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# Improved Understanding of the Inflammatory Response in Synovial Fluid and Serum after Traumatic Knee Injury, Excluding Fractures of the Knee: A Systematic Review

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## Abstract

*Background.* Traumatic knee injury results in a 4- to 10-fold increased risk of post-traumatic osteoarthritis (PTOA). Currently, there are no successful interventions for preventing PTOA after knee injury. The aim of this study is to identify inflammatory proteins that are increased in serum and synovial fluid after acute knee injury, excluding intra-articular fractures. *Methods.* A literature search was done according to the PRISMA guidelines. Articles reporting about inflammatory proteins after knee injury, except fractures, up to December 8, 2021 were collected. Inclusion criteria were as follows: patients younger than 45 years, no radiographic signs of knee osteoarthritis at baseline, and inflammatory protein measurement within 1 year after trauma. Risk of bias was assessed of the included studies. The level of evidence was determined by the Strength of Recommendation Taxonomy. *Results*. Ten studies were included. All included studies used a healthy control group or the contralateral knee as healthy control. Strong evidence for interleukin 6 (IL-6) and limited evidence for CCL4 show elevated concentrations of these proteins in synovial fluid (SF) after acute knee injury; no upregulation in SF for IL-2, IL-10, CCL3, CCL5, CCL11, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) was found. Limited evidence was found for no difference in serum concentration of IL-1β, IL-6, IL-10, CCL2, and tumor necrosis factor alpha (TNF-α) after knee injury. *Conclusion.* Interleukin 6 and CCL4 are elevated in SF after acute knee injury. Included studies failed to demonstrate increased concentration of inflammatory proteins in SF samples taken 6 weeks after trauma. Future research should focus on SF inflammatory protein measurements taken less than 6 weeks after injury.

## Keywords

osteoarthritis (OA), anterior cruciate ligament (ACL), meniscus, biomarkers, post-traumatic, inflammation

## Introduction

The knee is the most commonly injured joint with an estimated incidence of 2.29 per 1000 person years in the general population.[1] In the short term, knee injury results in decreased physical fitness and quality of life.[2,3] In the long term, it is strongly associated with the development of post-traumatic osteoarthritis (PTOA) of the knee at an early age.[4-6]

The risk of knee OA after an anterior cruciate ligament (ACL) or meniscus injury, is reported to increase 4- to ten-fold.[5,7] PTOA can affect a population as young as 30 to 40 years of age.[4,8] Thereby causing functional disability and lost productivity in a working-age population.[9] To date, there are no effective interventions for PTOA. Surgery, such as an ACL reconstruction or meniscus repair, has no

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protective effect against PTOA, while meniscectomy can even facilitate degeneration.[4,10-14]

Different types of knee injury can result in PTOA. Patients with intra-articular fractures of the knee have an estimated 23% to 44% chance of developing PTOA.[15,16] Acute mechanical damage and chronic abnormal joint loading are thought to be the main contributors to cartilage breakdown after intra-articular fractures.[17] For soft tissue injuries of the knee such as ligament tears and meniscal injury, several factors have been suggested in the pathogenesis of PTOA. Possible contributing factors are concomitant cartilage defect or acute tissue damage at time of injury and secondary biomechanical changes as a consequence of the structural damage.[16,18-20] Another suggested factor in the pathogenesis of PTOA is inflammation.[16,19-21]

Understanding the inflammatory response after knee injury is necessary to identify possible treatment strategies. Inflammatory biomarkers after trauma might predict the development of PTOA and subsequently become a target to prevent or treat PTOA development in an early stage. Because of the possible different pathways of PTOA after certain injuries, we included only knee injuries such as an ACL tear, posterior cruciate ligament (PCL) tear, meniscal tear, osteochondral fracture, or combined types and excluded knee fractures in this review. Osteochondral fractures are considered injuries where the cartilage is focally damaged with involvement of the direct underlying subchondral bone.[22] The bone damage does not exceed beyond the subchondral bone involvement in contrary to a knee fracture. Chondral fractures have focal cartilage damage without involvement of the subchondral bone. The aim of this systematic review is to identify inflammatory proteins that are increased in serum and synovial fluid after acute knee injury.

## Methods

The reporting in this systematic review was conducted according to the PRISMA statement and published in a protocol in the PROSPERO database (CRD42020189896).[23]

### *Data Sources and Searches*

With help of a health science librarian with extensive experience in conducting literature search for systematic reviews a search of the literature was performed for relevant articles up to December 8, 2021. Search terms included inflammation, knee, anterior cruciate ligament, posterior cruciate ligament, meniscus, trauma, injury, prognosis, and biological marker. The full electronic search strategy for the Medline (OvidSP) database is presented in Appendix 1 (see Supplemental Material online). Similar search strategies were used in Embase, Web-of-Science, PubMed publisher, Cochrane, and Google Scholar. Additionally, the reference lists of all eligible studies were manually screened.

### *Study Selection*

Two reviewers (M.N. and D.M.) independently assessed the studies for the eligibility criteria. Disagreements were solved by discussion and, if necessary, by a third author (M.R.). Additional citation tracking was performed by screening of the reference lists of the eligible studies.

