Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Garriga C, Goff M, Paterson E, et al. Clinical and molecular associations with outcomes at 2 years after acute knee injury: a longitudinal study in the Knee Injury Cohort at the Kennedy (KICK). Lancet Rheumatol 2021; published online June 24. https://doi.org/10.1016/S2665-9913(21)00116-8.
PPI. 3 participants were involved in the design and review process for this work (already enrolled in the study) - they offered to do this, having joined our registry which allows for involvement activities. Patient interviews supported the need for this study; they wanted information that helped to predict their risk of future OA. Patients during other PPI sessions run by our Centre supported SF aspiration for research at the time of procedures and supported the potential use of SF sampling for clinical testing or research. Many patients hoped for the option of a targeted intervention at the time of injury if they were found to be at high risk of OA, and identified that this type of study might improve prediction of their personal risk. The grant application to Versus Arthritis underwent lay review.
Supplementary Figure 1. Flow chart indicating timings of study visits in KICK over 2 years, and related injury and timing of its clinically indicated surgical interventions.

Completeness of KOOS data (baseline, 2 years) and sample collection (baseline, 14 days, 3 months) for the Full Available Dataset is shown. Baseline period is shown (from baseline to 3 month visit). PE = Primary Endpoint – time of primary outcome, 2 years. To be eligible for participation, participants had to fulfill eligibility criteria (see methods) including sustaining 1 or more of: meniscal tear, cruciate ligament rupture, collateral ligament tear, posterolateral corner injury, traumatic chondral defects, articular or periarticular fracture, patello-femoral or tibio-femoral dislocation within 8 weeks of baseline visit. Relative exclusion criteria (at the discretion of the investigator) were: Bony abnormality of the index knee; Injury of other body parts or surgery within last 3 months; Severe neurological/muscle/hip disease; Significant, active co morbidity; Contraindication for MRI.
Exploratory outcomes pre-defined for the study (to be investigated depending on sample size availability) as listed in Statistical Analysis Plan (27 November 2018)

- International Physical Activity Questionnaire (IPAQ) at 2 years.
- Short Form (SF-12) – physical and mental domains at 2 years.
- NHANES knee pain positive (which refers to ‘frequent knee pain’)
- ‘Clinical ACR OA’- NHANES knee pain positive (which refers to ‘frequent knee pain’; plus fulfilling ACR clinical criteria.
- ‘Early OA of the knee positive’ (Luyten et al criteria).
- KOOS subscale QOL and 2 of the 4 additional subscales should be equal to or less than the score obtained as follows: at least 50% of the questions within the subscale were answered with at least a 1-step decrease from the best response (indicating no pain/best possible function, etc.) on a 5-point Likert scale. After conversion to a 0–100 scale (0 = worst, 100 = best), the cut-offs are as follows: pain ≤ 86.1, symptoms ≤ 85.7, ADL ≤ 86.8, sport/rec ≤ 85.0, and QOL ≤ 87.5
- ‘Symptomatic Radiographic OA’ at 2 years: 1) NHANES knee pain positive AND 2) new radiographic OA or radiographic progression since baseline.
- X-ray change over 2 years:
  - change in minimum joint space width (mm) over 2 years (lateral, medial, total)
  - change in total OARSI osteophyte count over 2 years (lateral, medial, total)
- ‘Symptomatic MRI-defined OA’ at 2 years: 1) NHANES knee pain positive AND 2) MRI OA (OARSI MR Hunter et al criteria).
- Symptomatic imaging-based index knee OA at 2 years: 1) index NHANES knee/KOOS pain positive AND either X ray or MRI OA.
- Semi-quantitative magnetic resonance imaging (MRI) collective change over 2 years: ligaments, meniscus, synovosis, loose bodies, synovial cysts/bursae, cartilage signal and morphology, bone marrow abnormality, bone cysts, bone attrition, osteophytes.
- Quantitative MRI: Change in cartilage volume over 2 years in index knee in different zones.

Definitions of outcomes

- Where Kellgren Lawrence (KL) grade of tibiofemoral joint is based on X-rays scoring 0 to 4. KL grade in index knee at baseline is abbreviated to KL0 and KL grade in index knee at 2 years is abbreviated to KL2. Radiographic OA KL grade 2 or above will be considered ‘positive’. We will use a binary variable to define 2 groups, (0=kl 0 or 1, 1=kl 2, 3 or 4), assessed by a single reader).
- NHANES knee pain question: “Do you have pain, aching or stiffness in or around the knee on most days for at least 1 month in the last year?” Yes/No. Question retrieved from the National Health and Nutrition Examination Survey (NHANES) which is a programme of studies designed to assess the health and nutritional status of adults and children in the United States.
- Clinical ACR OA at 2 years:

  Knee pain (2 year NHANES index knee = yes) plus 3 or more of:
  - >50. Age at baseline (2 year visit date - baseline visit date)
  - <30 mins of stiffness
  - Crepitus on examination
  - Bony tenderness
  - Bony enlargement
  - Lack of warmth

- Early knee OA at 2 years

  Knee pain (as defined as Regular Knee pain (2 or more domains of KOOS <85) plus 1 of either:
  - Joint line tenderness
  - Crepitus
  - AND KL2 = 0 or 1
6 phenotypes (by X-ray) at 2 years (categorical)

1. Index knee pain and X-ray negative
2. Index knee pain and radiographic OA (new or progression)
3. Index knee pain free and radiographic OA (new or progression)
4. Index knee pain free and X-ray negative
5. Index knee pain free and no X-ray progression (pre-existing OA)
6. Index knee pain and no X-ray progression (pre-existing OA)

Where 'Knee pain' is defined as follows:
- Knee pain: 2 year NHANES index knee = yes
- Knee pain free: 2 year NHANES index knee = no
- X-ray negative (KL2=0 or 1)
- New radiographic OA (KL0=0 or 1 AND KL≥2 at 2 years)

And where X-ray progression is defined as follows:
- No X-ray progression (KL0>0 AND KL0 ≥KL2)
- X-ray progression (KL0>0 AND KL0 <KL2)

4 phenotypes (by MRI) at 2 years (categorical)

