finally collected, along with blood samples. Blood samples from 276 age and sex matched healthy volunteers were collected in each country to serve as a control for serum 25(OH)D levels.

Due to the circannual variations of vitamin D serum concentrations and its possible influence on disease activity/severity, it was decided to recruit the subjects only during the winter (beginning of December until end of March), known as characterized by the lowest seasonal 25(OH)D serum levels.

In all eligible RA patients, according to detailed study protocol and predefined case reported form, the following item were collected: demographic data, medical/clinical history, and disease activity using the DAS28 score [18]. The DAS28 was based on C-reactive protein (CRP) serum concentration. The DAS28-CRP combines information from the 28 tender and swollen joints, the CRP (in mg/dl) and the patient's general health status (PtGH), measured with a visual analogue scale (VAS 100 mm). Additionally, in order to test possible correlations among new and pre-existing validated PROs, RA patients were provided with three questionnaires: the Rheumatoid Arthritis Impact Diseases score (RAID) [10], the Health Assessment Questionnaire (HAQ) [3], and the new D-PRO (see below) at the time of serum sampling for 25(OH)D level measurement.

2.2. Serum sample collection and Vitamin D measurement

Serum samples for 25(OH)D serum level evaluation were performed centrally in a single laboratory at the Research Laboratory of Genova. Samples were obtained according to standard procedures, divided in 3 cryovial aliquots, labeled with pre-defined patient code and frozen (−80 °C) in each centre. After collection, frozen samples were shipped in dry ice thermal boxes to referring central laboratory where finally they were stored until analysis.

The evaluation of 25(OH)D concentrations was performed in a single run (testing 3 times each sample) by using a chemiluminescence immunoassay and an automatic analyser (LIAISON, DiaSorin, Italy). 25(OH)VitaminD serum concentrations were classified as normal (>30 ng/ml), insufficient (between 20 and 30 ng/ml) or deficient (<20 ng/ml), as already reported [23].

2.3. Canditate predictors of Vitamin D deficiency and D-PRO questionnaire

In line with standardized operational procedures (SOP) a systematic literature review was performed in computerized bibliographic databases (Pubmed, EBSCO, and Web of Science) and resulted in 10,906 publications mentioning vitamin D deficiency and clinical symptoms. Literature research limited by age (only adults), and disease (malignant and other chronic inflammatory diseases omitted) resulted in only few articles. Nevertheless, clinical manifestations most often mentioned in connection to vitamin D deficiency were muscle problems, bone pain and fractures, fatigue, mood changes, depression, anxiety, dementia, skin, finger nails and hair changes. Likewise, other vitamin D related items were physical activity, sun exposure and foods. Patient research partners (PRPs), namely representatives of national rheumatic patient organizations (PARE), were invited to add the benefit of their experiential knowledge about symptoms and nutritional habits concerning vitamin D.

Rheumatologists (expert opinionists) and patients from the different countries formed the panel that performed a consensus survey. When items proposed to be possible symptoms/cause of vitamin D deficiency reached 95% level of agreement they were considered valid and incorporated in Symptom Risk Score (SRS) or Habitus Risk Score (HRS) domains of the D-PRO questionnaire. The details related to the D-PRO questionnaire are reported in Table 1.

As a result of this process, the D-PRO questionnaire was finally constructed with two sets of questions regarding: a) skin, finger nails, muscle problems, hair changes, bone pain, fatigue, sleep irregularities and nervous system problems whose scoring provides the SRS; and b) daily physical activity, insolation, dietary habits, and vitamin D supplementation whose scoring provides the HRS. Sum of SRS and HRS enable calculation of Global Risk Score (GRS) which was the final D-PRO score value (Table 1).

The D-PRO questionnaire has been translated from English to local language in each country (by national rheumatologists), and administered to RA patients the day of blood sampling. Possible validity of D-PRO questionnaire was tested versus patient's 25(OH)D serum concentrations.

