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Marine ω-3, vitamin D levels, disease outcome and periodontal status in rheumatoid arthritis outpatients

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ABSTRACT

Objectives: Marine ω-3 fatty acids (FAs) and Vitamin D (VitD) are reportedly capable of down-regulating inflammation in rheumatoid arthritis (RA) and periodontal disease. This study was undertaken to relate marine FA and VitD status to RA disease status and periodontal conditions.
Methods: RA outpatients (age ≥35 y) were consecutively recruited. Rheumatologic clinical data were collected and periodontal status obtained. A food frequency questionnaire was used to estimate fish and supplement intake. FA profiles in whole-blood and serum VitD levels were determined.
Results: A total of 78 RA patients (age 57 ± 12 y, disease duration 15 ± 11 y) were included, 58% had active RA. Periodontitis was diagnosed in 82% of the patients, 18% had severe periodontitis. Seropositivity for rheumatoid factor and/or anticitrullinated protein antibodies was related to higher prevalence of periodontitis ($P = 0.008$). Seafood intake in accordance with nutritional recommendations was associated with better RA disease outcome (largest $P = 0.008$). An ω-3 index >8, present in 14% of the patients, correlated with a more desirable patient global health assessment scored on a visual analog scale (VAS; $P = 0.004$), lower periodontal probing depth (PD; $P = 0.021$), and ω-3 supplementation ($P = 0.001$). Serum VitD levels >50 nmol/L were found in 89%, of these 48% had VitD levels ≥75 nmol/L, no differences were found for RA disease activity and periodontal measurements.
Conclusions: Seropositive RA patients had a higher prevalence of periodontitis than seronegative patients. An ω-3 index >8 was related to ω-3 supplementation and more desirable VAS and lower PD. VitD status was satisfactory for most patients and was not associated with differences in RA severity or periodontal diagnosis.

Introduction

Rheumatoid arthritis (RA) and periodontitis are chronic progressive inflammatory diseases associated with soft and hard tissue destruction. Genetic, epigenetic, and environmental factors drive host responses in both diseases [1–3]. An association between periodontitis and RA has been described in systematic reviews [4] and meta-analyses [5]. The etiopathogenesis of RA is complex and the offending stimulus is still not clear. It is hypothesized that a second inflammatory "hit" is required to stimulate development of the disease [6]. Translocation and trapping of oral bacterial DNA from inflamed periodontal tissue into synovial fluid suggest the involvement of periodontitis in the etiopathogenesis of RA [7]. Although periodontal disease is clearly initiated by bacteria, the underlying mechanisms are hyperactive immune responses of the host to dysbiotic bacterial infection [8]. Periodontitis is a major cause of tooth loss in adults, which can exert a negative impact on nutritional status and health-related quality of life (HRQOL) [9]. In active RA, systemic inflammation is associated with multiple extraarticular comorbidities such as depression, asthma, cardiovascular events, malignancies, and chronic obstructive pulmonary disease, resulting in a significant reduction in HRQOL [10,11].

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Several studies have shown that fatty acid (FA) profiles of blood lipids reflects dietary fat intake and relate to health status [12,13]. Polyunsaturated FAs (PUFAs) in the ω-3 series and ω-6 series are precursors to potent lipid mediator signaling molecules, which have important roles in immune regulation and inflammation [14]. Marine ω-3 PUFAs are capable of down-regulating inflammation in RA and periodontitis [15,16]. In most populations, intake of ω-6 PUFA is increased, whereas the consumption of ω-3 PUFA is either insufficient or not effective at providing adequate tissue levels of the ω-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [13].

Vitamin D (VitD) might affect RA and periodontal disease through immunomodulatory effects. The active metabolite 1,25–dihydroxyvitamin D_3 was found to downregulate pro-inflammatory mediators such as TNF-α and RANKL in monocyte-derived macrophages from peripheral blood of RA patients [17] and to be inversely associated with severity of periodontitis in non-RA patients [18]. In RA patients, serum 25-hydroxyvitamin D (s-25[OH]D) levels were reduced and showed an inverse relationship with RA disease activity, interleukin (IL)-17/IL-23, and bone loss [19]. Whereas a higher VitD intake corresponded to lower odds of severe periodontal disease, moderate-to-severe alveolar bone loss has been linked to lower VitD intake [20], and insufficient VitD status has been related to decreased clinical periodontal attachment gain following periodontal surgery [21]. Genetic polymorphisms of the VitD receptor gene have also been related to severity of RA and chronic periodontitis [22,23], and enrichment of VitD response elements at RA-associated loci supports a role for VitD in development of this disease [24].

The aim of the present study was to examine the association between RA disease status and periodontal conditions in relation to intake and status of marine ω-3 FA and VitD.

Methods

Ethics

Study protocol and informed written consent of all participants, according to the Helsinki Declaration of 1975, version 2008 [25], were approved by the Institutional Medical Research Ethics Committee (2012/2212), University of Bergen, Norway.

Study design and patient selection

The data for this cross-sectional study were collected between May 2013 and March 2016 at the Department of Rheumatology at Haukeland University Hospital and at the Department of Clinical Dentistry at the University of Bergen (Bergen, Norway). A patient flow chart is presented in Figure 1. RA outpatients with chronic established RA were invited to participate (N = 140). RA disease was classified using the 2010 classification criteria of American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [26]. Inclusion criteria were chronic established RA, Caucasian ethnicity, and ≥35 y of age. Exclusion criteria were diabetes, malignancy, pregnancy, breast-feeding, and antibiotic use within 3 mo before the study. Demographic and behavioral characteristics were collected using questionnaires. Past medical history, clinical, and laboratory data on RA status and medication were obtained from medical records.