Where Hunter MRI OA score in index knee at 2 years = OAMRI2

- Knee pain and MRI negative
- Knee pain and any MRI OA
- Knee pain free and any MRI OA
- Knee pain free and MRI negative (2 year NHANES index knee = yes)
  - Knee pain (2 year NHANES index knee=yes)
  - Knee pain free (2 year NHANES index knee=no)
  - MRI negative (OAMRI2=No for patellofemoral AND No for tibiofemoral)
  - New MRI patellofemoral OA (Patellofemoral OAMRI2=yes)
  - New MRI tibiofemoral OA (Tibiofemoral OAMRI2=yes)
  - New MRI tibiofemoral and patellofemoral OA (Patellofemoral OAMRI2 AND Tibiofemoral OAMRI2=yes)
  - Any MRI OA (OAMRI2= yes to PF OR TF OR both)

Semi-quantitative imaging outcomes (semi-quantitative MR outcomes elsewhere, by WORMS)

- X-ray: Change in size and extent of osteophytes in index knee from baseline to 2 years (where total osteophytes = lateral femoral +lateral tibial + medial femoral +medial tibial OARSI osteophyte scores)
- Difference in change between index and contralateral knee in total osteophyte scores.

Continuous imaging outcomes

X-ray

- Change in cartilage thickness measures in index knee from baseline to 2 years
  - Lateral average joint space width
  - Bezier lateral minimum joint space width
  - Medial average joint space width
  - Bezier medial minimum joint space width
• Difference in change between index and contralateral knee in cartilage thickness measures

**MRI**

• Change in cartilage volume and thickness measures in index knee from baseline to 2 years, in 7 areas (highlighted in bold in the Appendix 5 table) plus summary tibial and femoral areas.
• Difference in change in cartilage volume and thickness in index knee compared with contralateral knee from baseline to 2 years, in summary tibial, femoral and patellofemoral areas
• Difference in change in MRI Worms score in index and contralateral knees
Supplementary Table 1. Categories of blood staining in synovial fluid

| SF Blood Staining Grade | Numerical Grade | SF Description |
|-------------------------|-----------------|----------------|
| None                    | 1               | No visible red staining of the SF |
| Mild                    | 2               | Visible red staining, high level of translucency (finger behind tube visible with low distortion) |
| Moderate                | 3               | Heavy red staining, low level of translucency (finger behind tube visible with high distortion) |
| Severe                  | 4               | Heavy red staining, opaque (finger behind tube not visible) |

The presence of blood staining in synovial fluid (SF) was graded subjectively in normal light conditions using a pre-defined visual grading scale.
Supplementary Table 2. Assays and performance

| Analyte                     | Assay                                          | Cat #, manufacturer       | Intra-assay c.v. (%) | Inter-assay c.v. (%) | SF Dilution (fold) |
|-----------------------------|------------------------------------------------|---------------------------|----------------------|----------------------|--------------------|
| Activin A                   | Human activin A Quantikine                     | R&D Systems DAC00B MSD    | 2·6                  | 6·9                  | 50                 |
| IL-6                        | Custom multiplex (IL-6 + MCP-1)                | MSD K15007C-1             | 10·8                 | 15·7                 | 5                  |
| IL-8                        | V-PLEX Human Cytokine                          | MSD (K151A0H-1) MSD      | 3·5                  | 8·9                  | 5                  |
| MCP-1                       | Custom multiplex (IL-6 & MCP-1)                | MSD K151AYC-1             | 6·4                  | 21·7                 | 5                  |
| IL-18                       | Human IL-18 ELISA                              | Invitrogen (7620) MSD    | 2·2                  | 21·3                 | 3                  |
| IL-18 Binding Protein       | Custom MSD Human IL-18BP                       | R&D Systems DY119 MSD     | 2·7                  | 20·7                 | 5                  |
| MMP-3                       | Human MMP 3-plex ultra-sensitive               | K15034C-1 MSD            | 6·2                  | 19·9                 | 400                |
| TIMP-1                      | Human TIMP-1                                   | K151JPC-1 MSD            | 6·5                  | 8·8                  | 200                |
| TSG-6                       | Custom human TSG-6                             | Prototype, MSD           | 8·6                  | 18·8                 | 4                  |
| FGF-2                       | V-PLEX Human (basic) FGF-2                     | MSD (K151MDD-1) MSD      | 4·0                  | 6·1                  | 4                  |
| TGFβ-1                      | Human TGFβ-1 ELISA                             | R&D (DB100B) IBL          | 3·7                  | 13·0                 | 4                  |
| Tenascin C                  | Human Tenascin C ELISA                         | IBL (27751)              | 3·4                  | 12·3                 | 10                 |

Immunoassays were by commercially-available plate ELISA, or by electrochemiluminescence (MSD, Rockville, USA). The latter included singleplex, multiplex or custom/prototype-printed assays, validated by us as indicated.

SF was centrifuged for 20min at 3000G. Whole blood (divided between EDTA and plain tubes for plasma and serum respectively) was centrifuged at 1600G for 15min at 20°C. Supernatants were stored in cryovials at -80°C in monitored freezers until use. Plasma or serum, depending on assay, or SF aliquots were brought up to RT and gently vortexed prior to assay.

FGF-2 and IL-6 were measured in plasma; MMP-3 and neuropeptide-Y (Millipore, US) in serum in addition. 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).

MSD=Mesoscale Discovery

An intra-assay and inter-assay coefficient of variation (c.v.) of <12% and <25% respectively was established for all assays. (N=20 for intra-assay c.v.; minimum N=4 for inter-assay c.v.). The values for SF (higher) are shown. Samples below the lower limit of quantitation were arbitrarily given half the lower limit of quantitation as their concentration during analyses. Spike recoveries within 80% and 120% were deemed acceptable. Linearity of dilution was confirmed for all assays across the dilution range used.

