Osteoarthritis and Cartilage 27 (2019) 71–79

Osteoarthritis and Cartilage

Exploring longitudinal associations of histologically assessed inflammation with symptoms and radiographic damage in knee osteoarthritis: combined results of three prospective cohort studies

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ARTICLE INFO

Article history:
Received 19 June 2018
Accepted 19 October 2018

Keywords:
Inflammation
Histology
Synovitis
Knee osteoarthritis
Symptoms
Radiographic severity/progression

SUMMARY

Objective: To explore the associations between different histologically assessed, inflammatory synovial characteristics and subsequent clinical and structural aspects in knee osteoarthritis (OA).
Design: Knee OA patients, ranging in stage from early to advanced, were recruited from three different ongoing studies. Synovial tissue biopsies were taken and histologically assessed for six features (four inflammatory related aspects, fibrosis and fibrin deposition). Clinical aspects (WOMAC pain, functioning and stiffness and SF-36 vitality) and structural aspects (Kellgren and Lawrence (KL)-grade, joint space narrowing (JSN; 0–3) and osteophytes (0–3), and reception of total knee replacement (TKR)) were repeatedly assessed during follow-up. Associations between histology and clinical and structural aspects were analysed using linear mixed model analyses and cox proportional hazards analysis.
Results: Biopsies of 83 patients (median complaint duration: 5 [2–8] years) were analysed. Follow-up was a median of 1.4 [0.8–2.7] years for clinical and 1.8 [0.2–5.2] years for structural aspects. Fibrosis and fibrin deposition were inversely correlated with the inflammatory features. A higher fibrosis score was associated with a lower scores for KL-grade, JSN and osteophytes, while higher scores for perivascular oedema, synovial lining thickness and vascularisation were associated with higher scores for structural aspects during follow-up. No associations were found between each of the histological features and any of the clinical aspects or the chance for TKR during follow-up.
Conclusions: Inflammatory related histological aspects are associated with subsequent increased radiological severity in knee OA, while fibrosis seems to protect against this, providing a potential therapeutic target for OA treatment.

Background

Current evidence suggests that synovial inflammation may play a role in osteoarthritis (OA) pathophysiology[1–5]. Inflamed synovium is thought to produce pro-inflammatory mediators which alter the balance of cartilage degradation and repair towards degradation. Cartilage degradation products, in turn, amplify synovial inflammation, resulting in a vicious cycle[2,4]. However, additional mechanisms are likely to be involved, as anti-inflammatory treatments that are effective in rheumatoid arthritis have shown negative results in OA[6,7].

Synovial inflammation can be assessed by multiple methods. Histological assessment is regarded as the preferred method, as basic inflammatory processes can be distinguished separately, as for example, increased synovial lining thickness, infiltration of immune cells and an increased number of blood vessels. Histologic assessments can only be performed after synovial tissue biopsy, an invasive procedure[4,8]. Therefore, more frequently used methods to assess synovial inflammation are imaging modalities as (contrast-enhanced) magnetic resonance imaging (MRI) and ultrasonography[4]. However, these methods assess parameters that are assumed

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https://doi.org/10.1016/j.joca.2018.10.014
1063-4584/

to indirectly reflect inflammation, such as effusion volume, thickening of the synovium, increased blood flow or altered infrapatellar fat pad intensity on MRI[4,7,9,10]. Therefore, to gain more insight into the pathophysiological processes of synovitis in OA, histological assessment is the preferred method.

Associations between synovitis and OA symptoms and radiographic severity have been studied, mostly using imaging techniques for synovial assessment, and not histology[7,11]. Overall, they show that synovitis is associated with worse pain, impaired functioning in daily life, and increased radiographic progression[7,11]. Two studies did use histological assessments of synovitis to investigate the association between inflammation and clinical aspects. Their assessments consisted of a composite score of several histological features and their results show no associations between inflammation and clinical outcomes[8,12]. However, they both had a relatively small study population ($n = 34$ and $n = 39$) with specific patient characteristics (i.e., end-stage OA and chronic inflammatory OA), representing only a fraction of the total knee OA population, impairing generalisability of their results. Furthermore, they had a cross-sectional design. One of these studies only used a mean inflammatory score for six individual histological features, ignoring contributions of individual features[12]. Therefore, there is a need for longitudinal studies using individual histologically assessed inflammatory synovial parameters in a broad range of knee OA patients, to gain more insight into the role of synovitis in the development of symptoms and radiographic damage.

The aim of the current study was to explore the longitudinal associations between different histologically assessed inflammatory synovial characteristics with clinical and structural aspects of knee OA.

Methods

Study design

This is a prospective, observational study. In short, knee OA patients were recruited from participants in three different studies, which were described previously[13–15]. Knee synovial tissue biopsies were taken after written informed consent. Clinical and radiographic data gathered during those studies, but after the biopsy, were used to assess the longitudinal association between histologically assessed parameters and 1) clinical aspects (pain, functioning, stiffness and fatigue) and 2) radiographic aspects (KL-grade, joint space narrowing (JSN), osteophytes). This study has been approved by the local ethics committee (reference nr.: 2004/009). Recommendations for reporting cohort studies (STrengthening the Reporting of OBservational studies in Epidemiology; STROBE) were followed in reporting the current study[16].

Patients

Patients participating in three ongoing studies at the Sint Maartenskliniek Nijmegen, The Netherlands, were subsequently invited to undergo mini-knee arthroscopy to collect synovial tissue samples. Patients using anticoagulants or with increased risk of infection (using immunosuppressive medication, having an immune disorder, or presence of a (skin) infection around the knee) were excluded.

