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Published in:
Scandinavian Journal of Immunology

DOI (link to publication from Publisher):
10.1111/sji.12704

Publication date:
2018

Document Version
Accepted author manuscript, peer reviewed version

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Article type : Regular Manuscript

TITLE PAGE

Article title: The D-vitamin metabolite 1,25(OH)$_2$D in serum is associated with disease activity and Anti-Citrullinated Protein Antibodies in active and treatment naïve, early Rheumatoid Arthritis Patients.

Short title for running head: Vitamin D in early Rheumatoid Arthritis

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Appropriate Scientific heading: Clinical Immunology

ABSTRACT

Rationale: Sufficient levels of vitamin D seem to be essential for proper immune function, and low levels might be associated to disease activity in Rheumatoid Arthritis (RA). Most studies investigate only 25OHD and not the physiologically active vitamin D metabolite, $1,25(OH)_2D$

Objective: To investigate associations between serum level of vitamin D metabolites and disease activity parameters in 160 inflammatory active and treatment naïve early RA patients. Serum level of vitamin D metabolites ($25OHD_2$, $25OHD_3$, and $1,25(OH)_2D$) were measured by isotope dilution mass spectrometry and radio-immunoassays at baseline. Disease characteristics were gender, number of tender

joints, number of swollen joints, DAS28-CRP, HAQ, VAS-scores, CRP, erosive status (Total Sharp Score; TSS), ACPA, and IgM-RF-status. Associations were evaluated using Spearman and Wilcoxon rank sum tests. The study was registered in Clinical trials; Trial registration number: NCT00209859.

Findings: Statistically significant inverse associations were found between the active metabolite $1,25(OH)_2D$ and DAS28-CRP (p=0.004, rho= -0.23), HAQ (p=0.005, rho= -0.22), CRP (p=0.001, rho= -0.25), $VAS_{patient\text{-}pain}$ (p=0.008, rho= -0.21), and a positive association was found to ACPA-status (p=0.04).

Conclusion: The vitamin D metabolite $1,25(OH)_2D$ was inversely associated with disease activity and positively associated with ACPA in treatment naïve and inflammatory active early RA. The results indicate that in RA, both the degree of inflammatory activity, and the diagnostic sensitivity and specificity might affect – or might be affected by the level of vitamin $1,25(OH)_2D$.

INTRODUCTION

Besides its well-known function in calcium homeostasis, vitamin D is also a potent immune-modulator, meant to affect the disease activity and course of several autoimmune diseases, including Rheumatoid Arthritis (RA)

Cells of the immunesystem contain enzymes essential for conversion of the “pre-hormone” 25OHD to the physiologically active metabolite $1,25(OH)_2D$, as well as vitamin D receptors, responsible for the altered gene transcription and thereby maturation and differentiation of the immune cells. Vitamin D orchestrates the immune system by affecting pivotal cytokines in the delicate balance which

maintains immunological homeostasis.[1-3] Briefly, sufficient levels of $1,25(OH)_2D$ leads to a self-accepting Th2 and Th-reg response, dominated by cytokines such as Interleukin(IL)4, IL5 and IL10,[4] whereas insufficient levels lead to a self-aggressive Th1 and Th17 response, dominated by interferon gamma, IL1, IL6 and IL17.[5]

In the classic calcium metabolism, most vitamin D circulates as the inactive metabolite 25OHD, which through hepatic and renal hydroxylation is converted to the physiologically active $1,25(OH)_2D$. Most 25OHD is bound to vitamin D Binding Protein (VDBP), which protects against degradation and renal elimination, thereby prolonging the half-life, but also sequesters 25OHD, which diminishes the bio-availability.[6] 25OHD has a relatively long half-life on 5-6 weeks, whereas the half-life of $1,25(OH)_2D$ is only hours.[7] The conversion of circulating 25OHD to $1,25(OH)_2D$ is tightly regulated by parathyroid hormone,hypocalcaemia, hypophosphatemia and $1,25(OH)_2D$ itself, thereby maintaining circulating $1,25(OH)_2D$ levels within the normal range (60-200 pmol/l, even in severe 25OHD insufficiency,[8] and the level of circulating 25OHD is therefore widely used as a marker of the body´s vitamin D reserves. Sufficient levels of 25OHD are above 50 nmol/l, while lower levels are leading to compensatory secondary hyperparathyroidism. Levels below 25 nmol/L are considered very low, as the secondary hyperparathyroidism is accelerated and the bone turnover increases.[8, 9] Though, in some chronic inflammatory conditions, such as sarcoidosis and RA, a local, non-renal production of $1,25(OH)_2D$ might contribute significantly to the systemic concentration.[10, 11]