TSG-6 (Tumour necrosis factor stimulated gene-6); IL (interleukin); BP (binding protein); TGFβ (transforming growth factor beta); TIMP-1 (tissue inhibitor of metalloproteinases-1); MMP-3 (metalloproteinase-3); FGF-2 (fibroblast growth factor-2); MCP-1 (monocyte chemoattractant protein-1); NP-Y (neuropeptide-Y)
Supplementary Table 3. Univariable associations of pre-defined clinical variables with KOOS4 in KICK participants at 2 years (including all predefined exploratory factors at baseline and 3 months)

| Pre-defined Variable | N   | Coeff. (95% CI) | P value | R²  |
|----------------------|-----|----------------|---------|-----|
| **Established baseline risk factors for OA** | | | | |
| Female sex (ref: male) | 92 | -0.4 (-8.9, 8.2) | 0.93 | 0.11 |
| Age (years) | 92 | 0.1 (-0.2, 0.4) | 0.59 | 0.12 |
| Body Mass Index (BMI) | 91 | 0.4 (-0.2, 1.0) | 0.17 | 0.13 |
| Index knee OA by Kellgren Lawrence grade at baseline | | | | |
| KL 1 (ref: grade 0) | 75 | 4.1 (-4.9, 13.1) | 0.36 | 0.12 |
| KL 2 (ref: grade 0) | | -2.4 (-12.1, 7.3) | 0.62 | |
| KL 3 (ref: grade 0) | | -2.2 (-11.9, 7.5) | 0.65 | |
| **Previous knee surgery to index knee** | | | | |
| Previous index knee meniscal surgery (ref: no) | 92 | -3.1 (-13.4, 7.1) | 0.55 | 0.12 |
| Previous index knee surgery - ligament (ref: no) | 92 | -1.1 (-11.1, 8.9) | 0.82 | 0.12 |
| Previous index knee surgery - other (ref: no) | | -6.8 (-17.2, 3.7) | 0.20 | |
| **Radiographic knee alignment at BL or 3m** | | | | |
| Any clinical malalignment BL or 3m (ref: no) | 92 | -2.1 (-9.6, 5.4) | 0.58 | 0.11 |
| Clinical varus (ref: no) | 62 | 13.8 (9.8, 17.7) | 0.08 | 0.08 |
| **No prior history of index knee injury (ref: yes)** | | | | |
| Family history of knee OA (ref: no) | 92 | -3.0 (-10.2, 4.2) | 0.41 | 0.12 |
| **Factors previously found to be important in our studies** | | | | |
| Time from joint injury (days) | 92 | -0.1 (-0.3, 0.0) | 0.070 | 0.13 |
| Extent/nature of joint injury | 92 | | | |
| Single complete ligament rupture (ref: meniscal tear only) | | 5.1 (-3.7, 14.0) | 0.25 | 0.14 |
| Single complete ligament rupture + meniscal tear (ref: meniscal tear only) | | 0.9 (-9.9, 8.0) | 0.84 | |
| Extended injury (ref: meniscal tear only) | | -4.9 (-18.2, 8.4) | 0.47 | |
| Non-professional physical activity pre-injury (ref: professional) | 92 | 0.2 (-7.2, 7.6) | 0.95 | 0.11 |
| **Other exploratory factors at baseline** | | | | |
| Medium/large clinical effusion (ref: none/small) | 91 | -7.2 (-13.5, -0.9) | 0.025 | 0.14 |
| Any meniscal tear (ref: none) | 92 | -3.2 (-9.8, 3.3) | 0.33 | 0.12 |
| Meniscal tear site (ref: none) | | | | |
| Medial | 92 | -0.16 (-8.1, 7.8) | 0.97 | 0.15 |
| Lateral | 92 | -3.17 (-11.3, 5.0) | 0.44 | 0.15 |
| Any chondral defects (ref: none) | 91 | -2.7 (-9.6, 4.1) | 0.43 | 0.10 |
| Any osteophytes (ref: none) | 62 | 2.0 (-16.6, 20.6) | 0.83 | 0.05 |
| Fracture or Dislocation (ref: no) | 92 | -8.6 (-23.6, 6.3) | 0.25 | 0.13 |
| Any meniscal extrusion (ref: none) | 63 | 4.2 (-1.3, 9.7) | 0.14 | 0.05 |
| MRE infra-patellar fat pad score 1 (ref: 0) | 63 | -3.7 (-12.6, 5.3) | 0.42 | 0.06 |
| MRE infra-patellar fat pad score 2 (ref: 0) | 63 | -3.0 (-12.2, 6.4) | 0.53 | 0.06 |
| NSAIDs between injury and baseline visit (ref: none) | 92 | -3.6 (-10.2, 2.7) | 0.25 | 0.12 |
| **Exploratory factors at 3 month visit** | | | | |
| Moderate index knee pain in last 30 days at 3 months (ref: none) | 83 | -4.1 (-10.8, 2.7) | 0.24 | 0.11 |
| Index knee locking at 3 months (ref: no) | 83 | 1.9 (-7.2, 11.0) | 0.68 | 0.09 |
| Index knee warmth at 3 months (ref: no) | 81 | -4.9 (-11.3, 1.5) | 0.13 | 0.10 |
| Variable                                                                 | N  | Coeff. | Lower CI | Upper CI | P value  | R squared |
|-------------------------------------------------------------------------|----|--------|----------|----------|-----------|-----------|
| Medium/large clinical effusion (ref:none/small)                         | 84 | -1.0   | -9.0     | 7.0      | 0.81      | 0.09      |
| Pain on index knee flexion at 3 months (ref:no)                         | 84 | -0.74  | -8.6     | 7.1      | 0.85      | 0.09      |
| Loss of full active extension at 3 months (ref:no)                      | 84 | 1.0    | -9.5     | 11.5     | 0.85      | 0.09      |
| Asymmetry of active range at 3 months (ref:no)                          | 68 | -4.7   | -13.6    | 4.3      | 0.30      | 0.11      |
| Asymmetry of single leg squat test at 3 months (ref:no)                 | 42 | 1.4    | -7.1     | 9.8      | 0.75      | 0.05      |
| Discrepancy in quads bulk at 3 months (ref:no)                          | 83 | -1.3   | -8.2     | 5.6      | 0.72      | 0.09      |
| Regular or recent use of painkillers/NSAIDs at 3 months (ref:no)        | 46 | -9.2   | -20.2    | 1.7      | 0.11      | 0.18      |