Assessment of RA disease activity

Recorded data included disease duration of RA, disease activity score (DAS28) [27,28], joint damage, modified health assessment questionnaire (MHAQ) [29], and a patient global health assessment scored on a visual analog scale (VAS). Routine laboratory analyses included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and/or anticyclic citrullinated peptides (anti-CCP) titers. Based on the laboratory reference level, all values >25 IU/mL for RF and ≥3 U/mL for anticitrullinated protein antibodies (ACPAs) were classified as seropositive for autoantibodies. VAS was measured on a horizontal visual analog scale of 10 cm with “no pain” at one end and “worst possible pain” at the other end. The patient was instructed to mark “X” on the scale by using the following phrasing: “We kindly ask you to review the activity of your rheumatic disease during the last week. When you take all the symptoms into account, how do you rate your condition?”. RA disease activity score (DAS28) was calculated using tender 28 joint score, swollen 28 joint score, ESR, and VAS. Active RA was defined as DAS28 ≥2.6 and remission as DAS28 <2.6 [30]. Low disease activity was confirmed if DAS28 score was ≤3.2, moderate if DAS28 score was >3.2 to ≤5.1, and high if DAS28 score was >5.1 [31].

Disease modifying antirheumatic drugs (DMARDs) were grouped as follows: Conventional DMARDs (methotrexate, leflunomide, hydroxychloroquine, sulfasalazine), biological DMARDs (tumor necrosis factor [TNF]-inhibitors, B-cell inhibitors, IL-6 inhibitors), and a combination of conventional and biological DMARDs.

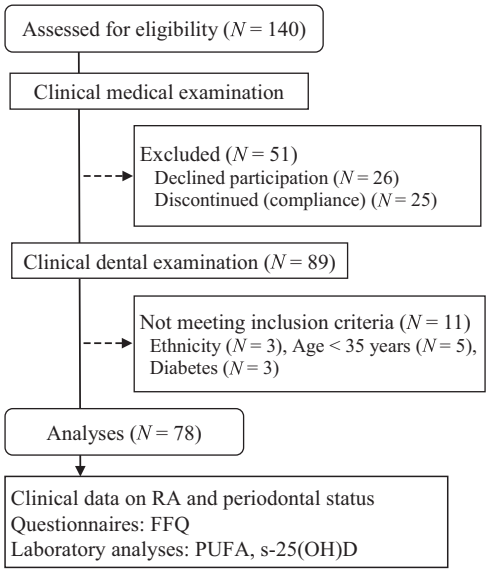

Fig. 1. Patient flow chart. FFQ, food frequency questionnaire; PUFA, polyunsaturated fatty acid; RA, rheumatoid arthritis; s-25(OH)D, serum levels of 25-hydroxyvitamin D.

Clinical oral examination

Clinical oral examination and periodontal data collection were performed under standardized conditions by a single dentist (K.B.). Detailed description is provided in Supplementary Methods. Prior to the study, calibration training for intraexaminer (K.B.) reproducibility of periodontal registrations was conducted. The intraclass correlation coefficient (ICC) ranged between 0.83 and 0.91 for probing depth (PD) and between 0.93 and 0.98 for clinical attachment level (CAL).

A comprehensive periodontal examination including registration of PD, CAL, bleeding on probing (BoP), and accumulation of bacterial plaque (plaque index, PI) was assessed at six sites per tooth by using a manual periodontal probe (PCP-26, Hu-Friedy, Chicago, Ilinois, USA). Third molar, if not in position of second molar, was excluded from periodontal registration.

Assessment of periodontal status

The periodontal status assessment was adapted from the Centers for Disease Control-American Academy of Periodontology clinical case definitions with some modifications [32]. Subjects were classified into three subgroups: 1) gingivitis: PD ≤3 mm, and BoP; 2) mild/moderate periodontitis: ≥ 2 interproximal sites with CAL ≥3 mm (not on the same tooth) and ≥2 interproximal sites with PD ≥4 (not on the same tooth) and BoP; or 3) severe periodontitis: ≥ 2 interproximal sites with CAL ≥6 mm (not on the same tooth) and ≥1 interproximal site with PD ≥5 mm and BoP.

Smoking status

Current smokers were defined as subjects who smoked at the time of study enrollment. Former smokers were subjects who had stopped smoking. The number

of pack-years was calculated by dividing the number of cigarettes smoked per day by 20 and multiplying by the number of years smoked.

Assessment of seafood intake and dietary supplement intake

RA patients completed a validated, short version food frequency questionnaire (FFQ), described in detail by Dahl et al. [33]. In brief, semiquantitative and retrospective habitual intake of seafood for dinner, as sandwich spread, in salads, or as snack meal during the last three months was investigated. Additionally, self-reported information about physical activity and sun exposure habits were registered. The FFQ was analyzed as described by Markhus et al. [34]. To compute the patients' total seafood index, composed of the seafood dinner index, seafood spread index, and ω-3 supplement index, the frequency data of the participants' indices were converted into numerical values. Adaptions to Markhus et al. [34] were made regarding the frequencies for total dinner seafood index as follows: "once a week" was converted to the numerical value 1, "two to three times per week" set to value 2.5, and "four or more times per week" was set to value 4. For the total seafood spread index, the frequencies were converted as follows: "three to five times per week" was set to the numerical value 4 and "more than five times per week" was set to value 5. The range of the total seafood index was 0 to 14. A standard Norwegian dinner seafood portion is defined as 150 g (e.g., one slice of salmon fillet, three fishcakes, or 2 dL of shrimp) [35]. According to the Norwegian food recommendations, 300 to 450 g/wk of fish for dinner and spread (fish filet and processed fish products) should be part of a healthy balanced diet, of which at least 200 g should be oily fish [36]. The total fish intake per week (including fish filet, fish as spread, and processed fish) corresponds to 54 g/d of raw fish and 35 g/d for oily fish [37].