The local vitamin D metabolism in T-lymphocytes, dendritic cells, monocytes and macrophages is affected by cell-activation status: Antigen presenting cells constantly express vitamin D Receptor, whereas it is inducible in T-lymphocytes upon activation

[3, 12-14]. Also enzymes responsible for conversion to active $1,25(OH)_2D$, and further inactivation (1-α-hydroxylase and 24-α-hydroxylase, respectively), are expressed by macrophages and dendritic cells when activated.[3] The local enzymatic activity is in most immune cells regulated in a negative feedback mechanism by $1,25(OH)_2D$ itself, thereby ensuring a balanced production of $1,25(OH)_2D$. In vitro studies indicate that 1-α-hydroxylase activity is increased in e.g dendritic cells and macrophages during activation, probably to modulate and direct the ongoing immune response.[3, 15] Concerning the $1,25(OH)_2D$ degrading enzyme; 24-α-hydroxylase, the local activity is inhibited in early activation of dendritic cells and macrophages, whereas the inhibition decreases during maturation, ensuring sufficient enzymatic activity to avoid over-stimulation by $1,25(OH)_2D$.[3, 16] However, it seems that despite the local ability to convert circulating 25OHD to $1,25(OH)_2D$, the availability of free 25OHD is limited by the binding to vitamin D binding protein, often stated as "the free hormone hypothesis".[6, 17, 18] Thus, a delicate interplay between binding proteins, enzymes, receptors and activation status of the implicated immune-cells impacts the immunomodulatory properties of vitamin D.

The main methods for measuring vitamin D status, assessed as D_{total}, the sum of $25OHD_2$ and $25OHD_3$, is either immunoassays (e.g radioimmunoassays (RIA)) or chromatographic methods (e.g. LC-MS/MS). In general there is reliable precision between RIA and LC-MS/MS,[19, 20] and especially the ability to detect severe vitamin D deficiency is sufficient sensitive.[20] LC-MS/MS is the golden standard when measuring 25OHD, but RIA might be a relevant alternative method that in some cases reach even lower detection limits than LC-MS/MS.[21] Though;

detection of D_2 might be lacking in immune-assay procedures, and thus the D_{total} might be underestimated, whereas LC-MS/MS detects $25OHD_2$ and $25OHD_3$ separately, and therefore more precisely measures D_{total}.[20] In Denmark, food is only rarely fortified with vitamin D, and in such cases, as well as in vitamin D supplementation, vitamin $25OHD_3$ is used, wherefore levels of $25OHD_2$ is low. Moreover, differences in vitamin D binding protein can lead to inaccuracy;[22] and must be taken into account, as vitamin D binding Protein seems to be a negative acute phase reactant, which is lowered during systemic inflammation.[23] Awareness of continuous bias in evaluation method is necessary; and Berry et al[24] recommend to statistically harmonize values, based on a small sample of duplicate-measurements, to ensure comparability between studies.

Concerning detection of $1,25(OH)_2D$ in circulation, methods are challenged by the very low concentrations (pmol/l) and the short half-life.[7] LC-MS/MS distinguish between $1,25(OH)_2D$ derived from $25OHD_2$ and $25OHD_3$ with high sensitivity and specificity, whereas RIA evaluates both metabolites as one. Both methods show reliable sensitivity in the normal range, whereas LC-MS/MS has higher sensitivity than RIA when evaluating very low levels. Generally, the correlation between RIA and LC-MS/MS is good when evaluating $1,25(OH)_2D$.[25]