The effect sizes (Coeff.) and 95% confidence intervals, associated P value and R squared (R^2) for the univariable associations of all pre-defined clinical variables with KOOS_4 at 2 years are shown when adjusted for KOOS_4 at baseline, in N (number with available data) of the 98 individuals where KOOS_4 was available at 2 years (and using KOOS_4 at 3 years where missing). Associations of variables (P≤0.05) which contributed to the core model are highlighted in **bold**.
Supplementary Table 4. Effects of other pre-defined variables in the core variable-adjusted model of outcome KOOS₄ at 2 years

| Selected variables                                      | N   | Univariable linear model (by baseline KOOS₄) | P Value | R squared |
|----------------------------------------------------------|-----|---------------------------------------------|---------|-----------|
|                                                          |     | Coeff. (95% CI)                             |         |           |
| Blood staining (moderate/severe)                         | 77  | -10.63 (-19.06, -2.20)                      | 0.0070  | 0.21      |
| Clinical Effusion (medium/large)                         | 77  | -9.05 (-15.5, -2.61)                        | 0.014   | 0.19      |
| Interaction, Effusion x blood staining                   | 77  | -20.5 (-34.8, -6.18)                        | 0.0060  | 0.30      |

| Other variables (tested post hoc)                        |     | Univariable linear model, adjusted by core variables (baseline KOOS₄, and effusion x blood staining) | Coeff (95% CI) |           |
|----------------------------------------------------------|-----|----------------------------------------------------------------------------------------------------------------|----------------|-----------|
|                                                          |     |                                                                                                               |                |           |
| Age (years)                                              | 77  | 0.09 (-0.29, 0.47)                                                                                               | 0.63           | 0.30      |
| Female sex (ref male)                                   | 77  | 1.00 (-6.94, 8.94)                                                                                               | 0.80           | 0.30      |
| Body mass index (Kg/m²)                                 | 77  | 0.4 (-0.22, 1.03)                                                                                               | 0.20           | 0.31      |
| Time from injury at baseline (days)                     | 77  | -0.15 (-0.35, 0.05)                                                                   | 0.14           | 0.13      |
| Extent/nature of knee injury                            | 77  |                                                                                                               |                |           |
| Meniscal tear                                           | 77  | 1                                                                                                               |                |           |
| Single ligament rupture only                            | 77  | 7.38 (-3.43, 18.19)                                                                                               | 0.18           | 0.31      |
| ACL + meniscal tear                                     | 77  | 2.92 (-5.75, 11.6)                                                                                 | 0.50           | 0.31      |
| Severe trauma                                            | 77  | 1.00 (-11.82, 13.83)                                                                          | 0.88           | 0.31      |

Given a lack of power to introduce all pre-defined clinical variables into a multivariable model simultaneously, individual clinical variables with P<0.05 were selected and introduced into a core model for association with the primary outcome 2 year KOOS₄ (as shown in Table 2). The selected variables for the core model (blood staining, clinical effusion and their interaction, when adjusted by baseline KOOS₄) are shown in the upper half of this table, with their associated 95% Confidence Intervals (CI), P Value and R squared.

Other predefined variables not reaching P<0.05 on univariable analysis (Table 2) which were felt on expert review to be important predefined clinical variables are shown in the lower half of this table. The univariable association with 2 year KOOS₄ of age, sex, BMI, time from injury to baseline and Extent/nature of the joint injury are shown, when adjusted for core model variables. 95% CIs, P Values and R squared are shown, so that their contribution can be directly compared with the existing core model.
Supplementary Table 5. Univariable associations of pre-defined clinical variables with Luyten early knee OA criteria in KICK participants at 2 years

| Pre-defined Variable                                                                 | N   | OR (95% CI)    | P value |
|--------------------------------------------------------------------------------------|-----|---------------|---------|
| Established baseline risk factors for OA                                             |     |               |         |
| Female sex (ref:male)                                                                | 81  | 0.97 (0.32, 2.92) | 0.96   |
| Age (years)                                                                          | 81  | 1.00 (0.95, 1.05)  | 1.00   |
| Body Mass Index (BMI)                                                                 | 81  | 0.97 (0.88, 1.08)  | 0.61   |
| Index Knee OA by Kellgren Lawrence grade at baseline                                 |     |               |         |
| KL 1 (ref: grade 0)                                                                  | 70  | 1.27 (0.32, 5.01)  | 0.73   |
| KL 2 (ref: grade 0)                                                                  |     | 2.72 (0.70, 10.63) | 0.15   |
| KL 3 (ref: grade 0)                                                                  |     | 3.40 (0.62, 18.75) | 0.16   |
| Previous knee surgery to index knee                                                 |     |               |         |
| Previous index knee meniscal surgery (ref:no)                                        | 81  | 1.66 (0.34, 8.04)  | 0.53   |
| Previous index knee surgery - ligament (ref:no)                                      | 81  | 4.90 (1.06, 22.72) | 0.042  |
| Previous index knee surgery - other (ref:no)                                         | 5.88 (0.99, 35.03) | 0.052 |
| Clinical malalignment BL or 3m (ref:no)                                              | 81  | 1.44 (0.23, 9.22)  | 0.70   |
| Prior history of index knee injury (ref:no)                                          | 81  | 0.38 (0.14, 0.99)  | 0.05   |
| Family history of knee OA (ref:no)                                                  | 80  | 1.21 (0.47, 3.11)  | 0.70   |
| Factors previously found to be important in our studies                               |     |               |         |
| Time from joint injury (days)                                                        | 81  | 1.01 (0.98, 1.03)  | 0.72   |
| Professional physical activity pre-injury (ref:non-professional)                     | 81  | 0.72 (0.27, 1.90)  | 0.50   |
| Moderate/severe synovial Fluid blood staining (ref:none/mild)                        | 67  | 3.78 (1.27, 11.19) | 0.016  |
| Any meniscal tear (ref:none)                                                         | 81  | 1.32 (0.47, 3.71)  | 0.60   |
| Meniscal tear site (ref:none)                                                        |     |               |         |
| Medial                                                                                | 81  | 1.19 (0.32, 4.37)  | 0.80   |
| Lateral                                                                              |     | 1.22 (0.37, 3.96)  | 0.74   |
| Both                                                                                 |     | 2.06 (0.42, 9.97)  | 0.37   |
| Any chondral defects (ref:none)                                                      | 81  | 1.89 (0.74, 4.85)  | 0.19   |
| Any osteophytes (ref:none)                                                           | 61  | 3.80 (0.32, 44.51) | 0.29   |
| Fracture or Dislocation (ref:no)                                                     | 81  | 1.06 (0.18, 6.21)  | 0.95   |
| Extent/nature of joint injury                                                        | 81  |               |         |
| Single complete ligament rupture (ref:meniscal tear only)                            |     | 0.33 (0.08, 1.43)  | 0.14   |
| Single complete ligament rupture + meniscal tear (ref:meniscal tear only)            |     | 0.41 (0.12, 1.44)  | 0.16   |
| Extended injury (ref:meniscal tear only)                                             |     | 0.5 (0.09, 2.84)   | 0.43   |
| Medium/large clinical effusion (ref:none/small)                                      | 81  | 1.92 (0.74, 4.97)  | 0.18   |
| Any meniscal extrusion (ref:none)                                                    | 63  | 2.00 (0.37, 10.87) | 0.42   |
| MRI: infra-patellar fat pad score 1 (ref:0)                                           | 63  | 0.68 (0.19, 2.43)  | 0.55   |
| MRI: infra-patellar fat pad score 2 (ref:0)                                           |     | 1.66 (0.47, 5.93)  | 0.43   |
| NSAIDs between injury and baseline visit (ref:none)                                  | 81  | 1.26 (0.48, 3.33)  | 0.64   |

