Watt, F., Hamid, B., Garriga, C., Judge, A., Hrusecka, R., Custers, R., ... Vincent, T. (2020). The Molecular Profile of Synovial Fluid Changes upon Joint Distraction and is Associated with Clinical Response in Knee Osteoarthritis. *Osteoarthritis and Cartilage*. https://doi.org/10.1016/j.joca.2019.12.005
The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis

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** Article history:
Accepted 22 December 2019

Keywords:
Osteoarthritis
Orthopaedic
Cytokines
Synovial fluid
Biomarker
Distraction

** SUMMARY

Objective: Surgical knee joint distraction (KJD) leads to clinical improvement in knee osteoarthritis (OA) and also apparent cartilage regeneration by magnetic resonance imaging. We investigated if alteration of the joint’s mechanical environment during the 6 week period of KJD was associated with a molecular response in synovial fluid, and if any change was associated with clinical response.

Method: 20 individuals undergoing KJD for symptomatic radiographic knee OA had SF sampled at baseline, midpoint and endpoint of distraction (6 weeks). SF supernatants were measured by immunoassay for 10 predefined mechanosensitive molecules identified in our previous pre-clinical studies. The composite Knee injury and OA Outcome Score-4 (KOOS4) was collected at baseline, 3, 6 and 12 months.

Results: 13/20 (65%) were male with mean age 54 ± 5yrs. All had Kellgren–Lawrence grade ≥2 knee OA. 6/10 analytes showed statistically significant change in SF over the 6 weeks distraction (activin A; TGFβ1; MCP-1; IL-6; FGF-2; LTBP2), P < 0.05. Of these, all but activin A increased. Those achieving the minimum clinically important difference of 10 points for KOOS4 over 6 months showed greater increases in FGF-2 and TGFβ-1 than non-responders. An increase in IL-8 during the 6 weeks of KJD was associated with significant molecular changes observed in SF following KJD, that are remarkably consistent between individuals. Preliminary findings appear to suggest that increases in some molecules are associated with clinically meaningful responses. Joint distraction may provide a potential opportunity in the future to define regenerative biomarker(s) and identify pathways that drive intrinsic cartilage repair.

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https://doi.org/10.1016/j.joca.2019.12.005

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Please cite this article as: Watt FE et al., The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2019.12.005
Introduction

Osteoarthritis (OA) affects all joint tissues, with articular cartilage loss being one of the hallmarks of progressive disease. It is likely that excessive mechanical load or loss of mechano-protective mechanisms in the joint is an underlying process in many cases of disease, but that there are other superimposed factors such as inflammation that modify its course. Longitudinal cohorts such as the Osteoarthritis Initiative and Clinical Assessment of the Knee (CAS-K) show that in ~40% of individuals with early knee OA, pain may stabilise or improve over time, suggesting that the disease may remit and is not inevitably progressive. Interventions that mechanically off-load the joint, such as strengthening exercises, weight loss, orthotics such as bracing or surgical interventions such as osteotomy or unloading devices all reduce knee symptoms.

It is often stated that adult articular cartilage is unable to repair but a body of literature is emerging that challenges this concept. This is best exemplified by traumatic focal cartilage defects that can repair spontaneously in young joints (reviewed in), but in individuals undergoing high tibial or distal femoral osteotomy for OA, structural modification has also been observed. The other evidence comes from studies of surgical knee joint distraction (KJD). The primary goal of this treatment is to improve symptoms sufficiently to delay knee arthroplasty. This is especially the case in younger patients, since these individuals have an increased risk of revision arthroplasty.

