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Investigating the value of serum biomarkers in predicting the development of post-traumatic osteoarthritis of the knee

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List of tables and figures

Publications arising from this thesis

Publications listed in order of appearance in the thesis.

Published manuscripts

(1) O'Sullivan O. Osteoarthritis: Pathophysiology and Classification of a Common Disabling Condition. In: Bennett, G., Goodall, E. (eds) *The Palgrave Encyclopedia of Disability*. Palgrave Macmillan, Cham, 2024. doi:10.1007/978-3-031-40858-8_286-1

(2) O'Sullivan O, *et al.* Osteoarthritis in the UK Armed Forces: a review of its impact, treatment and future research. *BMJ Mil Health* 2024;170**:**359-364 doi:10.1136/military-2023-002390

(3) O'Sullivan O. Electronic health records underreport the incidence and prevalence of osteoarthritis in the UK Armed Forces. *BMJ Mil Health* doi:10.1136/military-2025-003054

(4) O'Sullivan O, *et al.* Knee MRI biomarkers associated with structural, functional and symptomatic changes at least a year from ACL injury - A systematic review. *Osteoarthritis and Cartilage Open*, 2023;5(3);100385 doi:10.1016/j.ocarto.2023.100385

(5) O'Sullivan O, *et al*. Current status of catabolic, anabolic and inflammatory biomarkers associated with structural and symptomatic changes in the chronic phase of post-traumatic knee osteoarthritis– a systematic review. *Osteoarthritis and Cartilage Open* 2023:5(4):100412 doi:10.1016/j.ocarto.2023.100412

(6) O'Sullivan O, *et al*. Association of serum biomarkers with radiographic knee osteoarthritis, knee pain and function in a young, male, trauma-exposed population – Findings from the ADVANCE study. *Osteoarthritis and Cartilage* 2024:32(12);636-1646 doi:10.1016/j.joca.2024.07.016

(7) Behan F…O'Sullivan O. Osteoarthritis exercise interventions for a younger adult population – a narrative review. *Int J Sports Med* Published Online: 03 June 2025 doi:10.1055/a-2627-3277

(8) O'Sullivan O, *et al*. Prevention of Post-Traumatic Osteoarthritis in the Military: Relevance of OPTIKNEE and Osteoarthritis Action Alliance recommendations *BMJ Mil Health* Published Online: 08 October 2024. doi:10.1136/military-2024-00281

(9) O'Sullivan O. Management and prevention strategies for osteoarthritis in tactical athletes *BMJ Mil Health* Published Online: 11 June 2024 doi:10.1136/military-2024-002719

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(10) O'Sullivan O, *et al*. Insights into knee post-traumatic osteoarthritis pathophysiology from the relationship of serum biomarkers to radiographic features in the ADVANCE cohort. *Res Sq* doi:10.21203/rs.3.rs-6120483/v1

(11) O'Sullivan O, *et al*. Influence of Major Trauma and Lower Limb Loss on Radiographic Progression and Incidence of Knee Osteoarthritis and Pain: A Comparative and Predictive Analysis from the ADVANCE Study. *Res Sq* doi:10.21203/rs.3.rs-6119632/v1

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(14) O'Sullivan O. Preliminary analysis of validated serum osteoarthritis biomarkers in the ADVANCE cohort suggests a relationship between trauma exposure and osteoarthritis pathophysiology. *BMJ Mil Health* 2025;**171:**e1.

(15) O'Sullivan O, *et al*. 181 Serum biomarker associations with radiographic osteoarthritis in the Armed Service Trauma Rehabilitation Outcome (ADVANCE) study. *Osteoarthritis and Cartilage* 2024:32;S135-S136

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(17) O'Sullivan O, *et al.* Relationships between serum biomarkers and radiographic features give insight into knee osteoarthritis pathophysiology: The ADVANCE cohort. *Osteoarthritis and Cartilage* 2025:33(6);781

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List of abbreviations

6MWT - Six-Minute Walk-Tests
6MWD - Six-Minute Walk-test Distance
95% CI - 95% confidence intervals
AAOS - American Academy of Orthopaedic Surgeons
ABL - Affinity Biomarker Labs
ABSI - A Body Shape Index
ACF – Ante-Cubital Fossa
ACL (R) - Anterior Cruciate Ligament (Reconstruction)
ACLOAS - ACL OA Score
ACR - American College of Rheumatology
ADMR - Academic Department of Military Rehabilitation
ADAMTS - A Disintegrin and Metalloprotinease with Thrombospondon motifs
ADVANCE - Armed Services Trauma and Rehabilitation Outcome
AIC - Akaike Information Criteria
ANOVA - Analysis Of Variance
ARGS - Alanine, aRginine, Glycine, Serine
AT1B2 - sodium/potassium-transporting ATPase subunit beta-2
ATLAS - Arthritis Training, Learning And up-Skilling
AUC – Area Under the Curve
AUROC - Area Under The Receiver Operator Curve
AxSpA - Axial Spondyloarthropathy
BioMilOA - Biomarkers and Joint Pain in Military Osteoarthritis
BIC - Bayesian information criteria
BIPEDS - Burden of Disease, Investigative, Prognostic, Efficacy of Intervention, Diagnostic, Safety
BL - Baseline
BLOKS - Boston Leeds OA Knee Score
BMD - Bone Mineral Density
BMI – Body Mass Index
BML - Bone Marrow Lesions
BPG - Best Practice Guidance
C2M - Collagen Type-2 Specific Neoepitope
CAI - Combat-Associated Injury
CCL - Chemokine (C-C Motif) ligand
CENTRAL - Central Register of Controlled Trials
CLM9 - CMRF35-like module 9
CM - centimetres
CN - Charcot neuro-osteoarthropathy
CoC - Chain of command
COL2A1 - Collagen, Type II, Alpha 1
COMP - Cartilage Oligomeric Matrix Protein
CON - Control
(hs) CRP – (high sensitivity) C-Reactive Protein
CTX-I(II) - Carboxyl-Terminal Telopeptide of Collagen Type I(II)
CV – Cardiovascular
CXCL - Chemokine (C-X-C Motif) Ligand
DAMP - Disease-Associated Molecular Patterns
DEXA - Dual Energy X-ray Absorptiometry
DMICP - Defence Medical Information Capability Programme
DMOAD - Disease-modifying anti-OA drug
DMRC - Defence Medical Rehabilitation Centre
DMS - Defence Medical Services
ECM - Extracellular matrix
EHR - Electronic Health Records
ELISA - Enzyme-Linked Immunosorbent Assay
EMA - European Medicines Agency
ENPP5 - Ectonucleotide Pyrophosphatase/Phosphodiesterase Family Member 5
ESCEO - European Society of Osteoporosis, OA and MSK Diseases
EULAR - European Alliance of Associations for Rheumatology (formerly, European League Against Rheumatism)
Exp (+/-) – Exposure (present/absent)
Exp-A – Exposed to trauma and sustained an amputation
Exp-K – Exposed to trauma and sustained a local knee injury
Exp-NA – Exposed to trauma and did not sustain an amputation or local knee injury
FABPA - Fatty Acid Binding Protein For Adipocyte
FDA - Food and Drugs Administration
FNIH - Foundation for the National Institutes of Health
FMHS - Faculty of Medicine and Health Sciences
FOAR-score - Future OA Risk score