RA is a classic systemic, autoimmune disease that leads to joint swelling, erosions and disability. Patients are often affected by co-morbidities, such as osteoporosis, and systemic disease manifestations, mainly in the cardiopulmonal system.[26] The etiology of RA is not fully known, but is meant to be multifactorial, with genetic as well as environmental factors leading to lack of immunological self-acceptance, caused by an aggressive, Th1 driven immune response against the synovialis. Main

pro-inflammatory cytokines in RA are Tumor Necrotizing Factor, IL6 and IL17.[27] Owing to the immunomodulatory properties of vitamin D, associations between vitamin D metabolites and RA disease course is widely studied. Vitamin D levels in RA patients are often low, but it is not know if it is the cause or the consequence of the disease.[5] In RA cell cultures, $1,25(OH)_2D$ are shown to affect immune-cell maturation and differentiation,[5] and to directly affect main pro-inflammatory cytokines, such as TNF, IL1, IL6,[28] as well as IL17.[29] Human studies have mainly focused on the commonly measured 25OHD:[30] In chronic RA patients, treated with Disease-Modifying Anti-Rheumatic Drugs (DMARDs), studies found divergent associations between 25(OH)D levels and disease activity with a tendency towards an inverse association, confirmed in a recent meta-analysis.[30] On the contrary, the physiological active $1,25(OH)_2D$ is only rarely evaluated in vivo in RA cohorts, and a recent meta-analysis found studies evaluating $1,25(OH)_2D$ in RA too sparse to a separate meta-analysis.[31] Though, scientific work in the field of the physiological active metabolite seem to increase, and a recent study indicate that the in vitro association to a pro-inflammatory milieu is also found in human studies.[32]

The mounting evidence that vitamin D, and especially $1,25(OH)_2D$ is involved in the immune-homeostasis, and that low levels of vitamin D seem to predispose to a pro-inflammatory state, makes it relevant to evaluate associations between circulating levels of all vitamin D metabolites and their association to disease activity in RA patients with systemic inflammation.

The aim of this study was, in inflammatory active and treatment naïve early RA patients, to quantify serum levels of $25OH_2$, $25OH_3$, and $1,25(OH)_2D$ at the time of

diagnosis, and to test the pre-specified hypothesis that associations exist to RA disease-activity parameters.

MATERIALS AND METHODS

Patients: 160 patients were recruited from five Danish University Clinics from October 1999 to October 2002. They participated in the CIMESTRA study, eligibility criteria and selection of participants are described elsewhere,[33] baseline characteristics are listed in table 1. Patients were treatment naïve at the time of enrollment, and received no vitamin D or Calcium supplementation prior to inclusion. Vitamin D metabolites ($25OHD_2$, $25OHD_3$, and $1,25(OH)_2D$) were evaluated at baseline and D_{total} calculated as the sum of $25OHD_2$ and $25OHD_3$. Number of Tender Joints (NTJ), Number of Swollen Joints (NSJ), Visual Analogue Scores (VAS); $VAS_{global\text{-}doctor}$, $VAS_{global\text{-}patient}$, $VAS_{pain\text{-}patient}$, C-Reactive Protein (CRP), Disease Activity Score in 28 joints, calculated based on CRP (DAS28-CRP), Health Assessment Questionnaire (HAQ), Anti-Citrullinated Protein Antibodies (ACPA), Immunoglobulin M Rheumafactor (IgM-RF) were evaluated at time of inclusion.

Participant´s received oral and written information prior to inclusion. Written consent was given before enrolment. The study was in compliance with the Helsinki declaration, and approved by the national health authorities and local ethics committee (M-1959-98) and is registered at www.clinicaltrials.gov (Ref. number: NCT00209859).

Measuring vitamin D metabolites: Serum $25OHD_2$ and $25OHD_3$ were analyzed by isotope-dilution liquid-chromatography-mass-spectrometry (LC-MS/MS).[34] Mean coefficients of variation (CVs) for $25OHD_3$ were 8.1% at 48 nmol/l and 9.6% at 25 nmol/l, and for $25OHD_{2,}$ the CV were 8.5% at 23 nmol/l, and 8.0% at 64 nmol/l. $1,25(OH)_2D$ was analyzed by Radio immune assay (RIA)[35] after immune-extraction of the samples (1,25-dihydroxy vitamin D RIA, IDS, Boldon, UK). According to the supplier, the method co-determines $1,25(OH)_2D_2$, with a cross specificity of 92% compared to $1,25(OH)_2D_3$. Mean intra-assay CV of 6.8% and 9.0% were observed at levels of 90 and 220 pmol/l respectively.