The effect sizes (Coeff.) and 95% confidence intervals (CI) and associated P values are shown for the univariable associations of key predefined clinical variables with the outcome early knee OA at 2 years, in N (number with available data) of the 98 individuals where the outcome was available at 2 years. Variables with a P≤0.05 are shown in **bold**.
### Supplementary Table 6A. Univariable associations of pre-defined clinical variables with new radiographic knee OA in KICK participants at 2 years

| Pre-defined Variable                                                                 | N   | OR (95% CI)       | P value |
|--------------------------------------------------------------------------------------|-----|-------------------|---------|
| **Established baseline risk factors for OA**                                         |     |                   |         |
| Female sex (ref:male)                                                                | 41  | 0.40 (0.10, 1.56) | 0.19    |
| Age (years)                                                                          | 41  | 0.98 (0.92, 1.04) | 0.45    |
| Body Mass Index (BMI)                                                                 | 41  | 1.07 (0.89, 1.29) | 0.48    |
| Index Knee OA by Kellgren Lawrence grade at baseline                                 |     |                   |         |
| KL 1 (ref:grade 0)                                                                  | 33  | 0.69 (0.12, 3.97) | 0.62    |
| KL 2 (ref:grade 0)                                                                  |     | 1.0               | *       |
| KL 3 (ref:grade 0)                                                                  |     | 1.0               | *       |
| Previous knee surgery to index knee                                                 |     |                   |         |
| Previous index knee meniscal surgery (ref:no)                                        | 41  | 1.16 (0.63, 2.14) | 0.64    |
| Previous index knee surgery - ligament (ref:no)                                      | 40  | 1.0               |         |
| Previous index knee surgery -other (ref:no)                                          | 40  | 0.27 (0.03, 2.81) | 0.27    |
| Clinical malalignment BL or 3m (ref:no)                                              | 40  | 1.0               |         |
| Prior history of index knee injury (ref:no)                                          | 41  | 1.24 (0.34, 4.54) | 0.75    |
| Family history of knee OA (ref:no)                                                  | 41  | 0.95 (0.27, 3.31) | 0.94    |
| **Factors previously found to be important in our studies**                          |     |                   |         |
| Time from joint injury (days)                                                        | 41  | 1.01 (0.97, 1.05) | 0.62    |
| Professional physical activity pre-injury (ref:non-professional)                     | 41  | 0.79 (0.22, 2.77) | 0.71    |
| Moderate/severe synovial Fluid blood staining (ref:none/mild)                        | 34  | 0.30 (0.07, 1.25) | 0.10    |
| **Exploratory factors at baseline**                                                  |     |                   |         |
| Any meniscal tear (ref:none)                                                         | 41  | 5.70 (1.25, 25.92) | 0.024   |
| Meniscal tear site (ref:none)                                                        | 36  |                   |         |
| Medial                                                                               |     | 3.75 (0.59, 23.94) | 0.16    |
| Lateral                                                                             |     | 4.50 (0.85, 23.8) | 0.077   |
| Any chondral defects (ref:none)                                                      | 41  |                   |         |
| Any osteophytes (ref:none)                                                           | 39  | 1.05 (1.97, -27.02)| 0.87    |
| Fracture or Dislocation (ref:no)                                                     | 41  | 2.84 (0.27, 29.9) | 0.38    |
| Extent/nature of joint injury                                                        | 41  |                   |         |
| Single complete ligament rupture (ref:meniscal tear only)                            |     | 0.19 (0.01, 2.91) | 0.23    |
| Single complete ligament rupture + meniscal tear (ref:meniscal tear only)            |     | 0.83 (0.07, 10.55)| 0.89    |
| Extended injury (ref:meniscal tear only)                                             |     | 1.0 (0.18, 22.05) | 1.00    |
| Medium/large clinical effusion (ref:none/small)                                      | 41  | 1.99 (0.57, 6.9)  | 0.28    |
| Any meniscal extrusion (ref:none)                                                    | 39  | 3.18 (0.30, 33.58) | 0.34    |
| MRE: infra-patellar fat pad score 1 (ref:0)                                          | 39  | 2.14 (0.47, 9.70) | 0.32    |
| MRE: infra-patellar fat pad score 2 (ref:0)                                          | 39  | 1.22 (0.24, 6.31) | 0.81    |
| NSAIDs between injury and baseline visit (ref:none)                                  | 41  | 1.27 (0.36, 4.48) | 0.71    |
| **Exploratory factors at 3 month visit**                                             |     |                   |         |
| Moderate index knee pain in last 30 days at 3 months (ref:none)                      | 40  | 1.01 (0.29, 3.50) | 0.99    |
| Index knee locking at 3 months (ref:no)                                              | 40  | 3.00 (0.28, 31.63)| 0.36    |
| Index knee warmth at 3 months (ref:no)                                              | 38  | 1.00 (0.28, 3.57) | 1.00    |
| **Medium/large clinical effusion (ref:none/small)**                                 | 40  | 4.12 (1.02, 16.67) | 0.047   |
| Pain on index knee flexion at 3 months (ref:no)                                      | 40  | 0.86 (0.23, 3.15) | 0.82    |
| Loss of full active extension at 3 months (ref:no)                                   | 40  | 1.89 (0.16, 22.75)| 0.61    |
| Clinical Variable                                      | N  | Coefficient (95% CI) | P value |
|-------------------------------------------------------|----|----------------------|---------|
| Asymmetry of active range at 3 months (ref:no)        | 33 | 1.48 (0.32, 6.89)    | 0.62    |
| Asymmetry of single leg squat test at 3 months (ref:no)| 20 | 1.40 (0.23, 8.46)    | 0.23    |
| Regular or recent use of painkillers/NSAIDs (ref:no)   | 27 | 0.50 (0.11, 2.38)    | 0.38    |

