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Knee effusion-synovitis volume measurement and effects of vitamin D supplementation in patients with knee osteoarthritis

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SUMMARY

Objective: To develop a measure of knee joint effusion-synovitis volume and to examine the effect of vitamin D supplementation on effusion-synovitis in people with knee osteoarthritis (OA) and low vitamin D levels over 24 months.
Method: Symptomatic knee OA patients with low 25-(OH)D levels (12.5–60 nmol/l) were recruited for a multi-centre, randomised, placebo-controlled and double-blind trial. Participants (age 63 ± 7 years, 208 females) were allocated to either 50,000 IU monthly vitamin D_3 ($n = 209$) or placebo ($n = 204$) for 24 months. Knee effusion-synovitis volume in suprapatellar and other regions was measured on magnetic resonance imaging (MRI) using OsiriX software. The intra-class correlation coefficients (ICCs) were used to test inter- and intra-rater reliabilities. The least significant change criterion was used to define the increase/decrease in effusion-synovitis volume.
Result: The reproducibilities of effusion-synovitis volume measurement were high with ICCs ranging from 0.93 to 0.99. Over 24 months, effusion-synovitis volume remained stable in the vitamin D group but increased in placebos with a significant between-group difference (−1.94 ml, 95% confidence interval (CI): −3.54, −0.33). This effect was evident in those with baseline effusion-synovitis and with suprapatellar effusion-synovitis. The proportion with an increase in effusion-synovitis volume was lower in the vitamin D group than placebo (risk ratio (RR): 0.87, 95% CI: 0.77, 0.97).
Conclusion: This highly reproducible effusion-synovitis volume measurement could be a promising outcome measure in OA trials. Vitamin D supplementation could retard the progression of effusion-synovitis which can potentially benefit people with an inflammatory OA phenotype.

Introduction

Osteoarthritis (OA) was generally thought of as a 'non-inflammatory' type of arthritis; however, localised low-grade inflammation is now known to be an important factor in OA pathogenesis[1–3]. The development of chronic inflammation in OA following joint injury or metabolic dysfunction may contribute to the formation of a cycle of local tissue lesions, inflammation and repair[4]. Notably, synovial activation (effusion and/or synovitis) has been considered as a precursor of OA outcomes such as radiographic changes and total knee replacement[5,6]. It is independently associated with clinical symptoms, such as knee pain and physical function[7,8]. Studies have demonstrated the link between synovial inflammation and structural changes of knee OA[9–12], suggesting that reducing synovial inflammation may be a potential avenue for slowing disease progression in knee OA. This is extremely important, as there are no proven treatment options to modify disease progression in OA so far.

Joint effusion-synovitis (a magnetic resonance imaging (MRI) marker of synovial inflammation) has been assessed using MRI with high reproducibility and validity, but the scoring methods were often semi-quantitative and subjective even for experienced professionals[13,14]. This may be one of reasons why inconsistent

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findings regarding the association of structural alterations with severity of synovitis have been reported[15–17]. Quantitative measures of synovial membrane inflammation had been shown better correlated with clinical signs and histopathologic parameters in inflammatory arthritis[18]. Currently, very few studies have investigated effusion/synovitis volume using MRI in OA[19,20], and no study has yet practically investigated it as an outcome measure in clinical trials.

Importantly, compared to articular and bony alterations, synovial inflammation has a greater potential to regress or resolve[21] which creates a treatment opportunity. Pharmaceutical managements such as non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular steroid injection have been recommended for OA patients particularly for those with joint effusion[22]; however, these treatments can result in side-effects and drug intolerance during long-term use[23,24]. It is therefore important to identify safer and more cost-effective interventions targeting synovial inflammation in OA[25].

In observational studies vitamin D deficiency has been associated with cartilage loss and pain[26,27]. In animal models, vitamin D supplementation has a protective effect in OA by reducing the expression of pro-inflammatory cytokines[28]. Furthermore, an exercise-interventional study has found that vitamin D sufficiency increases anti-inflammatory cytokine response to muscular injury[29]. So far, randomised controlled trials (RCTs) on the efficacy of vitamin D supplementation for knee OA is limited and inconsistent. While one study suggested it had beneficial effects on symptoms[30], another showed no effects on symptoms and cartilage loss[31]. In our recent Vitamin D Effect on Osteoarthritis (VIDEO) study in patients with knee OA and low serum vitamin D levels, vitamin D supplementation over 24 months had no significant effect on knee pain or cartilage morphology but might have modest effects on knee function loss and bone marrow lesions[32]. However, none of these studies has investigated the effects of vitamin D on synovial inflammation. We hypothesised that vitamin D could reduce synovial inflammation in patients with knee OA.