Inclusion criteria for eligible studies were patients aged 45 years or younger at the time of biochemical collection or available data of an age-stratified <45-years group; acute knee injury (eg, ACL rupture, PCL rupture, meniscus tear, osteochondral fracture); inflammatory cytokines had to be measured within 1 year after injury; article had to be written in English, German, French, Spanish, or Dutch; and full text was available.

Exclusion criteria were history of knee trauma before current trauma; pre-existing radiological degenerative changes; knee fractures, except osteochondral fractures; no available control group, such as healthy controls or the contralateral knee; intra-articular anti-inflammatory treatment (eg, biologics, glucocorticoids); post-mortem studies; animal studies; not an original study (eg, reviews or editorials).

### *Data Extraction*

One reviewer (MN) extracted the data of the included studies. Extracted patient characteristics were age, sex, body mass index (BMI), time between injury and sample collection and type of injury. Time between injury and protein measurement was classified as acute (0-6 weeks) and subacute (>6 weeks to 1 year). The included inflammatory proteins are shown in **Supplementary Table S1**. For clarity in this paper, 1 synonym was consistently used for a biomarker. Concentrations of the included inflammatory proteins and measurement type were extracted. Of papers which reported the results of the inflammatory proteins only in figures, the concentrations were assessed by reading directly from figures. To collect missing data, study authors were contacted by email.

### *Risk of Bias Assessment*

To assess the potential risk of bias, 2 reviewers (M.N. and M.R.) independently assessed all included studies using the Cochrane Collaboration's tool for assessing risk of bias of prognostic studies. Both reviewers discussed their findings and asked a third reviewer (DM) for consensus if necessary.

The checklist consists of 12 criteria, which were divided into 4 categories: selection bias, information bias, confounding, and statistical bias. We considered a study to have a high risk of bias in case of 1 negative score in any of the categories, moderate risk of bias in case of a question mark in any of the categories and low risk of bias in case of no negative scores or question marks.

Selection bias was assessed by 2 items, namely "did the study have a clearly described population composed of patients in the same part of the disease timeline" (1) and "was there a long enough follow-up" (2).

Information bias was assessed by 4 items, namely "were outcomes described explicitly and objectively" (3), "were measurement of outcomes measured in a valid and reliable manner" (4), "were outcome assessors blinded for prognostic factors" (5), and "was there a sufficient proportion (≥80%) of follow-up available" (6).

Confounding was assessed by 4 items namely, "were prognostic factors described explicitly and objectively" (7), "were measurements of prognostic factors measured in a valid and reliable manner" (8), "was measurement of prognostic factors measured the same way for all patients and during a comparable part of the disease period" (9), and "were prognostic factors measured in a sufficiently large part (≥90%) of the study population" (10).

Statistical bias was assessed by 2 items, namely "were all patients included in the final analysis" (11), and "was statistical analysis done correctly" (12).

### *Statistical Analysis*

The clinical and methodological homogeneity of the included studies was checked to evaluate whether a meta-analysis could be performed. In case of heterogeneity a best evidence synthesis was performed. We divided extracted findings into 4 evidence levels: strong, limited, conflicting, and no evidence.[24] Therefore, we followed the Strength of Recommendation Taxonomy (SORT).[25] Strong evidence consisted from consistent findings (> 75%) from at least 2 studies with low or moderate risk of bias. Limited evidence consisted of 1 study with low or moderate risk of bias or consistent findings from studies with a high risk of bias. Conflicting evidence was defined as inconsistent findings (less than 75% consistency) in the studies. If none or only 1 study with a high risk of bias was available, we defined the outcomes as no evidence.

## Results

### *Study Characteristics*

A total of 7757 articles were identified of which finally 10 were included **(Fig. 1)**. The 10 studies published data from 9 unique trials **(Table 1)**. Struglics *et al.*,[33] and Larsson *et al.*,[31] both used data from the KANON trial. Baseline characteristics are found in **Table 1**. ACL injuries were evaluated in all studies. Six of the reviewed studies only assessed patients with ACL injuries.[26-29,32,33] One study evaluated patients with ACL tears, meniscal tears, cartilage injury or ACL, and meniscal injury and 1 study analyzed patients with ACL tears with or without concomitant cartilage injury.[27,30] Two studies included a heterogenous group of knee injuries combining ACL injuries, meniscal injuries and other injuries as a group.[34,35] Of the investigated studies 8 evaluated synovial fluid samples and 5 evaluated serum samples **(Table 2)**. An overview of the inflammatory biomarkers per study can be found in **Table 2**.