2.4. Statistical analysis

All data were entered into a Microsoft Excel database, which had been developed for management of cross-sectional multicentre data obtained. The data were analyzed using the SPSS version 22.0 (SPSS Inc., Chicago, IL), and the MedCalc® version 12.4.0 (MedCalc Software, Mariakerke, Belgium). Statistical analysis was carried out by non-parametric tests. The Mann–Whitney *U* test was used to compare unpaired groups of variables, and the Kruskal–Wallis test to compare continuous variables with nominal variables with more than two levels. Possible correlations between variables were assessed by Spearman rank correlation, simple and multiple regression tests. Any p values equal or lower than 0.05 was considered statistically significant. The results are reported as mean along with standard deviation (SD).

3. Results

3.1. Demographic data, laboratory and clinical parameters in the RA patients

In total 625 RA patients (529 women, 96 men) from 13 European countries were included from December 2013 to March 2014 to participate in the study. Mean age at enrolment was 55 ± 11 years and mean disease duration was 11 ± 9 years. Average serum CRP level at enrolment of RA patients was 11 ± 19 (mg/l), there were 484 (79.7%) IgM rheumatoid factor (RF) positive and 387 (73.3%) anti-citrullinated peptide antibodies (ACPA) positive patients. Detailed description of demographic data and laboratory parameters are reported in Table 2. Distributions of parameters evaluated among groups and among countries were similar.

Concerning treatment, 166 RA patients (27%) were treated with glucocorticoids (prednisone equivalent average dose 5.8 mg/day, max dose 7.5 mg/day). Regarding conventional synthetic DMARDs, 324 RA patients (53%) were receiving methotrexate (average dose 12.5 mg/week), 119 (19%) hydroxychloroquine (average dose 250 mg/day) and 50 (8%) leflunomide (average dose 20 mg/day), finally 9% biologic DMARDs. A total number of 223 RA patients (36%) reported to take >1000 IU/day vitamin D supplementation from at least 6 months. No data available about the healthy controls.

3.2. Vitamin D serum concentrations in rheumatoid arthritis patients

Mean serum concentration of 25(OH)D in total RA patients (17.62 ± 9.76 ng/ml) was found significantly lower if compared to the levels obtained in control subjects (18.95 ± 9.45 ng/ml) ($p = 0.01$). In both groups there were no difference among females and males (see Table 3 for further details).

25(OH)D deficiency (<20 ng/ml) was found in almost 66% of RA patients, and severe deficiency (<10 ng/ml) was detected in almost 25% of them; insufficiency (between 20 and 30 ng/ml) was found in 27% of RA patients (Fig. 1). Interestingly, only 6% of the RA patients were found within the concentrations considered normal (between 30 and 50 ng/ml). Male and female RA patients showed similar 25(OH)D values. Concerning the 25(OH)D serum concentrations in healthy subjects vitamin D deficiency (<20 ng/ml) was found in almost 53% of subjects, and severe deficiency (<10 ng/ml) was present in only 13% of them; insufficiency (between 20 and 30 ng/ml) was detected in 32% of controls, whereas 13% of the controls was found within the range of concentrations considered normal (between 30 and 50 ng/ml) (Fig. 1).

A statistically significant difference in vitamin D levels was found between some European countries, with significantly higher 25(OH)D serum concentrations in Spanish RA patients (Canary Islands) compared to patients from Latvia, Lithuania, Poland, Romania, Croatia, Russia and Italy ($p < 0.01$). Data are reported in Fig. 2 and Table 4.

Statistically significant difference in 25(OH)D serum levels were observed between RA patients (223, 36%) who stated to take vitamin D supplementation (>1000 IU/day) from at least 6 months, and those who stated not taking vitamin D supplementation (402. 64%) (20.20 ± 10.77 vs. 16.24 ± 8.97 ng/ml, respectively) ($p < 0.001$) (Table 3). There were no statistically significant differences in demographic characteristics among the groups.