FRZB – Frizzled-Related Protein
FU1(2) – Follow-Up 1 (2)
GAD-7 - Generalised Anxiety Disorder-7
GLP-1 - Glucagon-Like Peptide-1
(v) GRF – (vertical) Ground Reaction Force
GSV - Greater Saphenous Vein
H6ST3 - heparan-sulfate 6-O-sulfotransferase 3
HA - Hyaluronic Acid
HbA1c - glycated haemoglobin
IC – Initial Contact
IFN - Interferon
IGF - Insulin-like Growth Factor
IKDC - International Knee Documentation Committee
INJ – Injury, lower-limb, recent
IQR - Interquartile Range
IL – Interleukin
IRR – Incidence Rate Ratio
iOA – idiopathic OA
IPAQ - International Physical Activity Questionnaire
IRIS - Academic Unit of Injury, Recovery and Inflammation Sciences
JSN - Joint Space Narrowing
JTTR - Joint Theatre Trauma Registry
KAM - Knee Adduction Moment
KANON - Knee ACL, Nonsurgical versus Surgical
KCE1L - Potassium Voltage Gated Channel Subfamily E Regulatory Beta Subunit 5
KEM – Knee Extension Moment
KFA – Knee Flexion Angle
KFM – Knee Flexion Moment
KG - kilograms
KHJP - Knee and Hip Joint Pain
KL - Kellgren-Lawrence
KOA - Knee OA
KOALA - Knee OA Labelling Assistant
KOSS - Knee OA Scoring System
LASSO - Least Absolute Shrinkage And Selection Operator
LBP - lipopolysaccharide-binding protein
LFC - Lateral Femoral Condyle
LLOQ - Lower Limit Of Quantification
LSAMP - Limbic System-Associated Membrane Protein
LT - Lateral Tibia
LTP - Lateral Tibial Plateau
MCID - Minimum Clinically Important Difference
MDC - Minimal Detectable Change
MDT - Multidisciplinary
MFC - Medial Femoral Condyle
miRNA - microRNA small nucleolar RNA
MOAKS - MRI OA Knee Score
MoD – Ministry of Defence
MODREC - MoD Research Ethics Committee
MOST - Multicenter OA Study
MMP - Matrix Metalloproteinases
MRI - Magnetic Resonance Imaging
MSD - Meso Scale Discovery
MSKI - Musculoskeletal Injuries
MT - Medial Tibial
MTP - Medial Tibial Plateau
NAR3 - ecto-ADP-ribosyltransferase 3
NC-SEC - National statistics socio-economic classification
NCO – Non-Commissioned Officer
NICE - National Institute of Health Institute for Health and Care Excellence
NISS – New Injury Severity Scale
NOS - Newcastle-Ottawa Scale
NTRI - Neurotrimin
NTX-1 – N-Terminal Cross-Linked Type I Collagen
OA – Osteoarthritis
OAAA - OA Action Alliance
OAI - OA Initiative
OA LEAP - OA in ADVANCE Longitudinal assEssment of biomArkers and Proteomics
OP - Osteophytes
OR – Odds Ratio
OARSI - Osteoarthritis Research Society International

OBCAM - Opioid-Binding Protein/Cell Adhesion Molecule
OMERACT - Outcomes Measures in Rheumatology
OPTIKNEE - portmanteau of ‘optimise’ and ‘knee’
PACAP - Pituitary Adenylate Cyclase-Activating Polypeptide
PCL-5 - Post-traumatic stress disorder Checklist for DSM-5
PCR - Polymerase Chain Reaction
PF – Patellofemoral
PHQ-9 - Patient Health Questionnaire -9
PIIANP - Type II Collagen N-Propeptide
PIIINP – Procollagen III N-terminal peptide
PICO – Population, Intervention, Comparison, Outcome
PIL - Participant Information Leaflet
PPARg - Peroxisome Proliferator-Activated Receptor gamma
PPI - Public and Patient Involvement
PRISMA - Preferred Reporting Items for Systematic Review and Meta-Analyses
PROMs - Patient-Reported Outcome Measures
PsA - Psoriatic Arthritis
PTOA - Post-Traumatic OA
PTPRD - receptor-type tyrosine-protein phosphatase delta
QoL – Quality of Life
RA – Rheumatoid Arthritis
REC – Research Ethics Committee
REDCap - Research Electronic Data Capture
rOA (+/-) - Radiographic Osteoarthritis (present/absent)
ROM - Range Of Motion
RPE - Rate of Perceived Effort
RTD - Return to duty
SAP - serum amyloid P-component
Scl - Sclerosis
SD - Standard Deviation
SDF – Stromal-cell Derived Factor
SE - Standard Error
SES - Socio-Economic Status
SEZ6L - Seizure 6-Like Protein
SF – Synovial fluid
SHBG - sex hormone-binding globulin
sGAG - Sulphated Glycosaminoglycans
SOAR - Stop OsteoARthritis
SORE - Serum concentration of joint-specific biomarkers from the venous drainage of the lower limb may improve in the interpretation of acute and chronic degenerative knee and ankle joint disease
sRANKL - Soluble Receptor Activator of Nuclear Factor- KappaB Ligand
SSD - Side-to-Side Difference
SSM - Statistical Shape Modelling
SSV - Small Saphenous Vein
snoRNA - small nucleolar RNA
STOMP - Surveillance and Targeted interventions for OA in Military Personnel
SUPER-Knee - SUpervised exercise-therapy and Patient Education Rehabilitation
SWiM - Synthesis Without Meta-Analysis
T - Telsa
TAS - Tegner activity scale
TF - Tibiofemoral
TGF(-β) - Transforming Growth Factor (-beta)
TIMP - Tissue Inhibitors Of Metalloproteinases
TNF(-α) - Tumour Necrosis Factor (-alpha)
TR - Tibial Rotation
TT - Tibial translation
ULOQ – Upper Limit Of Quantification
UNC5H4 - netrin receptor UNC5D
UoN - University of Nottingham
UPF – Ultra-Processed Foods
USS – Ultrasound scanning
VAS – Visual Analogue Scale
WHO - World Health Organisation
WORMS - Whole Organ MRI Score
YO - Year Old

Abstract

Osteoarthritis (OA) significantly impacts the lives of those who live with it, physically, psychologically, and occupationally. Age, sex and body mass index are traditionally seen as the main risk factors for OA development, and with a global increasing life expectancy and increased obesity epidemic, the number of cases are expected to rise exponentially. However, injury accounts for a significant proportion of OA, commonly occurring in a far younger population, especially in high-risk groups such as sportspeople and military personnel. There is a requirement to identify those at the highest risk of OA, potentially with the use of biological markers (biomarkers), enabling interventions to prevent it or slow it. This PhD thesis entitled *Investigating the value of serum biomarkers in predicting the development of post-traumatic osteoarthritis of the knee* aims to understand the predictive utility of a panel of pre-selected serum candidate biomarkers in a cohort of young, male, British military personnel, half of whom sustained traumatic battlefield injuries.

