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Osteoarthritis and Cartilage

Association of serum biomarkers with radiographic knee osteoarthritis, knee pain and function in a young, male, trauma-exposed population – Findings from the ADVANCE study

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SUMMARY

Objective: The ArmeD SerVices TrAuma RehabilitatioN OutComE (ADVANCE) study is investigating long-term combat-injury outcomes; this sub-study aims to understand the association of osteoarthritis (OA) biomarkers with knee radiographic OA (rOA), pain and function in this high-risk population for post-traumatic OA.

Design: ADVANCE compares combat-injured participants with age, rank, deployment and job-role frequency-matched uninjured participants. Post-injury immunoassay-measured serum biomarkers, knee radiographs, Knee Injury and Osteoarthritis Outcome Scale, and six-minute walk tests are reported. The primary analysis, adjusted for age, body mass, socioeconomic status, and ethnicity, was to determine any differences in biomarkers between those with/without combat injury, rOA and pain. Secondary analyses were performed to compare post-traumatic/idiopathic OA, painful/painfree rOA and injury patterns.

Results: A total of 1145 male participants were recruited, aged 34.1 ± 5.4, 8.9 ± 2.2 years post-injury (n = 579 trauma-exposed, of which, traumatic-amputation n = 161) or deployment (n = 566 matched). Cartilage oligomeric matrix protein (COMP) was significantly higher in the combat-injured group compared to uninjured ($p = 0.01$). Notably, COMP was significantly lower in the traumatic-amputation group compared to non-amputees ($p < 0.001$), decreasing relative to number of amputations ($p < 0.001$). Leptin was higher ($p = 0.005$) and adiponectin lower ($p = 0.017$) in those with v without knee pain, associated with an

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increased risk of 22% and 17% for pain, and 46% and 34% for painful rOA, respectively. There were no significant differences between trauma-exposed and unexposed participants with rOA.
Conclusions: The most notable findings of this large, unique study are the similarities between those with rOA regardless of trauma-exposure, the injury-pattern and traumatic-amputation-associated differences in COMP, and the relationship between adipokines and pain.

Introduction

Osteoarthritis (OA) is a progressive deterioration of articular cartilage and subchondral bone, associated with low-grade inflammation, altered biomechanics, and other factors, leading to a clinical syndrome of pain, stiffness, loss of function, and increased mortality.[1,2] The estimated annual medical cost of OA in the US is $72billion,[3] with an OA-related two-fold indirect cost of lost workplace productivity,[4] and rising incidence; the approximate 600 million global cases of OA in 2020 are expected to double by 2050.[5]

Identifying pre-clinical OA enables appropriate interventions and guides drug discovery, with molecular and imaging biomarkers employed as proxy measures to identify pathophysiological mechanisms related to extracellular matrix (ECM), inflammation or metabolic dysregulation.[6–8] Cartilage-derived biomarkers, such as cartilage oligomeric protein (COMP) and C-terminal cross-linked telopeptide of type II collagen (CTX-II), are associated with OA progression and cartilage loss.[9] In addition, cytokines such as interleukins (ILs) or tumour necrosis factor-alpha (TNF-α) suggest ongoing inflammatory processes, with adipokines contributing to this and indicating concurrent aberrant metabolism and systemic processes.[10] A key research challenge is population heterogeneity, with biomarkers offering an opportunity for phenotyping, thus enabling personalised treatment and improved drug trial recruitment.[11,12]

Post-traumatic OA (PTOA) is a widely used paradigm for biomarkers studies as it commonly presents in younger individuals with fewer comorbidities after a clear initiating event.[13,14] In addition, certain populations, including tactical and professional athletes, are at higher risk of OA due to occupational factors, including trauma.[11,15–18] The prospective ArmeD SerVices TrAuma RehabilitatioN OutComE (ADVANCE) cohort study was initiated to investigate the long-term outcomes of combat injury in British service personnel following the Afghanistan conflict (2003–2014).[19] Earlier work in this cohort has demonstrated that the amputee sub-population is a distant population from a metabolic, musculoskeletal, and psychological perspective,[20–22] with an amputation conferring a 4x increased risk of OA, as did sustaining a local knee injury during the traumatic episode.[22] The ADVANCE cohort offers the opportunity to develop tools for identifying those at higher risk of sequelae, such as PTOA, perhaps due to injury pattern, severity or individual predisposition, which can be translated into clinical practice. Therefore, the nested sub-study, Biomarkers and Joint Pain in Military Osteoarthritis (BioMilOA) was established to investigate the predictive value of a panel of candidate serum biomarkers associated with cartilage and ECM turnover, inflammation, and metabolism in this high-risk population for PTOA. The BioMilOA hypothesis is three-fold: there will be significant differences between those exposed and not exposed to combat trauma, those with and without radiological features of OA, and those with and without knee pain.

Methods

Study population

Eligibility criteria included male British service personnel (≥18 years), deployed between March 2016 and August 2020 and sustaining any combat-related traumatic injury (defined as requiring aeromedical evacuation), were recruited (n = 579), with an uninjured comparison population frequency-matched for age, rank, service, deployment period, and job-role (n = 566). Due to very small numbers of female UK military combat casualties and physiological sex differences, which might confound the study hypothesis, only male participants were recruited. Further details on participant identification and recruitment are found here.[19,20]

Ethical approval

Favourable opinion for ADVANCE was granted by the MOD Research Ethics Committee (MODREC:357PPE12), with subsequent approval for BioMilOA from the University of Nottingham Faculty of Medicine and Health Sciences REC (FMHS 170–1122). Study participation was voluntary, and each participant provided written informed consent.

Public and patient involvement

Public and patient involvement (PPI) is regularly performed via thrice-yearly focus groups, feedback questionnaires at each visit, quarterly newsletters, participant-focussed study outcome impact reports and the ADVANCE website (www.advancestudydmrc.org.uk). It has helped shape study design, further research questions, outcome measure recording and study logistics.