KJD is a technique where, under anaesthesia, an external fixation frame is placed on both sides of the joint, allowing distraction (gradual pulling apart of the joint’s bony ends by ~5 mm for 6 weeks). During distraction, the patient is encouraged to weight-bear on the extended knee. Such weight-bearing creates intermittent joint fluid pressure changes, due to built-in springs in the frame enabling a maximal 3 mm axial displacement under full body weight. Studies of joint distraction have shown sustained and clinically significant improvement at a number of joint sites. For knee OA, joint distraction improved knee symptoms for 5–9 years in individuals with established OA. Remarkably, the 6 week intervention also led to apparent cartilage regeneration in the subsequent months and years, with increase in joint space width on X-ray, and increased articular cartilage thickness on magnetic resonance imaging (MRI). These studies suggest that, by temporarily off-loading the joint, KJD might somehow be responsible for ‘priming’ the joint to enable intrinsic cartilaginous repair. The biological mechanisms which underlie such a response are not understood but may include changes in the peri-articular bone and enhanced mesenchymal stem cell attachment to the damaged joint surface. KJD is therefore an attractive mechanistic model in which to investigate potential reparative pathways and identify novel associated markers of clinical response.

Synovial fluid (SF) represents an accessible fluid that contains molecules reflecting biological processes within the joint. These molecules are joint tissue-agnostic; likely being derived from all the tissues interfacing the joint cavity and can be sampled repeatedly to monitor change over time within an individual. SF the tissues interfacing the joint cavity and can be sampled molecules are joint tissue-agnostic; likely being derived from all

We hypothesised that over the course of KJD, changes in the joint’s mechanical environment modulate these candidate SF markers. We further hypothesised that changes in these mechanosensitive molecules either alone or in combination would be associated with clinical outcome. We set out to test these hypotheses in a proof-of-concept study in a group of individuals undergoing planned surgical KJD.

Method

Ethics

Approval for this study was given by a research ethics committee (#15-160/D; NL51539.041.15). Usual care clinical data was also accessed (#17-005). All participants gave written informed consent to participate prior to screening, according to the Declaration of Helsinki.

Participants

Potential participants were identified by the orthopaedic surgeon (RC) from a population with knee OA attending for consideration of KJD as part of their usual clinical care at a single site in Netherlands (University Medical Center Utrecht). Inclusion criteria were: age <65 years; knee OA fulfilling ACR clinical criteria; Kellgren and Lawrence (KL) grade ≥2 on X-ray; knee ligaments intact; preserved range-of-motion (flexion >120°; no loss of full extension); SF sample available at baseline. Exclusion criteria were: history of inflammatory arthritis affecting the index knee including rheumatoid arthritis; recent infection or systemic inflammatory disease; post-traumatic fibrosis; tibial plateau fracture; extensive bone-on-bone contact on X-ray; previous or planned knee arthroplasty during study period; surgery to the index knee within last 6 months; primary (isolated) patellofemoral OA; contralateral knee requiring surgical treatment; inability/contraindication/not consenting to provide SF; BMI ≥35 kg/m²; pregnancy.

Clinical outcomes

Knee Injury and Osteoarthritis Outcome Score (KOOS) was collected as part of usual hospital care electronically at baseline, 3, 6 and 12 months [Fig. 1(A)]. From this, KOOS4, a single composite score which has been validated as a single outcome in other clinical studies was calculated (the mean of 4 of 5 KOOS subscales: Pain, Symptoms, Sports/Recreation and Quality of Life).

Usual care intervention

A non-hinged, external proof-of-concept fixation joint distraction frame (Monotube Triax with pin clamps, Stryker) [Fig. 1(B)] was fitted to the index knee by an orthopaedic surgeon (RC) whilst the patient was under spinal or general anaesthesia (GA) and the joint surfaces distracted by 5 mm. The frame was then worn for 6–7 weeks.

Participant biological samples

A maximum of 2 ml of SF was aspirated by needle from the index knee at baseline visit (whilst participant under anaesthesia and prior to the distraction frame being fitted), subsequently at midpoint of distraction (3–4 weeks, under local anaesthesia) and at

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endpoint of distraction (at 6-7 weeks, immediately after the distraction frame was removed under anaesthesia) [Fig. 1(A)]. Within 2 h, all samples were centrifuged for 20 min at 3000G. Supernatants were stored in 200 μl aliquots in cryovials at -80°C in monitored freezers.