The initial part of the thesis sets the context with the definition, classification and epidemiology of OA, use of biomarkers and population of interest, before outlining the thesis hypothesis. The current evidence regarding molecular and imaging biomarkers a year or more from injury is reviewed, before a cross-sectional and three-year predictive analysis of serum biomarkers within the longitudinal Armed Services Trauma Rehabilitation Outcome (ADVANCE) cohort is performed for knee radiographic OA (rOA), knee pain and functional outcomes. The latter chapters will address new questions, the novel methodology and results of a pilot study to enhance biomarker sensitivity, and use of biomechanics, before the final chapter outlines clinical and research translation and implementation.

Acknowledgements

I have spent a long time imagining this section, and decided it would be the final component of this thesis that I would write. Here we are, on the 17th Feb 2025 (updated 3rd July), drawing a close on this section of my journey, which began in 2021.

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Chapter 1 : What is Osteoarthritis and why is it a problem?

Osteoarthritis (OA), the most common form of arthritis with a rising incidence and prevalence globally, is a heterogeneous, progressive whole-joint organ disease. It is associated with changes to the synovium, cartilage and bone, resulting in structural and functional alterations, leading to pain, stiffness, loss of function and increased inactivity (21,22). Whilst there is a complex pathophysiological process, there is also a prolonged asymptomatic prodromal stage, offering the opportunity for early identification and intervention (23). The lifetime risk of the most commonly disabling form, symptomatic knee OA, has been estimated at 45%, rising to 66% in those who are obese (24), with 1 in 3 people over the age of 65 affected by OA of any form (25).

There are many ways to describe this common condition, with implications for clinical care and research, with an understanding of each description required due to the variation in reported incidence and prevalence rates. In addition, different OA types are likely to have different underlying pathophysiology, require different optimal management and mitigation strategies, and can occur in different populations, therefore, we must understand the key populations to target these potential interventions in. The first chapter will discuss each of these key foundational components in turn, within the context of the overall PhD aims. Throughout this chapter, the potential use of molecular biological markers (biomarkers), as measured in the blood or other bodily fluids, will be introduced in preparation for further discussion in later chapters.

1.1 Osteoarthritis – Classification and epidemiology

Despite being one of the most common health problems for those aged >50 and a leading cause of global disability (26), there is no common definition for OA. These classifications depend on the required use of them, and include clinical, aetiological and research only methods. The UK National Institute of Health Institute for Health and Care Excellence (NICE), the European Alliance of Associations for Rheumatology (formerly known as European League Against Rheumatism, EULAR) and the American College of Rheumatology (ACR) all have different criteria-based clinical diagnoses or classification (27-30) (Table 1.1). There is similar discordance in the research domain, including radiographic features and patient-reported measures (31-34).

1.1.1 Clinical classification

Aetiologically, OA can be classified in several ways. The most common type, idiopathic, is classified as primary OA, with subtypes within primary OA including localised and generalised, or erosive (more common in hand OA) and inflammatory. Secondary types include those where the joint has undergone change, perhaps due to a co-existing pathology or injury, and OA has developed as a result. These subtypes include those secondary to congenital disorders, inflammatory arthropathies, such as Rheumatoid (RA) or Psoriatic Arthritis (PsA), or secondary to injury, such as post-traumatic OA (PTOA).

Table 1.1 Classification criteria for Osteoarthritis

	Age	**Symptoms**				**Examination**				
		Joint pain	Activity related joint pain	Morning stiffness none / <30min	Functional limitation	No palpable warmth	Crepitus	Bony enlargement	Restricted ROM	Bony tenderness
NICE	45		X	X						
ACR	50	X		X		X	X	X		X
EULAR	45		X	X	X		X	X	X	

NICE: National Institute of health and Care Excellence, ACR: American College of Rheumatology, EULAR: European League Against Rheumatism. ROM: Range of motion

NICE advise diagnosis if all three features are present. Radiological investigations should be performed in the presence of atypical features.(27)
ACR recommend classification of OA if knee pain plus three of the positive six features are present.(28)
EULAR state a 90% probability of radiological osteoarthritis if all six features are present. (29)

This latter type of OA is of particular interest to researchers and clinicians, including this one. It has been estimated that 13% of knee OA can be attributed to previous trauma (35). From a research point of view, the individuals are younger, with fewer co-morbidities, a clear initiating event, and an accelerated pathophysiological process, so they are useful to study from a mechanistic point of view. From a clinician position, as it occurs in a younger population, they are working age and are at risk of a potentially longer period of years lived with disability and, consequently, impact on the workforce. Certain injuries, such as a traumatic anterior cruciate ligament (ACL) rupture, meniscal injury, or intra-articular fracture, carry a significant risk of subsequent PTOA (36-38). In these cases, PTOA can present within a few years after injury, potentially with a period of intermittent symptomatic recovery prior to symptomatic and functional decline (36,37). Given the obvious starting point, this offers clinicians a chance to intervene with preventative strategies to slow or prevent the progression of OA in this high-risk population.

OA classifications differ internationally as demonstrated by those for knee OA. The UK-based NICE recommends clinical diagnosis in those aged 45 or more if they have activity-related joint pain and any morning joint stiffness lasting no more than 30 minutes (27). Previously, this was required to be present for three months or more, but this time period has been removed in the latest guidance. If an alternative diagnosis is suspected, it may be necessary to perform imaging to confirm the diagnosis. However, there has been a deliberate move away from using imaging for the diagnosis of OA due to the discordance between radiological signs and clinical symptoms, the reduction of potential harm from X-ray exposure and the cost of unnecessary medical procedures. In North America, ACR also uses an optional age category, >50, in the presence of knee pain, with further optional early-morning stiffness for <30mins, and the clinical signs of crepitus, bony enlargement, joint margin tenderness and no palpable warmth in the joint for the classification of OA in a research setting (28). Within Europe, EULAR

has six features: knee pain, early morning stiffness under 30 minutes, functional limitation, crepitus, restricted range of movement and bony enlargement, with the presence of all 6 features giving an estimated 90% probability of radiographic knee OA (29). These classifications are summarised in Table 1.1. The combination of patient-reported and clinician-assessed features in the latter two classifications suggests a more robust classification method; however, an analysis of over 13 thousand people suggested that NICE criteria identified the most cases (30). It is clear that in both the range of diagnostic criteria and lack of concordance between international agencies, OA is a heterogeneous condition that can present in various forms and, therefore, currently needs a broad diagnostic classification.