The FFQ also provided information about the use of supplements (marine ω-3, VitD, and calcium) during the last 3 mo. For ω-3 supplement, product names, amount (capsules or spoons), and frequency of supplement intake were recorded. Seasonal variation in ω-3 supplement intake during the year was registered.

The questionnaire was designed to be self-instructive; however, assistance was provided in case of item non-response.

Blood collection

Peripheral venous blood samples were obtained by venipuncture at the antecubital fossa (Vacutainer Blood Collection Set, BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, New Jersey).

Laboratory analyses

For analysis of s-25(OH)D levels, 4 mL venous blood was collected in Serum Sep Clot Activator vacutainers (Greiner Bio-One Gmbh, Kremsmuenster, Austria) and set to coagulate for minimum 30 min (maximum 60 min). After centrifugation at 1300 × g for 10 min, the supernatant was stored at –80°C until analysis. S-25(OH)D was determined by isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Hormone Laboratory at Haukeland University Hospital, Bergen, Norway [38]. S-25(OH)D levels were measured as sum of ergocalciferol (25[OH]D_2) and cholecalciferol (25[OH]D_3), whereas 25(OH)D_2 was found to be below the limit of quantification (LOQ). VitD status was evaluated as follows: Deficiency <20 ng/mL (<50 nmol/L), insufficiency 20 to 30 ng/mL (50–75 nmol/L), and sufficiency >30 ng/mL (>75 nmol/L) [39,40]; or deficiency <12 ng/mL (<30 nmol/L), insufficiency 12 to 20 ng/mL (30–50 nmol/L), and sufficiency >20 ng/mL [41].

Whole-blood samples for analysis of FA were collected in 3 mL EDTA vacutainers (Greiner Bio-One Gmbh, Kremsmuenster, Austria) and analyzed at Institute of Marine Research, Bergen, Norway, using ultrafast gas chromatography (UFGC) as an accredited laboratory method (NS-EN ISO/IEC 17025). The FA composition was calculated using an integrator (Chromeleon 6.80, Dionex Corporation, California) connected to the UFGC. The limit of quantification (LOQ) was 0.01, values <0.1 were recoded to the half of LOQ. Results were expressed as percentage of total FA by summing the absolute values of all analyzed FAs and dividing each FA by this value.

Evaluation of ω-3 FA status

The ω-3 index was used to evaluate ω-3 FA status and is defined as the sum of EPA and DHA, expressed as percentage of total FA [42].

Statistical analyses

Median and range were calculated for continuous variables. The Shapiro-Wilk test for normality was used to test for normality with a critical alpha value of 5%. The Wilcoxon rank-sum test was applied for continuous variables not normally distributed. Student *t* test (for continuous variables) and Pearson chi-square (for categorical variables) were used to test for differences between the groups. For the site-specific measures of PD and CAL, taken at each site for all teeth for each patient, mixed effect models with a random effect for patient and tooth were performed. For the relation between RA disease activity and the site-specific measures, logistic regression models with robust variance estimates adjusting for the clustering of tooth within patients were performed. A P value < 0.05 was considered statistically significant. All statistical data analysis was performed using the statistical software STATA version 14.0 for Microsoft Windows (StataCorp LP, Texas).

Results

The study population consisted of 78 RA patients (58% with active RA disease), ages 57 y (range 35.1–77.6 y). Categorical variables, behavioral and clinical characteristics, grouped by RA disease activity, are summarized in Table 1. Continuous variables and clinical characteristics, also grouped by RA disease activity, are summarized in Table 2. A description of the study subjects by sex is presented in Supplementary Tables S1 for categorical variables and Table S2 for continuous variables.

RA status

Active RA with low, moderate, or high disease activity (DAS28) was found in 23 (30%), 17 (22%), and 5 (6%) patients, respectively. In relation to disease duration, DAS28 differed in neither active RA nor remission ($P = 0.51$). Patients with active RA had

Table 1
Categorical variables and behavioral and clinical characteristics in RA patients, grouped by disease activity (N = 78)

	Remission (N = 33)	Active RA (N = 45)	*P* value
	n (%)	*n* (%)	
Females	24 (73)	33 (73)	0.95
Smoking status			
Never smokers	12 (36)	17 (38)	0.74
Former smokers	17 (52)	20 (44)	
Current smokers	4 (12)	8 (18)	
Periodontal conditions			
Gingivitis	5 (15)	9 (20)	0.68
Mild/moderate periodontitis	23 (70)	27 (60)	
Severe periodontitis	5 (15)	9 (20)	
Medical data on RA disease			
Seropositive	28 (85)	32 (71)	0.16
Seropositivity for IgM-RF	22 (67)	19 (42)	0.044
Seropositivity for ACPAs	26 (79)	29 (64)	0.11
Joint destruction	19 (58)	22 (49)	0.45
Antirheumatic medication	31 (94)	43 (96)	0.45
Conventional DMARDs	13 (39)	20 (44)	
Biological DMARDs	7 (21)	4 (9)	
Combination conventional and biological DMARDs	11 (33)	19 (42)	
Prednisolone	4 (12)	17 (38)	0.012
Other medication			
Oral bisphosphonates	3 (9)	5 (11)	0.77
Folic acid	19 (58)	28 (62)	0.68
Calcium	10 (30)	22 (49)	0.10
Supplements			
ω-3	17 (52)	26 (58)	0.58
Vitamin D	15 (45)	28 (62)	0.14

ACPA, anticitrullinated protein antibody; DAS28, disease activity score 28; DMARDs, disease-modifying antirheumatic drugs; RA, rheumatoid arthritis; RF, rheumatoid factor.
Values reported as number and percentage of patients. RA disease activity, diagnosed by DAS28; seropositive, positive tests for RF (N = 74) and/or ACPA (N = 77). Analyzed with Pearson chi-square.