The first study is an observational cohort study (Cohort of Optimal Non-invasively TReated Osteoarthritis of Lower extremities – Pain, function and Radiological Outcome; CONTROL-PRO), involving patients with knee and/or hip OA (clinical ACR criteria) following an evidence based, non-surgical stepped-care outpatient treatment program[15]. Patients with predominant symptoms in their knee were eligible for the current study. They can be regarded as patients with established knee OA.

The second study is a randomised controlled trial (Doxycycline in Knee OsteoArthritis; DKOA), assessing the effects of a 6-month doxycycline treatment vs placebo on pain and functioning in patients with knee OA (clinical and radiological ACR criteria)[14]. After 6 months, there was no difference in pain and functioning between the groups. Therefore, patients from both groups were eligible for the current study, after the 6-month follow-up period. Regarding both their structural progression and symptoms, these patients can be regarded as patients with advanced knee OA. Additional analyses showed no differences in characteristics, clinical and radiological aspects and histological grades between patients who received doxycycline and those who received placebo (supplementary file).

The third study is a nation-wide prospective cohort study of 1,002 individuals with early symptomatic OA of hip and/or knee (Cohort Hip & Cohort Knee; CHECK), of which 103 were included and assessed at the Sint Maartenskliniek Nijmegen[13]. Patients included at the Sint Maartenskliniek Nijmegen, with predominant knee symptoms, were eligible for the current study. They can be regarded as patients with early symptomatic knee OA.

Biopsies

Synovial knee tissue samples were collected under local anaesthesia during a single-trocar (Parker-Pearson) video-assisted mini-knee arthroscopy by three equally trained, experienced physicians. The equipment and approach was the same for all patents. First, synovial fluid was drained and anaesthetics were injected in the joint. Second, the synovial tissue adjacent to the patella tendon was visually inspected for the most inflamed areas, after retraction of the camera, blind synovial tissue samples were collected from these areas. A median [interquartile range (IQR)] of 3 [2–4] synovial tissue samples were taken per patient. Optionally, glucocorticoids were released at the end of the procedure. Unfortunately no records have been kept on which patient received glucocorticoids. Patients were advised to avoid loading of the knee during the first 24 h after biopsy.

Synovial tissue handling and staining

Directly after biopsy, synovial tissue samples were fixated in 4% formaldehyde. Subsequently, they were dehydrated and embedded in paraffin, grouped per individual patient. When all tissue samples were collected, 5 μm thick sections were sliced from these grouped tissue samples in a single batch. Sections from a single patient were mounted on a single microscope glass and stained with haematoxylin and eosin (H&E).

Synovial tissue assessment

Synovial tissue samples were scored per individual section according to a semi-quantitative scoring system, as described elsewhere (magnification 200×)[8,17,18]. Six parameters were assessed: 1) synovial lining thickness, 2) sub-synovial infiltration by lymphocytes and plasma cells, 3) surface fibrin deposition, 4) blood vessel dilatation and blood vessel proliferation, 5) fibrosis, and 6) perivascular oedema. Each parameter was scored on a 0–3 scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe) by a single experienced researcher (AB), who was blinded to all other data (Fig. 2).

Handling of histological grades

The median number of sections was 5 [4–5] per patient. For every section, the most severe scores of the individual histological aspects were recorded. For the different sections per patient, the median score was calculated for each histological feature as the