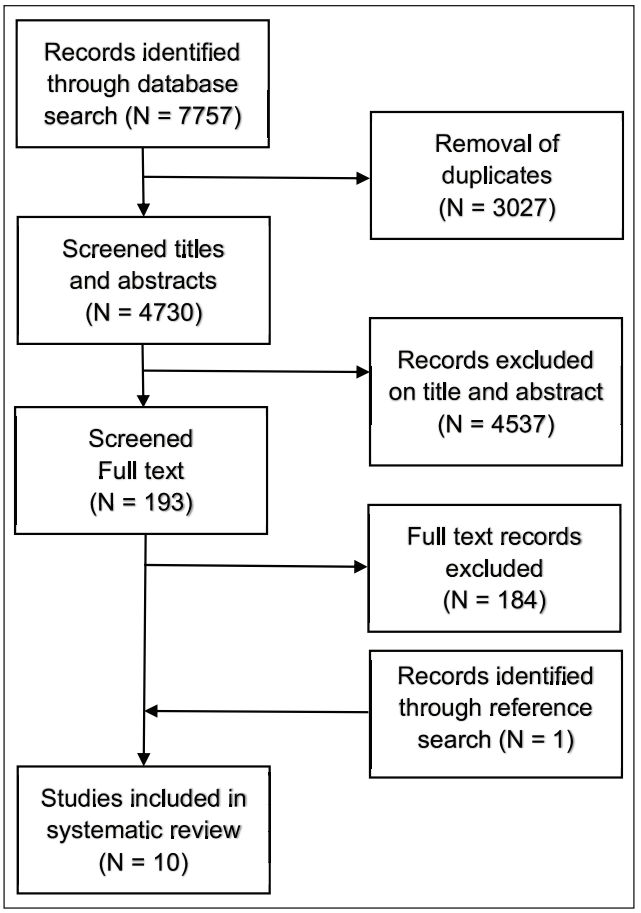


**Figure 1.** Flow chart of included and excluded studies.

### *Risk of Bias Assessment*

**Table 3** shows the risk of bias for the included studies. One study has a low risk of bias,[26] and 2 studies a moderate risk of bias.[29,34]

### *Synovial Fluid Biomarkers*

Several studies investigated the levels of IL-1β, IL-6, IL-10, CXCL8, CCL2, CCL4, TNF-α, and IFN-γ in synovial fluid after an acute knee injury, using a control group. **Tables 4 and 5** and **Supplementary Tables S2** to **S7**, summarize these studies per protein.

**Table 1.** Characteristics of the Included Studies.

| Publication | Study Type | Patients, n | Female, n (%) | Age, years[a] | BMI, kg/m$^{2a}$ | Time between Injury and Measurement[a] | Follow up[a] | Type of Injury | Type of Measurement |
|---|---|---|---|---|---|---|---|---|---|
| Cuellar *et al.*[26] | Prospective | 12 | 3 (25) | **33** (3) | ND | **22 days** {range: 13-39} | No | ACL injury | Multiplex assay. Human 17-plex inflammatory cytokine panel, BioPlex 200 System (Bio-Rad Laboratories, Hercules, CA), |
| | | 15 | 7 (47) | **43** (3) | ND | ND | No | Healthy controls | |
| Cuellar *et al.*[27] | Prospective | 22 (ICRS Grade 1) | ND | **28** (8) | ND | *3 months* {range: 1-264 months} | **17 months** (6) | ACL injury, meniscal injury, cartilage injury, ACL and meniscal injury Contralateral knee as healthy control | Multiplex bead assay. (Milliplex; Millipore, Billerica, MA) |
| Elsaid *et al.*[28] | Prospective | 30 | 11 (33) | **24** {range: 15-47} | ND | **103 days** {range: 32-364} | No | ACL injury Contralateral knee as healthy control | ELISA. (R&D systems, Minneapolis, MN) |
| Hagemans *et al.*[29] | Prospective | 152 | 52 (34) | **25** (IQR 21-32) | NC | 3-25 weeks after injury | 2 years | ACL injury | Mulitplex assay Proinflammatory Panel 1 Human Kit (No. K15049D-1; Meso Scale Diagnostics) |
| | | 16 | 7 (44) | **26** (IQR 21-36) | ND | | | Healthy controls | |
| Kaplan *et al.*[30] | Retrospective | 34 | ND | **34** (8.12) | ND | **5.5 weeks** {range: 3-12} | No | ACL injury without cartilage associated damage | Multiplex bead assay. (Milliplex, Millipore, Billerica, MA) |
| | | 28 | ND | **36.29** (9.04) | ND | **5.5 weeks** {range: 3-12} | No | ACL injury with cartilage associated damage | |
| | | 72 | ND | **41.06** (14.5) | ND | No | NA | Healthy controls | |
| Larsson *et al.*[31]; KANON trial | Retrospective | 119 | | | | | | | Multiplex assay. Multiplex Human Pro-inflammatory 7-plex immunoassay, Mesoscale Discovery (MSD, K15008C) |
| | | 60 | 12 (20) | **26.5** (5.1) | **24.4** (3.2) | 0-6 weeks | 5 years | ACL injury: Early ACL reconstruction | |
| | | 29 | 9 (31) | **26.4** (4.9) | **24.3** (3.1) | 0-6 weeks | 5 years | ACL injury: Rehabilitation alone | |
| | | 30 | 11 (37) | **25.2** (4.5) | **23.3** (2.0) | 0-6 weeks | 5 years | ACL injury: Delayed ACL reconstruction | |
| Palmieri-Smith *et al.*[32] | Prospective | 33 | ND | **25.25** (8.14) | ND | **31 days** (14) | **399 days** (61) | ACL injury | ELISA. (R&D Systems, Minneapolis, MN) |
| | | 4 | ND | **27.25** (4.11) | ND | ND | **425 days** (69) | Healthy controls | |
| Struglics *et al.*[33]; KANON trial | Prospective | 121 | 29 (24%) | **26** {range: 18-35} | ND | Synovial markers: **1.3 weeks** {range: 0.1-6.0 weeks} Serum and urine markers: **2.8 weeks** {range: 0.4-6.3} | 5 years | ACL injury | Multiplex assay. Multiplex Human Pro-inflammatory 7-plex immunoassay (#K15008C; Meso Scale Discovery) |
| | | 23 | | | | | | Healthy controls | |
| Swärd *et al.*[34] | Prospective | 111 | 28 (22%) | **27 {**range: 13-64} | ND | *1 day* {range: 0-23} | No | ACL injury, meniscal injury, other injury[b] | Multiplex assay. Multiplex Human Pro-inflammatory II 4-Plex Ultra-Sensitive ECLC immunoassay (#K11025C MSD) |
| | | 10 | 2 (20%) | **25 {**range: 16-47} | ND | NA | NA | Healthy controls | |
| Watt *et al.*[35] | Prospective | 150 | 29 (19%) | **25** {range: 16-50} | **26** {range 19-39} | *17 days* {range: 1-56} | 3 months | ACL injury, meniscal injury, other injury[c] | ELISA. R&D Systems. |
| | | 50 | 17 (35%) | **32** {range: 21-29} | ND | NA | No | Healthy controls (blood samples) | |
| | | 8 | 4 (50%) | **48** {range 41-68} | ND | NA | No | Healthy controls (SF samples) | |