The aims of this study were, therefore, to develop a measure of knee joint effusion-synovitis volume and to examine the effect of vitamin D supplementation over 24 months on effusion-synovitis as a post-hoc analysis in the VIDEO study.

Methods

Trial design

The VIDEO study was a randomised, double-blind, placebo-controlled clinical trial[32]. Participants were recruited in Tasmania and Victoria, Australia, using a combined strategy, including working with general practitioners, specialist rheumatologists and orthopaedic surgeons, and advertising through media and community groups. Eligible participants were randomly allocated to either treatment or matching placebo group in a 1:1 ratio. A telephone pre-screen assessed knee pain, anticipated knee and hip surgery, participation in other studies and comorbidities. Eligible participants were subsequently screened in a clinic visit including knee radiographs and a blood test for serum 25-(OH)D level.

Participants

Eligible participants were aged between 50 and 79 years with symptomatic knee OA for at least 6 months and pain of at least 20 mm on a 100 mm on a visual analogue scale (VAS) and were recruited from August 2010 to December 2011. All individuals were assessed according to the American College of Rheumatology (ACR) criteria for symptomatic knee OA[33]. Participants also had an ACR function class rating of I, II and III[34] and relatively good health, with a score of 0–2 on a 5-point Likert scale (from 0 indicating very good health to 4 indicating very poor health) according to the global investigator assessment of disease status. Participants were included if their serum 25-(OH)D levels >12.5 nmol/l or <60 nmol/l. Ethics approval was received from the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616). Informed written consent was obtained from all participants.

Exclusion criteria included grade 3 radiographic knee OA according to Altman's atlas[35], contraindication to MRI, rheumatoid or psoriatic arthritis, lupus, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, conditions affecting oral drug absorption, anticipated knee or hip surgery within the next 2 years, history of significant trauma of knees (e.g., arthroscopy or injury to ligaments or menisci within 1 year preceding the study) and history of taking vitamin D or an investigational drug within the last 30 days.

Interventions

Participants in the intervention group were given a monthly capsule of 50,000 IU (1.25 mg) vitamin D_3 (cholecalciferol) orally for 24 months[36]. The vitamin D_3 compound was purchased from Nationwide Compounding Pharmacy, Melbourne, Australia. Participants in the control group received an identical inert placebo provided by the same company. Serum 25-(OH)D was assayed by Liaison method utilizing a direct competitive chemiluminescent immunoassays (DiaSorin Inc., Stillwater, Minnesota, USA). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%.

Randomisation

Participants were allocated to either vitamin D or placebo arm at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a central automated allocation procedure with security in place to ensure allocation data could not be accessed or influenced by any person from the investigative team.

Blinding

Participants, research coordinators and investigators were all blinded to treatment assignment. The blinding procedure was maintained until all the data were collected, cleaned, confirmed for accuracy and statistical analyses were performed.

Outcome measures

The co-primary efficacy endpoint measures of the trial were MRI assessment of knee cartilage volume changes from baseline to month 24, as well as the Western Ontario and McMaster Universities Index of OA (WOMAC) score as have been reported[32]. This post-hoc analysis examined outcomes of volume of knee effusion-synovitis. The knee that met the inclusion/exclusion criteria was selected as the study knee for outcome measures. When both knees met the criteria, the less severe one was studied as it has more cartilage volume at baseline which would enable to observe the effect on loss of cartilage volume (the primary outcome) as large as possible.