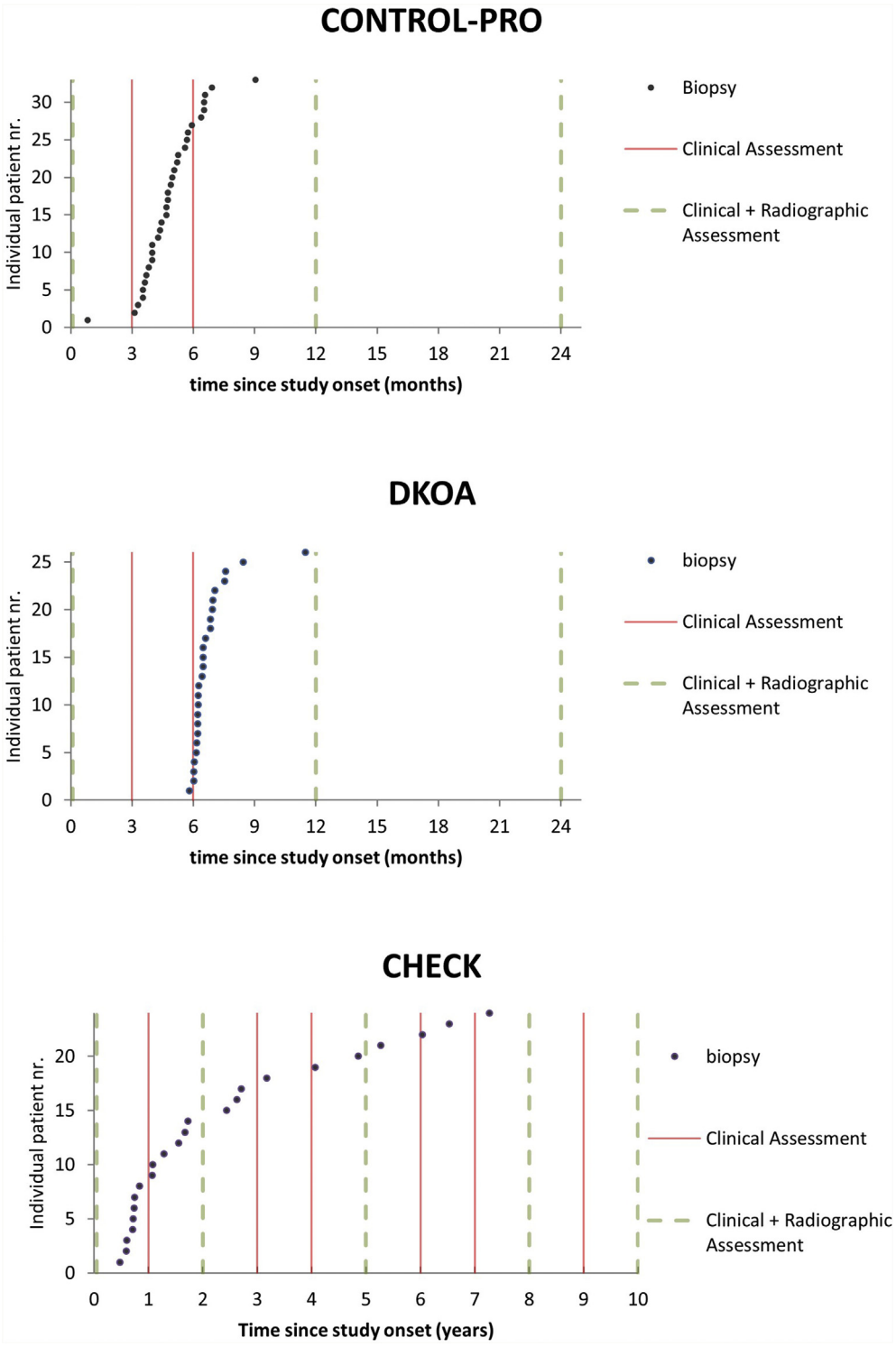

Fig. 1. Overview of moment of biopsy in relation to the start of the different studies and the standard clinical and radiographic assessments for the individual patients. Vertical lines represent standard moments of clinical and radiographic assessment.

patients' individual score for that feature. Furthermore, an inflammatory composite score was calculated as the mean of the four inflammatory histological parameters (synovial lining thickness, subsynovial infiltration, vascularisation, and perivascular oedema)[8].

Sensitivity of this scoring system on detecting inflammation has been shown[8,12]. Reliability assessment of the scoring was performed (supplementary file).

Clinical aspects

Patient demographics (age, sex, height, weight and date of symptom onset) and clinical data (Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Short-Form 36 (SF36)) were collected using standard questionnaires during assessments of the CONTROL-PRO, DKOA and CHECK studies. Body mass index