Comparator ranges

Normal: These were calculated in previously collected SF from patients undergoing amputation for treatment of lower limb tumour, at Royal National Orthopaedic Hospital (Stanmore), London, UK, or transplant donation, at Charing Cross Hospital, London, UK (REC 09/H0710/60), who had macroscopically normal knee articular cartilage at the time of surgery and no evidence of arthritis or tumour invasion into the joint23, OA: These were calculated from measurements in SF from research tissue bank samples of patients with a confirmed diagnosis of OA undergoing partial or total joint replacement surgery at the Nuffield Orthopaedic Centre, Oxford, UK (REC 09/H0606/11 + 5). SF had been processed and stored as above.

Reagents

General laboratory reagents were the best available grade from either Sigma–Aldrich (Dorset, UK) or BDH (Dorset, UK) unless otherwise stated. MesoScale Discovery (MSD) plates and MSD SULFO-TAG labelled Streptavidin (#R32AD-5) were from MSD (Rockville, MD, USA). Enzyme-linked immunosorbent assays (ELISAs) were from commercial providers (Table I).

Assays

Assays were conducted for 10 pre-defined candidate molecules listed in Table I. All assays were carried out as per manufacturers’ instructions unless stated otherwise. Each assay had either previously undergone validation by us23 or else underwent structured performance assessment and optimisation for SF for this project, and all also passed quality performance requirements during sample reads (Table I). ELISA plates were read using Berthold Mithras LB940 reader and MSD plates by MSD QuickPlex SQ120 reader (analysed with MSD Discovery Workbench software v4.0.12). For TSG-6, each plate well (MSD, Rockville, USA, L15XA) was custom-coated with 30 μl 10 μg/ml TSG-6 capture antibody (Merck, MABT108) in phosphate-buffered saline (PBS) overnight at 4°C. Methods were then as described23. Mean concentrations of analytes were calculated from duplicate assay reads for each participant for each timepoint. Inter- and intra-assay coefficients of variation (C.V.s) were calculated for all assays. The lower limit of quantitation (LLOQ) was calculated for all assays. Where a
measurement was below LLOQ, 50% of this value was used\textsuperscript{22,23}. Lower and upper limits were also calculated for all assays for normal ranges using the geometric mean ± 2 standard deviations.

**Statistical analysis**

All available data were analysed on all participants with sufficient SF at each of the 3 timepoints (and one patient with samples at baseline and 3 weeks). All SF analytes were above LLOQ (allowing attribution of endpoint measurements) except for one sample each for TGF\textsubscript{b}1 at baseline, FGF-2 at midpoint and activin A at endpoint. These values were considered as 50% of the LLOQ. Sample and KOOS completeness are shown in Fig. 1(A). Missing data were not imputed.

**Change in KOOS\textsubscript{4} over time**

Median differences between paired observations of KOOS\textsubscript{4} at baseline and either 3, 6 or 12 months were compared by Wilcoxon signed rank test.

**Change in analyte levels over time**

Median differences between paired observations (baseline vs 3 or 6 weeks) of analyte levels were compared by Wilcoxon signed rank test. Effect size (ES) was reported as the difference between medians. Correlations between the changes over 6 weeks for each analyte were assessed by Spearman’s R coefficient (range −1 to 1; where ±1 = strongest positive (or negative) correlation, 0 = no correlation).

**Association of change in analytes with KOOS\textsubscript{4}**

The clinical outcome variable was change in KOOS\textsubscript{4} over time (KOOS\textsubscript{4} at either 3, 6 or 12 months respectively – KOOS\textsubscript{4} at baseline). Linear regression was employed to model the relationship between continuous change in analyte levels (concentrations at 6 or 3 weeks – baseline concentrations) and change in KOOS\textsubscript{4}.

In a planned secondary analysis, linear regression also assessed change in KOOS\textsubscript{4} by categories of change in analytes. Concentrations of analytes (at baseline and 6 weeks) were classified into normal (≥25th and <75th percentiles), high (≥75th percentile) and low (<25th percentile) categories. The 25th and 75th centiles were calculated from measurements of these molecules in SF from 40 individuals with OA who had undergone either partial or total knee joint replacement (see Comparator ranges). These data were generated at same time as participant data, using the same assay batches. ‘Relevant change’ was defined as a movement between at least one category from baseline to 6 weeks (relevant increase, or relevant decrease), or as no relevant change.