MRI and image processing

MRI of the study knee was acquired with a 1.5 T whole-body magnetic resonance unit (Picker, Cleveland, OH, USA) using a commercial transmit-receive extremity coil. Image sequence

included the following: (1) T2-weighted sagittal fat suppressed fast spin echo (FSE), flip angle 90°, repetition time 3067 msec, echo time 112 msec, field of view (FOV) 16 cm, 45 slices, 228 × 256-pixel matrix, slices thickness of 2 mm; (2) Proton density-weighted coronal fat-suppressed, FSE, flip angle 90°, repetition time 3400 msec, echo time 64 msec, FOV 16 cm, 30 slices, 256 × 256-pixel matrix, acquisition time 5 min 26 s, 1 acquisition, slice thickness of 3 mm.

MRI measurements of knee joint effusion-synovitis

Quantitative measurement of effusion-synovitis volume. Effusion-synovitis was distinguished in the following subregions according to the anatomy of the knee joint synovial cavity[37]: (1) the suprapatellar pouch, extending superiorly from the upper surface of the patellar, between the posterior suprapatellar fat pad (quadriceps femoris tendon) and the anterior surface of the femur; (2) other cavity, which includes the area between the central femoral and tibial condyles, around the ligaments and menisci, and the area behind the posterior portion of each femoral condyle, inside of the joint capsule (Fig. 1). The volumes of individual joint subregions were isolated from the total volume by selecting each region of interest (ROI) according to the intra-articular fluid-equivalent signal on a section-by-section basis. The final 3-D volume rendering was generated using commercial in-house OsiriX Lite imaging software cursors (32-bit version 5.9, Pixmeo SARL, Geneva, Switzerland)[38] (Fig. 1). The readers were blinded to treatment allocation and patients' information. To analyse the reliability of measurement, two independent readers assessed 40 randomly selected images with at least a 4-week interval between readings.

Change in effusion-synovitis volume was calculated as follows:

$$\text{Absolute change (ml)} = (\text{follow-up volume}) - (\text{baseline volume});$$

$$\text{Percentage change per annum (\% p.a.)} = [(\text{absolute change})/(\text{baseline volume})]/(\text{time interval between two scans}) \times 100.$$

Semi-quantitative measurement. Effusion-synovitis in each subregion was scored individually according to Whole-Organ Magnetic Resonance Imaging Score (WORMS), grading collectively from 0 to 3 based on the estimated maximal distention of the synovial cavity