BMI = body mass index; ND = not defined; ACL = anterior cruciate ligament; ICRS = International Cartilage Repair Society; IQR = interquartile range; ELISA = enzyme-linked immunosorbent assay ; KANON = the Knee Anterior Cruciate Ligament, Nonsurgical versus Surgical Treatment study; ECLC = electrochemiluminescence; SF = synovial fluid.

[a]**Mean**/*median*, (SD), [95% CI], {range}

[b]Fifty-seven ACL tears, 10 with associated partial or total medial collateral ligament (MCL) tears, 3 with associated medial meniscus injury, 2 with associated lateral meniscus injury, 3 medial meniscus injuries (1 with an associated MCL tear), 1 posterior cruciate ligament tear (associated with an MCL tear), 5 patellar dislocations (1 with an associated MCL tear), 1 had a suspected tibial fracture; 3 MCL tears, and 2 lateral collateral ligament tears. In 39 patients, there was no specified diagnosis

[c]Twenty-seven meniscal tears, 28 single ligament ruptures, 61 ACL tears, and 34 severe trauma.

**Table 2.** Overview of the Used Inflammatory Proteins Per Study.

| Article | Specimen Type | Interleukines | Chemokines | TNF | IFN | CRP | CSF |
|---|---|---|---|---|---|---|---|
| Cuellar *et al.*[26] | SF | IL-1β<br>IL-2<br>IL-4<br>IL-5<br>IL-6<br>IL-7<br>IL-10<br>IL-12<br>IL-13<br>IL-17 | CXCL8<br>CCL2<br>CCL4 | TNF-α | IFN-γ | | G-CSF<br>GM-CSF |
| Cuellar *et al.*[27] | SF | IL-1β<br>IL-6<br>IL-10 | CCL2<br>CCL3<br>CCL4<br>CCL5<br>CCL11 | TNF-α | IFN-γ | | |
| Elsaid *et al.*[28] | SF | IL-1β<br>IL-6 | | TNF-α | | | |
| Hagemans *et al.*[29] | Serum | IL-10<br>IL-13 | CXCL8 | TNF-α | IFN-γ | | |
| Kaplan *et al.*[30] | SF | IL-1β<br>IL-6<br>IL-10 | CCL2<br>CCL3<br>CCL4<br>CCL5<br>CCL11 | TNF-α | IFN-γ | | |
| Larsson *et al.*[31] | SF | IL-6<br>IL-10 | CXCL8 | TNF-α | IFN-γ | | |
| Palmieri-Smith *et al.*[32] | Serum | | | | | CRP | |
| Struglics *et al.*[33] | SF and serum | IL-6<br>IL-10 | CXCL8 | TNF-α | IFN-γ | | |
| Swärd *et al.*[34] | SF | IL-1β<br>IL-6 | CXCL8 | TNF-α | | | |
| Watt *et al.*[35] | SF and serum | IL-1β<br>IL-6 | CCL2 | | | CRP | |

TNF= tumor necrosis factor; IFN = interferon; CRP = C-reactive protein; CSF = colony stimulating factor.

**Table 3.** Risk of Bias Assessment.

| | Type of bias | | | |
|---|---|---|---|---|
| | Selection | Information | Confounding | Statistical |
| Cuellar *et al.*[26] | 🟢 | 🟢 | 🟢 | 🟢 |
| Cuellar *et al.*[27] | 🔴 | 🔴 | 🔴 | 🔴 |
| Elsaid *et al.*[28] | 🟢 | 🔴 | 🔴 | 🟢 |
| Hageman *et al.*[29] | 🟢 | 🟡 | 🟢 | 🟢 |
| Kaplan *et al.*[30] | 🟢 | 🔴 | 🔴 | 🟢 |
| Larsson *et al.*[31] | 🟢 | 🔴 | 🟢 | 🔴 |
| Palmieri-Smith *et al.*[32] | 🟢 | 🔴 | 🟢 | 🔴 |
| Struglics *et al.*[33] | 🟢 | 🔴 | 🟢 | 🔴 |
| Swärd *et al.*[34] | 🟢 | 🟡 | 🟢 | 🟢 |
| Watt *et al.*[35] | 🟢 | 🔴 | 🟢 | 🟢 |

Green: low risk of bias; Yellow: moderate risk of bias; Red: high risk of bias.