3.3. Vitamin D serum levels and disease activity, disability and quality of life

Negative correlations were found between 25(OH)D serum levels and DAS28-CRP ($p < 0.0001$), RAID ($p = 0.04$) and HAQ ($p = 0.02$) scores in the whole RA patients group. The results were additionally confirmed in the subgroup of RA patients not taking vitamin D supplementation (DAS28-CRP $p = 0.002$, RAID $p = 0.05$, HAQ $p = 0.03$).

3.4. D-PRO questionnaire

Statistically significant negative correlations were found in the cohort of enrolled RA patients between 25(OH)D serum concentrations and SRS ($p = 0.04$), HRS ($p = 0.02$) and GRS ($p = 0.02$) domains of the D-PRO questionnaire. Same correlations between 25(OH)D serum levels and D-PRO domains were confirmed in the subgroup of RA patients not taking vitamin D supplementation (SRS $p = 0.01$, HRS $p = 0.03$, GRS $p = 0.02$).

There was also a statistically significant positive correlation between D-PRO-GRS and DAS28-CRP ($p = 0.001$), RAID ($p < 0.0001$) and HAQ ($p < 0.0001$), and between D-PRO -SRS domain and DAS28-CRP ($p = 0.0001$), RAID ($p < 0.0001$) and HAQ ($p < 0.0001$).

Table 1
D-PRO questionnaire design and calculations.
Symptom Risk Score (SRS) = sum of normalized scores of items 1–10 (0–10 each) (max score = 100): *, for each item, in first 4 questions calculate sum of collected scores × 5 (score range 0–10), i.e. (1 + 1) × 5 = 10 is the normalized score; §, score 0–10 for any item. Habitus risk score (HRS) = sum of normalized scores of items A–D (max score = 100): #, multiply for 2 the collected score for each item (i.e. 5 × 2 = 10 then 10 is the score normalized for physical activity); &, sum the scores of item 1–10 then divide by 3 (i.e. 30/3 = 10, then 10 is the normalized score for nutrition); $, multiply for 5 the collected score (i.e. 2 × 5 = 10 then 10 is the score normalized for vitamin D pharmacological supplementation).
Global Risk Score (GRS) = sum of normalized scores of SRS and HRS (max score = 100): i.e. (100 + 100)/2 = 100, then 100 is the normalized GRS score.

SYMPTOM RISK SCORE (SRS^{sum})

	yes	no
1. Do you notice SKIN changes?*		
Dry skin, flaking or cracks	1	0
Skin itching	1	0
2. Do you notice FINGERNAILS changes?*		
Brittle, dry or pitted fingernails	1	0
Slow fingernails growth	1	0
3. Do you notice MUSCLE problems?*		
Muscle weakness and/or hypotonia	1	0
Muscle twitching	1	0
4. Do you notice HAIR changes?*		
Hair loss	1	0
Slow hair growth	1	0

Please, score how often do you have?§	All the time									never
5. Bone pain (other than joint)	10	9	8	7	6	5	4	3	2	1
6. Fatigue	10	9	8	7	6	5	4	3	2	1
7. Sleep irregularities	10	9	8	7	6	5	4	3	2	1
8. Nervousness	10	9	8	7	6	5	4	3	2	1
9. Anxiety	10	9	8	7	6	5	4	3	2	1
10. Depression	10	9	8	7	6	5	4	3	2	1

HABITUS RISK SCORE (HRS^{sum})

	None	15 m'	30 m'	1 h	2 h	more
A. What is your average daily physical activity (running, swimming, walking etc.)?#	5	4	3	2	1	0
B. What is your average daily sun exposure?#	5	4	3	2	1	0

C. What are your average week food intakes?&	never	once/week	wice/week	every/week
1. Mushrooms	3	2	1	0
2. Mackerel fish	3	2	1	0
3. Salmon fish	3	2	1	0
4. Herring fish or sardines	3	2	1	0
5. Tuna fish	3	2	1	0
6. Cat fish	3	2	1	0
7. Cod liver oil	3	2	1	0
8. Eggs	3	2	1	0
9. Soymilk	3	2	1	0
10. Vitamin D fortified breakfast cereals	3	2	1	0