Routine laboratory assessments: IgM-RF was detected by enzyme-linked immunosorbent assay. ACPA IgG antibodies were determined by a second-generation enzyme-linked immunosorbent assay (Immunoscan RA kit, Euro-diagnostica AB, Malmo, Sweden) with the recommended 25 U/ml cut-off point. Serum CRP was measured using standard technique.

Reporting: Reporting of the results conform to the 'Strengthening the Reporting of Observational Studies in Epidemiology' (STROBE) statement for cross-sectional studies.

STATISTICS

Univariate associations between the independent variable, evaluated as continuous vitamin D metabolite levels, and the dependent variables; NTJ, NSJ, DAS28-CRP, CRP, HAQ, $VAS_{patient\text{-}pain}$, $VAS_{global\text{-}patient}$ and $VAS_{global\text{-}doctor}$, were evaluated using Spearman test. Wilcoxon rank sum test evaluated associations between vitamin D

metabolite levels and the sub-groups IgM-RF- positive versus negative patients, ACPA-status positive versus negative patients, erosive status evaluated as non-erosive disease (TSS = 0) or erosive disease (TSS > 0), and season of diagnosis, dichotomized as summer or winter (winter defined as diagnosis established from November to April). Significance was defined as the 5 % level. The "R" software package was used, and analyses were performed by an independent statistician.

RESULTS

69 patients (42%) had low D_{total}, (below 50 nmol/l). 15 patients (10 %) had very low levels below 25 nmol/l. 16 patients (10 %) had low $1,25(OH)_2D$ levels, (below 60 pmol/l). The majority of these (75 %) had concomitantly low D_{total}.

Statistically significant inverse associations were found between $1,25(OH)_2D$ (median 95 pmol/l, range 33–268), and RA disease variables as DAS28-CRP (rho=-0.23, p=0.004), HAQ (rho=-0.22, p=0.005), CRP (rho=-0.25, p=0.001), and $VAS_{patient-pain}$ (rho=-0.21, p=0.008).There were no significant associations to NSJ (rho=-0.09, p=0.24), NTJ (rho=-0.09, p=0.26), $VAS_{global-patient}$ (rho=-0.14, p=0.08) or $VAS_{global-doctor}$ (rho=-0.14, p=0.09). Significant positive association was found between $1,25(OH)_2D$ and ACPA-status (p=0.04); but not to IgM-RF status (p=0.35), nor to erosive or non-erosive status at baseline (p=0.29). Please see figure 1.

Median $25OHD_2$ was 0 nmol/l (range 0–37). This was significantly inversely associated with NTJ (rho=-0.17, p=0.03) and not to other variables, see figure 1 and table 2.

Median $25OHD_3$ was 52 nmol/l (range 0–145), this was not associated to any variables, see table 2.

Seasonal variation was found for $25OHD_3$, with significantly lower levels in patients diagnosed during the winter (p=0.003). No significant seasonal variation in levels of $25OHD_2$ (p=0.46) and $1,25(OH)_2D$ (p=0.15) was found.

For all results, see table 2.

Concerning missing data, 3 patients had missing values of vitamin D metabolite levels at baseline, and 3 patients had missing values of CRP and therefore of DAS28. There are no indications that these data were not missing at random, and therefore these patients were not excluded during the analyses.

DISCUSSION

This study is one of few human studies investigating all vitamin D metabolites. Main finding is, in 160 early diagnosed, treatment-naive RA patients that low serum levels of $1,25(OH)_2D$ are associated to several parameters for RA disease activity, including CRP, HAQ, $VAS_{patient\text{-}pain}$ and DAS28-CRP. Despite 90 % of the cohort had $1,25(OH)_2D$ in the normal range, there were several strong associations to markers for disease activity when evaluating the metabolite as a continuous variable. This was chosen prior to analyses, to investigate if levels lower in the normal range was associated to markers of disease activity. We find this approach to be relevant because of the gap in knowledge concerning cut-offs in vitamin D metabolites and proper immune-function. Even though $1,25(OH)_2D$ is closely regulated under normal

physiological conditions,[3] our results indicate that the regulation might be disturbed during systemic inflammation.

Activation status and cytokine production of T-lymphocytes and antigen presenting cells affect the local vitamin D metabolism, leading to increased local $1,25(OH)_2D$ synthesis.[3, 12-14, 36] It is somewhat contra-intuitive to our results, where lower levels of $1,25(OH)_2D$ are associated to elevated markers of disease activity, including markers of systemic inflammation, such as CRP and DAS28CRP, and denotes that much is still unknown concerning vitamin D and inflammation in RA- as well as in non-RA subjects.