*Upregulation after trauma.* Strong evidence was found for increased concentrations of SF biomarker IL-6 **(Table 4)** in acute injuries of the knee (0-6 weeks after tra uma).[26,27,30,31,33-35] Limited evidence was found for increased concentrations of SF biomarkers CCL4 **(Table 5)** in acute injuries of the knee.[26,27,30] For IL-6, the available evidence for the subacute phase is conflicting showing no significant difference after 6 weeks between injured knees and controls in the KANON trial and a significant difference at 3 months in the study by Cuellar *et al.* (see **Table 4**).[26,27,30,31,33-35] Regarding subacute measurements not enough data are available for CCL4.

*No increase in concentration after knee injury.* Limited evidence by 1 study with a low risk of bias was found for similar IL-2, G-CSF, and GM-CSF concentrations in patients with or without an acute knee-injury **(Table 6)**.[26] No evidence was available regarding the subacute measurements for IL-2, G-CSF, and GM-CSF.

Limited evidence was found for no increase of concentration after knee injury for CCL3, CCL5, and CCL11 concentrations in 2 separate studies with high risk of bias at, respectively, 5.5 weeks (range 3-12 weeks) and 3 months after trauma (range 1-264 months).[27,30] When subdividing in acute and subacute measurements, no conclusions could be made, because of the low quality of included studies and only 1 study available per group.

For IL-10 limited evidence showed no difference between the control group and knee injury group (**see Suppl. Table S2**).[26,27,30,31,33] More than 75% of available studies using different datasets showed no difference between the acute knee injury group and the control group. Subdividing in acute and subacute measurement groups showed no increased concentration of SF IL-10 for the subacute measurements (limited evidence). For acute injuries, this statement is conflicting with 2 studies showing no significant difference and 1 study showing a significant difference **(see Suppl. Table S2)**.

*Conflicting findings.* Conflicting findings were found for IL-1β, CXCL8, CCL2, TNF-α, and IFN-γ (see **Suppl. Tables S3 to S7**). When subdividing in acute and subacute measurements there was limited evidence of no increased concentration of IFN-γ in the subacute setting (see **Suppl. Table S7**). For the other inflammatory proteins, results remained conflicting for acute and subacute measurements.[27,31,33]

*No evidence.* For IL-4, IL-5, IL-7, IL-12, IL-13, and C-reactive protein (CRP), not enough evidence was available.[26,35] Concerning IL-1α, IL-1Ra, IL-1, IL-3, IL-9, IL-15, CCL7, CCL8, CCL12, CCL13, CCL22, CX3CL1, CXCL1, CXCL2, CXCL3, CXCL10, and M-CSF no evidence was available.

### *Serum Measurements*

Limited evidence was found for no upregulation of IL-6 and CRP in serum after knee injury.[32,33,35] Also IFN-γ, CXCL8, and IL-10 concentrations were not different in serum between the knee injury group and healthy control group.[29,33] Conflicting evidence was found for TNF-α.[29,33,34] For the other serum biomarkers included in this review, no evidence was available.[35,36]

## Discussion

### *Summary of Synovial Fluid Biomarkers after Knee Trauma*

In this review, we found that IL-6 and CCL4 are upregulated in SF after acute knee injury. All studies which included IL-6 and CCL4 found a significant difference in concentration between the knee injury group and control group (See **Table 4 and 5)**.[26,27,30,31,33-35] The evidence was determined as strong evidence for IL-6 with 1 low risk of bias study by Cuellar *et al.* and 1 moderate risk of bias study by Swärd *et al.* and 5 high risk of bias studies all confirming higher SF IL-6 concentrations after knee injury. For CCL4 the evidence was determined as limited evidence with 1 low risk of bias study by Cuellar *et al.* and 2 high risk of bias studies conforming higher SF CCL4 concentrations after knee injury.[26,27,30] In the subacute setting, at least 6 weeks after trauma, this finding is not as clear, with contradictory findings for IL-6 and not enough evidence for CCL4.

In contrast, we found unchanged concentrations in SF after trauma for IL-2, IL-10, CCL3, CCL5, CCL11, G-CSF, and GM-CSF in SF. For IL-10, the evidence was conflicting, but >75% of available studies using different datasets, showed no significant difference between the control group and knee injury group.[26,27,30,31,33] For IL-2, G-CSF, and GM-CSF 1 high-quality study was available.[26] No significant difference between healthy controls and injured knees was shown in this study.