D. Do you take vitamin D pharmacological supplementation?$	None	Yes<1,000 UI/day	Yes >1,000 UI /day
	2	1	0

GLOBAL RISK SCORE (GRS) (0 -100) **= [$SRS^{normalized\ sum}$ + $HRS^{normalized\ sum}$] / 2**

Table 2
Description of demographic data, laboratory and clinical parameters in rheumatoid arthritis patients. ACPA = Anti-Citrullinated Peptide Antibodies; DAS28-CRP = Disease Activity Score based on C Reactive Protein; HAQ = Health Assessment Questionnaire; RAID = Rheumatoid Arthritis Impact Diseases score; D-PRO = vitamin D Patient Reported Outcome; SRS = Symptoms Risk Score; HRS = Habitus Risk Score; GRS = Global Risk Score.
Score ranges are described in Table 1 and text.
The p values refer to the comparison between vitamin D supplemented and vitamin D not supplemented patients. * Statistically significant values.

Parameters	All RA patients (N = 625)	Non vitamin D supplemented (N = 402)	Vitamin D supplemented (N = 223)	p significance
Age (years)	55 ± 11	53 ± 11	57 ± 9	0.382
Gender M/F	93/517	71/322	22/195	0.013*
IgM RF+ N (%)	484 (79.7)	314 (80.7)	170 (78.0)	0.484
ACPA+ N (%)	387 (73.3)	252 (73.7)	135 (72.6)	0.864
CRP (mg/l)	11.1 ± 19.2	11.8 ± 21.1	9.7 ± 14.4	0.799
25(OH)VitD (ng/ml)	17.6 ± 9.8	16.2 ± 9.0	20.2 ± 10.8	<0.001*
DAS28-CRP score	3.7 ± 1.5	3.8 ± 1.5	3.4 ± 1.5	0.009*
HAQ score	0.9 ± 0.9	0.9 ± 0.8	0.9 ± 0.8	0.605
RAID score	4.4 ± 2.3	4.5 ± 2.3	4.6 ± 2.3	0.836
SRS (D-PRO) score	66.1 ± 17.1	73.9 ± 13.6	52.4 ± 14.1	<0.001*
HRS (D-PRO) score	8.3 ± 1.1	8.3 ± 1.0	8.2 ± 1.1	0.220
GRS (D-PRO) score	55.2 ± 13.1	58.5 ± 12.2	49.1 ± 12.5	<0.001*

Almost similar patterns of correlations were found in the subgroup of RA patients not supplemented with vitamin D.

4. Discussion

Present investigation is the first pan-European study documenting serum vitamin D level status in RA patients from different latitudes, with homogenous sampling obtained in winter time and analyzed in a centralized laboratory. Sixty-four percent of the RA patients, not vitamin D supplemented, showed deficient serum 25(OH)D levels.

Sufficient vitamin D body concentrations seem generally to lower the risk of autoimmune disorders, such as type I diabetes mellitus, multiple sclerosis, systemic lupus erythematosus (SLE) and RA, by exerting immune-modulatory effects [5,7,24–28]. Currently, uncontrolled studies suggested that vitamin D supplementation can be useful for preventing the development of autoimmune diseases, and for reducing the severity of the pre-existing disease [29–32].

The measurement of serum 25(OH)D provides partial insight into D hormone status (active form = $1.25(OH)_2D_3$) because of its tight physiologic control [7,8,33]. The deficiency of the "hormonal" active form of vitamin D and of its precursor 25(OH)D is extremely common, at least during winter, in all Europe and particularly in Northern countries like a "silent seasonal epidemic" [11].

Numerous studies from different continents and countries and their recent meta-analysis suggest significantly lower levels of vitamin D to be common in RA patients [10,34–36]. An inverse relationship between serum levels of vitamin D metabolites and disease activity or disability in RA patients has been reported [3,37]. These facts justify need for a specific questionnaire to evaluate vitamin D sufficiency/insufficiency-related clinical consequences in RA patients.