The effect sizes (Coeff.) and 95% confidence intervals (CI) and associated P values are shown for the univariable associations of all pre-defined clinical variables with the outcome new radiographic knee OA at 2 years, in N (number with available data) of the 98 individuals where the outcome was available at 2 years and baseline. Variables with a P≤0.05 are shown in **bold**.
### Supplementary Table 6B. Univariable associations of pre-defined clinical variables with new symptomatic and radiographic knee OA in KICK participants at 2 years

| Pre-defined Variable                                                                 | N   | OR (95% CI)          | P value |
|--------------------------------------------------------------------------------------|-----|----------------------|---------|
| Established baseline risk factors for OA                                             |     |                      |         |
| Female sex (ref:male)                                                               | 24  | 0.75 (0.13, 4.22)    | 0.74    |
| Age (years)                                                                         | 24  | 0.92 (0.83, 1.02)    | 0.12    |
| Body Mass Index (BMI)                                                               | 24  | 0.85 (0.63, 1.16)    | 0.32    |
| Index Knee OA by Kellgren Lawrence grade at baseline                                 |     |                      |         |
| KL 1 (ref:grade 0)                                                                  | 19  | 0.53 (0.09, 3.31)    | 0.50    |
| KL 2 (ref:grade 0)                                                                  |     | 1.00                 |         |
| KL 3 (ref:grade 0)                                                                  |     | 1.00                 |         |
| Previous knee surgery to index knee                                                |     |                      |         |
| Previous index knee meniscal surgery (ref:no)                                       | 24  | 0.60 (0.26, 1.37)    | 0.23    |
| Previous index knee surgery - ligament (ref:no)                                     | 24  | 1.75 (0.10, 31.96)   | 0.71    |
| Previous index knee surgery - other (ref:no)                                        |     | 0.57 (0.24, 1.36)    | 0.21    |
| Clinical malalignment BL or 3m (ref:no)                                             | 24  | 0.60 (0.26, 1.37)    | 0.23    |
| Prior history of index knee injury (ref:no)                                         | 24  | 0.45 (0.08, 2.60)    | 0.38    |
| Family history of knee OA (ref:no)                                                 | 24  | 0.43 (0.07, 2.81)    | 0.38    |
| Factors previously found to be important in our studies                              |     |                      |         |
| Time from joint injury (days)                                                       | 24  | 1.01 (0.97, 1.06)    | 0.50    |
| Professional physical activity pre-injury (ref:non-professional)                    | 24  | 7.00 (0.69, 70.74)   | 0.10    |
| Moderate/severe synovial Fluid blood staining (ref:none/mild)                       | 19  | 0.75 (0.11, 4.90)    | 0.76    |
| Exploratory factors at baseline                                                     |     |                      |         |
| Any meniscal tear (ref:none)                                                        | 24  | 4.00 (0.62, 25.96)   | 0.15    |
| Meniscal tear site (ref:none)                                                        |     |                      |         |
| Medial                                                                               | 22  | 1.33 (0.09, 20.71)   | 0.84    |
| Lateral                                                                             |     | 4.00 (0.50, 31.98)   | 0.19    |
| Any chondral defects (ref:none)                                                     | 24  | 0.44 (0.08, 2.44)    | 0.35    |
| Any osteophytes (ref:none)                                                          | 23  | 0.53 (0.23, 1.26)    | 0.15    |
| Fracture or Dislocation (ref:no)                                                     | 23  | 1.00                 |         |
| Extent/nature of joint injury                                                       | 22  |                      |         |
| Single complete ligament rupture (ref:meniscal tear only)                           |     | 0.25 (0.036, 1.70)   | 0.16    |
| Single complete ligament rupture + meniscal tear (ref:meniscal tear only)           |     | ..*                 |         |
| Extended injury (ref:meniscal tear only)                                            |     | ..*                 |         |
| Medium/large clinical effusion (ref:none/small)                                     | 24  | 4.00 (0.69, 23.09)   | 0.12    |
| Any meniscal extrusion (ref:none)                                                   | 23  | 2.00 (0.11, 36.95)   | 0.64    |
| MRI: infra-patellar fat pad score 1 (ref:0)                                          | 23  | 2.22 (0.24, 20.17)   | 0.48    |
| MRI: infra-patellar fat pad score 2 (ref:0)                                          | 23  | 5.00 (0.55, 45.39)   | 0.15    |
| NSAIDs between injury and baseline visit (ref:none)                                 | 24  | 3.06 (0.47, 19.88)   | 0.24    |
| Exploratory factors at 3 month visit                                                |     |                      |         |
| Moderate index knee pain in last 30 days at 3 months (ref:none)                     | 24  | 0.91 (0.17, 4.81)    | 0.92    |
| Index knee locking at 3 months (ref:no)                                              | 23  | 1.00                 |         |
| Index knee warmth at 3 months (ref:no)                                               | 23  | 1.50 (0.27, 8.45)    | 0.65    |
| Medium/large clinical effusion (ref:none/small)                                     | 24  | 14.00 (1.86, 105.27) | 0.010   |
| Pain on index knee flexion at 3 months (ref:no)                                      | 24  | 2.20 (0.38, 12.57)   | 0.38    |
| Loss of full active extension at 3 months (ref:no)                                   | 24  | 4.00 (0.31, 52.06)   | 0.29    |
Asymmetry of active range at 3 months (ref:no) & 20 & 7.33 (0.88, 61.33) & 0.070 \\
Asymmetry of single leg squat test at 3 months (ref:no) & 9 & 2.00 (0.11, 35.81) & 0.64 \\
Regular or recent use of painkillers/NSAIDs (ref:no) & 16 & 2.67 (0.35, 20.51) & 0.35 