Table 2
Continuous variables, clinical characteristics of all rheumatoid arthritis patients and grouped by disease activity (N = 78)

	Total	Remission	Active RA	*P* value
	N = 78	N = 35	N = 43	
Age (y)	57.1 ± 11.5	56.5 ± 11.6	57.5 ± 11.6	0.70
BMI (kg/m^2)	26.1 ± 4.3	25.9 ± 4.4	26.3 ± 4.4	0.63*
Oral data				
Teeth, *n*	25 ± 5	25 ± 4	24 ± 6	0.22
PD (mm)	2.8 ± 0.4	2.7 ± 0.3	2.8 ± 0.4	0.65
CAL (mm)	3.1 ± 0.8	3.0 ± 0.7	3.1 ± 0.8	0.57
BoP (% of sites)	31 ± 16	33 ± 14	30 ± 17	0.49
PI (% of sites)	33 ± 16	36 ± 19	31 ± 14	0.20
RA data				
RA debut (age)	43 ± 14	41 ± 13	45 ± 15	0.30
RA duration (y)	15 ± 11	16 ± 12	14 ± 11	0.37*
DAS28 (score)	3.0 ± 1.1	2.1 ± 0.3	3.7 ± 1.0	<0.001*
VAS (score)	28 ± 21	17 ± 14	36 ± 21	<0.001*
MHAQ (score)	0.37 ± 0.38	0.17 ± 0.25	0.52 ± 0.39	<0.001*
Laboratory data				
ESR (mm/h)	19.8 ± 15.5	12.7 ± 8.3	25.0 ± 17.5	<0.001*
CRP (mg/L)	8.8 ± 13.5	5.0 ± 10.0	11.6 ± 15.1	0.001*
S- 25(OH)D (nmol/L)	74 ± 19	78 ± 19	71 ± 19	0.071
Supplementation				
Vitamin D (μg/d)†	27 ± 12	30 ± 13	25 ± 12	0.25*

BMI, body mass index; BoP, bleeding on probing; CAL, clinical attachment level; CRP, C-reactive protein; DAS28, disease activity score 28; ESR, erythrocyte sedimentation rate; MHAQ, modified health assessment questionnaire; PD, probing depth; PI, plaque index; RA, rheumatoid arthritis; s-25(OH)D, 25-hydroxy vitamin D; VAS, visual analog scale.
Values reported as mean ± standard deviation.
Analyzed with Student *t* test.
* Analyzed with Wilcoxon rank-sum.
† Vitamin D users only (N = 45).

significantly elevated clinical and laboratory parameters related to RA disease than patients in remission ($P < 0.001$, Table 2). Of note, 54% (N = 22) of the RF positive patients and 30% (N = 10, $P = 0.044$) of the RF negative patients were in RA disease remission. Prednisolone treatment (mean dose 7.4 μg/d, range 2.5–30), was found in a higher number of patients with active RA disease (N = 17, 38%) than in RA patients with remission (N = 4, 12%; $P = 0.012$); no differences were found for DMARDs.

Regarding the relation of smoking on RA disease activity, no differences were found between active disease and remission ($P = 0.74$; Table 1). In current smokers, the mean smoking pack-years were 14 y (range 5–30 y). Differences in smoking status were found in ACPA seropositive patients compared with seronegative patients ($P = 0.049$). A higher proportion of ACPA seropositive (N = 23, 42%) than seronegative patients (N = 6, 27%) were never smokers and more seronegative patients were current smokers (N = 7, 32%) compared with seropositive patients (N = 5, 9%; $P = 0.043$). Smoking status did not differ in RF seropositive patients ($P = 0.63$).

Periodontal status

Gingivitis was diagnosed in 18%, mild to moderate periodontitis in 64%, and severe periodontitis in 18% of the patients. Periodontal disease status worsened with increased age ($P = 0.008$). The mean ages of patients with gingivitis, mild to moderate, or severe periodontitis were 51, 58, and 63 y, respectively. For the severity of periodontal disease, significant differences for PD ($P < 0.001$), CAL ($P < 0.001$), BoP (largest $P = 0.033$), and PI (largest $P = 0.010$) were found (Supplementary Table S3). Periodontitis was found in a higher number of patients who tested seropositive for RF and/or ACPA (N = 53, 88%) compared with seronegative patients (N = 11, 61%; $P = 0.008$).

At patient level for PD and CAL, respectively, a median (range) of 3 mm (1–10 mm) and 3 mm (1–14 mm) was found. At tooth level, mandibular molars had the highest mean PD (3.3 mm) and mean CAL (3.6 mm). Mandibular incisors (PD 2.4 mm, CAL 2.7 mm) and premolars (PD 2.5 mm, CAL 3.0 mm) had a lower PD/CAL compared with their respective maxillary teeth (PD 2.6 mm, CAL 2.8 mm; and PD 2.8 mm, CAL 3.2 mm, respectively). Compared with mandibular incisors, all other tooth types in both jaws had significantly deeper PD ($P < 0.001$) and CAL ($P < 0.001$).

At site level, the majority of sites showed PD and CAL ≤3 mm (90% and 83%, respectively). Sites with PD and CAL of 4 mm were found in 7% and 9.5%, and PD and CAL ≥5 mm were found in 3% and 7.5%, respectively.

Dichotomizing study subjects by remission and active RA disease (DAS28 <2.6 or DAS28 ≥2.6), no differences in PD, CAL, BoP, PI at site level, or number of missing teeth were found (highest $P = 0.11$). Patients diagnosed with active RA and periodontitis (N = 36) had a higher mean CRP (12.9 ± 16.4) and ESR (26.5 ± 18.0) compared with RA patients in remission having periodontitis (N = 28; CRP 5.4 ± 10.8, $P = 0.043$ and ESR 12.6 ± 8.6, $P < 0.001$). In patients without periodontitis, no differences were found between active RA and remission for CRP ($P = 0.13$) and ESR ($P = 0.48$).