Study visits

Study visits occurred at the Defence Medical Rehabilitation Centre Headley Court (2015–2018) or Stanford Hall (2018–2020). A trained research nurse collected a range of assessments, including demographic data, medical history and traumatic injury, anthropometric data (height, weight, waist circumference), patient-reported outcome measures, radiographic assessment, serum sampling and functional tests. All participants were fasted and absent from caffeine and alcohol for at least 8 h before the visit. Body mass was corrected using an appropriate formula for missing limbs.[23] The functional six-minute walk tests (6MWT) were performed on a linear, flat 20 m course, with verbal instructions before and during, asking them to walk as far as they could between two cones, but not run, for six minutes, with regular time updates. Participants could use aids and stop to rest if required, with distance recorded to the nearest 0.5 m – further details on protocols used can be found here.[24]

The Knee injury and OA Outcome Score (KOOS) were recorded with 5 subscales scored from 0 (very severe) to 100 (no problem), which has divergent and convergent construct validity and high test-retest reliability.[25,26] Due to an error during data entry, only the KOOS Pain and KOOS Symptoms sub-scores were correctly recorded. The KOOS Pain subscale was used to determine the presence of pain in the index knee, with participants categorised as Pain+ or Pain−. Participants were dichotomised using a cut-off of 86.1, a threshold developed by consensus in a cohort of 155 participants aged 54 ± 12, 16 years post-meniscectomy, and validated in a prospective cohort of 1761 aged 23 (17–35) years old with an anterior cruciate ligament reconstruction.[27,28]

Self-reported, contemporaneous combat-injury and electronic health records (EHR) were used to classify injury patterns, including amputation (presence and number of) and local knee injury. Injured participants were coded as injured non-amputation (Inj-NA), injured amputation (Inj-A), knee injury (K-I) (when both Inj-A and K-I were present, they were coded as K-I, inline with previous analysis[22]). Study data were collected and managed using REDCap, hosted at Imperial College London, a web-based platform enabling secure and auditable data capture, export and integration into statistical and analytical software.[29]

Radiographic scoring

Semi-flexed (7–10°) posterior-anterior views of all possible participant knees were taken using a Synaflexer X-ray positioning frame (Synarc Inc, San Francisco, California). The tibiofemoral joint was scored using the Kellgren-Lawrence (KL) method, graded 0 ('none'), 1 ('doubtful'), 2 ('minimal'), 3 ('moderate') and 4 ('severe')[30] The US Food & Drugs Agency approved Knee OA Labelling Assistant (KOALA, Image Biopsy Lab, Vienna, Austria) was utilised, with manual checking, which offers an accuracy of 82%, the sensitivity of 78% and specificity of 88% for KL grades ≥1.[31] When participants had two variables for KL grade (from both knees), the index knee score signifying more advanced radiographic OA (rOA) was selected (higher KL grade). For those with an above-knee amputation, the single variable was used. Radiological OA was categorised as a KL score of ≥1, with participants classified as rOA+ or rOA−. This would not be expected in a population of this age and is the strongest predictor of confirmed OA.[12]

Biomarker assessment

Fasted blood was taken and centrifuged at 3500 rpm for 10 min, with serum aliquoted and stored in cryovials in monitored freezers at −80°. Sera underwent analysis for selected cartilage turnover biomarkers (COMP, CTX-II, N-propeptide of collagen IIA (PIIANP)), pro-inflammatory cytokines (IL-1β, IL-17α, TNF-α) and metabolic markers (leptin and adiponectin) using enzyme-linked immunosorbent assay (ELISA) or Meso Scale Discovery (MSD) by Affinity Biomarkers Lab (London, UK). This panel was selected to offer insights into some of the different pathological mechanisms underlying OA, including aberrant inflammation, tissue turnover and metabolism. Each plate included two kit controls and three internally identified quality control samples. The worst reported intra- or inter-variability coefficient of variation for each biomarker was; MSD: IL-17α CV < 9.5%, IL-1β < 7%, TNF-α < 15%; ELISA: COMP < 12%, Leptin < 7%, Adiponectin < 8%, CTX-II < 11%, PIIANP < 6%. For biomarker concentrations below the lower limit of quantification (LLOQ), a value halfway between zero and LLOQ threshold was selected, and for those above the upper LOQ (ULOQ), it was ULOQ threshold + 1. This was performed for IL-17α (< 0.54 = 0.27, n = 24), PIIANP (< 5.9 = 2.95 n = 23, > 1000 = 1001, n = 9), CTX-II (< 0.1 = 0.05, n = 421), and IL-1β (< 0.043 = 0.0215, n = 713).

Statistical analysis

Data were screened for normality visually using histograms, with parametric and non-parametric testing used accordingly. Univariate analysis was performed, depending on normality and groups (Mann-Whitney-U and Student's t, or Kruskal-Wallis and analysis of variance, ANOVA). Parametric data are presented as mean ± standard deviation (SD) and non-parametric data as median (interquartile range, IQR).

Unadjusted analyses were initially performed, followed by adjusted, with the confounders age, body mass, time from injury/deployment, rank (proxy for socio-economic status, SES[32]) and ethnicity adjusted for. This was performed by transforming the biomarkers using their natural logarithm and adjusting for the confounders using a regression model, with studentised residuals created and taken forward for analysis.[33] Trauma-exposure status was additionally controlled for in the pain analysis. Within an athletic population, the body mass index (BMI) can 'overscore' individuals with a high muscle mass; therefore, a body shape index (ABSI), calculated with BMI and waist circumference, was utilised.[34] Time from injury/deployment was measured from the participant's index deployment. Patient-reported and EHR were reviewed to identify those with knee pathology prior to index injury/deployment, of which there were 93 participants, 37 of whom had a specific injury associated with increased risk of OA (meniscal, cruciate, fracture), and the remainder reported persistent knee pain. Using the more conservative measure, the chi-squared test showed there were no differences between the two exposure groups, with correlation analysis showing no notable association with biomarkers; therefore, other confounders were prioritised in the model.

Primary analysis: Do serum biomarker concentrations differ between those with/without trauma exposure, knee rOA or pain?

- Two-group unadjusted and adjusted univariate analysis (Mann-Whitney-U/Student's t) of biomarkers, dichotomised by the presence of Exp, rOA or Pain. Specifically;
- Differences between those with and without trauma-exposure (Exp+/Exp-), knee rOA (rOA+/rOA-) and knee pain (Pain+/Pain-)

Secondary analyses: Do serum biomarker concentrations differ between those with idiopathic v PTOA, painful v painfree OA, or with different injury patterns?