Although several factors are involved in the vitamin D deficiency observed in RA patients, the association between disease activity and lower 25(OH)D levels seems to remain a statistically significant reality as confirmed by recent meta-analysis [34]. Present study reported in a large RA patient cohort, the existence of negative correlations between 25(OH)D level and DAS28-CRP, RAID and HAQ scores.

A previous small study also reported an inverse association between baseline vitamin D serum levels and the quality of life measured by HAQ score [33]. Additionally, vitamin D deficiency (25(OH)D values <20 ng/ml) was found in 43% of a large RA patient cohort from south Europe, whereas ranging from 30 to 63% in other study cohorts [3,34,38].

Several studies indicated that serum vitamin D levels can influence disease outcome measures which contain RA patient reports such as DAS28 and HAQ [39,40]. In particular, 25(OH)D serum concentrations, <16 ng/ml, have been found to be associated with substantially poorer leg muscle function, and additionally, there are evidences that vitamin D deficiency might be implicated in mechanisms of chronic pain, importantly influencing HAQ score [41].

Table 3
Mean ± standard deviations of 25(OH)D serum concentrations (ng/ml) in rheumatoid arthritis (RA) patients and healthy subjects (controls). 25(OH)D serum levels in RA patients supplemented (D Suppl) or not (Not Suppl) with 1000 IU/day vitamin D; 25(OH)D serumconcentrations in male and female heatly mathced controls are also reported.

	25(OH)-Vitamin D (ng/ml)	p significance
Total RA patients	17.6 ± 9.8	0.01
Controls	19.0 ± 9.4	
D Suppl RA patients	20.2 ± 10.8	0.001
Not Suppl RA patients	16.2 ± 9.0	
Male RA patients	18.6 ± 9.6	0.16
Female RA patients	17.4 ± 9.8	
Male controls	19.3 ± 8.0	0.42
Female controls	18.8 ± 9.9	

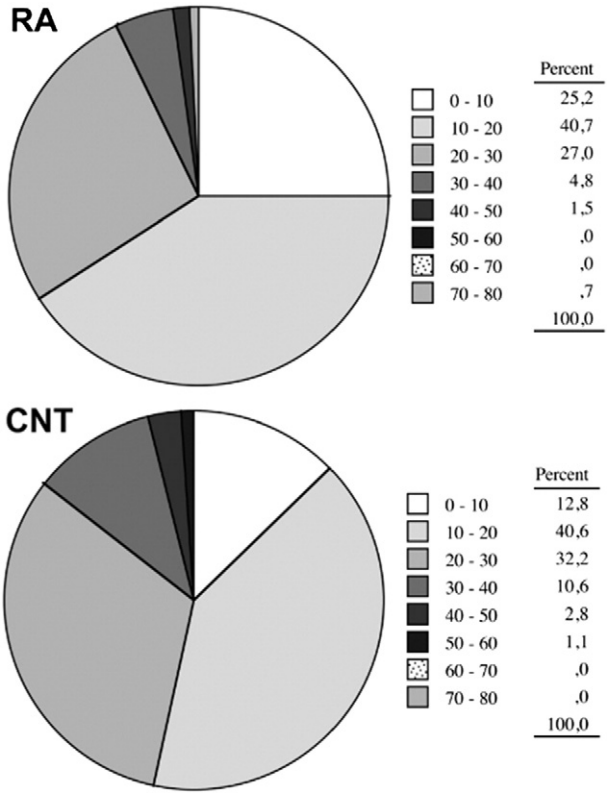

Fig. 1. Percentage of subjects with insufficiency or deficiency of 25(OH)D in RA patients (RA) and age matched healthy controls (CNT) reported as ng/ml (left column).

On the other hand, statistically significant negative correlations were found in our cohort of RA patients between 25(OH)D serum concentrations and SRS, HRS and GRS scores of the domains of the D-PRO questionnaire.