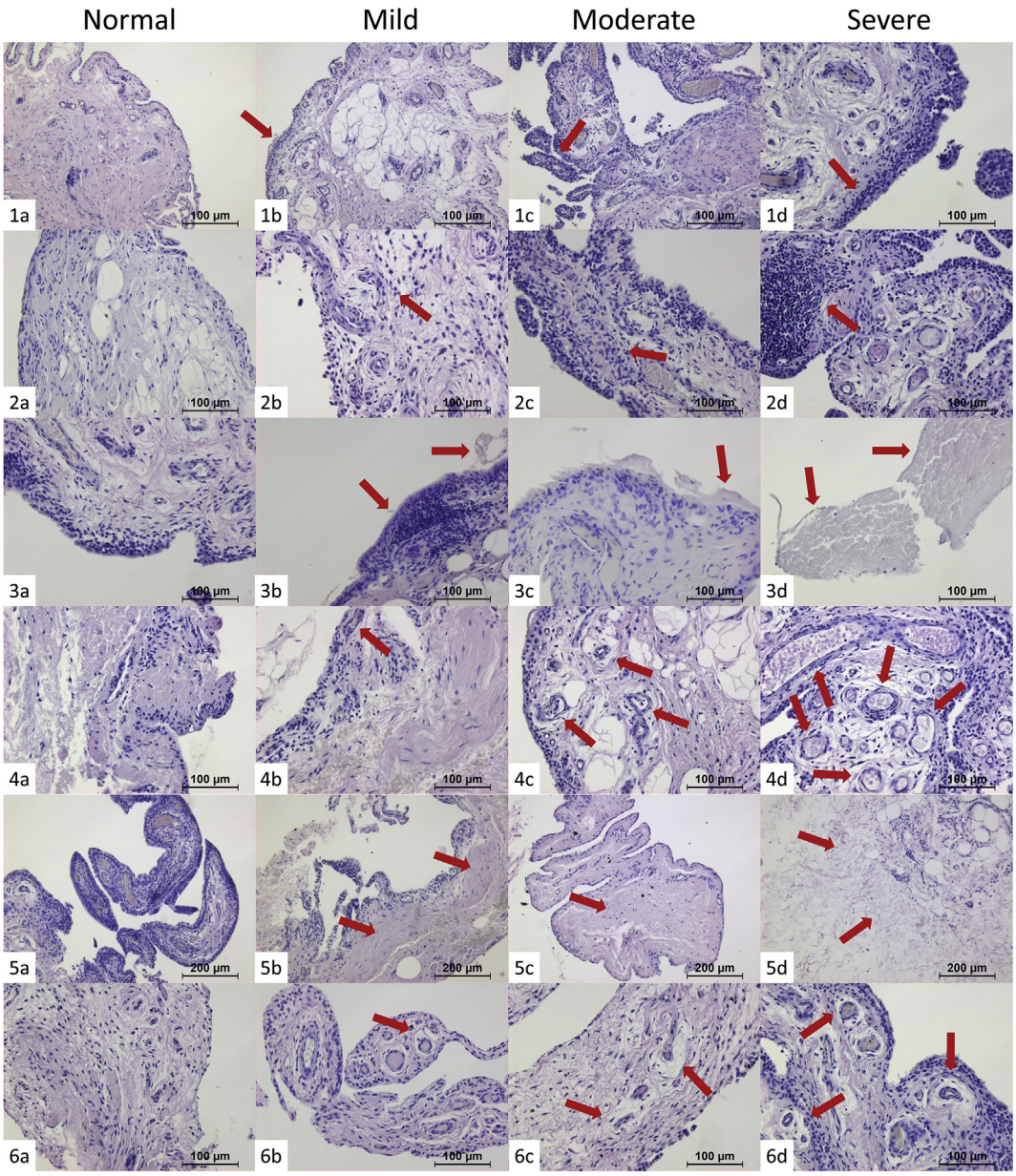

Fig. 2. Examples of the different grades of the histological features. Magnification 200× (fibrosis 100×). a: normal (score 0); b: mild (score 1); c: moderate (score 2); d: severe (score 3). 1: synovial lining thickness; 2: sub-synovial infiltration; 3: fibrin deposition; 4: vascularisation; 5: fibrosis; 6: perivascular oedema. 1) Synovial lining thickness: scored by counting the number of synovial cell layers in the lining layer. a. ≤2 cell layers was regarded as normal (score: 0)[33]. b. The remaining numbers of cell lining layers were divided in tertiles. i. 1: 3 layers. ii. 2: 4 layers. iii. 3: 5–8 layers. 2) Sub synovial infiltration: quantification of the infiltrating cells. a. 0: no infiltrating cells. b. 1: only mild infiltration mostly near the blood vessel and sub-lining. c. 2: moderate infiltration. d. 3: maximal infiltration, diffusely throughout the tissue. 3) Surface fibrin deposition: estimation of the amount of fibrin adjacent to the synovium, or as isolated "islands" of fibrin. a. 0: no fibrin deposition. b. 1: some small isolated clots of fibrin on or close to the synovial lining. c. 2: more and bigger clots on or near the lining. d. 3: large clots of fibrin covering a big part of the synovium, or as isolated fibrin islands further away from the tissue). 4) Vascularisation: the presence of blood vessels in the sub-lining layer (arbitrary). a. 0: just a few blood vessels, normal aspect. b. 1: somewhat increased number of blood vessels, still a relatively normal mix in small vs large vessels. c. 2: moderately increased number of blood vessels in the sub-lining, more small vessels. d. 3: maximal amount of blood vessels, mostly small, throughout the tissue. 5) Fibrosis: Assessment of the volume of fibrotic tissue, judged by relatively absence of cells, containing only fibroblasts and directional extracellular matrix, in comparison to the total volume. a. 0: no fibrotic areas. b. 1: some small fibrotic areas, often deeper in the tissue, less than 10% of the total tissue area. c. 2: larger areas of fibrotic tissue, but still over 60% area with tissue with a non-fibrotic aspect. d. 3: large areas of fibrosis, generalized throughout the tissue, with often only a thin synovial lining layer identifying it as synovial tissue. 6) Perivascular oedema: absence or presence and size of void "halo's" around the blood vessels. a. 0: no or hardly any halo's around the blood vessels. b. 1: Some small halo's around blood vessels, other, adjacent blood vessels have a normal aspect. c. 2: Halo's around roughly half of the vessels, some quite wide. d. 3: Most (or all) of the vessels show halo's, many relatively wide.

(BMI), age and symptom duration at time of biopsy were calculated. WOMAC pain, stiffness and functioning subscales were calculated (0–100; higher scores indicate better health status)[19]. SF-36 vitality was used as a measure for fatigue (0–100; higher score indicates a better health status)[20].

Clinical assessments were performed at baseline and after 3, 6, 12 and 24 months of follow-up for patients included from CONTROL-PRO and DKOA, and at baseline and every 12 or 24 months of follow-up (depending on disease severity), up to 10 years, for patients included in CHECK. For the current study, only clinical data collected at the time of biopsy (up to 2 weeks prior) and after biopsy were used. As the moment of biopsy during the different studies varied between the individual patients, the number of clinical assessments included in the current study can vary among individuals

(Fig. 1). The median time between biopsy and the nearest clinical assessment was 32 [18–51] days for CONTROL-PRO, 2 [0–6] days for DKOA, and 107 [63–148] days for CHECK.

Structural aspects

Structural aspects used for the current study were Kellgren and Lawrence (KL-) grade (0–4)[21], and scores for tibiofemoral (TF) (JSN; 0–3) and osteophytes at four locations (medial and lateral femur, medial and lateral tibia; 0–3) according to the Osteoarthritis Research Society International (OARSI) scoring system[22], and single scores for patellofemoral (PF) JSN (0–3) and osteophytes (0–3). KL-grade and TF outcomes were assessed on weight bearing, fixed flexion anterior–posterior (AP) radiographs. PF outcomes were assessed on skyline radiographs, taken in supine position. Mean scores for JSN and osteophytes were calculated from the individual TF and PF scores. Structural aspect scores were used if the radiographs were taken at a maximum of 6 months prior to biopsy or at any time after biopsy. Furthermore, additional radiographs from electronic medical files were assessed by a trained researcher (MM) to extend available data.

When no radiographs were available within 6 months of the biopsy ($n = 28$), baseline KL-grades were imputated by extrapolation of available KL-grades.

Total knee replacement (yes/no; TKR) and radiographic progression (yes/no), defined as an increase in KL-scale $\geq$1 or reception of TKR after biopsy, were determined. TKR was assessed during the clinical assessments and, for CONTROL-PRO and DKOA, using medial files and follow-up questionnaires after 60 months, after completing the study.