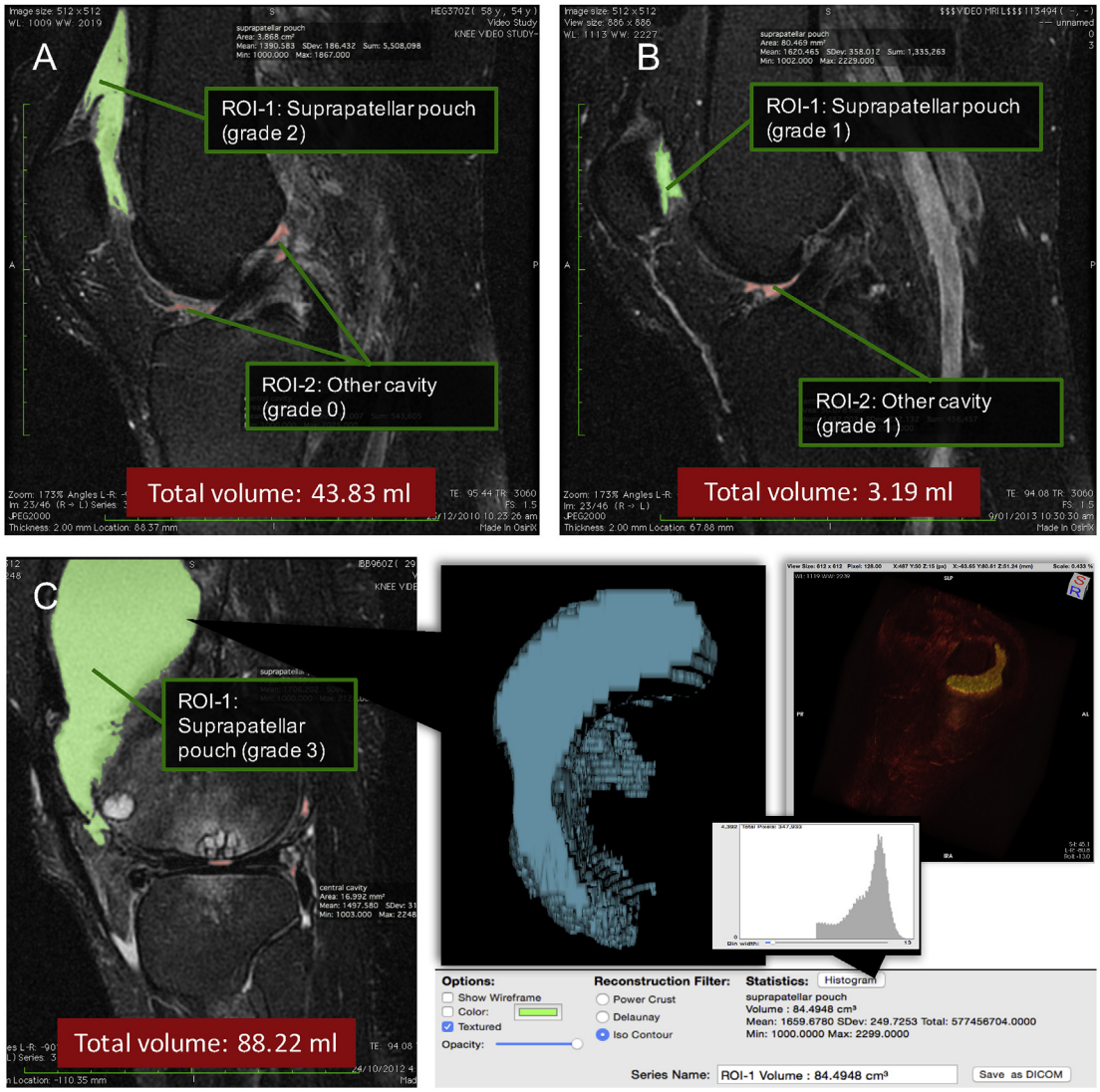

Fig. 1. MRI acquired from the knee, with superimposed colour data showing the area of high signal. The images were obtained before (A) and 24 months after (B) intervention. Data were analysed in two ROIs, which were located in the suprapatellar pouch (ROI-1, pixels shown in green) and the other joint cavity (ROI-2, pixels shown in red), respectively. The total volume was generated from the area of each ROI in the entire series of images using OsiriX software (C). Semi-quantitative grades for each ROI were also provided accordingly.

(Fig. 1): 0 = normal; 1 = ≤ 33% of maximum potential distention; 2 = 33–66% of maximum potential distention; 3 = ≥ 66% of maximum potential distention[14]. Total effusion-synovitis score of the whole joint was defined as the maximum score of each subregion, ranging from 0 to 3. Change of effusion-synovitis was calculated by subtracting the baseline score from the follow-up score, and change of effusion-synovitis score of ≥1 was defined as an increase in effusion-synovitis volume. The inter-rater reliability was 0.63–0.75 and intra-reader reliability was 0.60–0.75 (weighted κ) in different subregions as described previously[39].

As the MRI sequence used to determine synovitis at the site in Victoria was obtained in the coronal plane, subregional effusion-synovitis was unable to be differentiated in Victorian participants. Hence, subregional analyses were only performed in participants from Tasmania.

Statistical methods

Baseline characteristics were compared between two groups with the use of Student's *t*-tests or Chi-square tests. Independent *t*-tests were used to compare changes in effusion-synovitis volume from baseline to follow-up between groups. In secondary analyses, the minimal clinically important difference (MCID) was estimated for effusion-synovitis volume (both the absolute and the relative annual change). A reduction of mean WOMAC function score ≥7 was used as an anchor to determine the cut-off of effusion-synovitis volume in patients who actually experienced clinically significant improvement[40]. A least significant change (LSC) criterion was used to define an increase, stable or a decrease in effusion-synovitis volume. This takes into account measurement error and the correlation between the baseline and follow-up measurements[41]. The formula was as follows:

$$LSC = 1.96 \times \sigma\sqrt{2(1-\rho)}$$

(σ = the standard error of the mean; ρ = the serial correlation). For example, LSC of total effusion-synovitis volume was calculated to be 1.81 ml (where $\sigma = 1.17$ and $\rho = 0.69$) in this study. Therefore, participants were categorised as having an increase in effusion-synovitis volume if change in effusion-synovitis volume was ≥+1.81 ml, having a decrease if change in effusion-synovitis was ≤−1.81 ml, and having a stable effusion-synovitis if change in effusion-synovitis volume was between −1.81 and +1.81 ml. Generalised linear regression model with a log-binomial link for binary outcomes (e.g., improvement vs no improvement, increase vs stable, and decrease vs stable) and ordinal logistic regressions for ordinal outcomes (e.g., decrease, stable and increase) were applied, respectively. The proportional odds assumption was tested for the ordered logistic regressions.