**Change in analytes by responders and non-responders in KOOS\textsubscript{4}**

Responders (those whose change in KOOS\textsubscript{4} over 6 months (the latest point at which there was clinical change from baseline), [Fig. 1(C)] was ≥10 points, i.e., the minimal clinically important difference (MICD) for KOOS\textsubscript{4})\textsuperscript{32}; and Non-Responders (those whose KOOS\textsubscript{4} change over 6 months was <10 points) were categorized. Differences between molecular changes in these 2 groups were compared by Mann-Whitney U test.

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**Table I**

| Analyte     | Assay                              | Manufacturer (Catalog No.) | Intra-Assay CV (%) | Inter-Assay CV (%) | Lower Limit of Normal (pg/ml) | Upper Limit of Normal (pg/ml) | Diln Factor (SF) |
|-------------|------------------------------------|---------------------------|-------------------|-------------------|-------------------------------|-------------------------------|-----------------|
| Activin A   | Human/Mouse/Rat Activin A          | R&D (DAC00B)              | 3.9               | 11.7              | 1,028                         | 5,253                        | 50              |
| MCP-1       | V-PLEX Human MCP-1                 | MSD (K151NND-1)           | 3.1               | 5.1               | 60                            | 493                          | 5               |
| FGF-2       | V-PLEX Human (basic) FGF-2         | MSD (K151MDD-1)           | 4.0               | 6.1               | 2                             | 411                          | 4               |
| IL-6        | V-PLEX Custom Human Cytokine       | MSD (K151A0H-1)           | 4.1               | 9.7               | 1                             | 20                           | 5               |
| IL-8        | V-PLEX Custom Human Cytokine       | MSD (K151A0H-1)           | 3.5               | 8.9               | 2                             | 39                           | 5               |
| TSG-6       | Human TSG-6 Ultra-Sensitive        | MSD (K151FCZ-1)           | 4.7               | 13.6              | 3,742                         | 231,000                      | 50              |
| MMP3        | Human TSG-6 Ultra-Sensitive        | R&D (DB100B)              | 3.7               | 13.0              | 257.3                         | 1,545                        | 4               |
| TIMP-1      | Human TIMP-1 Ultra-sensitive       | MSD (K151FZC-1)           | 9.1               | 10.3              | 143,000                       | 744,700                      | 200             |
| TGF\textsubscript{b}1 | Human TGF\textsubscript{b}1 Quantikine ELISA | R&D (DAC00B) | 6.6               | 17.3              | 1,887                         | 13,630                       | 4               |
| LTBP2       | Human LTBP2 ELISA                 | Abhexa (abh 152242)       | 6.6               | 17.3              | 1,887                         | 13,630                       | 4               |
| IL-8        | V-PLEX Human (basic) FGF-2         | MSD (K151A0H-1)           | 4.1               | 9.7               | 1                             | 20                           | 5               |
| FGF-2       | V-PLEX Custom Human Cytokine       | MSD (K151A0H-1)           | 3.5               | 8.9               | 2                             | 39                           | 5               |
| TSG-6       | Human TSG-6 Ultra-Sensitive        | MSD (K151FCZ-1)           | 4.7               | 13.6              | 3,742                         | 231,000                      | 50              |
| MMP3        | Human TSG-6 Ultra-Sensitive        | MSD (K151FCZ-1)           | 4.7               | 13.6              | 3,742                         | 231,000                      | 50              |
| TIMP-1      | Human TIMP-1 Ultra-sensitive       | MSD (K151FZC-1)           | 9.1               | 10.3              | 143,000                       | 744,700                      | 200             |

Inter- and intra-assay coefficients of variation (C.V.s) were calculated for all assays. Lower and upper limits were also calculated for all assays for normal ranges using the geometric mean ± 2 standard deviations.

Abbreviations: CV = coefficient of variation; Diln = Dilution; MSD Mesoscale Discovery.