- Four-, three- or two-group unadjusted and adjusted univariate analysis (Mann-Whitney-U/Student's t or Kruskal-Wallis/ANOVA) of biomarkers, dichotomised by the presence of Exp and rOA, Pain and rOA, traumatic-amputation, or the pattern of traumatic-injury, specifically;
- OA aetiology: Trauma- v non-trauma-associated OA (Exp-/rOA-, Exp-/rOA+, Exp+/rOA-, Exp+/rOA+)
- Painful OA: Painful v pain-free rOA (Pain-/rOA-, Pain+/rOA-, Pain-/rOA+, Pain+/rOA+)
- Injury pattern, amputation status: traumatic amputation vs non-traumatic amputation, stratified by number of amputations(0-3);
- Injury pattern, local knee injury: Inj-NA vs Inj-A vs K-I

When the adjusted analyses were significant, odds ratios (OR) were calculated using binary or multinominal logistic regression models containing the same confounders, using standardised biomarkers units (mean = 0, SD = 1), reported with 95% confidence intervals (95% CI). Finally, correlation analysis (using Spearman's or Pearson's, accordingly) was undertaken between the adjusted biomarkers and the rOA, pain, and 6MWT distance (6MWD). As all hypothesis and statistical tests were pre-planned, adjustment for multiple testing was not required.[35] Significance was set at 0.05. Analyses were performed in Stata 18 (StataCorp LLC, Texas) and GraphPad Prism 10 (Dotmatics, Boston).

Results

1145 male participants were recruited, aged 26.1 ± 5.2 years at the time of injury (cases) or deployment (comparison) and 34.1 ± 5.4 years old at baseline assessment. The mean average time from deployment or injury was 8.9 ± 2.2 years. Within the cohort, 579 suffered combat trauma (Exp+) and 566 were recruited as comparison participants (Exp-). 161 sustained a traumatic amputation (28% of Exp+); number of amputations 1 n = 85, 2 n = 65 and 3 n = 12, respectively. Demographic data for all participants can be found in Table I (additional data on ethnicity and injury type can be found in Supplementary Table 1 and 2). Complete biomarker data were available for 1118 participants, and radiographic data for 1074, which are presented, alongside KOOS Pain and 6MWD, in Table II.

	Total	Unexposed	Exposed (Exp+)		
		(Exp-)	All	No Amputation	Amputation
	N = 1145	N = 566	N = 579	N = 418	N = 161
Age, Mean (SD)	34.1 (5.4)	34.2 (5.4)	34.0 (5.3)	34.4 (5.6)	33.0 (4.6)
Body Mass Index, Mean (SD)	27.8 (3.7)	27.4 (3.4)	28.1 (3.9)	27.9 (3.7)	28.8 (4.4)
Abdo. Circum.(cm), Median (IQR)	93.5 (88.0–101.0)	92.0 (87.0–100.0)	94.0 (88.0–102.0)	94.0 (88.0–102.0)	95.0 (89.0–104.0)
Caucasian, N (%)	1008 (88%)	494 (87%)	514 (89%)	370 (89%)	144 (89%)
Rank, N (%)					
Junior NCO	754 (66%)	340 (60%)	414 (72%)	286 (68%)	128 (80%)
Senior NCO	253 (22%)	147 (26%)	106 (18%)	86 (21%)	20 (12%)
Officer	138 (12%)	79 (14%)	59 (10%)	46 (11%)	13 (8%)
NISS, Median (IQR)	12 (5–22)	-	12 (5–22)	9 (4–17)	25 (17–24)
Time from injury/deployment, Mean (SD)	8.9 (2.2)	8.8 (2.2)	8.9 (2.2)	9.2 (2.2)	8.1 (2.1)

SD – standard deviation, IQR – interquartile range, Abdo. – abdominal, circum. – circumference, cms – centimetres, NCO – non-commissioned officer, NISS – New Injury Severity Scale.

Table I Osteoarthritis and Cartilage

Demographic data for all participants, stratified by exposure (Exp-/Exp+) and amputation status.

	Total	Unexposed (Exp-)	Exposed (Exp+) No Amputation	Amputation	p-value Unadj./Adj.
	N = 1118	N = 553	N = 409	N = 156	
IL-1β (ng/l) Median (IQR)	0.0 (0.0–0.1)	0.0 0.0–0.1)	0.0 0.0–0.1)	0.0 0.0–0.1)	0.592~ 0.528~
TNF-α (ng/l) Mean (SD)	1.9 (± 0.6)	1.9 (± 0.6)	1.9 (± 0.5)	1.9 (± 0.4)	0.818^ 0.736^
IL-17α (ng/l) Median (IQR)	1.3 (1.0–1.8)	1.3 (1.0–1.8)	1.3 (1.0–1.8)	1.3 (1.0–1.9)	0.756~ 0.945~
CTX-II (ug/l) Median (IQR)	0.2 (0.1–0.6)	0.2 (0.1–0.7)	0.2 (0.1–0.6)	0.1 (0.1–0.7)	0.661~ 0.771~
Leptin (ug/l) Median (IQR)	5.7 (3.0–9.3)	5.5 (3.0–8.8)	5.7 (3.2–9.6)	6.3 (3.2–11.8)	0.070~ 0.361~
COMP (ug/l) Mean (SD)	263.6 (± 88.6)	267.1 (± 88.8)	279.5 (± 85.6)	209.3 (± 74.3)	< 0.001^ < 0.001^
Adipo (mg/l) Mean (SD)	6.3 (± 4.5)	6.2 (± 4.0)	6.6 (± 5.1)	6.1 (± 4.2)	0.350^ 0.731^
PIIANP (ug/l) Median (IQR)	109.1 (73.9–160.1)	109.2 (74.6–157.3)	110.5 (73.8–168.3)	105.8 (71.5–151.1)	0.333~ 0.364~
KL ≥1, N (%)	250 (23.8)	96 (17.4)	114 (28.3)	40 (42.6)	< 0.001~
Index knee KL Grade, N (%)					< 0.001~
0	799 (71)	456 (82)	289 (71)	55 (35)	
1	147 (13)	59 (11)	65 (16)	23 (15)	
2	74 (7)	26 (5)	35 (9)	13 (8)	
3	26 (2)	9 (2)	13 (3)	4 (3)	
4	3 (0)	2 (0)	1 (0)	0 (0)	
Missing	69 (6)	1 (0)	6 (1)	62 (40)	
Index knee KOOS Pain Median (IQR)	92 (78–100)	94 (83–100)	89 (72–100)	92 (78–100)	< 0.001~
KOOS Pain < 86.1, N (%)	405 (38)	179 (33)	185 (46)	41 (39)	< 0.001~
6MWD Mean (SD)	599 (± 117)	631 (± 96)	593 (± 118)	488 (± 121)	< 0.001^

IL – Interleukin, TNF – Tumour Necrosis Factor, CTX-II – C-terminal cross-linked telopeptide of type II collagen, COMP – cartilage oligomeric protein, PIIANP – N-propeptide of collagen IIA, Adipo – Adiponectin, KL – Kellgren-Lawrence, KOOS – Knee Injury and Osteoarthritis Outcome Score, 6MWT – Six-minute walk-test distance, 95% CI – 95% confidence interval, Unadj. – unadjusted, Adj. – Adjusted.
^threegroup oneway analysis of variance ~three group Kruskal-Wallis, dichotomised by exposure and amputation status.