Statistical analyses

To assess differences in patient characteristics between the studies, analysis of variance (ANOVA) with post hoc *t*-tests were used for continuous outcomes and Kruskal–Wallis with post-hoc Wilcoxon rank-sum tests were used for ordinal data. To assess the associations between the histological features (independent) and 1) clinical aspects and 2) structural aspects during the time following biopsy (dependent), linear mixed model analyses were used, allowing inclusion of multiple assessments of clinical and structural aspects per individual patient.

Hazard Ratios for radiographic progression and receiving TKR were analysed using cox proportional hazards analysis.

As time between biopsy and the different assessments varied between individuals and assessments, all analyses were adjusted by including the time (years) between biopsy and the assessment. Furthermore, analyses were adjusted for age, sex, symptom duration (>5 years; yes/no), BMI and nonsteroidal anti-inflammatory drug (NSAID) use (yes/no) (non-adjusted results presented in supplementary file). STATA 13.1 was used for statistical analyses; *P*-values <0.05 were considered significant. Descriptive statistics are provided as mean (standard deviation (SD)) or median [interquartile range (IQR)] when appropriate.

To assess the influence of one particular group on the associations of the complete population, separate analyses were performed for patients from the three individual studies.

Results

Patients

Between May 2005 and November 2011, synovial tissue biopsies were taken from 96 patients. To assess selection bias, included patients were compared to not-included patients. Patients included from DKOA had more frequently a symptom duration >5 years. Patients included from CHECK and CONTROL-PRO were more frequently male. Other characteristics were comparable (supplementary file). Biopsy quality was insufficient for analyses in 13 (14%) patients, which were excluded. Of the remaining 83 patients, 33 (40%) originated from CONTROL-PRO, 26 (31%) from DKOA and 24 (29%) from CHECK (Table I). Patients from CONTROL-PRO were younger, were more frequently male and a higher proportion used pain medication than patients from other groups, while patients from DKOA had a longer symptom duration, a higher BMI and were older.

Synovial tissue biopsy scores

The histological scores are shown in Table II and the supplementary file. Vascularization was in the majority of biopsies

Table I
Baseline characteristics

	CONTROL-PRO	DKOA	CHECK	Total
n (%)	33	26	24	83
Age (years), mean (sd)	52.8 (8.7)	60.6 (9.9)	58.0 (5.0)	56.8 (8.8)
Sex (m/f), *n* (%)	19/14 (58/42)	9/17 (35/65)	10/14 (42/58)	38/45 (46/54)
Symptom duration (years), median [25–75%]	4.1 [2.1–8.1]	8.2 [5.7–20.2]	2.7 [1.5–5.1]	4.7 [2.4–8.2]
Symptom duration >5 year, *n* (%)	15 (47)	19 (76)	6 (25)	40 (49)
Time since diagnosis, median [25–75%]	1.1 [0.7–3.4]	5.9 [2.2–11.0]	N.A.	2.2 [0.9–8.5]
BMI kg/m^2, mean (sd)	28.5 (4.8)	30.9 (6.3)	27.5 (3.5)	29.0 (5.2)
Any medication use, yes (%)*	31 (94)	7 (27)	10 (42)	48 (58)
Paracetamol use, yes (%)	27 (81)	4 (15)	8 (33)	39 (47)
NSAID use, yes (%)	28 (85)	4 (15)	2 (8)	34 (41)
Other medication use, yes (%)	6 (18)	1 (4)	1 (4)	8 (10)
KL-score: *n* (%)				
0	14 (42)	0 (0)	7 (29)	21 (25)
1	7 (21)	3 (12)	13 (54)	23 (27)
2	4 (12)	13 (50)	4 (17)	21 (25)
3	8 (24)	8 (31)	0 (0)	16 (19)
4	0 (0)	2 (8)	0 (0)	2 (2)
KL $\geq$ 2, *n* (%)	12 (36)	23 (88)	4 (17)	39 (47)
Mean JSN score (0–3), median [25–75%]	1 [0.7–1.0]	1.2 [0.7–1.5]	0.3 [0.3–0.7]	0.67 [0.33–1]
Mean osteophytes score (0–3), median [25–75%]	0.8 [0.4–1.0]	1 [0.8–1.4]	0.4 [0.2–0.8]	0.8 [0.4–1]
Time since start of cohort (months), median [25–75%]	4.7 [4.0–5.7]	6.5 [6.2–6.9]	19.3 [8.9–43.5]	6.4 [5.1–8.8]
Location of biopsy (medial/lateral/combined/not distinct), *n* (%)	1/4/8/20 (3/12/21/61)	0/0/0/26 (0/0/0/100)	1/0/1/22 (4/0/4/92)	2/4/9/68 (2/5/11/82)
Number of TKP, yes (%)	11 (35)	6 (23)	3 (13)	20 (25)

* Medication use as self-reported during the clinical assessment prior to the biopsy.