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**Table II**

| Baseline Characteristic | N (%), or mean, SD |
|-------------------------|--------------------|
| Sex                     |                    |
| Male                    | 13 (65%)           |
| Female                  | 7 (35%)            |
| Age (years)             | 55, 5              |
| Body Mass Index (BMI)   | 29, 3              |
| Kellgren and Lawrence grade |               |
| 2                       | 2 (10%)            |
| 3                       | 10 (50%)           |
| 4                       | 8 (40%)            |
| KOOS\textsubscript{4}   | 30, 11             |

Abbreviations: SD Standard deviation; KOOS\textsubscript{4}, Knee injury and OA outcome score-4.
Measurement of synovial fluid analytes during knee joint distraction. Synovial fluid from study participants immediately prior to distraction (‘baseline’), after 3 weeks of knee joint distraction (‘midpoint’) and at 6 weeks after knee joint distraction (‘endpoint’) were assayed for pre-defined markers of interest by electrochemiluminescence or ELISA (see Table I). Measurements for each of 10 analytes are shown, with mean concentrations for each analyte plotted on a log 10 y axis. \*P < 0.05, \*P < 0.01, \*P < 0.001 by Wilcoxon signed rank test, comparing paired levels at end point or midpoint vs baseline (individual P values are given in Supplementary Table 1). A, shows 6 analytes with change at endpoint vs baseline. B, shows 4 analytes without change at endpoint (although upper 2 showed change at midpoint). ULN and LLN of normal ranges were calculated for each analyte as described in methods and Table I. Abbreviations: LLOQ, lower limit of quantification; ULN, upper limit of normal; LLN, lower limit of normal; LTBP2, latent-transforming growth factor beta-binding protein 2; TGFβ-1, transforming growth factor beta 1; FGF-2, basic fibroblast growth factor; TIMP-1, tissue inhibitor of metalloproteinases 1; TSG-6, tumour necrosis factor-inducible gene 6 protein; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein 1; IL-8, interleukin 8; MMP3, matrix metalloproteinase-3.
Data were stored on a secure database (OpenClinica). Analysis was performed in STATA IC 13.1 and Graphpad Prism 6.03.

Results

13/20 (65%) participants were male with mean age 55 ± 5 years (Table II). All had KL grade ≥ 2: 18 (90%) grade 3/4, with substantial knee pain at baseline (KOOS pain 38.6 ± 16.0; where 100 is no pain, normal function). As expected from previously published studies, there was an improvement in KOOS4 in the subsequent months following the intervention [Fig. 1(C)].

6/10 SF analytes showed changes between baseline and 6 weeks (IL-6, ES = 56.6, P = 0.0043; MCP-1, ES = 155.1, P = 0.0016; FGF-2, ES = 164.7, P = 0.0123; TGFβ-1, ES = 21, P = 0.0003; LTBP2, ES = 0.4, P = 0.0475; activin A, ES = −6.8, P = 0.0002) [Fig. 2(A)] and (Supplementary Table 1). Of these, IL-6, MCP-1, FGF-2 and TGFβ-1 showed a predominant increase in levels, while activin A mainly decreased (to within normal range for most individuals). There was variation in response between individuals, exemplified by LTBP-2. For several analytes, change was detectable within 3 weeks of distraction (activin A, TGFβ-1 and IL-6) (Supplementary Table 1). 2 further molecules, IL-8 and TIMP-1 were different at 3 weeks (ES = 73.5 and ES = 389, respectively), but not at 6 weeks (ES = 10.5, ES = 115.2, respectively) [Fig. 2(B)], (upper panels). The remaining 2 analytes (MMP3, TSG-6) did not change over the distraction period (ES = −75.3, P = 0.53 and ES = 41508, P = 0.21 respectively, Fig. 2[B], lower panels).

Several analytes correlated with each other in their change over the 6 week distraction period (Fig. 3). Associations between changes in markers could also be seen over the initial 3 weeks of knee joint distraction (Supplementary Figure 1). Higher correlations were found for TGFβ-1 and FGF-2 (R = 0.68); IL-6, TIMP-1 and either MMP3 or TSG-6; (all pairs R > 0.5). LTBP2 and activin A had low correlation with other analytes over time. TGFβ-1 and IL-6 were negatively correlated (R = −0.43).