1.1.2 Research classification

Similar to clinical classification criteria, there is no single endpoint or diagnostic criteria used for recruitment or outcome within the research domain. Radiological and structural features are approved by some agencies, such as the European Medicines Agency (EMA) (39), but not in isolation by others, including the US Food and Drugs Administration (FDA) (40). This is due to the difficulties of understanding how the radiographic image relates to the individual. The FDA recognises that OA is a serious disease requiring therapies that modify the underlying pathophysiology (40), echoing the language seen in the literature elsewhere (41,42); however, they describe the several challenges that must be overcome before radiological measures can be employed solely. These include the complex pathophysiology of OA, the interaction of the risk and other modulating factors, and the discordance of structurally and clinically meaningful symptomatic changes. In the absence of standard definitions, or proxy markers, of disease progression, the FDA has invited relevant stakeholders to address these concerns and collaboratively arrive at structural outcomes that

enable substantial confidence in their ability to predict a clinical outcome of interest reliably to address this unmet need (40).

On the contrary, the EMA does approve imaging using radiological evidence such as Kellgren-Lawrence grade or degree of joint space narrowing (39). However, this must be in conjunction with either ACR or EULAR diagnostic criteria to mitigate discordance (28,29,39). Furthermore, the EMA advocate that pain and functional disability attributable to the target joint are the primary outcomes for any drug trials, using validated methods such as the visual analogue scale (VAS), multidimensional assessment tools or disease- and joint-specific instruments and additional tools, such as magnetic resonance imaging (MRI), ultrasound scanning (USS) or biochemical measurements (molecular biomarkers, measured in serum, urine, joint fluid) alongside this (29). Molecular biomarkers offer the potential opportunity to categorise using disease stage, from the pre-clinical molecular phase to end-stage disease, especially when combined with imaging metrics (43-45).

In addition to the regulatory research criteria, efforts have been made across stakeholders and agencies to draw consensus around creating new composite tools. Research organisations, including the Osteoarthritis Research Society International (OARSI) and Outcomes Measures in Rheumatology (OMERACT) have aligned workstreams on clinical and research outcome measures with regulatory agencies, pharmaceutical corporations and patient groups to co-develop future endpoints. Notable collaborations include the OARSI-FDA initiative on disease definition and structural change assessment (46,47), OMERACT-OARSI responder criteria (48,49), and the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium, which are building an evidence base on alternative measures, such as MRI measures and molecular biomarkers to use as future research endpoints (33,50-53).

1.1.3 Genotype / Phenotype

OA is a heterogeneous condition, with a variety of risk and modulating factors, and the balance of each of these contributing factors is likely to be different. This is described as an individual's endotype, which can be seen clinically with differences in how their condition presents, described as their phenotype (Table 1.2). Studying differing phenotypes allows an understanding of their underlying inflammatory pathway and metabolic and biomechanical changes, setting the conditions for optimised and personalised interventions.

Genotyping, phenotyping and endotyping have been suggested to categorise OA sub-groups and those at high-risk to target appropriate investigations, interventions, and enhance drug study recruitment (Table 1.2) (2). A consensus definition for phenotypes has been agreed upon as '*subtypes of OA that share distinct underlying pathobiological and pain mechanisms and their structural and functional consequences*' (54). Phenotyping categories have been suggested, including chronic pain, inflammatory, metabolic, bone and cartilage, mechanical and minimal joint (55), further expanded into secondary, extra-articular and age-related or systemic (56).

Table 1.2 Applicability of typing to osteoarthritis

Genotype	The complete genetic material of an individual, inclusive of specific variants
Phenotype	Observable characteristics - interaction between genotype and environment. Can include physical characteristics such as symptoms, biochemical, and physiological characteristics
Endotype	Condition subtype distinguished by distinct causes or mechanisms, allowing identification of subgroups who require different treatments

The discovery of discrete endotypes and phenotypes may enable a better understanding of the pre-clinical molecular phase, enhanced through the use of biomarkers (57,58). Dividing the disease process in this manner enables prevention strategies at each level, primary, secondary, and tertiary, with the hope of early diagnosis and better

intervention opportunities. Figure 1.1 outlines this concept for those who are exposed to increased risk, such as athletes or military, which might include both injury prevention but also total activity management and exposure to specific risks such as driving or vibration (59). Secondary prevention should focus on those with an injury with the aim to delay or prevent PTOA development (60,61), using a patient-centred approach including patient education, strength and conditioning, and weight management. Tertiary prevention strategies should focus on the how the person experiences their condition to minimise potential disability (62). Figure 1.1 also visualises the discrepancy between symptomatic and radiological change.

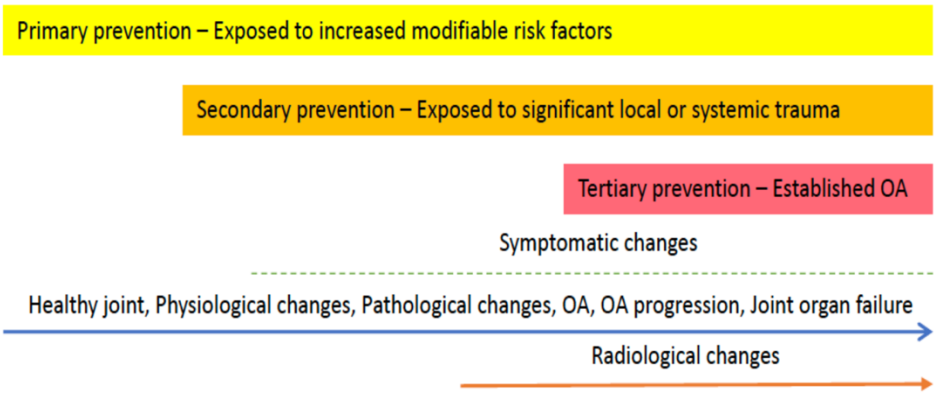

Figure 1.1 Primary, secondary and tertiary osteoarthritis prevention (9)

1.1.4 Further challenges

There is a well-established discordance between structural changes and patient-reported outcomes, leading to a further classification of radiological OA and symptomatic OA. It is unclear if this discordance reflects separate mechanistic processes, if individual predispositions influence the clinical picture, if there are other influences, such as co-morbidities, or if it is due to a combination of all those factors. Another way to frame this discordance is to describe either the OA *disease* (underlying pathophysiology and cellular biology, including structural change) or the OA *illness* (an individual's experience, characterised by symptoms, function and quality of life) (46,60,63). This phenomenon has implications for the design of clinical research studies, understanding pathways and markers associated with disability, dysfunction and pain, as well as future OA diagnostic criteria. In addition, it has real implications for patient care, as interventions can be implemented targeting the 'illness', i.e., pain and other symptoms, thus improving quality of life, which are separate to those which may target the underlying 'disease' process.