Table II
Scores for histological features

$n = 83$	CONTROL-PRO	DKOA	CHECK	Total
Synovial lining thickness	0.5 [0–1.5]	0.5 [0–1]	0 [0–1]	0 [0–1]
Sub-synovial infiltration	1 [1–2]	1 [0–2]	1 [0–1.5]	1 [0–2]
Fibrin deposition	0 [0–0.5]	0 [0–0]	1 [0–2]*	0 [0–1]
Vascularisation	2 [1–2]	2 [1–2]	1 [1–2]	2 [1–2]
Fibrosis	1 [1–2]	0 [0–1]*	2 [0–2]	1 [0–2]
Perivascular oedema	1 [0–1]	1 [0–1]	0 [0–0.25]*	0.5 [0–1]
Inflammatory composite score	1.0 [0.5–1.8]	1.0 [0.5–1.5]	0.6 [0.4–1.3]	0.9 [0.5–1.5]

Al scores range from 0–3, presented in median [interquartile range].
* Significantly different from other groups; analysed by Kruskal–Wallis test with post-hoc Wilcoxon rank-sum test.

graded as mild or higher, whereas synovial lining thickness, fibrin deposition and perivascular oedema were most frequently graded as 'normal'. Patients from CHECK had a significantly higher score for fibrin deposition and a significantly lower score for perivascular oedema than patients from the other studies, while patients from DKOA had a significantly lower score for fibrosis than patients from the other studies. The other histological scores were similar for all groups. The four inflammatory histological features were moderately correlated (range $r = 0.40$ to $r = 0.65$), while these features showed inverse correlations with fibrin deposition and fibrosis (range $r = -0.28$ to $r = -0.17$; supplementary file).

Longitudinal associations between histological features and clinical aspects

For three patients, no clinical data was available within 2 weeks before, or at any time after biopsy; one patient received a total knee prosthesis only 11 weeks after biopsy. Therefore, these were excluded from this analysis; 79 patients were included. Clinical aspect scores at the assessment closest to biopsy are shown in the supplementary file; patients from CONTROL-PRO had worse scores for pain, functioning and stiffness compared to patients from CHECK. Other clinical aspects were similar for all groups. Follow up was available for a median of 1.4 [0.8–2.7] years after biopsy. 294 clinical aspect observations were included, with a median of 3 [2–4] per patient. Clinical data at time of biopsy (within 14 days of biopsy) was available for only 31 (39%) patients. Therefore, no cross-sectional analyses were performed. Mixed model analyses showed no significant associations between each of the histological features and any of the clinical outcomes during follow-up (Table III).

Longitudinal association radiographic aspects

For three patients no AP radiographs were available within 6 months of biopsy; for one patient no radiographs before TKR were available. Therefore, these patients were excluded from this analysis; 79 were included. No skyline radiographs were available for 2 (3%) patients. In total, skyline radiographs were unavailable for 24 (14%) of the radiographic assessments. Radiographic follow-up was available for 77 patients for a median of 1.8 [0.2–5.2] years after biopsy. 188 radiographic observations were included, with a median of 2 [1–3] per patient. Associations between each of the histological features and any of the radiographic aspects during follow-up are shown in Table IV. A higher score for fibrosis was associated with a lower KL-grade and mean osteophyte score during follow-up, while higher scores for perivascular oedema, vascularisation and the inflammatory compound score were associated with a higher KL-grade during follow-up. No other significant associations were found.

When the analyses were performed separately for each of the three individual studies, most of these associations were not found. Only associations were found between vascularisation and the inflammatory compound score and KL-grade during follow-up, and between vascularisation and mean JSN score during follow-up in CONTROL-PRO. Therefore, it is unlikely that our results were caused by strong associations in a single subgroup.

Twenty out of 77 patients (26%) received TKR during follow-up after a median of 2.9 [1.3–6.0] years after biopsy; 3 (13%) for CHECK, 11 (35%) for CONTROL-PRO, and 6 (26%) for DKOA. Hazard ratios are shown in Table V. No significant associations were found between any of the histological features and receiving TKR. Thirty-nine out of 63 patients (62%) showed radiographic progression after a median of 1.9 [1.3–4.4] years after biopsy; 13 (57%) for CHECK, 21 (70%) for CONTROL-PRO and 5 (50%) for DKOA. No associations were found between any of the histological features and radiographic progression (Table V).

Discussion

This is the first study to assess the longitudinal association between inflammation and clinical and radiographic aspects, using histologically assessed synovial tissue biopsies, in a relatively large population of patients with early to advanced knee OA. Fibrosis and inflammatory signs were consistently inversely correlated. We found no associations between any of the histologically assessed parameters and the clinical aspects during follow-up. However, we did find that higher fibrosis scores were associated with lower radiographic severity during follow-up, while higher scores for several inflammatory features were associated with a higher radiographic severity during follow-up. Although these associations may be small, they give insight into the potential role of inflammation and fibrosis formation in OA pathophysiology. These findings could not be supported by our findings on receiving TKR or radiographic progression, as no associations were found between the histologically assessed parameters and these outcomes.

Table III
Longitudinal associations between inflammation and clinical outcomes

$n = 79$	Pain*	Functioning*	Stiffness*	Vitality†
Synovial lining thickness	0.50 [−3.26 to 4.27]	0.55 [−3.32 to 4.42]	−0.65 [−4.80 to 3.51]	0.63 [−2.90 to 4.16]
Sub-synovial infiltration	3.54 [−0.78 to 7.87]	2.79 [−1.68 to 7.27]	0.58 [−4.27 to 5.44]	0.53 [−3.59 to 4.65]
Fibrin deposition	0.56 [−3.89 to 5.01]	1.12 [−3.46 to 5.70]	−0.75 [−5.65 to 4.16]	1.15 [−3.01 to 5.32]
Vascularisation	1.69 [−2.92 to 6.3]	2.06 [−2.67 to 6.79]	0.98 [−4.12 to 6.08]	1.17 [−3.15 to 5.49]
Fibrosis	−1.23 [−5.34 to 2.88]	−1.64 [−5.86 to 2.59]	−2.32 [−6.85 to 2.20]	−2.79 [−6.61 to 1.03]
Perivascular oedema	−0.48 [−5.16 to 4.19]	−0.25 [−5.05 to 4.56]	−1.91 [−7.06 to 3.25]	0.37 [−4.00 to 4.74]
Inflammatory composite score	2.03 [−3.38 to 7.43]	1.98 [−3.59 to 7.54]	−0.42 [−6.41 to 5.57]	1.06 [−4.02 to 6.13]

0: worse health status; 100: better health status.
Linear mixed model: β-coefficient [95% CI].
Adjusted for time between biopsy and assessment, age, sex, symptom duration (>5 years; yes/no), BMI and NSAID use.
* WOMAC subscale.
† SF36 subscale.