Table II Osteoarthritis and Cartilage

Biomarker, radiographic, patient-reported and functional outcomes for all participants, stratified by exposure (Exp-/Exp+) and amputation status.

Participant refusal, sampling or analysis errors, and individuals with amputations account for all missing values.

Exposure status

Exposed participants have higher rates and more severe grades of rOA, with worse KOOS Pain scores and shorter 6MWD (all $p < 0.0001$). Unadjusted analysis showed that COMP was significantly higher in combat-trauma exposure ($p = 0.03$) (Fig. 1A), remaining after adjustment for age, SES, ethnicity and time from injury ($p = 0.02$). No other biomarkers were different.

Radiographic OA change

955 participants had paired radiographic and serum data (rOA+ n = 210, rOA- n = 745). Those with rOA were older, had a higher BMI,

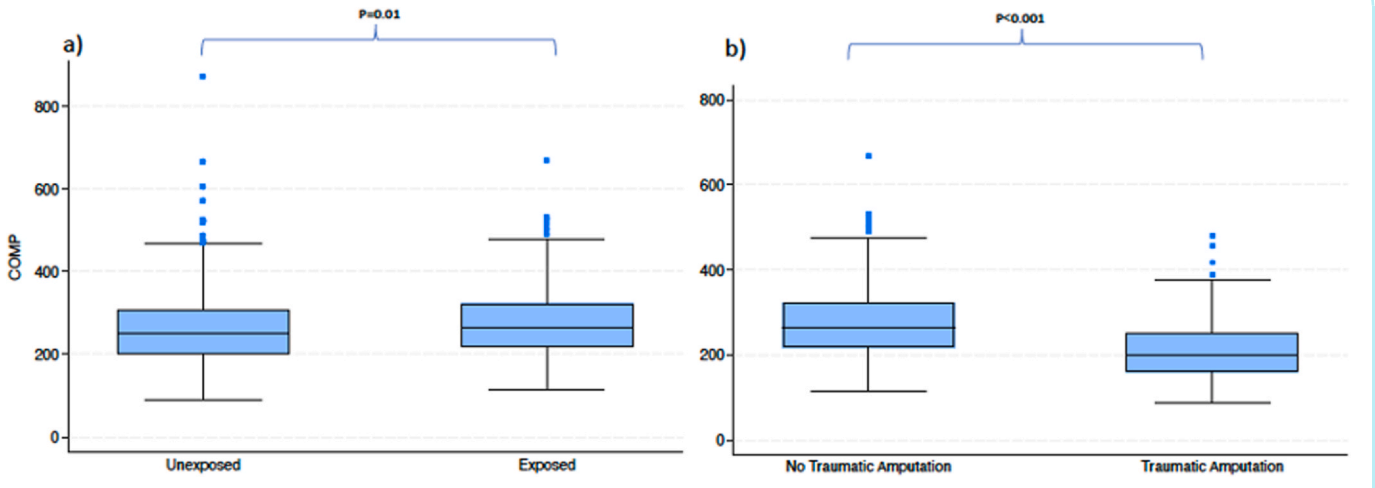

Fig. 1

Osteoarthritis and Cartilage

Differences in concentrations of cartilage oligomeric matrix protein (COMP) between those unexposed and exposed to combat trauma (a) and within those exposed to combat trauma, those without and with a traumatic amputation (b). Values in (ug/l), shown with median (IQR), test used: Student's t test.

and had worse KOOS Pain than those without (all $p < 0.001$). Those with rOA had significantly higher levels of leptin ($p < 0.001$) and COMP ($p = 0.005$) and significantly lower levels of PIIANP ($p = 0.001$) than those without (Table III). After adjustment for age, body mass, ethnicity, SES and time from injury, no biomarker remained significant.

Exposure and radiographic OA change

Four groups were created, dichotomised by exposure and rOA status:

1. Exp-/rOA− (n = 456, reference group)
2. Exp-/rOA+ (n = 96)
3. Exp+/rOA− (n = 289)
4. Exp+/rOA+ (n = 114)

Unadjusted analysis revealed significant between-group differences in leptin ($p = 0.003$), COMP ($p = 0.009$) and PIIANP ($p = 0.01$). Specifically, leptin was significantly higher in Exp−/rOA+ ($p = 0.05$) and Exp+/rOA+ ($p < 0.001$) v reference, and between Exp+/rOA− and Exp+/rOA+ ($p = 0.001$); PIIANP was significantly lower in Exp−/rOA+ ($p = 0.01$) and Exp+/rOA+ ($p = 0.027$) v reference, and between Exp−/rOA+ ($p = 0.003$) and Exp+/rOA+ ($p = 0.009$) v Exp+/rOA−; and COMP significantly higher in Exp+/rOA+ ($p = 0.031$) v reference.

After adjustment for age, body mass, SES, ethnicity and time from injury, no biomarker remained significant.

Knee pain status

Participants with pain were older, had increased and more severe OA, and shorter 6MWD than those without (all $p < 0.001$).

Unadjusted analysis demonstrated significantly higher leptin ($p < 0.0001$) and significantly lower adiponectin ($p = 0.01$) in those with pain compared to those without (Table IV, Fig. 2). Both remained significant after adjustment for age, body mass, SES, ethnicity, time from injury and exposure status, leptin ($p = 0.001$) with an OR of 1.22 (95% CI: 1.06,1.41) and adiponectin ($p = 0.004$), OR 0.83 (95% CI: 0.71,0.98).