Concerning the effect of smoking on PD and CAL, ever smokers had higher mean PD (2.9 ± 0.4 mm) and mean CAL (3.2 ± 0.9 mm) compared with never smokers (PD 2.6 ± 0.2 mm, $P = 0.002$ and CAL 2.8 ± 0.3 mm, $P = 0.015$); no difference was found between active RA and remission RA. With regard to sex, males who were ever smokers had a higher mean PD (3.2 ± 0.6 mm) and CAL (4.0 ± 1.4 mm) compared with females (PD 2.8 ± 0.1 mm, $P = 0.005$ and CAL 2.9 ± 0.4 mm, $P < 0.001$). Moreover, male sex and smoking were associated with higher prevalence and more severe periodontitis ($P = 0.037$), fewer teeth ($P < 0.001$), and more dental plaque ($P = 0.017$) compared with female sex in ever smokers. Between former and current smokers, no differences were found.

Food frequency questionnaire

All patients reported seafood consumption as dinner, and 96% reported seafood as spread. The frequency of seafood intake as dinner and as spread, the number of portions as dinner, and the corresponding amount of seafood intake in grams per day are presented in Table 3.

The mean intake of seafood as dinner was 1.9 ± 0.9 meals per week. The consumption of seafood as a spread, such as on a sandwich, in a salad, or as a snack, was 1.4 ± 1.4 meals per week. A seafood intake as dinner with ≥2 to 3 servings per week and a meal size corresponding to the standard Norwegian seafood meal size or higher was found in 45 patients (58%). Of them, 27 (60%) reported 1 to 2 weekly serving with oily fish as dinner. The relative amount of seafood meals as dinner per week was 38% oily fish, 32% lean fish, 16% manufactured fish product, 5% intermediate oily fish and shellfish each, and <5% fresh water fish and sushi.

Regarding RA disease severity, females consuming two or more fish meals per week (N = 33) were found to have lower mean MHAQ (0.26) and VAS (22.6) compared with females having a lower fish intake (N = 24, MHAQ 0.51, $P = 0.008$ and VAS 38.4, $P = 0.003$). No differences were found in males (MHAQ: $P = 0.59$; VAS: $P = 0.22$).

Table 3
Frequency, number of portions, and estimated intake of seafood in RA patients, and grouped by sex and RA disease activity (N = 78)

	Total (N = 78)	Females (N = 57)	Males (N = 21)	*P* value	Remission (N = 33)	Active RA (N = 45)	*P* value
Seafood for dinner							
Never	0	0	0		0	0	
<1 time/mo	2	2	0	0.44	2	0	0.31
1 to 3 times/mo	3	1	2		2	1	
1 time/wk	28	21	7		9	19	
2 to 3 times/wk	43	32	11		19	24	
≥4 times/wk	2	1	1		1	1	
Portion size of dinner							
≤0.5 portion	3	3	0	0.010	2	1	0.32
1 portion	45	38	7		22	23	
1.5 portions	22	13	9		7	15	
2 portions	8	3	5		2	6	
3 portions	0	0	0		0	0	
Seafood as spread							
Never	3	3	0		2	1	
<1 time/mo	13	9	4	0.71	6	7	0.91
1 to 3 times/mo	25	19	6		11	14	
1 to 2 times/wk	24	16	8		10	14	
3 to 5 times/wk	10	7	3		3	7	
>5 times/wk	3	3	0		1	2	
Seafood intake (g/d)							
Total seafood	52 ± 47	50 ± 52	57 ± 29	0.067*	42 ± 30	60 ± 55	0.063*
Dinner seafood	47 ± 44	46 ± 49	53 ± 28	0.058*	37 ± 28	54 ± 53	0.045*
Oily fish	18 ± 22	18 ± 24	19 ± 15	0.23*	12 ± 11	22 ± 27	0.030*
Intermediate/lean fish	17 ± 20	18 ± 20	22 ± 18	0.24*	16 ± 15	21 ± 22	0.32*

RA, rheumatoid arthritis.
Values reported as number of patients and mean ± standard deviation. Portion size = 150 g.
Analyzed with Pearson chi-square.
* Analyzed with Wilcoxon rank-sum.

Elderly patients (N = 44, >60 y of age) had higher median total seafood intake (64 ± 57 g/d, range 13–345) than younger patients (N = 34, ≤60 y of age, 43 ± 35 g/d, range 1–213; *P* = 0.009).

Fish consumption of ≥54 g/d for dinner was found in 31% of the patients (89 ± 59 g/d, range 55–331). Between sex, fish consumption ≥54 g/d for dinner was found in 53% of the males (74 ± 16 g/d, range 57–107) and 23% of the females (102 ± 79 g/d, range 55–331 g/d; *P* = 0.27), whereas ≥34 g/d oily fish for dinner was consumed by 14% (43 ± 0 g/d, range 43–43) and 5% (107 ± 37 g/d, range 64–129; *P* = 0.040) of males and females, respectively.

Use of marine ω-3 supplement was reported by 43 patients (55%); of them, 19 (44%) took marine ω-3 supplements only during wintertime (mean 5.3 mo). The mean intake was 3.9 ± 2 times per week. At the time of examination, 37 (86%) were using an ω-3 supplement. No differences in use of marine ω-3 supplements for RA disease status and periodontal conditions were found.

Intake of cholecalciferol (VitD3) supplement from ω-3 and/or calcium supplement was reported by 43 patients (Tables 1 and 2). VitD3 originating from ω-3 supplement (17 ± 6 μg/d) was registered in 32 patients (41%). The estimated intake of VitD3 from calcium supplement was 20 ± 5 μg/d. No differences for high or low ultraviolet light exposure regarding RA disease activity or between sex were found.