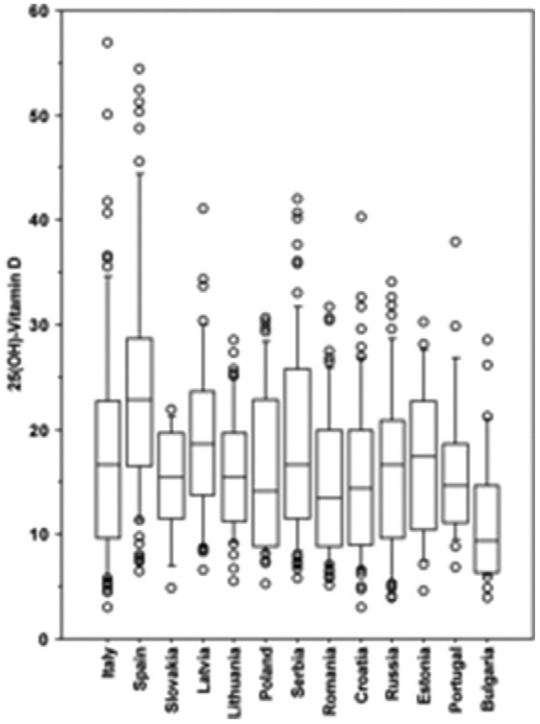

Fig. 2. 25(OH)D serum concentrations in RA patients from different European countries. See Table 3 for statistical significances. Data are given as 5th, 10th, 50th (median), 90th, and 95th percentiles.

Table 4
Statistical significances from comparison of 25(OH)D serum concentrations between different European countries.

Country comparison	p significance	Country comparison	p significance
Italy vs Spain	<0.0001	Latvia vs Estonia	n.s.
Italy vs Slovakia	n.s.	Latvia vs Portugal	n.s.
Italy vs Latvia	n.s.	Latvia vs Romania	0.0006
Italy vs Lithuania	n.s.	Lithuania vs Poland	n.s.
Italy vs Poland	n.s.	Lithuania vs Serbia	n.s.
Italy vs Serbia	n.s.	Lithuania vs Romania	n.s.
Italy vs Romania	n.s.	Lithuania vs Croatia	n.s.
Italy vs Croatia	n.s.	Lithuania vs Russia	n.s.
Italy vs Russia	n.s.	Lithuania vs Estonia	n.s.
Italy vs Estonia	n.s.	Lithuania vs Portugal	n.s.
Italy vs Portugal	n.s.	Lithuania vs Bulgaria	0.0006
Italy vs Bulgaria	0.0006	Poland vs Serbia	n.s.
Spain vs Slovakia	0.0004	Poland vs Romania	n.s.
Spain vs Latvia	0.005	Poland vs Croatia	n.s.
Spain vs Lithuania	<0.0001	Poland vs Russia	n.s.
Spain vs Poland	<0.0001	Poland vs Estonia	n.s.
Spain vs Serbia	<0.0001	Poland vs Portugal	n.s.
Spain vs Romania	<0.0001	Poland vs Bulgaria	0.004
Spain vs Croatia	<0.0001	Serbia vs Romania	0.02
Spain vs Russia	<0.0001	Serbia vs Croatia	0.05
Spain vs Estonia	0.0002	Serbia vs Russia	n.s.
Spain vs Portugal	<0.0001	Serbia vs Estonia	n.s.
Spain vs Bulgaria	<0.0001	Serbia vs Portugal	n.s.
Slovakia vs Latvia	n.s.	Serbia vs Bulgaria	<0.0001
Slovakia vs Lithuania	n.s.	Romania vs Croatia	n.s.
Slovakia vs Poland	n.s.	Romania vs Russia	n.s.
Slovakia vs Serbia	n.s.	Romania vs Estonia	n.s.
Slovakia vs Romania	n.s.	Romania vs Portugal	n.s.
Slovakia vs Croatia	n.s.	Romania vs Bulgaria	0.001
Slovakia vs Russia	n.s.	Croatia vs Russia	n.s.
Slovakia vs Estonia	n.s.	Croatia vs Estonia	n.s.
Slovakia vs Portugal	n.s.	Croatia vs Portugal	n.s.
Slovakia vs Bulgaria	0.05	Croatia vs Bulgaria	0.007
Latvia vs Lithuania	n.s.	Russia vs Estonia	n.s.
Latvia vs Poland	n.s.	Russia vs Portugal	n.s.
Latvia vs Serbia	n.s.	Russia vs Bulgaria	0.004
Latvia vs Romania	0.02	Estonia vs Portugal	n.s.
Latvia vs Croatia	0.04	Estonia vs Bulgaria	0.008
Latvia vs Russia	n.s.	Portugal vs Bulgaria	0.005