Table IV
Longitudinal association between inflammation and radiographic severity with 95% CI

$n = 79$	KL-grade (0–4)	Mean joint space narrowing (0–3)	Mean osteophytes (0–3)
Synovial lining thickness	0.18 [−0.03 to 0.39]	0.02 [−0.09 to 0.13]	0.04 [−0.08 to 0.15]
Sub-synovial infiltration	0.14 [−0.11 to 0.39]	−0.01 [−0.14 to 0.12]	0.04 [−0.09 to 0.18]
Fibrin deposition	−0.16 [−0.40 to 0.08]	−0.02 [−0.15 to 0.1]	−0.09 [−0.22 to 0.04]
Vascularisation	**0.34 [0.08 to 0.61]**	0.03 [−0.12 to 0.17]	0.13 [−0.01 to 0.28]
Fibrosis	**−0.39 [−0.60 to −0.17]**	−0.09 [−0.21 to 0.03]	**−0.19 [−0.31 to −0.07]**
Perivascular oedema	**0.36 [0.09 to 0.64]**	0.1 [−0.04 to 0.25]	0.15 [0.00 to 0.30]
Inflammatory composite score	**0.38 [0.08 to 0.69]**	0.05 [−0.12 to 0.21]	0.13 [−0.04 to 0.30]

Linear mixed model: β-coefficient [95% CI].
Adjusted for time between biopsy and assessment, age, sex, symptom duration (>5 years; yes/no), BMI and NSAID use.
Bold: Statistically significant.

Table V
Hazard ratios for radiographic progression and receiving TKR with 95% confidence interval (CI)

Hazard ratio's	Radiographic progression (yes/no) $n = 63$	TJR (yes/no) $n = 77$
Synovial lining thickness	1.33 [0.93–1.91]	1.18 [0.70–2.01]
Sub-synovial infiltration	1.38 [0.91–2.11]	1.30 [0.68–2.49]
Fibrin deposition	0.86 [0.59–1.26]	0.71 [0.37–1.38]
Vascularisation	1.57 [0.99–2.49]	1.05 [0.60–1.86]
Fibrosis	0.87 [0.59–1.27]	0.73 [0.39–1.34]
Perivascular oedema	1.19 [0.79–1.78]	1.30 [0.72–2.33]
Inflammatory composite score	1.47 [0.93–2.34]	1.31 [0.63–2.69]

Adjusted for age, sex, symptom duration (>5 years; yes/no), BMI and NSAID use.

This study has several limitations. First, the moment of biopsy was not synchronised with the clinical assessments that were planned. Therefore, clinical scores at the time of biopsy could not be determined in a large part of our population and cross-sectional analyses or correction for symptom severity at time of biopsy were not possible. Nevertheless, using multi-level analyses, we were able to optimally use the data that was available. Second, synovial tissue biopsies were taken blindly after visual inspection of the synovium. Therefore the most inflamed areas could have been missed during the actual biopsy, leading to a potential underestimation of the histological scores in high-inflammatory patients. Inaccurate biopsy taking probably also underlies the relatively large number of biopsies with insufficient quality for accurate assessment. Additionally, for pragmatic reasons no specific histological staining was used for the different features (e.g., von Willebrand factor for blood vessels or trichrome staining for fibrosis). This may have caused an underestimation of some histological grades. Third, no data were recorded on the macroscopic severity of inflammation. Synovitis in OA is characterised by a non-diffuse, patchy nature, frequently found near joint margins[23]. Conceivably, a moderate diffuse inflammation has more severe consequences than a locally severe inflammation, as the total amount of expressed inflammatory mediators might be much higher in that situation. Fourth, due to the explorative nature of this study, a large number of statistical analyses were performed, without correction for multiple testing. Therefore, it is likely that a number of the associations we found were falsely positive. However, the associations we found were consistent over different outcomes. Also, the analyses were performed on data with dependency, using multiple observations per patients, decreasing the chance for spurious findings. Fifth, radiographic scores from different individual studies were used. Therefore, scores from the different studies might be systematically different. However, we were unable to assess the overall intra-observer reliability. But, as observers for the different studies were well trained, we assume that the radiographic scores used in the current study were accurate. Finally, the administration of glucocorticoids after biopsy was not recorded. Therefore, possible effects on the course of symptoms could not be included. However, it is highly unlikely that an effect of glucocorticoids exceeds 6 months[24], while this study comprised a follow-up of a median of 1.4 [0.8–2.7] years. Overall, these limitations all introduced some noise into our data, and, thus, might have led to underestimation of the associations in our results. Nevertheless, we still found associations between fibrosis and structural aspects during follow-up, pleading that these associations truly exist.

The internal validity of our findings is supported by the use of validated outcome measures assessing various clinical domains. Also, three measures for radiographic severity were used, assessing multiple aspects of radiographic OA changes. Lastly, a relatively large number of biopsies was analysed in this study, increasing the power. Therefore, we believe that our results have good internal validity. The external validity of our findings is strengthened by the inclusion of patients with different stages of OA, ranging from the early symptomatic OA patients from CHECK, to the established OA patients of CONTROL-PRO, and the advanced OA patients of DKOA. It could be argued, however, that only patients with relatively severe symptoms might be willing to undergo a synovial tissue biopsy. However, included patients had similar characteristics and symptoms as patients who were not, suggesting that selection was unbiased.