For intention-to-treat analysis, multiple imputations were used to address missing data due to loss to follow-up and non-response. We performed 20 imputations on missing values in effusion-synovitis volume for each treatment arm using a truncated regression model which includes baseline variables such as age, gender, BMI and serum 25-(OH)D level. All the data analyses were performed on Stata V13.0 (Stata Corp., College Station, Texas, USA). A two-sided *P* value of 0.05 was considered statistically significant.

Results

Participants

The flow of study participants is described in Fig. 2. Of 599 participants were screened for eligibility, 413 subjects (211 had both knees affected) were randomly assigned to either vitamin D or placebo group. Over 24 months, 28 (13.4%) in the vitamin D group and 45 (22.1%) in the placebo group withdrew. Three hundred and forty patients (82.3%) completed the follow-up. More patients in the placebo group discontinued treatment allocation than in the

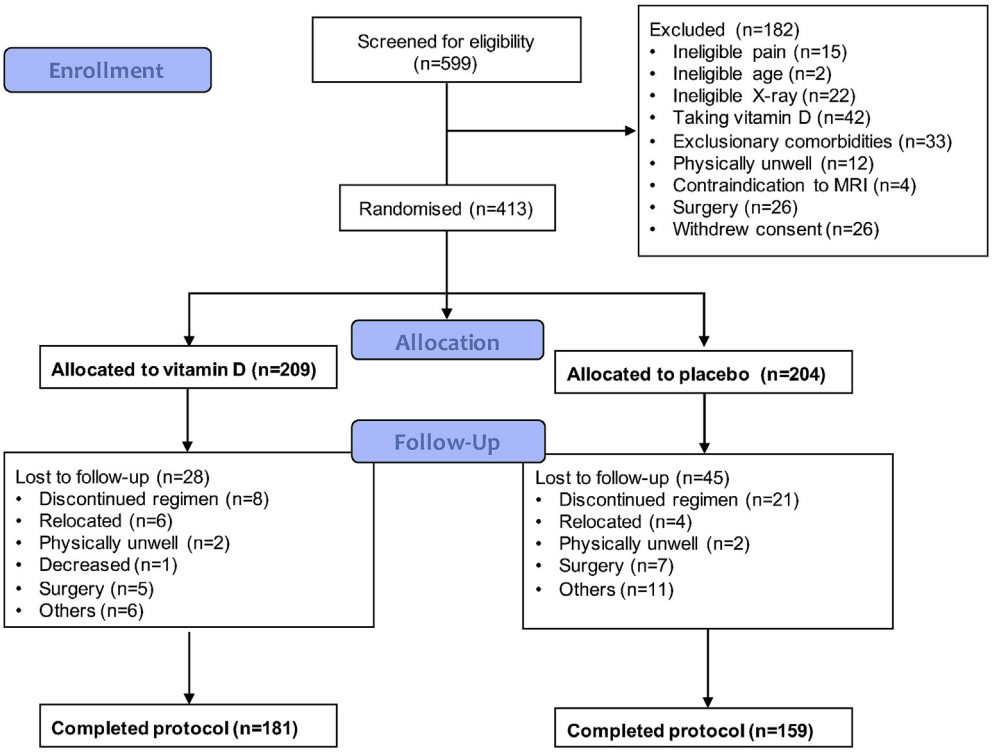

Fig. 2. The flowchart of the study.

vitamin D group (21 vs 8). The major reason for discontinuation was non-adherence to the protocol when low 25-(OH)D levels were disclosed to the participants by their general practitioners. The mean age of participants was 63.2 ± 7.0 years, with 208 (50%) females and a mean BMI of 29.6 ± 5.0 kg/m^2, and baseline characteristics were comparable between the vitamin D and placebo groups (Table I). Using a semi-quantitative grading assessment, baseline prevalence of effusion-synovitis (score ≥ 2) was 48%, which was similar in both groups (49% in vitamin D vs 47% in placebo) (Table I). Baseline characteristics of the participant were also comparable between treatment groups in the subgroup (e.g., participants with or without baseline effusion-synovitis or participants from different study sites) analyses except for age in the subgroup with baseline effusion-synovitis (65.5 vs 63.4, $P = 0.04$).