The association of change in candidate molecules over the distraction period with subsequent change in KOOS4 was examined. Change in 4 molecules was associated with change in KOOS4 over the first 3 months: activin A, TGFβ-1, FGF-2 and MCP-1 [Fig. 4(A)]. For all except activin A, an increase in the analyte was associated with greater improvement in KOOS4, but the effects were weak (Supplementary Table 2). The low effect sizes were primarily because the unit of change of a marker within the regression model was per 1 pg/ml, whereas often much larger changes in markers than 1 pg/ml were seen. Similar associations persisted at 6 months for all 4 molecules. For example, for the effect of change in FGF-2 over 6-weeks, on change in KOOS4 over 6-months, for a 1-unit increase in FGF-2 change per pg/ml, the change in KOOS4 is 0.03 points. To interpret the 95% CI, the underlying effect in the population could lie between 0.004 and 0.057. To aid interpretation, the median increase of FGF-2 over 6 weeks is 165 pg/ml. Hence for a 165 pg/ml unit increase in FGF-2, the change in KOOS4 is 4.95 points (95%CI 0.66 to 9.41). IL-8 had the largest and increasing effect size (0.28 by 12 months), but the confidence intervals at all timepoints were wide.

To test the relevance of these findings, we categorised participants’ molecular measurements as having no relevant change, a relevant increase or a relevant decrease over the 6 week distraction period (see methods) and examined the association of these categories with change in KOOS4. Those with a relevant increase in SF IL-8 during the distraction period had a greater improvement in KOOS4 over 12 months than those with no change (regression coefficient 17.6 [12, 34.0]; P = 0.04). However, no other molecular changes were associated with clinical outcome when categorised in this way (Supplementary Table 3). Furthermore, the confidence intervals for this observation are wide and given that the other

![Correlation between change of analytes in the synovial fluid of participants over period of knee joint distraction.](https://doi.org/10.1016/j.joca.2019.12.005)
findings for IL-8 did not reach significance [Fig. 4(A)], this could be a chance finding).

We also compared the change in analyte levels over the 6 weeks of distraction in those making the MCID of 10 points or more by KOOS4 (responders) with those who did not improve by this amount (non-responders). The clinical response to joint distraction was most pronounced at 6 months, with 11/15 (73%) of individuals with available data reaching a MCID on KOOS4. Responders at 6 months had a greater increase in TGF-β1 and FGF-2 during the distraction period than non-responders [Fig. 4(B)]. (Supplementary Table 4 and Supplementary Figure 2). Similar analyte changes were also seen in responders and non-responders at 3 months, when TIMP-1 levels were also different between the 2 groups (ES = 497 ng/ml, P = 0.02, Supplementary Figure 3).

Discussion

Easily detectable, substantial changes in levels of 8 putative mechanosensitive molecules of the inflammatory response (activin A, LTBP2, TGF-β1, FGF-2, TIMP-1, IL-6, MCP-1, and IL-8) were seen in SF over the period of KJD. There were also associations between several of these molecules over time. These changes would not
appear to be due to SF volume change because whilst some analytes increase, others stay the same or even decrease. Of the regulated molecules, whilst IL-6 and MCP-1 (also known as CCL-2) have been associated with degeneration or pain in the osteoarthritic joint, FGF-2 and TGFβ-1 are more typically associated with repair. It is perhaps not surprising that a mechanically-induced inflammatory response should include both catabolic and reparative processes. But that an intervention which apparently leads to net articular cartilage repair involves the activation of traditionally inflammatory pathways would go against current convention. Overall there was substantial variation between individuals for certain molecules in the extent and sometimes direction of this response. This supports the notion that an individual's biological response to the intervention could vary and be related to their clinical response. On the other hand, some molecules like TGFβ-1 and Activin A showed very consistent directional changes following KJD.

Our proof-of-concept study appears to suggest an association between this measurable biological response to joint distraction and subsequent clinical outcome. The clinical response to joint distraction was most pronounced at 6 months. Several of the associations between change in analytes and KOOS4 at 6 months were also apparent at 3 and 12 months, and when individuals were stratified, either by their molecular response or their clinical response. This supports that elements of this biological response to distraction appeared to be associated with a clinically meaningful response: for example, FGF-2 and TGFβ-1, typically associated with cartilage anabolism/anti-catabolism, were raised in responders.