For IL-1β, CXCL8, CCL2, TNF-α, and IFN-γ the findings were conflicting. Regarding IL-1β, CXCL8, and TNF-α, 2 studies had a low or moderate risk of bias (see **Suppl. Tables S3, S4 and S6)**. Cuellar *et al.*[26] (low risk of bias) showed no difference between the control group and knee injury group and in contrast, Swärd *et al.*[34] (moderate risk of bias) showed a significant difference for all 3 biomarkers. This study had more patients than Cuellar's study (111 vs 12). For TNF-α, the KANON trial (high risk of bias) demonstrated a significant difference between the control group and knee injury group during the entire follow-up until 1 year.[31,33] For CCL2 and IFN-γ, 1 low risk of bias study was available showing a significant difference between the control group and knee injury group.[26] This finding was inconsistent in other studies with high risk of bias for both inflammatory proteins (see

**Table 4.** Synovial Fluid IL-6 Outcomes.

| | | | | Knee Injury Group | | Control Group | | | Measurement Type |
|---|---|---|---|---|---|---|---|---|---|
| | Injury Type | Time between Injury and Measurement[a] | Time Group | N | IL- 6 concentration (pg/ml)[a] | N | IL- 6 concentration (pg/ml)[a] | *P*-value | |
| Cuellar *et al.*[26] | ACL injury | **22 days** {range: 13-39} | Acute | 12 | **105** (72) | 15 | **0** (0) | <0.001 | Multiplex assay |
| Cuellar *et al.*[27] | ACL injury and meniscal injury | *3 months* {range: 1-264} | Subacute | 22 | **1312** (184) | 22 | **21** (10) | <0.01 | Multiplex bead assay |
| Kaplan *et al.*[30] | ACL injury | **5.5 weeks** {range: 3-12} | Acute | 62 | ACL with cartilage injury: **400** [95% CI 158.1- 642.8] ACL without cartilage injury: **191** [95% CI 58.0- 323.3] | 72 | **21** [95% CI 1.1-41.3] | ACL with cartilage injury; $P = 0.009$ ACL without cartilage injury; $P = 0.038$ | Multiplex bead assay |
| KANON Trial | ACL injury | 0-6 weeks | Acute | 47 | **1271.1** [95% CI: 271.5-3877.7] | 21 | **1.21** [95% CI: 0.30- 9.81] | <0.001 | Multiplex assay |
| Larsson *et al.*[31]; Struglics *et al.*[33] | ACL injury | 16 weeks | Subacute | 64 | **11.21** [95% CI: 2.85- 33.64] | | | 0.007 | |
| | ACL injury | 30 weeks | Subacute | 63 | **3.25** [95% CI: 0.30- 17.04] | | | 0.316 | |
| | ACL injury | 1 year | Subacute | 63 | **1.26** [95% CI: 0.30- 5.90] | | | 0.748 | |
| Swärd *et al.*[34] | Different types of injury | *1 day* {range: 0-23} | Acute | 111 | **3386.4** {range: 17.4 -66099.5} | 10 | **2.1** {range: 0.1 - 503.9} | <0.001 | Multiplex assay |
| Watt *et al.*[35] | ACL injury, meniscus injury, severe trauma | *17 days* {range 1-57} | Acute | 150 | **175** {range: 2-100000} | 8 | **9 {**range: 2-11}) | <0.001 | ELISA |

ACL = anterior cruciate ligament; CI = confidence interval; KANON = the Knee Anterior Cruciate Ligament, Nonsurgical versus Surgical Treatment study; ELISA = enzyme-linked immunosorbent assay.
[a]**Mean***/median*, (SD), [95% CI], {range}.

**Table 5.** Synovial Fluid CCL4 Outcomes.

| | | | | Knee Injury Group | | Control Group | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Injury Type | Time between Injury and Measurement[a] | Time group | N | CCL4 concentration (pg/ml) | N | CCL4 concentration (pg/ml) | *P* value | Measurement Type |
| Cuellar *et al.*[26] | ACL injury | **22 days** {range: 13-39} | Acute | 12 | **16** (3.8) | 15 | **0.3** (0.2) | <0.001 | Multiplex assay |
| Cuellar *et al.*[27] | ACL injury and meniscal injury | *3 months* {range: 1-264} | Subacute | 22 | **15** (8) | 22 | **9** (1.3) | <0.01 | Multiplex bead assay |
| Kaplan *et al.*[30] | ACL injury | **5.5 weeks** {range: 3-12} | Acute | 62 | ACL with cartilage injury: **15** [95% CI 12.1- 18.1] ACL without cartilage injury: **13** [95% CI 11.4- 16.7] | 72 | **9** [95% CI 6.8- 10.6] | ACL with cartilage injury; $P = 0.003$ ACL without cartilage injury; $P = 0.045$ | Multiplex bead assay |

ACL = anterior cruciate ligament; CI = confidence interval.
[a]**Mean***/median*, (SD), [95% CI], {range}.

**Table 6.** Synovial Fluid IL-2, G-CSF and GM-CSF Outcomes.

| | Injury Type | Time between Injury and Measurement[a] | Time Group | Protein | Knee Injury Group | | Control Group | | *P* value | Measurement Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | N | Protein concentration (pg/ml)[a] | N | Protein concentration (pg/ml)[a] | | |
| Cuellar *et al.*[26] | ACL injury | **22 days** {range: 13-39} | Acute | IL-2 | 12 | **18** (14) | 15 | **0** (0) | No significant difference | Multiplex assay |
| | | | | G-CSF | 12 | **0** (0) | 15 | **1** (1) | No significant difference | |
| | | | | GM-CSF | 12 | **0** (0) | 15 | **4** (5) | No significant difference | |

ACL = anterior cruciate ligament.
[a]**Mean**/*median*, (SD), [95% CI], {range}.

**Suppl. Tables S5 and S7).**[27,30,31,33,35] Based on these studies, the effect of an acute knee trauma on IL-1β, CXCL8, CCL2, TNF-α, and IFN-γ in the synovial fluid remains unclear.