Our study adds to the literature that 25OHD, a commonly used marker for the body`s vitamin D reserve, is not associated to RA-disease activity or diagnostic criteria in early, treatment-naive RA patients. Further, the tradition of only measuring 25OHD when assessing vitamin D status in RA patients, is challenged by our results.

As expected, 25OHD levels showed seasonal variation, owing to increased cutaneous production during sun exposure in the summer.[37] This is a factor that must be taken into consideration, whenever studying associations to 25OHD. In small studies, this weakens the power, whereas our finding of no seasonal variation in $1,25(OH)_2D$ in newly diagnosed RA patients might be useful knowledge when assessing vitamin D in future studies.

Associations between $1,25(OH)_2D$ and disease activity in early RA is rarely assessed in human studies. Our finding of a negative association between $1,25(OH)_2D$ and DAS28 is in agreement with the findings of Liu et al [32] in 208 treatment naïve RA patients. In 206 early, inflammatory poly-arthritis patients treated with DMARD up to 6 weeks, Patel et al finds significant inverse associations

between baseline 25OHD and NTJ, CRP, HAQ and DAS28-CRP, whereas the only association regarding $1,25(OH)_2D$ was for the HAQ-score.[38] Differences in study populations, treatment and statistical methods are possible explanations for the discrepancies when compared to our results: Liu et al evaluates patients fulfilling ACR1987 classification criteria with a mean disease duration of 9 years, Patel et al evaluates undifferentiated poly-arthritis patients with a mean disease-duration of 4 months, whereas symptom-duration in the CIMESTRA cohort was 3.2 months and all patients full-filled the ACR1987 classification criteria.[33] Liu et al evaluates DMARD- and steroid naïve patients like the CIMESTRA cohort, whereas Patel et al accept DMARD treatment 6 weeks prior to inclusion. Moreover, Liu et al evaluates vitamin D as dichotomized variable, whereas Patel et al evaluates changes in disease activity markers per 10 unit change in vitamin D metabolites.

Our finding of an inverse association between $1,25(OH)_2D$ and CRP has also been observed in healthy subjects.[39] A possible explanation is interaction between vitamin D, calcium-homeostasis and IL-6, affecting the production of acute-phase reactants.[40]

The association of $1,25(OH)_2D$ to CRP and ACPA, and of $25OH_2D$ to NTJ add credit to the notion that the level of vitamin D metabolites may influence on diagnostic specificity and sensitivity associated with the ACR/EULAR2010 classification criteria for RA.[26] Though, other studies evaluating vitamin D and ACPA departs from our results: Liu et al[32] found a negative association between ACPA and 25OHD, and similarly, Kerr et al found association between low levels of 25OHD and ACPA-positivity in multivariate analyses.[41] On the contrary, Feser et al found no

association between 25OHD and ACPA in subjects susceptible to RA.[42] To our knowledge, no previous studies have evaluated associations between ACPA and the active vitamin D metabolite $1,25(OH)_2D$. The first step in the citrullination process is calcium-dependent, and $1,25(OH)_2D$ is essential in maintaining calcium homeostasis, so it can be hypothesized that the association might be linked to the calcium-homeostasis. However, the association between $1,25(OH)_2D$ and ACPA needs further investigation before conclusions can be reached.

We found no significant associations between any of the vitamin D metabolites and presence of erosions at the time of RA diagnosis. Human RA studies evaluating associations between vitamin D metabolites and erosions have only evaluated 25OHD and not the active metabolite, and found no associations.[43, 44] Receptors responsible for the action of vitamin D are present in the RA synovialis, and cell culture studies have shown that $1,25(OH)_2D$ can affect chondrolysis,[17] and that low levels of $1,25(OH)_2D$ might increase the risk of bone erosions.[45] This is not confirmed in human studies. In our human RA study, the serum level of vitamin D metabolites were not associated with the TSS, thereby supporting that no association exists between vitamin D metabolites and RA erosions.

This study has some limitations. The cross-sectional design does not investigate causality, and it can be argued that the associations of low vitamin D metabolite levels to RA disease activity are the consequence rather than the cause of the disease. Moreover, vitamin D status is the result of a delicate interplay with both

immune-cells and calcium-homeostasis, and a limitation in our study is the lack of measuring calcium, phosphate, parathyroid hormone and albumin.