One molecule, activin A, strikingly fell in all individuals to what we estimate are normal levels in human SF. Activin A is produced by osteoarthritic and injured articular cartilage and promotes skin wound healing. Its direction of change (opposite to that of FGF-2/TGFβ-1) is perhaps surprising: activin A is a TGFβ superfamily member and is strongly FGF-2-dependent in the joint in our pre-clinical studies. It may be that these apparent paradoxes are because different joint tissues are involved: FGF-2 and TGFβ may derive from the capsule, say, whereas joint-offloading may reduce the cartilage injury response, reducing activin A. Whilst activin A appears to be a highly sensitive read-out of the intervention, it does not show association with patient-reported outcomes. This observation supports the accurate measurement of this response in SF as both possible and informative. Ways of finding associations with a positive outcome to distraction are currently limited. These observations show the potential to define biomarker(s) associated with positive clinical responses to this and similar interventions aiming to off-load the joint surfaces. Biomarker stratification, identifying individuals most likely to respond in clinical trials or usual care would increase the utility of this already apparently cost-effective intervention. Experimental studies of joint distraction represent a novel way of identifying potential regenerative pathways that drive intrinsic connective tissue repair; these pathways might be amenable to augmentation, by pharmacological or other means to treat symptomatic OA.

**Studies involving humans/animals**

The procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

**Data availability**

Data generated by this research will be made available on reasonable request to the authors where this is legally and ethically possible.

**Author contributions**

All authors fulfilled the ICMJE criteria below. All authors have made substantial contributions to all three of sections (1), (2) and (3) below:

1. the conception and design of the study, or acquisition of data, or analysis and interpretation of data
2. drafting the article or revising it critically for important intellectual content
3. final approval of the version to be submitted

Specifically:

Planning and design of study TV, FW, SM, FL, RC, CG, AJ.
Conduct, data collection and biomarker analysis MJ, RC, SM, FW, RH, BH.
Statistical analysis and reporting CG, AJ, BH, FW, TV, SM, RC, FL.

Fiona Watt (Fiona.watt@kennedy.ox.ac.uk), Tonia Vincent (tonia.vincent@kennedy.ox.ac.uk) and Simon Mastbergen (s.mastbergen@umcutrecht.nl) take joint responsibility for the integrity of the work as a whole, from inception to finished article.
Conflict of interest
Fiona Watt: no conflicts of interest; other unrelated funding received from Pfizer Ltd, USA clinical study (AZR00860) and from Astellas European Foundation clinical study (AZR00850).
Benjamin Hamid: no conflicts of interest.
Cesar Garriga: no conflicts of interest.
Andrew Judge: no conflicts of interest.
Renata Hrusceka: no conflicts of interest.
Roel Custers: no conflicts of interest.
Mylene Jansen: no conflicts of interest.
Floris Lafeber - is co-founder, shareholder, and co-director of ArthroSave BV, and is consultant for Synerkine Pharma BV, both spin-off companies of the UMC Utrecht involved in treatment of osteoarthritis.
Simon Mastbergen: no conflicts of interest.
Tonia Vincent: no conflicts of interest.

Role of funding source
This work was supported by coordinated project grants from Versus Arthritis project grant (20783), Centre for OA Pathogenesis Versus Arthritis (grants 20205 and 21621) and the Kennedy Trust for Rheumatology Research (all of the UK), and also ReumaNederland, the Dutch Arthritis Society (ISP14-3-301/16-1-404) for the work in the UK and The Netherlands for the study respectively. FEW is supported by a UKRI Future Leaders Fellowship and was supported during this work in part by the NIHR Oxford Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

The study funders/study sponsors had no involvement in the study design, data collection, analysis or interpretation of the study, or in the writing of, or decision to submit the manuscript.

Acknowledgements
The authors wish to thank Charlotte Kerr for administrative support. The authors also wish to thank the study sites and the participants.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2019.12.005.

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Please cite this article as: Watt FE et al., The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2019.12.005.
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