Painful knee radiographic osteoarthritis

Four groups were formed, dichotomised by rOA and pain:

1. Pain−/rOA− (n = 483, reference group)
2. Pain+/rOA− (n = 258)
3. Pain−/rOA+ (n = 103)
4. Pain+/rOA+ (n = 104)

Those with Pain+/rOA+ had higher BMI and reduced 6MWD (both $p < 0.001$) (Table IV). Unadjusted analysis demonstrated significant between-group differences for COMP ($p = 0.05$), adiponectin ($p = 0.01$) and leptin ($p < 0.001$). Specifically, there were non-significant differences for COMP; significantly lower adiponectin in Pain+/rOA+ v reference ($p = 0.03$) and Pain−/rOA+ ($p = 0.02$); and significantly higher leptin in Pain+/rOA− ($p = 0.001$) and in Pain+/rOA+ v all groups (reference $p < 0.001$, Pain+/rOA− and Pain−/rOA+ both $p = 0.006$).

After adjustment for age, body mass, SES, ethnicity, time from injury and exposure status, adiponectin ($p = 0.017$) and leptin ($p = 0.006$) remained significant, with significantly lower adiponectin in Pain+/rOA+ ($p = 0.028$), and significantly higher leptin in Pain+/rOA− ($p = 0.007$) and Pain+/rOA+ (v reference $p = 0.001$, and Pain−/rOA+ $p = 0.05$). Leptin and adiponectin ORs for Pain+/rOA+ from the reference group were 1.46 (95% CI: 1.19,1.79) and 0.66 (95% CI: 0.45,0.98), respectively.

Associations between the biomarkers and radiographic change, pain and function

There were statistically significant correlations between four biomarkers (adjusted for age, body mass, SES, ethnicity, and time from injury), and rOA, pain and/or 6MWD. Leptin had a correlation coefficient of 0.12 ($p < 0.001$) with knee pain and −0.11 ($p < 0.001$) with 6MWD, adiponectin of −0.08 ($p = 0.01$) with pain, TNF-α of −0.08 ($p = 0.01$) with 6MWD, and PIIANP −0.06 ($p = 0.05$) with rOA (Fig. 3).

	Total	rOA-	rOA+	p-value Unadj./Adj.	Exp-/rOA-	Exp-/rOA+	Exp+ /rOA-	Exp+ /rOA+	p-value Unadj./Adj.
	N = 955	N = 745	N = 210		N = 456	N = 96	N = 289	N = 114	
Age Mean (SD)	34.3 (± 5.5)	33.7 (± 5.2)	36.5 (± 5.9)	< 0.001#	33.7 (± 5.2)	36.7 (± 6.0)	33.6 (± 5.3)	36.3 (± 5.8)	< 0.001^
BMI Mean (SD)	27.6 (± 3.5)	27.3 (± 3.3)	28.7 (± 3.8)	< 0.001#	27.2 (± 3.3)	28.3 (± 3.5)	27.4 (± 3.4)	29.0 (± 3.9)	< 0.001^
IL-1β (ng/l) Median (IQR)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.469" 0.405	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.788~ 0.735~
TNF-α (ng/l) Mean (SD)	1.9 (± 0.6)	1.9 (± 0.6)	1.9 (± 0.5)	0.686# 0.798	2.0 (± 0.7)	1.9 (± 0.4)	1.9 (± 0.6)	2.0 (± 0.5)	0.784^ 0.863^
IL-17α (ng/l) Median (IQR)	1.3 (1.0–1.8)	1.3 (1.0–1.8)	1.3 (1.0–2.0)	0.071" 0.172	1.3 (0.9–1.8)	1.3 (1.0–1.9)	1.3 (1.0–1.7)	1.4 (1.0–2.1)	0.331~ 0.535~
CTX-II (ug/l) Median (IQR)	0.2 (0.1–0.6)	0.2 (0.1–0.6)	0.2 (0.1–0.7)	0.186" 0.709	0.2 (0.1–0.7)	0.4 (0.1–0.8)	0.2 (0.1–0.6)	0.2 (0.1–0.6)	0.412~ 0.819~
Leptin (ug/l) Median (IQR)	5.6 (3.0–9.1)	5.3 (2.9–8.7)	6.6 (4.0–10.1)	< 0.001" 0.075	5.3 (2.8–8.7)	6.1 (3.5–9.1)	5.3 (2.9–8.7)	7.0 (4.2–11.6)	0.003~ 0.227~
COMP (ug/l) Mean (SD)	272.5 (± 87.8)	268.2 (± 86.3)	287.4 (± 91.6)	0.005# 0.789	263.4 (± 86.8)	285.6 (± 96.0)	275.9 (± 85.0)	289.0 (± 88.1)	0.009^ 0.116^
Adipo (mg/l) Mean (SD)	6.4 (± 4.5)	6.4 (± 4.4)	6.1 (± 5.0)	0.289# 0.151	6.3 (± 4.2)	5.8 (± 3.1)	6.7 (± 4.7)	6.3 (± 6.2)	0.386^ 0.353^
PIIANP (ug/l) Median (IQR)	109.9 (73.9–162.5)	115.0 (77.0–168.5)	96.0 (70.2–144.2)	0.001" 0.045	114.7 (75.8–163.9)	95.5 (71.0–141.1)	115.6 (81.8–178.3)	96.8 (68.7–150.1)	0.010~ 0.579~
Index knee KOOS Pain Median (IQR)	94 (81–100)	94 (81–100)	86 (69–100)	< 0.001"	97 (83–100)	89 (75–100)	92 (75–100)	86 (67–100)	< 0.001~
6MWD Mean (SD)	615 (± 106)	617 (± 106)	609 (± 106)	0.35#	629 (± 95)	637 (± 100)	597 (± 119)	585 (± 106)	< 0.001^

IL – Interleukin, TNF – Tumour Necrosis Factor, CTX-II - C-terminal cross-linked telopeptide of type II collagen, COMP - cartilage oligomeric protein, PIIANP - N-propeptide of collagen IIA, KL - Kellgren-Lawrence, KOOS – Knee Injury and Osteoarthritis Outcome Score, 6MWD – Six-minute walk test distance, rOA – radiographic osteoarthritis, Unadj. – unadjusted, Adj. - adjusted.
#Two-group Student's t "two-group Mann-Whitney-U, dichotomised by presence of radiographic osteoarthritis.
^Four-group oneway analysis of variance ~four-group Kruskal-Wallis, dichotomised by presence of radiographic osteoarthritis and exposure status.