VitD3 users had higher s-25(OH)D levels (78 ± 17 nmol/L) compared with non-users (68 ± 20 nmol/L, *P* = 0.021). VitD3 users having oily fish for dinner ≥1 to 2 times per week had higher s-25(OH)D levels (80 ± 18 nmol/L) compared with VitD3 non-users having less oily fish (63 ± 15 nmol/L, *P* = 0.009). A s-25(OH)D < 50 nmol/L was found in females, only (N = 6, 42 ± 7 nmol/L), five of six were not using VitD3.

There were no differences for s-25(OH)D between remission and active RA disease (Table 2), periodontal diagnosis (data not shown), nor sex (supplementary Table S2).

FAs and ω-3 index

The relative weight percentage of selected whole blood FA in all patients and grouped by RA disease activity are presented in Table 4. Selected whole blood FA (wt%), grouped by sex, and according to the use and no use of marine ω-3 supplement are presented in Supplementary Table S4.

The mean ω-3 index was 6.2 ± 2.1 (range 3–12). The majority of the study population had an ω-3 index from 4 to ≤8, 14%

Table 4
Relative weight percentage of selected whole-blood FAs in RA patients and grouped by disease activity (N = 78)

	Weight percentage of FA (wt%)			
	Total	Remission	Active RA	*P* value
	N = 78	N = 35	N = 43	
Saturated FA	37.5 ± 1.9	38.0 ± 2.1	37.3 ± 1.8	0.15
Unsaturated FA	20.6 ± 2.6	20.1 ± 2.1	20.9 ± 2.9	0.24*
Polyunsaturated FA	39.2 ± 2.5	39.2 ± 2.0	39.2 ± 2.8	0.99
Sum ω-6 FA	30.7 ± 2.5	30.7 ± 2.5	30.6 ± 2.5	0.94
Sum ω-3 FA	8.5 ± 2.3	8.5 ± 2.1	8.5 ± 2.4	0.92*
ω-3 index	6.2 ± 2.1	6.2 ± 2.0	6.2 ± 2.2	0.98*
LA, 18:2 ω-6	18.9 ± 2.1	18.9 ± 2.3	18.9 ± 1.9	0.74*
AA, 20:4 ω-6	9.3 ± 1.4	9.2 ± 1.3	9.3 ± 1.5	0.98
ALA, 18:3 ω-3	0.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.2	0.74*
EPA, 20:5 ω-3	1.7 ± 1.1	1.7 ± 1.2	1.7 ± 1.1	0.96*
DPA, 22:5 ω-3	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	0.08
DHA, 22:6 ω-3	4.5 ± 1.1	4.4 ± 0.9	4.4 ± 1.2	0.87*

AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linolenic acid; RA, rheumatoid arthritis; wt%, weight percentage of total FA.
Fatty acids (FAs) are reported as mean ± standard deviation in weight percentage (wt%) calculated from total FA (wt%). Limit of quantification (LOQ) 0.1, values <0.1 were recoded to the half of LOQ.
Analyzed with Student *t* test.
* Analyzed with Wilcoxon rank-sum.

Table 5
ω-3 index in relation to selected whole-blood FAs, total seafood intake, and clinical parameters on periodontal conditions and RA (N = 78)

	ω-3 index <4	ω-3 index 4 to ≤8	ω-3 index >8
	N = 9 (12%)	N = 58 (74%)	N = 11 (14%)
Age (y)	48 ± 12	57 ± 11	64 ± 9
FAs (wt%)			
ω-3 index	36 ± 0.3	5.9 ± 1.2 [A]	10.2 ± 1.1 [B,C]
Sum ω-6 FA	31.8 ± 2.6	30.8 ± 2.3	28.8 ± 2.4 [B,C]
AA, C20:4 ω-6	9.5 ± 2.1	9.3 ± 1.4	8.5 ± 0.6 [C]
GLA; C20:3 ω-6	1.5 ± 0.3	1.4 ± 0.3	1.0 ± 0.3 [B,C]
C18:1	20.6 ± 3.0	18.0 ± 2.0 [A]	16.1 ± 1.4 [B,C]
C20:1	0.17 ± 0.05	0.18 ± 0.06	0.25 ± 0.07 [B,C]
FFQ			
Total seafood intake (g/d)	34 ± 20	51 ± 35	75 ± 92
Periodontal data			
PD (mm)	2.7 ± 0.3	2.8 ± 0.4	2.5 ± 0.3 [C]
CAL (mm)	2.9 ± 0.6	3.1 ± 0.9	2.8 ± 0.3
BoP (% of sites)	37 ± 15	31 ± 16	24 ± 14
PI (% of sites)	38 ± 10	32 ± 18	32 ± 13
RA data			
RA duration, y	13 ± 8	14 ± 11	17 ± 15
DAS28 (score)	3.2 ± 0.9	2.9 ± 1.2	3.2 ± 1.1
VAS (score)	47 ± 15	27 ± 21 [A]	20 ± 15 [B]
MHAQ (score)	0.60 ± 0.41	0.35 ± 0.39	0.31 ± 0.23
Laboratory data			
ESR (mm/h)	14 ± 13	19 ± 16	28 ± 15 [C]
CRP (mg/L)	5 ± 10	10 ± 15	6 ± 6
s-25(OH)D (nmol/L)	65 ± 25	75 ± 19	74 ± 16

AA, arachidonic acid; BoP, bleeding on probing; CAL, clinical attachment level; CRP, C-reactive protein; DAS28, disease activity score; ESR, erythrocyte sedimentation rate; FA, fatty acid; FFQ, food frequency questionnaire; GLA, gamma-linolenic acid; MHAQ, modified health assessment questionnaire; PD, probing depth; PI, plaque index; RA, rheumatoid arthritis; s-25(OH)D, 25-hydroxyvitamin D; VAS, visual analog scale; wt%, weight percentage of total FA.
Values reported as mean ± standard deviation. Analyzed with Student *t* test and Wilcoxon rank-sum. Letters in superscript (A, B, and C) denote a significant difference ($P < 0.05$) between the ω-3 index groups: [A] ω-3 index <4 versus ω-3 index 4- ≤ 8; [B] ω-3 index <4 versus ω-3 index >8; [C] ω-3 index 4 to ≤8 versus ω-3 index >8.

had an ω-3 index >8, and 12% had an ω-3 index <4. Older age (>60 y of age) was related to a higher ω-3 index (5.5 ± 1.8) compared with younger age (≤60 y of age, 7.1 ± 2.1; $P < 0.001$). ω-3 supplement users had a higher ω-3 index compared with non-users ($P < 0.001$; Supplementary Table S3).