Furthermore, a common challenge in vitamin D studies is the vitamin D measurement method, as great variability between methods exists, thereby affecting the between-studies comparability.[19, 20] In this study, $25OHD_2$ and $25OHD_3$ were measured using LS-MS/MS, which detects D_2 and D_3 separately, whereas $1,25(OH)_2D$ was measured with RIA, which detects both D_2 and D_3-derived $1,25(OH)_2D$.[35] We find the variation coefficients provided by the suppliers acceptably low across the low and normal range.[34, 35]

Possible confounders are adiposity, physical capacity, age and renal disease, which all are associated to both vitamin D deficiency and RA disease activity. The lipophilic vitamin D clusters in adipose tissue, and leads to lower levels of circulating vitamin D, and adipose tissue is known to contribute to inflammation in RA.[46] Physical inactivity is common in RA, and is associated with inflammatory activity, pain and fatigue,[47] whereas some studies also associate physical inactivity to lower vitamin D levels, including $1,25(OH)_2D$.[48] Low vitamin D levels are common in the elderly,[8] and in patients with renal insufficiency. The exclusion of patients over 75 years of age and patients with renal insufficiency could lead to underestimation of the prevalence of vitamin D deficiency in our cohort, compared to the general population, thereby weakening the external validity.

The strengths of our study are the well characterized, early and treatment-naïve RA patients and the measurement of both 25OHD and $1,25(OH)_2D$ serum levels.

The novelty of this study is the evaluation of all vitamin D metabolites, compared to the vast amount of reports in 25OHD only. We find it relevant to investigate this gap in knowledge, as the $1,25(OH)_2D$ is the physiologically active metabolite, which is responsible for the immunological actions of vitamin D, as well as in the calcium homeostasis. That said, knowledge concerning calcium homeostasis suggests that sufficient levels of circulating D_{total} is necessary for local conversion to $1,25(OH)_2D$.[5, 18] Off note, our results show that most patients have $1,25(OH)_2D$ in the normal range, even when circulating D_{total} is extremely low. This finding is expected, as the circulating levels of $1,25(OH)_2D$ is strictly regulated by calcium, parathyroid hormone and $1,25(OH)_2D$ itself, as part of the calcium homeostasis, even in severe vitamin D deficiency. We find that the evaluation of $1,25(OH)_2D$ as a continuous variable shows a clear trend toward an inverse association to several RA disease activity parameters. However, the study design does not allow conclusions concerning causality, and the question; does low levels of vitamin D; especially $1,25(OH)_2D$, lead to increased RA disease activity, or does the systemic inflammation lead to lower levels of circulating $1,25(OH)_2D$, is still open. Preferably, this should be evaluated in large, prospective cohorts.

In summary, our study revealed biological plausible associations between circulating $1,25(OH)_2D$ and several parameters relevant for diagnosis and assessment of disease activity in a cohort of inflammatory active and treatment-naïve early RA patients. These results are likely applicable in early RA patients of Scandinavian ancestry. In future studies we recommend measurement of all vitamin D metabolites as well as calcium, phosphate, parathyroid hormone, and albumin, to evaluate the interplay between vitamin D metabolites and calcium metabolism in RA.

ACKNOWLEDGEMENT

The authors are grateful to Niels Steen Krogh for expert assistance with the statistical analysis.

FUNDING:

This work was supported by The Danish Rheumatism Association, who provided financial support to scientific personnel and vitamin D metabolite analyses [grant number R117-A2898-B553, R115-A2764-B553]. Silkeborg Region Hospital Research Fond and Research Foundation at Regional Hospital Midt provided financial support to vitamin D metabolite analyses. PhD fund at Region Southern Denmark [grant number 14/24417] and Faculty Scholarship of University of Southern Denmark provided financial support to scientific personnel. Novartis Healthcare Denmark A/S provided the ciclosporine (SandimmunNeoral) and placebo-ciclosporine and sponsored an independent GCP monitor. Nycomed provided methotrexate (Emthexate), folic acid (Apovit) and calcium/vitamin D (CaviD) supplementation. Schering-Plough provided betamethasone (Diprospan) and MSD provided alendronate (Fosamax). Pfizer Denmark provided an “unrestricted grant” to the project [grant number WS2583631]. The sponsors were not involved in the study design, data collection, analysis or interpretation, and had no influence on the publishing of data.