## *Summary of Serum Biomarkers after Knee Trauma*

There was limited evidence for no change in serum levels for IL-6, IL-10, CXCL8, IFN-γ, and CRP between controls and the knee injury group reported for each protein by at least 2 independent studies.[29,33,35,37] Other serum biomarkers such as IL-1β, IL-2, IL-13, CCL2, CCL3, CCL4, and CCL5, were also not higher in the knee-injury group than in the control group. This was only reported by 1 study for each separate protein, therefore no conclusions could be made about these proteins.[33,35,37] For TNF-α, the data were conflicting.[29,33] Hagemans *et al.*[29] found a significant increase of concentration in serum after ACL rupture (3-25 weeks after trauma). This remained significantly higher than the healthy control group until the 2-year follow-up. This was not supported by data from Struglics *et al.* The low number of available studies and high risk of bias requires further research, but the absence of any increased concentration of inflammatory cytokines in serum debates the use of these serum markers in future research considering knee injury and onset of PTOA.

## *Time between Injury and Measurement*

With the limited available evidence, subgroup comparison in terms of acute (0-6 weeks after trauma) and subacute measurements (6 weeks to 1 year after trauma) further decreases the amount of evidence.

Higher concentrations of IL-6 and CCL4 are only found in the acute setting, whereas we could not prove this for the subacute setting **(see Tables 4 and 5).** For most of the cytokines, differentiating between acute and subacute measurements did not influence the outcome as in no change after trauma or conflicting findings. Except for IL-10 and IFN-γ, we found no difference in concentrations between groups in the subacute setting and conflicting evidence when measured in acute injuries (see **Suppl. Tables S2 and S7**).

The study by Swärd *et al.* did the harvest of SF mostly on the first day after trauma. They found increased concentrations in SF for IL-1β, IL-6, CXCL8, and TNF-α in the knee injury group, where other studies failed to show a significant difference with longer time intervals between injury and sampling **(see Suppl. Tables S3, S4 and S6).**[34] This finding is supported by Irie *et al.*,[38] where they found immediate increase of inflammatory cytokines in SF for TNF-a, IL-1b, IL-6, IL-8, IL-1ra, and IL-10 after ACL injury until approximately 1 week after injury. Within 1 week nearly all of the inflammatory cytokines measured decreased to the

level of that in the chronic arthritis group. Other studies also described this low-grade chronic inflammation phase after joint injury.[28,33,39-42] At a later harvest of SF, the inflammatory cytokines may have already disappeared from the joint by either usage or diffusion.

We therefore question the use of SF sampling for inflammatory proteins in a subacute phase after knee injury. Our findings imply only increased concentrations of IL-6 and CCL4 in the acute phase and not in the subacute phase (see **Tables 4 and 5)**. In addition, IL-10 and IFN-γ, show conflicting results when measured in the acute phase and no difference between groups in the subacute phase (see **Suppl. Tables S2 and S7**). Future research should focus on SF inflammatory protein measurements taken in the acute phase (less than 6 weeks) after knee injury.

## *Inflammatory Markers and PTOA*

A first step to prevent PTOA is to understand the role of inflammation in the pathogenesis. This has been thoroughly investigated with animal model studies. A variety of animal studies can be used to provoke joint injury either surgically, traumatic or chemically.[43] An ovine model was used to compare ACL detachment and direct reconstruction to prevent instability, with sham surgery and healthy controls.[44,45] Cartilage changes and osteophyte formation were found 2 weeks after ACL repair when compared to healthy controls.[21,44,45] At 20 weeks from ACL repair changes were consistent with early OA, although progression from 2-week follow-up was minimal. Changes were significantly different with early osteophyte formation and cartilage damage in the ACL reconstruction group versus sham surgery and healthy controls. Synovium samples were taken at 2 and 20 weeks. Messenger RNA expressions of IL-1β and IL-6 were both significantly upregulated in synovium for ACL repaired knees versus the healthy contralateral control. The inflammatory response normalized at 20 weeks. This article shows that in absence of instability the immediate postinjury inflammatory response in an ovine model contributes to early cartilage degeneration.[44] In another rabbit model, 2 holes were drilled into subchondral bone in the intercondylar notch.[46] Creating an injury which neither changes joint loading or mechanics of the joint. Synovium was examined for histology and changes in mRNA expression for IL-1β, IL-1Ra, IL-6, IL-8 and TNF-α at 72 hours, 3, 6, 8, and 52 weeks compared to sham surgery and unoperated healthy controls. All surgical damaged joints showed gross and histological cartilage damage after surgery with significant worsening until 52 weeks. The sort-term synovial inflammatory expression of IL-1β, IL-1Ra, IL-6, IL-8, and TNF was increased 3 to 4 times at 72 hours. This resolved to baseline levels by 3 weeks. The authors conclude that intraarticular bone injury creates an early joint inflammation with progressive cartilage damage consistent of OA in a rabbit model.[46] Both studies show that without altering joint loading, stability, or mechanics of the joint an early inflammation phase exist after knee injury. Resolving in cartilage changes consistent with early OA in animal models.