Table III

Osteoarthritis and Cartilage

Demographic and outcome differences between those with and without knee radiographic osteoarthritis changes (rOA-/rOA+), further stratified by exposure status (Exp-/Exp+).

Amputation status

Within the exposed group, data were available for those with (n = 156) and without (n = 409) traumatic amputation. Those with a traumatic amputation achieved a lower 6MWD ($p < 0.001$).

Unadjusted analysis revealed significantly lower COMP in traumatic amputation ($p < 0.001$), which remained significant after adjustment for age, body mass, SES, ethnicity and time from injury ($p < 0.001$), compared to those without a traumatic amputation (Fig. 1b).

The levels of COMP reduced relative to the number of amputations; none (n = 409) 264.2 (217.6–322.8), one (n = 81) 232.8 (180.4–280.4), two (n = 63) 167.4 (137.9–206.9), three (n = 12) 132.9 (118.6–184.2), $p < 0.001$ (Fig. 4).

In those with an amputation who had radiographs (n = 94), those with rOA (n = 40) showed significantly higher CTX-II ($p = 0.009$) and significantly lower IL-17α ($p = 0.03$) than without (n = 54) in unadjusted analysis (Supplementary Table 3). Only IL-17α remained significant after adjustment for age, body mass, SES, ethnicity and time from injury ($p = 0.02$).

In those with an amputation, serum and KOOS Pain scores (n = 105) showed no differences between those with (Pain+, n = 41) or without pain (Pain-, n = 64).

Specific knee injury

Within the exposed group with serum (n = 565), three groups were formed:

1. Inj-NA (n = 389, reference group)
2. Inj-A (n = 141)
3. K-I (n = 35)

Two biomarkers were significant on unadjusted analysis (COMP, $p < 0.001$ and leptin, $p = 0.02$) (Supplementary Table 4). COMP was significantly lower in Inj-A (209.0 ± 76.1 ug/l, $p < 0.001$) and K-I (243.3 ± 69.0 ug/l, $p = 0.03$) v reference (280.3 ± 86.2 ug/l), which remained significant after adjustment (Inj-A $p < 0.001$, K-I $p = 0.05$). Those with K-I had significantly higher leptin (8.68 ug/l, IQR: 4.57–12.86) than reference (5.68 ug/l, IQR: 3.16–9.37, $p = 0.003$) and Inj-A (6.1 ug/l, IQR: 3.26–11.67, $p = 0.02$), which after adjustment, remained significant for K-I v reference ($p = 0.003$) and Inj-A ($p = 0.009$). COMP had a non-significant OR, and leptin had an OR of 1.33 (95% CI: 1.09,1.63) for rOA after K-I.

Discussion

This is the largest study investigating candidate biomarkers of early OA in a young, physically active, homogenous male population at high risk for PTOA. It has several key findings. COMP was significantly higher following combat injury compared to non-injured participants, but significantly lower in those with a traumatic amputation compared to non-amputees, proportional to the number of amputations. To the author's knowledge, this is the first description of such a finding. Remarkably, there were no differences between those with trauma-exposed rOA v non-trauma-exposed rOA. Increased leptin and decreased adiponectin were associated with an increased risk of knee pain of 22% and 17%, and painful rOA 46% and

	Total	Pain-	Pain+	p-value Unadj./Adj.	Pain-/rOA-	Pain+/rOA-	Pain-/rOA+	Pain+/rOA+	p-value Unadj./Adj.
	N = 949	N = 587	N = 362		N = 483	N = 258	N = 103	N = 104	
Age Mean (SD)	34.3 (± 5.5)	34.1 (± 5.3)	34.6 (± 5.7)	0.268#	33.7 (± 5.1)	33.7 (± 5.4)	36.0 (± 5.9)	36.8 (± 5.8)	< 0.001^
BMI Mean (SD)	27.6 (± 3.5)	27.2 (± 3.3)	28.3 (± 3.7)	< 0.001#	27.1 (± 3.3)	27.8 (± 3.5)	27.7 (± 3.4)	29.6 (± 3.8)	< 0.001^
IL-1β (ng/l) Median (IQR)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.195" 0.910"	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.458~ 0.418~
TNF-α (ng/l) Mean (SD)	1.9 (± 0.6)	1.9 (± 0.6)	1.9 (± 0.7)	0.887# 0.790#	1.9 (± 0.6)	2.0 (± 0.7)	1.9 (± 0.4)	1.9 (± 0.5)	0.955^ 0.987^
IL-17α (ng/l) Median (IQR)	1.3 (1.0–1.8)	1.3 (1.0–1.8)	1.3 (1.0–1.9)	0.802" 0.995"	1.3 (1.0–1.7)	1.3 (1.0–1.8)	1.3 (1.0–2.1)	1.4 (1.0–2.0)	0.758~ 0.533~
CTX-II (ug/l) Median (IQR)	0.2 (0.1–0.6)	0.2 (0.1–0.6)	0.2 (0.1–0.7)	0.739" 0.407"	0.2 (0.1–0.6)	0.2 (0.1–0.6)	0.2 (0.1–0.6)	0.2 (0.1–0.8)	0.574~ 0.816~
Leptin (ug/l) Median (IQR)	5.6 (3.1–9.0)	5.2 (2.5–8.3)	6.0 (3.8–10.0)	< 0.001" 0.001"	5.1 (2.5–8.3)	5.7 (3.5–9.4)	5.8 (3.2–8.5)	7.4 (4.8–11.2)	< 0.001~ 0.008~
COMP (ug/l) Mean (SD)	272.6 (± 87.8)	270.6 (± 85.4)	275.9 (± 91.7)	0.367# 0.615#	267.7 (± 85.2)	269.8 (± 88.2)	284.0 (± 85.2)	290.9 (± 98.8)	0.045^ 0.904^
Adipo (mg/l) Mean (SD)	6.4 (± 4.6)	6.7 (± 4.9)	5.9 (± 3.8)	< 0.010# 0.004#	6.6 (± 4.5)	6.2 (± 4.2)	7.0 (± 6.4)	5.2 (± 2.8)	0.012^ 0.019^
PIIANP (ug/l) Median (IQR)	110.2 (74.3–162.5)	113.2 (76.3–163.9)	106.8 (72.9–160.2)	0.115" 0.192"	118.3 (77.3–169.6)	110.5 (76.3–166.9)	97.2 (71.6–144.2)	90.8 (65.2–146.1)	0.206~ 0.122~
6MWD Mean (SD)	615 (± 106)	629 (± 99)	593 (± 113)	< 0.001#	629 (± 99)	595 (± 114)	631 (± 98)	587 (± 111)	< 0.001^

IL – Interleukin, TNF – Tumour Necrosis Factor, CTX-II - C-terminal cross-linked telopeptide of type II collagen, COMP - cartilage oligomeric protein, PIIANP - N-propeptide of collagen IIA, rOA – radiographic osteoarthritis, KL - Kellgren-Lawrence, 6MWD – Six-minute walk test distance, Unadj. – unadjusted, Adj. – adjusted.
#Two-group Student's t "two-group Mann-Whitney-U, dichotomised by presence of pain.
^Four-group one-way analysis of variance ~four-group Kruskal-Wallis, dichotomised by the presence of pain and radiographic osteoarthritis.