Fish consumption as dinner ≥2 to 3 times per week was related to a higher ω-3 index (6.6 ± 2.0) compared with less fish consumption (5.5 ± 2.1, $P = 0.019$). An additional increase of ω-3 index was found in patients having oily fish ≥1 to 3 times per week (7.1 ± 1.9, $P = 0.018$). Patients with an ω-3 index >8 were ω-3 supplement users and 83% of them had ≥2 to 3 weekly seafood servings as dinner.

Smoking was related to a lower ω-3 index in current and former smokers (5.7) compared with never smokers (7.1, $P = 0.003$). Former smokers had an ω-3 index of 0.03 higher than current smokers ($P = 0.96$).

The relations of the different levels of ω-3 index to selected FA, total seafood intake, periodontal conditions and RA disease are presented in Table 5.

Discussion

This study investigated marine ω-3 and VitD levels, disease outcome, and periodontal status in RA outpatients. Periodontitis was more prevalent in RA patients seropositive for RF and/or ACPA. The RA disease parameters MHAQ and VAS were lower in females consuming seafood as recommended. The use of ω-3 supplement was associated with an ω-3 index >8.

In the present study, females were approximately three times more preponderant and appeared at a younger age than males when diagnosed with RA. Previous reports confirm this finding, describing a female dominance, especially in the premenopausal age group. This relationship has been interpreted in terms of both genetic and non-genetic risk factors [43].

Periodontitis was diagnosed in more than three-quarters of the RA patients. Data from a meta-analysis have shown that RA patients were more likely to have a higher prevalence and severity of periodontitis than non-RA controls, and patients with periodontitis had a higher prevalence of RA than those without periodontitis [5]. The prevalence of periodontitis in our group of RA patients was almost twice as high for moderate and severe periodontitis compared with American non-RA adults (ages ≥30 y), using similar diagnostic criteria for periodontitis [44]. Compared with an epidemiologic study on oral health of inhabitants in Jönköping, a Swedish city, the prevalence of periodontitis in RA patients in our study was 2.6 times higher, and twice as many patients were diagnosed with severe periodontitis [45]. Importantly, the classification systems applied were different in the studies. Overall, in our study, applying the classification system by Eke et al. [32], severe periodontitis was found in a slightly higher proportion than the commonly reported range of 5% to 15% in any population [46]. Nevertheless, PD and CAL ≥4 mm were identified in only 10% and 17% of the sites, respectively. The low number of sites with severe periodontitis and narrow interindividual differences might be the reason why we were not able to show differences for periodontal measurements between active RA and remission.

Smoking and male sex have been reported as risk factors for more severe periodontitis [44,46]. Accordingly, male smokers had significantly higher mean PD and CAL compared with their respective counterparts. Furthermore, periodontitis worsened with age, reflecting lifetime disease accumulation [46].

One of the strengths of this study is the performance of comprehensive periodontal measurements at site level including both PD and CAL. Prevalence and severity estimates of periodontitis depend on clinical assessment for periodontal measures and case definitions used for periodontitis. The assessment of both PD and CAL at six sites per tooth is the gold standard in clinical periodontal examination to estimate the entire extent of periodontitis [44]. As a limitation and similar to the majority of the published studies, third molars were excluded to make our results best comparable, but extent and severity of periodontitis may have been underestimated [44].

Seropositivity to ACPA has been found to have a strong correlation with RA disease severity [47] and is also associated with a higher frequency of periodontitis compared with healthy controls [5]. In the present study, patients seropositive to ACPA and/or RF were significantly more frequently diagnosed with periodontitis.

Smoking is a major preventive risk factor for RA [48]. Especially in seropositive patients, smoking has been found positively associated with the risk of RA [48,49]. In a Swedish population-based case-control study, a dose-response association between cumulative dose of smoking and risk of developing ACPA positive RA has been observed [50]. In our study, a higher proportion of ACPA seropositive patients were non-smokers, and fewer patients were current smokers compared with seronegative patients. The high number of never and former smokers in this study may explain the inability to show significant differences between smoking status and RA disease activity.

Seafood consumption was found at a high frequency in this group of RA patients. This finding may be explained by a long tradition of Norwegian seafood consumption. However, seafood consumption in complete fulfilment of the Nordic nutrition recommendations (NNR) [36] was found in only 35% of the patients. Comparing our data with sex specific data from the latest national dietary survey (Norkost3) [37], we found that daily fish consumption was 1.4 times higher in male RA patients compared with females.

Females eating seafood at recommended frequency had better health assessment (MHAQ and VAS) compared with females eating less seafood. This is in line with a previous study on fish consumption in RA patients, where significantly lower disease activity (DAS28) was observed in subjects consuming fish ≥2 times/wk compared to never or <1 time/mo fish intake [51].

The use of a validated FFQ questionnaire to estimate seafood intake is a strength of this study. As a limitation, the FFQ is not applicable to calculate ω-3 supplement intake.