The effect sizes (Coeff.) and 95% confidence intervals (CI)) and associated P values are shown for the univariable associations of all pre-defined clinical variables with the outcome new symptomatic and radiographic knee OA at 2 years, in N (number with available data) of the 98 individuals in the 2 year Dataset where the outcome was available at 2 years and baseline, compared with those no symptomatic or radiographic knee OA. Variables with a P≤0.05 are shown in **bold**.

*a*Omitted because of collinearity. (6 participants with new symptomatic and radiographic knee OA vs. 6 participants with no symptomatic or radiographic knee OA).

*b*There was a single participant with new symptomatic and radiographic knee OA for the category of extended injury. These data were omitted because it did not allow prediction of an odds ratio. There were no participants with extended injury at baseline and no symptomatic or radiographic knee OA at 2 years.
Supplementary Table 7A. Univariable associations of baseline synovial fluid and plasma/serum biomarkers with new radiographic knee OA in KICK participants at 2 years

| Analyte       | N  | OR (95% CI)     | P Value |
|---------------|----|-----------------|---------|
| SF            |    |                 |         |
| TSG-6         | 37 | 1.00 (0.99, 1.00)| 0.38    |
| IL-18 BP      | 37 | 1.00 (1.00, 1.00)| 0.74    |
| IL-18         | 35 | 0.99 (0.97, 1.00)| 0.12    |
| Tenascin C    | 32 | 1.01 (1.00, 1.02)| 0.41    |
| TGFβ-1        | 36 | 1.00 (1.00, 1.00)| 0.95    |
| TIMP-1        | 37 | 1.00 (1.00, 1.00)| 0.79    |
| MMP-3         | 37 | 1.00 (1.00, 1.00)| 0.13    |
| Activin A     | 37 | 1.00 (1.00, 1.00)| 0.07    |
| IL-8          | 37 | 1.00 (0.98, 1.01)| 0.48    |
| FGF-2         | 37 | 1.00 (0.98, 1.00)| 0.43    |
| MCP-1         | 37 | 1.00 (1.00, 1.00)| 0.37    |
| IL-6          | 37 | 1.00 (1.00, 1.00)| 0.37    |
| Blood         |    |                 |         |
| MMP-3         | 41 | 1.09 (0.98, 1.22)| 0.12    |
| NP-Y          | 41 | 1.03 (0.96, 1.11)| 0.42    |
| IL-6          | 41 | 0.77 (0.31, 1.91)| 0.57    |
| FGF-2         | 41 | 0.99 (0.97, 1.01)| 0.22    |

The effect sizes (Coeff.) and 95% confidence intervals (CI) and associated P values are shown for the univariable associations of all pre-defined molecules with the outcome new radiographic knee OA at 2 years, in N (number with available data) of the 98 individuals in the 2 year Dataset where the outcome was available at 2 years and baseline.

TSG-6 (Tumour necrosis factor stimulated gene-6); IL(interleukin); BP (binding protein); TGFβ (transforming growth factor beta); TIMP-1 (tissue inhibitor of metalloproteinases-1); MMP-3 (metalloproteinase-3); FGF-2 (fibroblast growth factor-2); MCP-1 (monocyte chemoattractant protein-1); NP-Y (neuropeptide-Y)
Supplementary Table 7B. Univariable associations of baseline synovial fluid and plasma/serum biomarkers with new symptomatic and radiographic knee OA in KICK participants at 2 years

| Analyte       | N  | OR (95% CI)     | P Value |
|---------------|----|----------------|---------|
| SF            |    |                |         |
| TSG-6         | 22 | 1.00 (0.99, 1.00) | 0.64    |
| IL-18 BP      | 22 | 1.00 (1.00, 1.00) | 0.38    |
| IL-18         | 21 | 1.00 (0.97, 1.03) | 0.98    |
| Tenascin C    | 20 | 1.02 (1.00, 1.05) | 0.09    |
| TGFβ-1        | 21 | 1.00 (1.00, 1.00) | 0.17    |
| TIMP-1        | 22 | 1.00 (1.00, 1.00) | 0.21    |
| MMP-3         | 22 | 1.00 (1.00, 1.00) | 0.20    |
| Activin A     | 22 | 1.00 (1.00, 1.00) | 0.55    |
| IL-8          | 22 | 1.01 (0.97, 1.05) | 0.62    |
| FGF-2         | 22 | 1.01 (0.90, 1.05) | 0.48    |
| MCP-1         | 22 | 1.00 (1.00, 1.01) | 0.36    |
| IL-6          | 22 | 1.00 (1.00, 1.00) | 0.51    |
| Blood         |    |                |         |
| MMP-3         | 24 | 1.02 (0.88, 1.08) | 0.80    |
| NP-Y          | 24 | 0.96 (0.82, 1.13) | 0.65    |
| IL-6          | 24 | 1.59 (0.07, 37.80) | 0.78    |
| FGF-2         | 24 | 0.96 (0.90, 1.02) | 0.20    |

The effect sizes (Coeff.) and 95% confidence intervals (CI) and associated P values are shown for the univariable associations of all pre-defined molecules with the outcome new symptomatic and radiographic knee OA at 2 years, in N (number with available data) of the 98 individuals where the outcome was available at 2 years and baseline.