This study shows no associations between histologically assessed synovial inflammation and clinical outcomes. This is in line with previous, cross-sectional research using histologic synovial assessments[8,12]. Nevertheless, other studies, using imaging modalities for synovitis assessment, have shown associations between inflammation and clinical outcomes[7,11]. Furthermore, synovitis is known to fluctuate, and this fluctuation has shown correlations to fluctuations in clinical outcomes[25,26], although conflicting evidence exists[27,28]. The discrepancy with our results may be caused by the fact that inflammation was only assessed once, while clinical aspects were assessed multiple times during follow-up. Possible fluctuations in inflammation could therefore not be detected in this study. Additionally, clinical outcomes are less stable than, for instance, radiological outcomes[29]. These factors increase the difficulty of finding associations with clinical outcomes over time. Therefore, our results on clinical outcomes need to be interpreted with care.

An unexpected finding from this study is that increased fibrosis was associated with lower radiological damage in knee OA during follow-up. Furthermore, fibrosis was inversely correlated with inflammatory histological features. However, these findings were not supported by our findings on the associations with TKR and with radiographic progressions. This might be due to the limited follow-up time with a median of roughly 3 years, which may be too short to show associations on these outcomes. Furthermore, 20 (25%) patients received TKR which may have resulted in a low power to

detect associations. Additionally, although follow-up was shortest for the established and advanced OA patients, they contributed most to the number of arthroplasties. This may have confounded these results.

It is conceivable that a transition may occur from an inflammatory state, during which the disease is active and structural changes are facilitated, to a state where fibrosis has been formed, during which there is low disease activity and structural changes are ceased; analogue to healing processes in damaged tissue. Although this theory may not be fully supported by the results of this study, as fibrosis was most commonly present in early OA and least common in advanced OA, it is possible that the rate and occurrence of this transition varies between individuals. It is possible that fibrosis formation has not been (fully) initiated in our advanced OA patients, allowing OA to progress, while fibrosis formation was initiated early in our early OA patients, hampering their OA progression. The difference of the initiation of this process between individuals may also explain the discrepancy with findings from Oehler *et al.*, who found increased fibrosis in end-stage OA and low fibrosis in early OA[30]. Possibly, fibrosis has only started to form in a late stage of the disease, while its formation was not initiated yet in their (very) early OA patients. The theory of transition from inflammation to fibrosis is supported by the findings of Felson *et al.*, who found that patients who had shown recent progression are more likely to continue progressing, while patients who were stable, were more likely to remain stable[31]. Furthermore, Haraoui *et al.* reported that the formation of fibrosis is inversely related to subsynovial infiltration, a sign of active inflammation[32]. Longitudinal histological studies are warranted to give insight into the course of different features over time and confirm this theory. Overall, this would imply that the formation of fibrosis, or a factor underlying the formation of fibrosis, might protect against radiographic changes in knee OA.

When similar research is conducted in the future, the following aspects need to be assured. First, accurately taking tissue samples from the intended location should be assured. This lowers the chance for insufficient biopsy quality and justifies the patients burden on participating in the study. Second, during biopsy, macroscopic assessments of the synovial inflammation should be collected, in order to judge the spread of the inflammation. Third, clinical and radiographic assessments should be performed at the day of biopsy, in order to accurately relate these outcomes with inflammation at that particular moment. And last, study follow-up should be of adequate length to be able to assess the associations over time, ideally including recurrent biopsies taken.

In conclusion, our results show that higher scores for fibrosis are associated with lower radiographic severity in knee OA over time. Furthermore, we found that fibrosis is inversely related to these inflammatory features cross-sectionally. We hypothesise that a transition from an inflammatory state to a fibrotic, non-inflammatory state occurs in OA. However, to replicate our findings and confirm this hypothesis, further well-designed longitudinal studies are necessary.

Author contributions

The authors declare the following contributions to the preparation of the manuscript: AAB, CvdE, PvdK and FvdH had the idea for the study. Study conception and design (MM, AB, AAB, CvdE); inclusion and data collection (MM, AB, GS, HC); literature search, data analysis, tables, and figures (MM) and interpretation of data (all authors); drafting of the manuscript (MM); critical revision of the manuscript for important intellectual content (all authors); final approval of the manuscript (all authors). All authors take responsibility for the integrity of the work and agreed to submit the article for publication.

Conflict of interest

There are no conflicts of interest.

Role of the funding source

There was no external funding source for this study. The study costs were jointly covered by Sint Maartenskliniek and Radboud university medical center.

Acknowledgements

We thank Birgitte Walgreen for her efforts on the histological scoring of the synovial tissue samples and Lenny Geurts, Yvonne de Punder and Mark Wenink for performing the synovial tissue biopsies. The CHECK-cohort study is funded by the Dutch Arthritis Foundation. Involved are: Erasmus Medical Center Rotterdam; Kennemer Gasthuis Haarlem; Leiden University Medical Center; Maastricht University Medical Center; Martini Hospital Groningen/ Allied Health Care Center for Rheumatology and Rehabilitation Groningen; Medical Spectrum Twente Enschede/Ziekenhuisgroep Twente Almelo; Reade Center for Rehabilitation and Rheumatology; Sint Maartenskliniek Nijmegen; University Medical Center Utrecht and Wilhelmina Hospital Assen.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2018.10.014.

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