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Table 1: Baseline demographic, clinical and immunological characteristics of 160 early RA patients who were inflammatory active and treatment naïve.

	Baseline n = 160
Age (years)	53.0 (20.4–75.2)
Female sex (%)	67
Disease duration (weeks)	14.1 (6.1–26.6)
IgM-RF positive baseline (%)	66
ACPA positive baseline (%)	58
NTJ (0-40)	14 (0–39)
NSJ (0-40)	11 (2–37)
$VAS_{global\text{-}doctor}$ (0-100 mm)	58 (3–98)
$VAS_{patient\text{-}pain}$ (0-100 mm)	46 (2–100)
$VAS_{global\text{-}Patient}$ (0-100 mm)	50 (3–100)
CRP (mg/l)	20.1 (0.0–253.1)
DAS28-CRP	5.5 (2.23–8.38)
HAQ (0–3)	0.937 (0–2.875)
$1,25(OH)_2D$ (pmol/l)	95 (33–268)
$1,25(OH)_2D$ below 60 pmol/l (%)	9,3
$25OHD_3$ (nmol/l)	52 (0-145)
D_{total} (nmol/l)	53 (0–145)
D_{total} below 50 nmol/l (%)	43.1
Erosive disease (TSS> 0) (%)	61

Values are median, unless otherwise stated. Values in parentheses are range.

Abbreviations: IgM-RF: Immunoglobulin M Rheuma factor, ACPA: Anti Citrullinated Protein Antibodies, NTJ: Number of Tender Joints. NSJ: Number of Swollen joints. VAS: Visual Analogue Scores. CRP: C-reactive Protein. DAS28-CRP: Disease Activity Score calculated in 28 joint counts, based on CRP. HAQ: Health Assessment Questionnaire.D_{total}: The sum of $25OHD_2$ and $25OHD_3$ TSS: Total Sharp Score

Table 2: Baseline D vitamin levels and associations with RA disease activity parameters and immunological status in 160 early RA patients who are inflammatory active and treatment naïve.

	$1,25(OH)_2D$		**$25OHD_2$**		**$25OHD_3$**	
	rho	p	rho	p	rho	p
NTJ	-0.09	0.26	**-0.17**	**0.03**	-0.04	0.59
NSJ	-0.09	0.24	-0.06	0.48	-0.07	0.40
VAS_{doctor}	-0.14	0.09	0.03	0.74	-0.13	0.10
VAS_{global}	-0.14	0.08	-0.04	0.59	0.02	0.79
$VAS_{patient}$	**-0.21**	**0.01**	-0.03	0.67	-0.04	0.61
CRP	**-0.25**	**0.001**	0.03	0.74	-0.15	0.06
DAS28-CRP	**-0.23**	**0.004**	-0.05	0.54	-0.06	0.44
HAQ	**-0.22**	**0.010**	-0.004	0.96	-0.04	0.62
ACPA-status		**0.04**		0.52		0.38
IgM-RF-status		0.35		0.96		0.96
Baseline TSS		0.29		0.73		**0.32**
Season of diagnosis		0.148		0.463		**0.003**

Spearman test was used for univariate associations of D vitamin metabolite levels to NTJ, NSJ, $VAS_{global\text{-}doctor}$, $VAS_{global\text{-}patient}$, $VAS_{patient\text{-}pain}$, CRP, DAS28-CRP and HAQ. P-value and regression coefficient presented.

Wilcoxon rank sum test was used to evaluate ACPA-status, IgM-RF-status, baseline erosive status,and season of diagnosis.

Significant p values in bold

Abbreviations: NTJ: Number of Tender Joints. NSJ: Number of Swollen joints. VAS: Visual Analogue Scores. CRP: C-reactive Protein. DAS28-CRP: Disease Activity Score calculated in 28 joint counts, based on CRP. HAQ: Health Assessment Questionnaire. ACPA: Anti Citrullinated Protein Antibodies. IgM-RF: Immunoglobulin M Rheuma factor.TSS: Total Sharp Score D_{total}: The sum of $25OHD_2$ and $25OHD_3$

FIGURE 1