Even for confirmed OA (regardless of classification criteria), there are joint-dependent differences, which determine the clinical presentation and are likely to be linked to the underlying mechanisms. As an example of the relevance of this, the EMA has issued guidance advising against extrapolating findings from one site (i.e. hand) to another (i.e. hip or knee) due to upper and lower limb pathophysiology and functional differences (64). They also advise that research study inclusion criteria should be specific to a single joint and age range to improve the homogeneity of patient recruitment and groupings.

All in all, there are significant challenges in disease definition in the OA research field, regardless of whether this is clinically, radiologically or pathologically determined. Work is ongoing, aiming to unify this; a future solution might be using composite measures involving molecular

biomarkers, imaging, and/or patient and clinician reports (with the recognition of the increased cost and complexity required for this multi-modal approach). Ultimately, this could mean that OA as a unified concept needs to be reviewed. It could actually be a variety of conditions with diverse underlying pathological mechanisms but similar clinical phenotypes, or vice versa, a common pathophysiology resulting in a range of clinical syndromes. The final consideration may be the most important – what is the research question being asked and therefore, why is the definition/classification required; a clinical study might value pain and other symptoms as outcomes, but a disease-modifying anti-OA drug (DMOAD) trial might value structural outcomes, signifying disease change.

1.2 Epidemiology of Osteoarthritis

OA is extremely common, affecting 10 million individuals in the United Kingdom and nearly 600 million individuals globally (26,65). It is one of the leading global causes of disability; in 2015, it accounted for 3.9% of years lived with disability, with this rising, and it now comprises the seventh-ranked cause of disability for over 70-year-olds (26,66). OA contributes to one million hospitalisations in the USA annually (the vast majority for joint replacement surgery) (67), with the knee, in particular, is the most common joint impacted, encompassing approximately 85% of the burden (26,68). In the UK, there are over 6 million individuals with knee OA, over 3 million with hip OA, rising by approximately 350,000 new cases per year, with 49% of females and 42% of males over 75 having a diagnosis of OA (65).

1.2.1 Incidence and prevalence

Unsurprisingly, due to the range of possible diagnostic and classification criteria discussed above, there is variation in the levels of prevalence and incidence of OA reported (69,70). Both prevalence and incidence rates of OA are joint-dependent, and influenced by age, sex and geographic location. Geographic location is possibly due to genetic differences, joint morphology specific to ethnic groupings, socio-economic factors, national healthcare provision (or reporting systems), and other lifestyle and environmental factors (70). Socio-economic status has also affected OA risk, with knee and hip OA prevalence seen to be higher in those who are from a lower socio-economic category (65,71).

The method of classification also contributes to the variation in reported rates. Radiographic OA is the most widely-used criterion, used in over half of prevalence studies conducted between 1995-2011, with symptomatic OA (combined radiological and patient-reported

measures) and self-reported OA being the next most utilised criteria (70). Using radiological measures in isolation leads to a higher rate of estimated OA, with symptomatic and self-reported OA both returning much more similar estimates (Table 1.3) (70).

Table 1.3 Primary idiopathic osteoarthritis prevalence estimates, stratified by location and sex

Location	Self-reported (F/M)		Symptomatic (F/M)		Radiographic (F/M)	
Knee	7.9-16.5%	5.7-13.0%	8.6-38.0%	3.7-13.5%	9.9-78.6%	4.3-64.5%
Hand	5.8-9.5%	2.3-2.5%	3.4-75.4%	0.5-80.5%	20.6-82.5%	30.3-82.9%
Hip	6.2-12.3%	2.2-6.5%	1.5-8.0%	0.3-6.7%	0.7-46.9%	1.1-42.9%

F: female, M: male

Both the location of the OA and age of the patient contribute significantly the rates described, reflecting both the increased frequency of certain locations and the contribution of age to the disease process. When comparing joints, hip OA has the lowest prevalence and hand OA the highest disease prevalence (70), despite knee OA having the most significant burden of illness (68). Similar sex estimates were found for primary idiopathic hand and hip radiographic OA between males and females, with knee OA prevalence higher in females, all-age female/male OA prevalence for hip of 11.6% & 11.5%, knee 27.3% & 21%, and hand 43.3% & 44.5%, respectively (70). Another study reports the sex discrepancy in knee OA, with 40% of males and 47% of females developing knee OA across their lifetime (31). As we age, the rate at which our joints change increases with multiple national-level studies showing that this is both joint and sex-dependent. For example, the risk of hand and knee risk increases rapidly between 50 and 75 years of age, with the highest peak of incidence at age 75 (65,68,72). Specifically, within the knee, those <45 have an estimated prevalence rate of 19.7% (95% confidence interval, CI: 19.2,20.2) and 17.4% (95% CI: 6.8,8.4) for females and males, increasing to 36.9% (95% CI: 35.7,38.0) and 26.9% (95% CI: 25.6,28.1) for those aged 45-59, and 33.6% (95% CI: 32.8,34.5) and 24.3% (95% CI: 23.3,25.3) once over

60 years old (70). These estimates were computed by the DerSimonian-Laird method, to combine self-reported, radiographic and symptomatic estimates in a meta-analysis (70).

1.2.2 OA-related economic burden

The economic costs of OA are vast. These take the form of both healthcare utilisation and direct medical care, but also the indirect costs resulting from occupational impact (work-absenteeism), loss of productivity (work-presenteeism), and early retirement on medical grounds (25,68). The medical cost of OA has been estimated at between 1-2.5% of a high-income country's gross domestic product (GDP), with the bulk of this due to appointments for pain management, hospital admissions, joint surgery and all their associated costs (68). Indirect costs, such as work loss and early retirement, at a national scale, surpass the medical cost (68,71,73,74) In the US in 2003, arthritis-related costs were estimated at approximately $128 billion (41), and by 2010, 10% of ambulatory care visits were due to arthritis (58% for symptomatic OA) (75). A similar picture is seen in the UK, where the treatment of OA and RA is estimated to have cost £10.2 billion in 2017, and it is projected that the cost of loss of working days due to OA and RA will be £3.43 billion per year by 2030 (65).

1.2.3 Individual morbidity and mortality

On an individual level, OA can be life-changing. Typically of a slow onset, there is a gradual build-up of symptoms, reduction in function and increase in pain levels. Within the UK, OA is the 8th highest cause of years lived with disability, with 20% of those with OA experiencing anxiety and depression (65). For those with end-stage symptomatic OA, the burden can be unbearable, with constant severe pain, minimal ability to perform activities of daily living, and a corresponding busy schedule of polypharmacy and healthcare appointments (71).

There are increased rates of physical co-morbidities too, with 59-87% having at least one co-morbidity and >30% at least five (31). Specifically, those with OA have a 61% increased chance of diabetes, are 3x more likely to have ischaemic heart disease or heart failure, and overall, are 1.2x more likely to have an additional long-term condition. It, therefore, is not a surprise that there is a 20% increased risk for all-cause mortality in those with comorbidities (31), and there is growing consensus around OA being a serious disease with increased associated mortality as well as morbidity (41,42). Of relevance to the population studied in this PhD, this effect is also seen with military populations, with those with OA having an increased 1.7x risk of diabetes, increased lipids or high blood pressure compared those without OA (76).