Table IV Osteoarthritis and Cartilage

Demographic and outcome in whole population data stratified by the self-reporting of knee pain (Pain-/Pain+), further stratified by the presence of radiographic osteoarthritis (rOA-/rOA+).

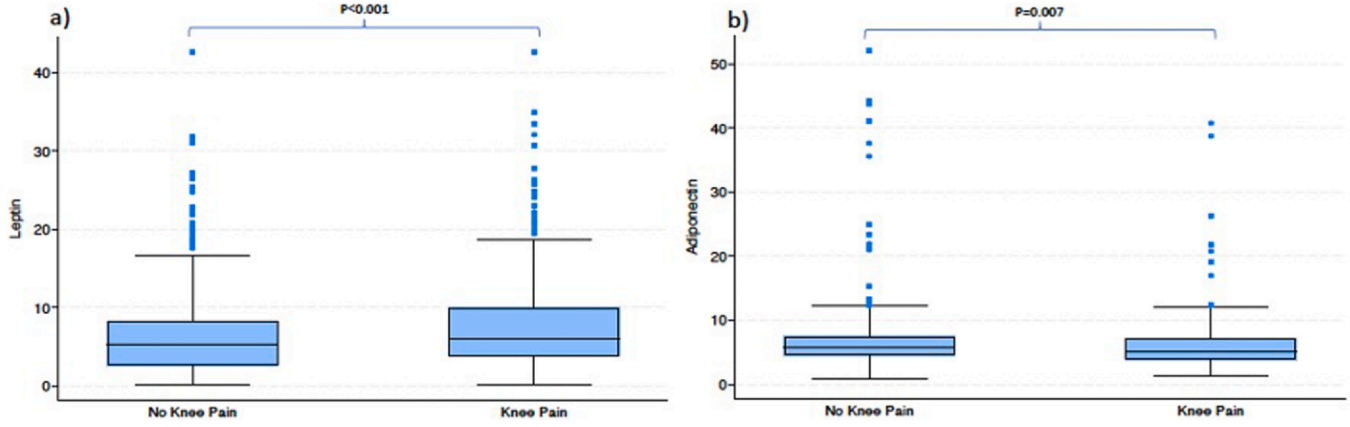

Fig. 2 Osteoarthritis and Cartilage

Differences in leptin (a) and adiponectin (b) between those not reporting and reporting knee pain. Leptin values in ug/l, adiponectin mg/l, shown with median (IQR), test used: Student's t and Mann-Whitney-U.

34%, respectively. There were weak correlations between PIIANP with rOA, leptin and adiponectin with pain, and leptin and TNF-α with 6MWD. An initial local knee injury influenced outcomes, with those sustaining a traumatic knee injury having higher levels of leptin compared to other trauma-exposed participants, associated with a 33% risk of rOA, and lower levels of COMP compared to trauma-exposed non-amputees.

Understanding the molecular picture following trauma is important, which, given the frequency and severity similarities of polytrauma following road traffic accidents, is a generalisable research question.[36] Only one biomarker, COMP, was significantly different, suggesting that COMP may play a role in the body's response to injury and maintenance of cartilage integrity. COMP, a collagen-network stabiliser binding type I and type II collagen fibres

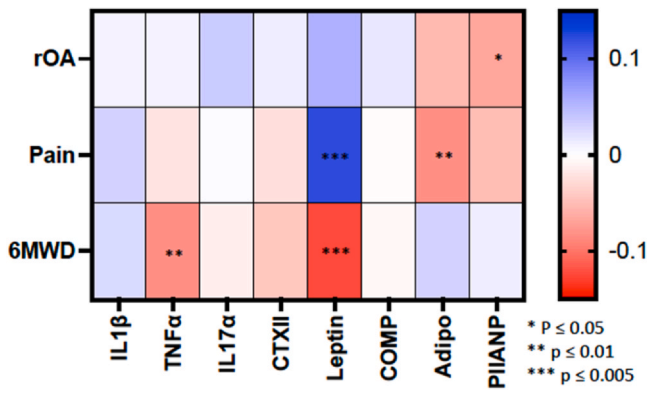

Fig. 3 Osteoarthritis and Cartilage

Heatmap demonstrating the correlations between the panel of fully adjusted biomarkers and the presence of early radiographic osteoarthritis change, the presence of knee pain and the distance achieved on the six-minute walk test. Spearman or Pearson's correlation, depending on normality.

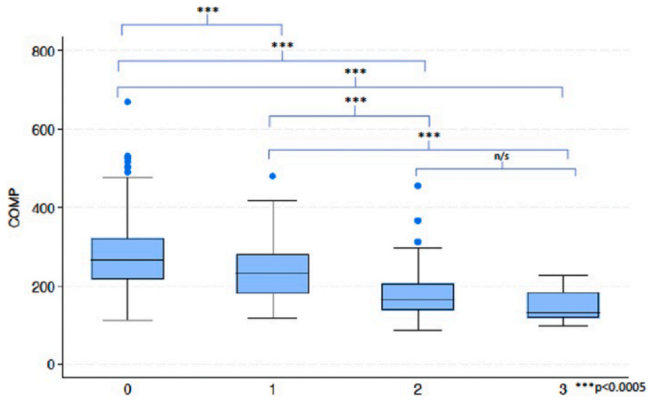

Fig. 4 Osteoarthritis and Cartilage

Differences in levels of cartilage oligomeric protein stratified by quantity of traumatic limb amputations. Values in (ug/l), shown with median (IQR), test used: oneway analysis of variance.