Second, we need to identify important biomarkers which could be a potential target for treatment in humans. Which is in the scope of this review. Third, correlation between these inflammatory markers and PTOA should be assessed. The lack of available data correlating biomarker data and long-term follow-up radiographic data makes this difficult. One study is available correlating biomarkers taken after knee trauma followed by ACL reconstruction with radiological follow-up.[47] Biomarkers were measured 64 days (standard deviation [SD] = 27.1) after trauma and correlated with magnetic resonance imaging (MRI) findings of different time points after surgery (6 months, 1, 2, and 3 years). The authors performed a cluster analysis with 2 groups. One high inflammatory group (with increased concentrations of IL-rα, IL-1α, IL-6, IL8, IL-10, TNF-α, and IFN-γ**).** This was compared to a group with high sulfated glycosaminoglycans (with high concentrations of sGAG) group, which is related to cartilage degradation. During follow-up measurement, higher T1 and T2 relaxation times were found in group that had high sGAG concentrations compared to the high inflammatory group. None of the inflammatory biomarkers analyzed in this study could be correlated with cartilage degradation on MRI. This is in contrast with the well-known hypothesis that more inflammation would lead to more cartilage breakdown.[21,48-52]

A possible explanation may be the time course of the inflammatory markers. Biomarkers were measured at 64 days (SD = 27.1), which is relatively late after trauma, the level of these markers may have normalized by then. For IL-10, CXCL8, and IFN-γ the available data from especially the KANON trial showed these markers were elevated up to 6 weeks after knee injury.[31,33] After this timeframe of 6 weeks, no significant difference were found when comparing the knee injury group with healthy controls (see **Suppl. Tables S3, S4, S6 and S7**). Supporting the hypothesis that these inflammatory markers follow a specific time course. For IL-6 and TNF-α, we found no support of this theory, since they were elevated up to, respectively, 3 months for IL-6 and 5 years for TNF-α in multiple studies (see **Table 5 and Suppl. Table S7**).[26,27,30,31,33] The concentrations of other inflammatory markers could have already declined by the time of synovial fluid harvest at 64 days. Higher concentrations of inflammatory cytokines may already influence cartilage homeostasis. Earlier studies reported that IL-6, IL-10, and CXCL8 are significantly correlated with matrix metalloproteinase 1 (MMP-1) and MMP-3, enzymes involved in the degradation of cartilage proteins, directly after ACL injury.[53-55]

No other studies available determined the association between inflammatory markers and the radiographical development of PTOA. Further research with radiological follow-up is needed to clarify the relationship between the inflammatory response after knee injury and PTOA.

### *Future Treatment Options for PTOA*

Instead of late-stage treatment of PTOA, there has been a shift in focus toward preventing or delaying early disease progression.[56] By understanding the inflammatory response after knee injury, we could investigate potential treatment strategies in the prevention of PTOA. Different treatment strategies and approaches for treatment are currently studied. For example, disease-modifying OA drugs (DMOADs), such as dexamethasone and triamcinolone acetonide or intra articular anti-inflammatory treatments such as hylaruonic acid, inhibitors of TNF-α, IL-1ra, IL-1β, IL-6, the complement system or anti-inflammatory cytokines IL-4, IL-10, and IL-13.[57]

In this review, we have summarized the direct inflammatory response after knee injury from clinical studies. Two out of 37 included inflammatory proteins were found to be elevated after knee injury and can act as a biomarker. For 13 other inflammatory proteins, results were conflicting or not enough data were available, as while for 15 inflammatory proteins no evidence was available.

In absence of a direct clinical relation between PTOA and most of the included inflammatory proteins combined with the current lack of clinical data, further research is needed to identify the role of these proteins in the development of PTOA.

### *Limitations*

Unfortunately, there was high heterogeneity between studies. The time between injury and measurement of biomarkers varied a lot in different studies. Furthermore, biomarkers were measured with different techniques based either on a protein level (enzyme-linked immunosorbent assay (ELISA), multiplex bead assay, multiplex assay) and with different calibrators resulting in different concentrations. Because of these differences, directly comparing results between studies was not possible. Therefore, we focused our research on studies using a control group, so measurement type and calibration is the same for injury and control groups. The control group could be a healthy control or the contralateral knee. Using the contralateral knee as a healthy control is controversial because of the possible systemic inflammatory reaction. By using the reported statistically significance per study, underpowering and study sizes may influence our outcomes. Also, the overall risk of bias of the included studies was high.

## Conclusion

There is strong evidence for IL-6 and limited evidence for CCL4 supporting elevation of these proteins in SF after acute knee injury up to 6 weeks. Further studies are needed to identify the role of these proteins in the development of PTOA. For IL-2, CCL3, CCL5, CCL11, G-CSF, and GM-CSF in SF, there is limited evidence that these biomarkers are not elevated after an acute knee injury. Interleukin 10 and IFN-γ show conflicting results in the acute phase and no increased concentrations after injury in the subacute phase. For other included inflammatory proteins there is not enough available data. Further research must resolve this question considering these inflammatory markers and should focus on SF measurements taken in the acute phase (less than 6 weeks) after knee injury.

There is limited evidence available concluding no increased concentrations of inflammatory proteins IL-6, IL-10, CXCL8, IFN-γ, and CRP in serum after acute knee injury.

In absence of a direct clinical role for most of the included inflammatory proteins and current lack of clinical data, we must not rule out the potential role of inflammatory proteins as a predictor or treatment modality for PTOA.

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Ethics approval was not required for this systematic review.

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