This increased mortality is likely multifactorial, with OA and cardiometabolic disease likely to share some underlying pathological mechanisms, such as fat metabolism and innate immune system dysregulation (68,77). The 24-78% increased risk of cardiovascular (CV) disease, demonstrated by meta-analysis involving millions of patients (78,79), is likely multi-factorial, including an increase in atherosclerosis (78), and a decrease in physical activity (80) and OA-related loss of walking (81). There is also likely to be a bidirectional relationship between OA and cardiometabolic disease, with each element worsening outcomes for the other – the presence of a co-morbidity is predictive of faster progression of pain or structural change, with worsening pain likely to reduce function and increase physical inactivity, thereby increasing CV risk (82). Consequently, there is a link between metabolic syndrome and OA - increased physical inactivity leads to an increased body mass index (BMI), subsequent glucose intolerance and associated risks, and increased BMI also makes both the OA ‘disease’ worse. This is as a result of multiple pathways, including increased load through the abnormal joint but increases in the release of adipokines which cause OA progression, as well as

worsening OA ‘illness’ due to increased pain and symptomatic perception in those with a higher BMI (41,68,83,84).

This section has highlighted the many challenges of disease diagnosis and classification. These challenges are likely to have contributed to the lack of successful disease-modifying therapy as it is possible that different disease groups or phenotypes might respond differently to therapies, especially if they are slow-progressing (85). It is for this reason that many clinical-academic-industry consortia are being established to standardise the research streams and develop specific biomarkers to help identify and stratify phenotype – one example of this are the FNIH Biomarker Consortium (32,86). However, in order to understand what these biomarkers might actually be identifying, we need to explore the pathophysiology of OA.

1.3 Pathophysiology and risk factors

Typically, OA takes years to develop, either after an initiating injury or in response to chronic over-use, modulated by the interaction of physical, immunological and mechanical factors, with an asymptomatic and pre-radiographic molecular phase prior to radiographic and symptomatic phases (21). The function of the joint is to enable load transfer across an articulation whilst retaining the flexibility of motion. Using this paradigm, it is intuitive that OA is a biomechanical disease caused by abnormal or excessive loads. The abnormal load transfer is likely compensated up until a point with physiological adaptions; however, in the presence of significant joint changes, such as following injury, these adaptions are overwhelmed, and pathology occurs.

However, this explanation is only part of the picture, and from previously being thought of as a progressive "wear and tear" degenerative disease, it is now understood to be a heterogeneous process with distinct underlying pathophysiological pathways and the interaction between modifiable and non-modifiable risk factors and individual features (Figure 1.2). The joint should be considered as an organ, with multiple different tissue types working together for a common purpose (87). These tissue types include the articular cartilage, bone, synovium (and lubricating synovial fluid, SF), and the meniscus and ligaments within the knee. The pathophysiological process is likely triggered by such an event (such as injury or chronic overload), and is further modulated by inflammation and metabolic changes, especially in those with a genetic or immunological predisposition.

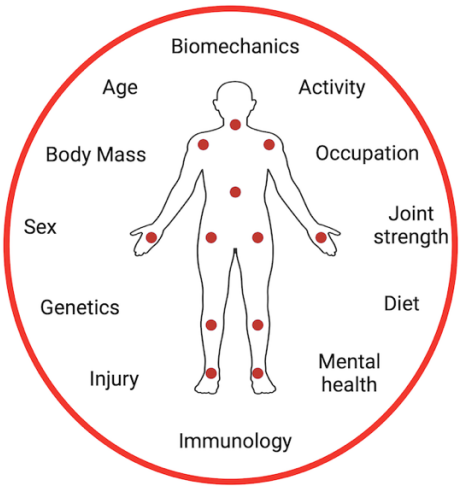

Figure 1.2 Risk factors for Osteoarthritis development
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The articular cartilage has received the most attention, as for a long time, it was felt to be the predominant contributing tissue type for OA. Articular cartilage, two to four-millimetre thick, mechanically compliant, flexible connective tissue, is formed from hyaline cartilage composed of an extracellular matrix (ECM) with chondrocytes, water, collagen, and proteoglycans (21,88). Collagen is the most abundant macromolecule, with collagen type II representing 90-95% or ECM collagen, forming fibrils intertwined with proteoglycan aggregates (88). Proteoglycans are synthesised and maintained by chondrocytes, regulated by peptides including insulin-like growth factor (IGF), transforming growth factor (TGF)-β, interleukin (IL)-1 and tumour necrosis factor-alpha (TNF-α) (88). The ECM protects the chondrocytes, with ECM homeostasis maintained by balancing continuous catabolism and anabolism. The presence of water in cartilage provides its unique properties due to its ability to manage and absorb pressure using a ‘fluid phase’ (88), and it provides a smooth, lubricated articular surface, facilitating load transfer with low frictional coefficients to the underlying subchondral bone (21,88).

Unfortunately, changes in cartilage composition secondary to OA lead to a loss of integrity and its material quality, such as force absorption

and, despite cartilage being extremely robust and able to tolerate high stress/load, OA-related changes increase the potential for physical damage (68). The pathological situation is exacerbated by the poor ability of cartilage to heal due to limited chondrocyte replication potential and poor vascularisation. Hypertrophic chondrocytes increase synthesis but also trigger a proinflammatory and anabolic cascade (68,88), and as a result, even minor injuries can lead to irreversible articular cartilage structure or functional changes and ultimately lead to OA.

In addition, changes to the subchondral bone, meniscus, other components of the ECM, and the synovium also contribute to the development of OA (87,89-92). The proinflammatory cascade, triggered by chondrocytes, leads to complementary proliferation and proinflammatory mediator release by the adjacent synovium, which results in tissue hypertrophy and increased vascularisation (68). Subchondral bone turnover is also increased, with a corresponding increase in vascularisation, leading to subchondral bone marrow lesions and osteophytes. Changes in the SF composition due to synovial injury, hypoxia and haemarthrosis, are also involved, with these alterations resulting in insufficient lubrication of the joint boundary, further diminishing joint function (91).

As previously described, there is a particular research focus on the secondary form of OA following an acute injury, PTOA. After an injury, the joint undergoes activation of multiple signalling pathways, resulting in cartilage matrix degradation and synovial inflammation, leading to a process of repair, remodelling, and adaptation. However, aberrant mechanistic pathways can contribute to a lack of repair and remodelling, inadequate adaptation, and the subsequent development of OA, with altered joint biomechanics, metabolism, and low-grade inflammation all playing crucial roles.

These aberrant mechanistic pathways result in disturbed joint homeostasis as a result of changes in joint loading triggered by pain or structural changes, or alterations in the production of inflammatory mediators, growth factors, and ECM components, with the suggestion that structural changes influence local bone and cartilage compositional changes (36,93,94). Those processes, and the resultant imbalance between anabolism and catabolism, are likely to represent a failure of initial injury repair and/or remodelling. They involve the generation of new, and adaptation of existing, tissue, including cartilage matrix macromolecule synthesis or subchondral bone resorption, mediated by cytokines, and leave signals which can be detected at a molecular level.