mainly expressed by cartilage,[37] is associated with OA[9]and other conditions including liver, lung, and skin fibrosis.[37,38] It is possible, therefore, that the increase in COMP is representative of fibrosis following trauma, which might have implications for future joint and cardiovascular health.[20] While a traumatic amputation is likely to lead to significant fibrosis, it will also reduce the cartilage volume to synthesise it. This study reports, for the first time, significantly lower COMP following traumatic amputation, quantified by the number of amputated limbs. This is a notable finding, providing evidence of cartilage volume in COMP synthesis, which, by extension, also includes cartilage health, and this is supported by those with a local knee injury having lower COMP levels. The lack of significant difference between those who sustained two and three amputated limbs is likely due to upper-limb involvement, with resultant reduced cartilage loss volume. COMP levels seen in traumatic amputations are lower than a US military combat amputee population, although this could be due to their far smaller numbers (n = 31 v n = 161 in this study).[39]

One of this study's most striking results comes from the analysis stratified by exposure and rOA. The pre-specified hypothesis was that there would be a difference between those developing OA following trauma exposure compared to the unexposed, predicated on the traumatic-dominant mechanism displaying a significantly different molecular pattern to an idiopathic-dominant (albeit accelerated) pattern. This was not what was found. However, these surprising results were in keeping with genetic studies that have yet to find differences between idiopathic and PTOA[40,41] and animal models postulating idiopathic and PTOA share a common 'mechanoinflammation' mechanism.[42] This finding, if validated in other populations, would enable results from PTOA studies to be extrapolated across all OA research fields.

The next area was the differences in serum biomarkers between those with and without rOA. KL ≥1 was selected purposefully as the criteria as the strongest predictor of diagnosed OA,[12] especially in this young population where rOA should not be present. Although leptin, COMP and PIIANP were significantly different in unadjusted analysis, no biomarkers remained significant after adjustment for age, body mass, SES and ethnicity (all independent risk factors for OA development and progression[1,11,43]). The lack of significant results might reflect their fluid type or be related to the time from injury, as the evidence is poor regarding the utility of serum biomarkers over a year from injury.[6] A recent review by the study authors synthesised all biomarkers measured a year or more from injury, reporting eight studies with 879 participants.[6] 38 serum biomarkers were measured, and only three (cleavage of type II collagen, hyaluronic acid, N-telopeptide of type I collagen) had a relationship to rOA, and one (TNF) to pain (also, like this study, measured by KOOS).[6] This suggests that biomarkers acknowledged to have value in the early stage following injury might not have the same value later in the disease course, and further evidence is required to understand this.[6,8,44] In addition, CTX-II, selected by the Foundation for the National Institutes of Health (FNIH) OA Biomarkers Consortium (BC) as a candidate marker[8] was measured using the serum, not the urinary form, which might explain the results.

Early identification of individuals with painful OA is essential, given pain is the primary symptom, the leading cause of medical consultation and very complex to manage.[45,46] In this study, both leptin and adiponectin were associated with an increased risk of developing knee pain and painful knee rOA. Leptin is believed to have a role in cartilage degeneration through cytokine mediation and synthesis of cartilage proteoglycan,[40] with animal studies demonstrating that deletion of the leptin gene prevents OA development.[47] Adiponectin has been seen to have an anti-inflammatory effect and, in addition, analgesic properties through inhibition of p-p38 MAPK signalling.[48] These findings are consistent with the understanding that adipokines can influence pain via serotonin, inflammatory and metabolic pathways[48,49]; therefore, potentially, they could be used to identify and categorise a painful OA phenotype. The increased leptin levels in those with a local knee injury suggest that this biomarker may offer some increased value for the knee joint specifically. It is important to note that these differences, whilst statistically significant, are small and therefore of uncertain clinical significance but do generate hypotheses for further analysis.

Ultimately, the value of any biomarker is the ability to detect predetermined outcomes and quantify the pathophysiological process in question. Correlation analyses between all biomarkers and key outcome measures, rOA, knee pain, and 6MWD, showed limited cross-sectional value. Only PIIANP correlated with rOA, leptin and adiponectin to pain, and leptin and TNF-α to function, with all

correlations weak (r = −0.11–0.12). As described above, these findings could be partly explained by both time from injury and biomarker type, with further work required to understand their value and potential roles.[6,44]

The key strength of this study is its design; large numbers (n = 1145), and frequency-matched comparison population. Additionally, the median time from injury is beyond the five-year period suggested by UK BioBank as highest risk for PTOA.[40] The study's key limitation is that all participants are young and male. This population is relatively under-researched in OA research with many studies involving postmenopausal females, and therefore fills an unmet need, however, validation of findings might be challenging. Outside of the amputee groups, populations drawn from recreational or elite sports or other military populations, including the US[39] are comparable and potentially able to validate findings. Further weaknesses are the ELISA floor and ceiling effect, particularly CTX-II and IL-1β, which had 37% and 60% of values below the LLOQ, and the single radiographic view of the knee, which may underscore rOA.

This study reports the differences between those exposed and not exposed to combat trauma, rOA, and pain in a young, physically active population at high risk for PTOA. Significant findings include, contrary to the predetermined hypothesis, a lack of difference between those with early-onset idiopathic OA and a PTOA presentation, a quantification of the relationship between traumatic amputation (and therefore cartilage synthesis) to COMP, and the potential value of adipokines for both painful OA phenotyping and identification of pain and functional outcomes.

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Author contributions

ANB, CJB, NTF, and AMJB conceived the study. OOS and ANB gathered data. JS undertook study logistics. OOS analysed the data with support from SS, AV, JS, and SK. OOS drafted the manuscript, with critical input from all authors. FW and JB, alongside SK and ANB, provided strategic direction and senior guidance. All authors agreed the final version and are accountable for accuracy and integrity. OOS acts as the guarantor and corresponding author.

Declaration of competing interest

There are no competing interests to declare.

Declaration of Generative AI and AI-assisted technologies in the writing process

No AI technology was used during the study or preparation of this manuscript.

Data Availability

Data relate to serving and ex-serving military personnel and thus are sensitive and have not been made widely available. Requests for data can be made via the corresponding author can will be considered on a case-by-case basis and are subject to UK Ministry of Defence clearance. The ADVANCE study protocol is available online. The code used for analysis will be shared on request to the corresponding author.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.joca.2024.07.016.

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