The ω-3 index is originally considered a risk stratification tool for sudden cardiac death and has been used as a surrogate for assessing tissue levels of EPA plus DHA [12]. An ω-3 index >8 has been considered as favorable, and an index of <4 may be regarded as undesirable [42]. Oily fish and fish oil supplement are the primary source of EPA and DHA. Dose-response studies showed increasing levels of ω-3 PUFA by increasing amount of fish oil supplementation in a dose dependent manner [12]. In this study, all patients having an ω-3 index >8 were identified as ω-3 supplement users, and of them 83% had a seafood consumption in accordance with NNR. Comparing our data with a study on FA composition and presence of acute coronary syndrome in the US, a group of Caucasian control patients showed an average 1.2 to 1.4 lower ω-3 index [52]. These results may reflect the sparse fish intake in the typical American diet. In Japan, a country with a strong tradition of fish consumption and low prevalence of cardiovascular diseases, in older Japanese, an average ω-3 index between 8 and 10 was reported [53]. These findings are in accordance with the data from our study, where the mean age was highest for those with an ω-3 index >8 and the ω-3 index, ranging from 9 to 13, was even higher than in the Japanese group. We found that an ω-3 index >8 was related to more desirable patient global health assessment (VAS) and periodontal conditions compared with a lower ω-3 index. This ω-3 group consisted of ω-3 supplement users, exclusively. Our findings are consistent with data of a prospective, randomized trial on fish oil supplementation that showed decreased VAS and DAS28 compared with no supplementation [54].

Another strength of this study is the performance of FA analysis in whole-blood because the estimation of EPA and DHA intake from seafood and/or ω-3 supplement is thought to be difficult due to differences in EPA and DHA content depending on season, the fish's diet, and food preparation methods [52]. Conventionally, FAs were measured in plasma or serum reflecting short-term fat intake or in red blood cells (RBCs) reflecting long-term fat intake. Whole-blood contains FAs from all lipid classes and reflects FA status [55]. The EPA and DHA content of whole-blood samples, which is used to calculate the ω-3 index, was found to be highly correlated with that of RBC [42].

According to the Norkost3 study, the VitD3 supplement intake was 6.7 μg/d in males and 4.9 μg/d in females [37]. In our study, the mean VitD3 supplement intake was higher in females compared with males and higher compared with sex specific data from Norkost3. Compared with the reference values for recommended intake of VitD3 supplement, the intake in patients <75 y in our study was twice as high in males and almost three times higher in females [36]. Calcium supplementation containing VitD3 as a prophylactic adjuvant against osteoporosis in glucocorticoid treatment and intake of ω-3 supplement may reasonably explain the higher intake in this patient group.

Assessing VitD status by measuring circulating s-25(OH)D is generally considered as the best single marker of VitD status [40]. Currently, no general consensus on the required s-25(OH)D for an adequate VitD status has been established. Some research indicates that s-25(OH)D should be >50 nmol/L [41], but other evidence suggests s-25(OH)D levels >75 nmol/L to cover both the skeletal and non-skeletal benefits of VitD [56].

VitD status is reported to be higher in Northern Europe compared with Southern Europe, despite higher latitude having shorter periods of ultraviolet radiation from sun exposure [57]. Accordingly, in the present study 88% had an VitD status within the recommended range and approximately half of them had s-25(OH)D level of ≥75 nmol/L. Norway's coastal location with strong tradition of fatty fish and cod liver oil consumption may explain these findings. An inverse association between s-25(OH)D concentrations and RA disease activity and severity has been reported [19], but this was not seen in the present study. The overall good VitD status in this group of RA patients may explain the inability to show differences for RA disease activity and periodontal diagnosis. Less intake of oily fish and lower/no VitD3 supplementation was associated with lower VitD levels.

There are some limitations to the present study. The high percentage of low RA disease activity/remission and low prevalence of deep periodontal pockets seen here may be the result of enrollment taking place among patients attending a rheumatologic outpatient clinic at a university hospital with a treat-to-target treatment regime and good access to synthetic and biological DMARDs. Furthermore, over the last 40 y, a continuous improvement in oral health was seen, with increased number of individuals with no marginal bone loss and a decrease in the number of subjects with moderate alveolar bone loss [45]. Good systematic dental care, reflected by a high attendance rate and frequency of dental recall visits as well as a high degree of self-performed oral hygiene procedures may explain the overall low severity of periodontitis (results not shown). On the other hand, the perceived ability to pass through the examinations may have influenced the patient's decision to participate in the study and may have resulted in refusal by patients with more severe RA disease. The RA disease treatment mode with synthetic and/or biological DMARDs showed large interindividual differences resulting in subgroups with few patients. The limited interindividual differences in outcome measurement of RA disease and periodontal disease, in addition to subgroups with small numbers of patients, could be responsible for the inability to detect significant differences. The patient recruitment from urban catchment implies good access to medical care as well as the earlier mentioned participation bias may challenge the validity and generalizability of the results. Therefore, the results of this study should be evaluated with caution due to the small sample size in the resulting groups.

Conclusions

In this group of RA patients, the overall range of RA disease activity was low, with a high percentage of patients in remission. Despite this, periodontitis was more prevalent and severe in RA patients and showed demographic and RA disease specific differences compared with what has previously been reported. The prevalence of periodontitis was higher in seropositive versus seronegative patients. The use of ω-3 supplement was related to

a higher ω-3 index. An ω-3 index >8 was associated with better RA disease outcome. In general, RA patients in this study had a good VitD status, which may explain the inability to show differences for RA disease activity and periodontal diagnosis related to VitD.

A novelty of this study was the simultaneous assessment of RA disease parameters, periodontitis measurements, marine ω-3 status by a FFQ and blood FA, and serum VitD levels in the same group of patients. Longitudinal and interventional studies are needed to further evaluate the effects of ω-3 and VitD3 on RA and periodontitis.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nut.2018.03.054.

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