Therefore, it is not solely irreversible mechanical damage that leads to PTOA, but a combination of enduring chronic inflammation, hypoxia, biomechanical changes, genetic factors, and individual predispositions (37,95,96), such as age, sex, genetics and immunological predisposition, body composition, inflammation, joint architecture, and occupation (37,68,84,97). The risk factors for new (incident) OA may differ from those related to OA progression. The next section will briefly summarise the implications of each.

1.3.1 Age

The impact of age and sex on the incidence and prevalence of OA has previously been reported (Section 1.2), and can be explained by increased accumulation of exposure to risk factors and natural age-related biological changes to joints (68,98). Age plays a role in the development of OA, partially due to increased cumulative load but also due to ageing ('senescence') of the cartilage itself ('chrondrosenescence') and subsequent physiological changes, including deep movement of chondrocytes, reduction in ECM hydration and reduction of proteoglycan aggregate size (88,99). These effects are more pronounced in OA-cartilage, thus forming a negative feedback loop, with increased expression of senescence-associated markers, chondrocyte telomere reduction and oxidative damage to mitochondria with resulting dysfunction (21,100). OA-cartilage ageing changes are more pronounced due to the interaction of co-existing inflammatory, mechanical and oxidative stress leading to hypertrophic differentiation and premature chrondrosenescence (21,101). The sum of these changes is an increased sensitivity to pro-catabolic and pro-inflammatory mediators, reinforcing and compounding the negative microenvironment. It is likely that other factors, including increased adiposity, sedentary behaviour and inflammation, also lead to premature chrondrosenescence (21,99,102).

1.3.2 Sex

Females are both more likely to develop OA, accounting for 60% of cases after age 40 (103), and to suffer sporting injuries, particularly ACL rupture (98,104). Worldwide pooled data suggest an OR of 1.39 (95% CI: 1.23-1.56) and 1.69 (95% CI: 1.59-1.80) corresponding to OA incidence and prevalence, respectively, for women compared to men (66), with the sex differences most apparent at the knee (105). There are many likely contributing factors to the difference in OA incidence and prevalence, including anatomical and resulting biomechanical differences (103,106), muscle strength (107), reduction of cartilage volume (independent of body/bone size) (108), genetic haplotypes (including frizzled related protein, FRZB, and collagen, type II, alpha 1, COL2A1) (109), and the effect of hormones (110) (although the lack of clear improvement following hormone replacement therapy makes this hard to quantify (111)). In addition, there appears to be a difference in the presentation of OA illness in females when compared to males, with more frequent and severe pain, functional limitation and disability, possibly due to biological (hormonal and inflammatory), psychosocial and pain processing differences (105,112,113).

1.3.3 Body Mass

Body mass is a significant risk factor for OA. High BMI increases OA risk by up to 30%, often preceding the onset, with both conditions influencing each other. If OA were purely a biomechanical issue, with structural changes and symptoms resulting from abnormal load transfer across the joint, then weight reduction would reduce the load, and the condition would improve. However, it is more complex than that, as demonstrated by the relationship between body mass and hand OA, given that there is no sustained load transfer across the hand joints, with increased body mass impacting both the disease and the illness (114).

For each increase in one BMI unit, there is an increased risk of between 1 and 5% for OA (65,115), and a five-point change confers a 35% increased risk (116). Adipose tissue generates and releases cytokines and other mediators, such as leptin, which have been seen to contribute to OA progression and, therefore, are independently associated with the disease (117-119). Furthermore, those with a higher body mass can experience a heightened perception of pain and reduced quality of life, thereby leading to a worse illness presentation (114).

International guidance advocates for weight loss as first-line therapy for OA, however, this is an unnuanced view, with the loss of adipose tissue important, but the retaining or improvement of muscle mass as important (if not more so). The risk of indiscriminate weight loss, without appropriate resistance training to maintain muscle bulk, is sarcopenic obesity, with high adiposity and low skeletal muscle mass, associated with reduction in function and slower recovery time (120), adversely impacting on both the development of OA and the ability to perform functional tasks (121,122). Therefore, weight needs to be considered through the lens of body composition, not just absolute weight or metrics such as BMI.

1.3.4 Genetic

The genetic component of OA is yet to be fully understood, however, the contribution is estimated to be between 40-80%, depending on the joint. For example, there is a weaker genetic contribution for knee OA than for hand and hip OA (68). Genome wide association studies (GWAS) have aimed to understand what components make up this risk specifically with mixed results. One study using nearly half a million participants from UK BioBank was unable to detect a difference, with the authors stating that this might be due to under-powering (123). A subsequent study, with over 800,000 participants, demonstrated commonality in mechanisms between different joint areas (including weight-bearing and non-weight-bearing), predominantly related to signals related to bone and cartilage changes, with some additional insights into pain mechanisms (124). Two studies have suggested a genetic difference in those developing PTOA when compared with those developing idiopathic OA, suggesting a difference for an individual's risk profile and also the factors which initiate the development of different OA mechanisms (123,125). Overall, it is felt that early-onset OA, in the absence of a more likely cause (such as trauma), could be due to a larger genetic effect (such as a monogenetic mutation), whereas late-onset OA is likely multifactorial with the genetic component of this (due to many common DNA variants) is relatively smaller.

1.3.5 Inflammation

It is highly likely that inflammation, both local and systemic, plays a key role in the initial pathogenesis and ongoing progression of OA, as demonstrated by the common presence of joint synovitis and effusion (126,127). The inflammatory response is sometimes triggered by an acute event, such as an injury, but otherwise in response to gradual changes (21,94). Once the homeostasis between pro-inflammatory mediators (such as IL-1β, -6 or TNF-α) and anti-inflammatory (such as IL-4, -10, -13), has been breached, there is a subsequent imbalance of pro-degradative proteases, such as matrix metalloproteinases (MMPs), leading to further disease (128). Recent work suggests that OA pathogenesis involves failure of the innate immune system and an unresolved acute phase reaction, including in the interaction with haemostasis (related to fibrin clot formation and fibrinolysis and the coagulation cascade), and the role of disease-associated molecular patterns (DAMPs) in ECM breakdown and reduction in boundary lubrication (23). The net result of increased and sustained pro-inflammatory mediator release by the cartilage, subchondral bone and synovium (128,129), is articular cartilage and subchondral bone degeneration, new bony osteophyte formation and persistent synovitis (21,128).