TSG-6 (Tumour necrosis factor stimulated gene-6); IL (interleukin); BP (binding protein); TGFβ (transforming growth factor beta); TIMP-1 (tissue inhibitor of metalloproteinases-1); MMP-3 (metalloproteinase-3); FGF-2 (fibroblast growth factor-2); MCP-1 (monocyte chemoattractant protein-1); NP-Y (neuropeptide-Y)
Supplementary Figure 2. Synovial fluid analytes in synovial fluid and blood at the time of acute knee injury

A

B

IL-8

Tenascin C

FGF-2

TGFβ-1

IL-18

IL-18 Binding Protein
Levels of analytes were measured by ELISA, or by electrochemiluminescence in duplicate in synovial fluid (SF) and in plasma or serum (blood) by assays as detailed in Table 2. Bars show the median and interquartile range.

A. Data is shown for 6 analytes previously measured in KICK participants at baseline (injured) and healthy controls (Control). This is a reproduced figure from Watt et al 2016. Differences between groups were tested by Mann-Whitney U test, ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05

B. Data is shown for 6 analytes in KICK participants in SF and blood at Baseline and in blood at 3 months (3m).

Watt FE, Paterson E, Freidin A, Kenny M, Judge A, Saklatvala J, et al. Acute Molecular Changes in Synovial Fluid Following Human Knee Injury: Association With Early Clinical Outcomes. Arthritis Rheumatol. 2016;68(9):2129-40.

Watt FE, Paterson E, Freidin A, Kenny M, Judge A, Saklatvala J, et al. Acute Molecular Changes in Synovial Fluid Following Human Knee Injury: Association With Early Clinical Outcomes. Arthritis Rheumatol. 2016;68(9):2129-40.
Supplementary Figure 3. Association between KOOS-4 and KOOS pain in KICK participants at 2 years

Measurements of KOOS pain subscale and KOOS-4 composite measure in individual participants at 2 years (and 3 years where 2 years not available) were plotted and compared by Spearman R (SpR) test in all available data from participants with baseline and 2 year KOOS (n=98).
## Imaging protocol for 3T MRI of knee

| Sequence            |
|---------------------|
| Localisers x2       |
| t2_dce3d_we_sag_p2_iso |
| t1_vibe_we_sag_iso  |
| t2_tse_cor_p2_fs    |
| pd+t2_tse_sag_fs    |
| T2-star_p2_anatomical |
| t2_tse_cor_p2_fs    |
| pd+t2_tse_sag_fs    |
| t1_tirm_cor         |
CONSORT 2010 Flow Diagram

Enrollment
Assessed for eligibility (n=184)
Excluded (n=34)
- Not meeting eligibility criteria (n=2)
- Declined to participate (n=32)
Eligible for inclusion (n=150)

Baseline
Included (n=150)
- Excluded at baseline (n=0)

Follow-Up
Lost to follow-up (not responding to contact/not completing 2/3 year outcomes) (n=50)
Withdrawn (ineligibility after baseline) (n=2)

Analysis
Analysed in 2 year Dataset (n=98)
STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at [http://www.plosmedicine.org/](http://www.plosmedicine.org/), Annals of Internal Medicine at [http://www.annals.org/](http://www.annals.org/), and Epidemiology at [http://www.epidem.com/](http://www.epidem.com/)). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

| Section and Item             | Item No. | Recommendation                                                                 | Reported on Page No. |
|------------------------------|----------|--------------------------------------------------------------------------------|----------------------|
| **Title and Abstract**       | 1        | (a) Indicate the study’s design with a commonly used term in the title or the abstract | 1-3 (intro)          |
|                              |          | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 3-4 (intro)          |
| **Introduction**             |          |                                                                                  |                      |
| Background/Rationale         | 2        | Explain the scientific background and rationale for the investigation being reported | 1-2                  |
| **Objectives**               | 3        | State specific objectives, including any prespecified hypotheses                  | 2                    |
| **Methods**                  |          |                                                                                  |                      |
| Study Design                 | 4        | Present key elements of study design early in the paper                           | 3-5                  |
| Setting                      | 5        | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 3-4                  |
| Participants                 | 6        | (a) **Cohort study**—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 3                    |
|                              |          | Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls | N/A                  |
|                              |          | Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants | N/A                  |
(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed

Case-control study—For matched studies, give matching criteria and the number of controls per case

| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 4-8 |

| Section and Item | Item No. | Recommendation | Reported on Page No. |
|------------------|----------|----------------|---------------------|
| Data Sources/ Measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 4-8 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 6-8 |
| Study Size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative Variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 6-8 |
| Statistical Methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | 6-8 |
| | | (b) Describe any methods used to examine subgroups and interactions | 6-8 |
| | | (c) Explain how missing data were addressed | 6-8 |
| | | (d) Cohort study—If applicable, explain how loss to follow-up was addressed | 8 |
| | | Case-control study—If applicable, explain how matching of cases and controls was addressed |
| | | Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy |
| | | (e) Describe any sensitivity analyses | N/A |

Results

| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 9 (also Web Appendix p 17) |
| | | (b) Give reasons for non-participation at each stage | 9 (also Web Appendix p 17) |
(c) Consider use of a flow diagram  

**Descriptive Data**  

14*  

(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders  

(b) Indicate number of participants with missing data for each variable of interest  

(c) **Cohort study**—Summarise follow-up time (e.g., average and total amount)  

**Outcome Data**  

15*  

**Cohort study**—Report numbers of outcome events or summary measures over time  

**Case-control study**—Report numbers in each exposure category, or summary measures of exposure  

**Cross-sectional study**—Report numbers of outcome events or summary measures  

| Section and Item | Item No. | Recommendation | Reported on Page No. |
|------------------|----------|----------------|---------------------|
| Main Results     | 16       | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 9-12, Tables 2-3 |
|                  |          | (b) Report category boundaries when continuous variables were categorized | N/A |
|                  |          | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | N/A |
| Other Analyses   | 17       | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses | 11-12 |
| Discussion       |          |                |                     |
| Key Results      | 18       | Summarise key results with reference to study objectives | 13 |
| Limitations      | 19       | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 16 |
| Interpretation   | 20       | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-16 |
| Generalisability | 21       | Discuss the generalisability (external validity) of the study results | 16-17 |
| Other Information|          |                |                     |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |
|---------|----|----------------------------------------------------------------------------------------------------------------------------------|

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.*

Once you have completed this checklist, please save a copy and upload it as part of your submission. **DO NOT** include this checklist as part of the main manuscript document. It must be uploaded as a separate file.