Reliability and validity of the measurement

Intra- and inter-rater reliabilities for effusion-synovitis volume measurement were assessed using the intra-class correlation coefficient (ICC). The intra-rater reliability was 0.97 in the whole joint (0.98 in suprapatellar pouch and 0.95 in central portion). The inter-rater reliability was 0.99 in the whole joint (0.99 in suprapatellar pouch and 0.93 in central portion). Effusion-synovitis volume was highly correlated with effusion-synovitis score (*rho* = 0.77, $P < 0.01$ for total, *rho* = 0.91, $P < 0.01$ for suprapatellar pouch, and *rho* = 0.77, $P < 0.01$ for central portion).

Outcomes

Serum 25-(OH) D levels increased by an average 40.6 ± 19.5 nmol/l in the vitamin D group but only 6.7 ± 17.9 nmol/l in the placebo group throughout the study period, as described elsewhere[32]. In the total study sample, total effusion-synovitis volume increased from baseline (8.0 ± 8.5 ml) to follow-up (9.0 ± 10.5 ml). The mean effusion-synovitis volume increased from 8.0 ± 9.2 ml to 10.0 ± 12.3 ml in the placebo group ($P = 0.08$), but remained stable in the vitamin D group (8.0 ± 7.8 ml to 8.0 ± 8.4 ml).

There were statistically significant differences in absolute and relative effusion-synovitis volume changes between groups (−1.94 ml over 24 months or −45% p.a.) (Table II). These statistically significant differences were only evident in patients who had baseline effusion-synovitis (score ≥ 2), not in those without baseline effusion-synovitis (Table II). Additional subgroup analyses in patients with vitamin D level of <50 nmol/l showed a greater effect of vitamin D supplementation with a between-group difference of −2.42 over 24 months.

In subregional analyses, the absolute changes in volume of effusion-synovitis in suprapatellar pouch were less in the vitamin D than the placebo group, and the between group difference was statistically significant for suprapatellar pouch but not for other joint cavity (Table III).

Clinical improvements in effusion-synovitis volume (absolute and percentage changes) were further defined using MCID. The proportions of participants with improvements in percentage changes of total effusion-synovitis were significantly higher in the vitamin D compared to placebo group (Table IV). When the change in effusion-synovitis volume was categorised as an increase or a decrease by LSC, the percentages of subjects with decreasing effusion-synovitis were 19%, and with increasing effusion-synovitis was 32% in the whole sample. The proportions with decreasing, stable and increasing effusion-synovitis was significantly different between groups ($P = 0.03$). The proportions with an increase in effusion-synovitis volume were lower in the vitamin D group ($P = 0.01$), while the proportion with a decrease in effusion-synovitis volume was not significantly different between groups (Table IV).

Per protocol analysis comparing those reached a 25-(OH)D level over 60 nmol/l at month 3 to those who did not (253 vs 146) showed similar results of change in effusion-synovitis volume (data not shown).

Table I
Characteristics of the participant at baseline

Total Sample	Vitamin D ($N = 209$)	Placebo ($N = 204$)
Age (y)	63.55 (6.88)	62.85 (7.22)
Women (%)	51	50
Body mass index (kg/m^2)	29.57 (5.39)	29.64 (4.62)
Radiographic OA (%)	96	96
Plasma 25-hydroxyvitamin D (nmol/l)	43.74 (11.80)	43.81 (12.66)
Effusion-synovitis volume (ml)	7.93 (7.81)	7.98 (9.20)
Effusion-synovitis prevalence (%)	49	47

Results are shown as mean (SD) or percentage unless stated otherwise.
Student *t*-test or χ^2-test was used for the comparison.