Further important result of the present study is the positive correlation of D-PRO-GRS with DAS28-CRP, RAID and HAQ scores, implicating a role for vitamin D deficiency/insufficiency in RA patient's outcomes. This is in accordance with previously mentioned studies performed in much smaller sample of patients.

There are some limitations in the present investigation. Mainly the construction of the questionnaire without adequate statistical testing of single items to prior confirms the construct validity.

The heterogeneous food intake among different population, that compromised the item enclosed into the questionnaire, is another bias. The sample size and clinical heterogeneity of RA patients recruited, limited the significance of some correlations, increasing the standard deviations. Furthermore, primary cross-sectional study design and lack of interventional prospective part of the study, biased the study potential to estimate D-PRO questionnaire responsiveness.

However, our study provided evidence that low 25(OH)D serum concentrations may influence RA activity outcome measure (DAS28) and PROs (RAID and HAQ).

This could be of great importance, in understanding and interpretation of the PROs used as outcome measures.

A recent study, after 12 months follow-up in patients with early onset RA and basal hypovitaminosis D, found out a reduction of disease activity, percentage of remission and response to treatment, that were significantly lower than observed in patients with normal vitamin D serum concentrations [42].

Very recently, a further study supported a role for vitamin D as a clinical biomarker for RA, and baseline 25(OH)D levels were suggested even to have potential as a predictor of disease severity again in early onset RA patients [43].

Practically, the D-PRO instruments could potentially be used in interventional longitudinal study investigations with aim to find out optimal vitamin D treatment doses at least in RA patients. This would be in accordance with recent publications and increasing interest for vitamin D supplementation possibilities in RA patients and for related potential clinical implications [23,44–46].

Future applications exist for use of touch-screen versions of D-PRO questionnaire. This format may facilitate patient assessment in clinical practice and can easily be adapted to other specialties and expanded to additional information on other PROs.

5. Conclusions

Present multicentre European survey adds new evidences that vitamin D serum levels correlate with quality of life, disease activity and disability in RA patients. Furthermore, vitamin D insufficiency/deficiency is confirmed as frequent in RA patients, and statistically significant differences in serum concentrations among several European countries are now evident.

This first D-PRO questionnaire was found valid in the cohort of RA patients evaluated in the present pilot survey. A statistically significant negative correlation was found between 25(OH)D serum concentrations and D-PRO Global Risk Score.

A further analysis and selection of the D-PRO items seems necessary in order to better define the questionnaire construct, due to the persistent need to estimate the risk for vitamin D insufficiency/deficiency and clinical-related consequences in RA patients.

Take home messages

Vitamin D status correlates with quality of life, disease activity and disability in rheumatoid arthritis patients. Patient Reported Outcome questionnaire (D-PRO) need to be validated in an even larger number of RA patients to further estimate the risk of vitamin D insufficiency/deficiency and clinical-related consequences.

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Ethics

The study was performed in accordance with GCP and Helsinki Declaration and local Ethical Committee. Patient Informed Consent was obtained and aims and possible consequences of the study had been fully explained before patient enrolment at each national centre.

Declaration of interest

All authors disclose no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

Submission declaration

Authors declare the work described has not been published previously (except in the form of an abstract), that it is not under consideration for publication elsewhere, that its publication is approved by all

Authorship declaration

Authors have made contributions with different involvement to the following steps in the study production: (1) conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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