Discussion

In this study we developed a method to measure effusion-synovitis volume in patients with knee OA and found that this method was reproducible and valid. This post-hoc analysis suggested that vitamin D supplementation retarded the progression of effusion-synovitis over 24 months in vitamin D deficient knee OA patients. This is the first study using quantitative effusion-synovitis as an outcome measure in a clinical trial of OA. Most importantly, effusion-synovitis is able to regress or resolve suggesting its potential as a target for OA treatment.

In our previous observational studies, we reported that effusion-synovitis score, particularly in suprapatellar pouch, predicted worsening of knee pain, independent of other joint structural changes[42]. In addition, it was significantly associated with cartilage defects and bone marrow lesions[39] over time. Effusion-synovitis score in other regions was inconsistently associated with the progression of structural abnormalities, indicating effusion-synovitis in other regions was less clinical relevant possibly due to the limited joint space[39]. Therefore, therapies targeting suprapatellar effusion-synovitis are most likely to have effect on disease progression and symptoms in knee OA. In this study, we measured effusion-synovitis volume in a way which was highly reproducible and had very good criterion validity. Most importantly, effusion-synovitis volume in suprapatellar pouch responded well to vitamin D treatment in this trial. Its capacity to resolve over time indicates that it is sensitive to change. Our findings suggest that suprapatellar pouch effusion-synovitis can be used as an outcome measure in future clinical trials.

The initial report from our clinical trial suggested that vitamin D supplementation over 2 years did not have a major effect on cartilage morphology and knee pain in OA patients with low serum vitamin D levels[32]. However, post-hoc analyses showed that vitamin D supplementation had significant but small effects in reducing knee pain assessed using VAS, improving physical function and slowing the progression of bone marrow lesions[32]. The effect size was smaller than expected, and the major reason would be that vitamin D might have a slow-acting effect on disease progression. It is possible that small incremental benefits could take more than 24 months to be measured as seen in an observational study (up to 5 years[43]). Results from this secondary analysis suggest a beneficial effect of vitamin D supplementation on effusion-synovitis particularly in the suprapatellar region in knee OA

Table II
2-Year changes in total knee effusion-synovitis between vitamin D and placebo groups

Effusion-synovitis measures	Vitamin D	Placebo	Between-group difference*	*P* value
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	
Whole sample	$N = 209$	$N = 204$		
Volume, absolute change (ml)	0.26 (−0.82, 1.34)	2.20 (1.01, 3.38)	−1.94 (−3.54, −0.33)	**0.02**
Volume, relative change (p.a.)†	16% (−8%, 39%)	60% (31%, 89%)	−45% (−82%, −7%)	**0.02**
Grade, increase vs no increase (%)	18% (10%, 26%)	23% (14%, 32%)	−5% (−17%, 7%)	0.40
Those with baseline effusion-synovitis	$N = 106$	$N = 108$		
Volume, absolute change (ml)	0.13 (−1.09, 1.35)	2.17 (0.88, 3.46)	−2.04 (−3.83, −0.25)	**0.03**
Volume, relative change (p.a.)†	9% (−1%, 18%)	28% (17%, 38%)	−19% (−33%, −5%)	**0.01**
Those without baseline effusion-synovitis	$N = 103$	$N = 96$		
Volume, absolute change (ml)	0.80 (−0.22, 1.82)	1.97 (0.64, 3.30)	−1.17 (−0.50, 2.85)	0.17
Volume, relative change (p.a.)†	60% (−144%, 264%)	351% (69%, 632%)	−290% (−57%, 638%)	0.10

Bold *P* value indicates statistically significant difference at $\alpha = 0.05$.
* The quantitative results in this table were generated on imputed datasets.
† Relative change = (absolute change/baseline value)/time interval.

Table III
2-Year changes in regional knee effusion-synovitis between vitamin D and placebo groups

Effusion-synovitis measures	Vitamin D ($N = 129$)	Placebo ($N = 132$)	Between-group difference*	*P* value
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	
Suprapatellar pouch				
Volume, absolute change (ml)	0.04 (−1.46, 1.53)	2.53 (0.84, 4.22)	−2.49 (−4.74, −0.25)	**0.03**
Volume, relative change (p.a.)†	19% (−111%, 149%)	148% (−6%, 302%)	−129% (−330%, 72%)	0.21
Other cavity				
Volume, absolute change (ml)	0.12 (−0.13, 0.38)	0.40 (0.13, 0.67)	−0.28 (−0.65, 0.09)	0.14
Volume, relative change (p.a.)†	10% (−15%, 34%)	39% (11%, 68%)	−30% (−67%, 8%)	0.12

Bold *P* value indicates statistically significant difference at $\alpha = 0.05$.
* The results in this table were generated on imputed datasets.
† Relative change = (absolute change/baseline value)/time interval.

Table IV
Changes in knee effusion-synovitis defined using MCID or LSC over 2 years

Effusion-synovitis measures	Vitamin D	Placebo	Between-group difference in change	*P* value
	Percentage (*N*)	Percentage (*N*)	RR (95% CI)	
Improvement in absolute change by MCID	53% (96)	44% (76)	1.22 (0.98, 1.52)*	0.07
Improvement in relative change‡ by MCID	70% (126)	60% (104)	1.16 (1.00, 1.36)*	**0.05**
			OR (95% CI)	
Changes by LSC			0.64 (0.43, 0.95)†	**0.03**
			RR (95% CI)	
Decrease	21% (38)	18% (31)	1.03 (0.59, 1.81)*	0.91
Stable	53% (95)	43% (75)		
Increase	26% (47)	39% (68)	0.87 (0.77, 0.97)*	**0.01**

Bold *P* value indicates statistically significant difference at $\alpha = 0.05$.
* Generalised linear regression model with a log-binomial link for binary outcomes (decrease or increase vs stable).
† Ordinal logistic regressions for the ordinal outcomes (decrease, stable and increase).
‡ Relative change = (absolute change/baseline value)/time interval.

patients with vitamin D deficiency. The effect size (1.9 ml) was small but statistically significant. Not surprisingly, the effect was only evident in those with baseline effusion-synovitis. The results were largely consistent when an improvement defined by MCID or changes defined by LSC was used as an outcome, suggesting that effect of vitamin D supplementation on effusion-synovitis was not due to a measurement error and is large enough to be of clinical importance. The possible biological mechanism is that vitamin D could alter the inflammatory status by modulating pro-inflammatory mediators[29] through vitamin D receptors signalling pathways in the inflammatory OA phenotype.

There were several limitations to our study. First, as this is a post-hoc analysis it requires confirmation in further studies[44]. Nonetheless, the results of the current study are biologically plausible. Further, the sample size in the original trial had sufficient power to address the research question in the current study. Indeed, we were able to detect quite small changes in effusion-synovitis even if the sample size decreased by 50% after exclusion of those without baseline effusion-synovitis. Second, MRI coronal planes were used at one clinical site so regional effusion-synovitis was unable to be measured in all participants. This may reduce the power to detect significant effects of vitamin D supplementation on regional effusion-synovitis. Lastly, low vitamin D levels at baseline and over time potentially made participants in the placebo group taking vitamin D during the trial which caused potential contamination/non-adherence issues and might dilute the treatment effects in our intention-to-treat analysis. However, the serum levels of 25-(OH)D increased by 40.6 nmol/l in the vitamin D group but only 6.7 nmol/l in the placebo group, suggesting that potential contamination/non-adherence issues would not be a concern.

In conclusion, the assessment of effusion-synovitis volume is highly reproducible and responsive to treatment, and should be a

promising outcome measure in OA trials. Vitamin D supplementation could retard the progression of effusion-synovitis in patients with knee OA and low 25-(OH)D levels, suggesting vitamin D can potentially improve outcomes in people with an inflammatory knee OA phenotype.

Trial registration

ClinicalTrials.gov identifier: NCT01176344; https://clinicaltrials.gov/ct2/show/NCT01176344.

Australian New Zealand Clinical Trials Registry: ACTRN12610000495022.

Protocol

Cao Y, Jones G, Cicuttini F, *et al*. Vitamin D supplementation in the management of knee osteoarthritis: study protocol for a randomized controlled trial. Trials 2012;13:131.

Contribution

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. All authors had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. XW, CD and GJ designed and carried out data analyses, interpreted the results and drafted the manuscript. FC, XJ, ZZ, AEW, WH, LB, BA and TW collected the data, designed the data analyses, interpreted the results, and revised the manuscript for important intellectual content.

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and no competing interests to declare.

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