When the local low-grade inflammation becomes established, other systemic processes contribute to maintaining it. These include altered load transfer across the joint, with the damaged articular surfaces inducing the activation of mechano-sensitive receptors and release of proteases, so-called ‘mechanoinflammation’ (93), processes resulting from metabolic syndrome, central obesity and adipokine dysregulation, also known as ‘meta-inflammation’ (130), and the inflammatory changes that result from physiological ageing, ‘inflammaging’ (131). Animal models have demonstrated components of these interactions, including the role of chondrocytes (132), signalling pathways (133), key proteases (134), and metabolic mediators (117). In contrast to

inflammatory arthritides, such as RA or PsA, the inflammation is ‘low-grade’, with standard histological staining revealing low-moderate inflammation in OA synovial tissue (127). This ongoing inflammation is a potential target for phenotyping and pharmacological intervention.

1.3.6 Joint injury

Certain specific joint injuries, such as anterior cruciate ligament (ACL) rupture, increase OA risk, especially when poly-structural injuries occur, with a concurrent meniscal injury significantly increasing this risk (37). The initial traumatic episode can result in localised disruption of articular cartilage, cartilage fissures, and chondrocyte death, accompanied by a post-traumatic inflammatory response and synovitis, as well as concurrent damage affecting the biomechanical function of the joint, such as fractures (94). Preceding joint injury, both traditional musculoskeletal injuries (MSKI) and lower-limb amputations, contribute through altered loading, changes in muscle bulk and strength, and by increasing physical inactivity, BMI and impairing physical function (21,37,60,135-139). Concurrent joint disease can similarly lead to poor biomechanics resulting in mechanoinflammation (2,139-141).

The type of injury relates to the risk of future OA development. It is well established that an ACL injury can lead to PTOA (142), although estimates of the magnitude of this risk vary (between 10-90% at ten years) (143). This risk is increased if multiple structures are injured, including cartilage (two-fold), meniscus (three-fold) or combined (six-fold) (37,142). Echoing the challenges outlined in Section 1.2, there is variation in rates between symptomatic and radiographic OA after ACL injury (144). The severity of injury might also influence the risk, but also the speed of OA development, with 100% of individuals experiencing major trauma to their knee developing OA within two years (36).

1.3.7 Joint structure

The joint structure itself can influence both the risk and progression of OA. Muscles and tendons acting to transfer loads through tension and position – when non-uniform load transfer occurs due to structural changes, there is a heightened chance of cartilage damage (145). At the knee joint, joint alignment (including valgus or varus), damage from previous injury, joint morphology and surrounding muscle strength can contribute (37), with conditions such as cam deformity and dysplasia in the hip conferring an increased risk, with severe dysplasia in particular linked to early hip OA (68). These changes can increase local stress on cartilage, due to abnormal load transfer, and pre-dispose individuals to developing OA (145).

Joint surface incongruity and instability (against which, periosteal cells and the synovium form osteophytes) also contributes, leading to joint biomechanical changes, influencing weight bearing, gait and overall joint function (94). This is reinforced by activation of mechano-signalling pathways, and resultant mechanoinflammation (93). Joint laxity might contribute to OA development, due to shear stress and inappropriate distribution of force (146). Muscle strength is becoming a highly studied component, in part due to its modifiable nature and, therefore, potential for improvement, with recent studies highlighting quadriceps strength as a key modifier (107,147).

1.3.8 Systemic factors

Further mechanisms relate to metabolic processes, including the interactions between glucose and lipid pathways (148,149). As mentioned previously (Section 1.2.3), metabolic syndrome has a bidirectional relationship with prolonged inflammation and activation of the innate immune system, further contributing to the ongoing dysregulation and pathological processes, causing accelerated OA progression and increased pain modulation (68,84,150). Animal studies also suggest hypercholesteremia, dysregulated lipid metabolism and cholesterol accumulation are associated with the development and progression of OA (150).

In addition, the OA illness can be influenced further by additional factors such as pain processing, sensitisation and modulation, and anxiety, depression, and other mental health conditions can affect the individual's experience of OA (84). Socioeconomic status has also been seen to have an association with a higher incidence of OA, which is explained by a variety of reasons, including higher rates of obesity, lower educational attainment (and corresponding reduced health literacy and ability to find health-promoting interventions) and manual labour (151-154).

Given the number of risk factors leading to OA, there is an opportunity for interventions tailored to the individuals, including preventative strategies, which can minimise their impact and potentially slow or halt the development or progression of OA. In particular, these should be proactively considered for certain populations, such as the Military, due to their increased risk of OA, as demonstrated in Figure 1.1. Identification of those at high risk to enable modification of their risk factors is a research priority and is the focus of this PhD. The use of molecular biomarkers might identify and stratify individuals.

1.4 What are biomarkers, and what do they show?

1.4.1 Biomarker categorisation

The OA biomarkers network created a classification system to improve biomarker development and use, employing five categories: burden of disease, investigative, prognostic, efficacy of intervention, and diagnostic (155), with a sixth, safety, subsequently added, resulting in the BIPEDS classification (86). Biomarkers can be divided into ‘wet’ (serum, plasma, urine, or SF) or ‘dry’ (USS or MRI) and are used to provide an early signal of joint changes, demonstrate specific mechanisms (such as inflammation, boundary lubrication, and changes in glucose metabolism), and judge the impact of management (including diet, exercise or the effect of DMOADs).

Wet biomarkers can identify specific pathological changes in cartilage, bone and synovium, and concurrent inflammatory and metabolic responses (86). All these measures are components of a continuum extending from genome proximity to a clinical phenotype of a painful joint limiting function, and therefore, potentially can identify pathological processes, such as catabolism or anabolism of cartilage and collagen or active inflammation, before any symptomatic, structural, or functional impact (58). In addition, they allow the identification of homogeneous subgroups/phenotypes based on shared clinical, epidemiological, and biochemical characteristics (Table 1.2) (58).

Given the non-invasive nature of imaging, allied with the development of new modalities, more options are now available for dry biomarkers. Unlike XR, USS actively assesses joint changes, including irritation, recovery, and OA development by reliably monitoring synovitis and synovial thickening (156), with a standardised protocol developed by Outcome Measures in Rheumatology (OMERACT) (157). USS is safe, non-invasive, quick and cheap, providing real-time information to a

trained practitioner. Whilst more expensive and slower, MRI allows both sequential and functional imaging to assess, in greater detail, the changes of multiple individual tissue pathologies related to OA development in different joint components, offering increased sensitivity for early disease change (44,158,159). Offering both the ability for qualitative and quantitative measures, a range of metrics can be assessed, depending on the tissue of interest, with semi-quantitative scoring systems, including whole organ MRI score (WORMS) (160), knee OA scoring system (KOSS) (161), Boston Leeds OA knee score (BLOKS) (162) and MRI OA knee score (MOAKS) (163), among others.

Over the last two decades, there has been a focus on elucidating underlying molecular changes and their relationship to abnormal pathophysiological processes to create phenotypes and endotypes. In addition, understanding at what time-period these occur from a temporal relationship point of view can help determine which biomarkers can be used as proxy measures to monitor both the appearance and development of PTOA. This is essential for diagnosis, prognostication, and judging the effect of interventions. It was with this concept in mind, '*that disease starts when detected by the best marker available to define it*', that the OARSI and FDA Biomarker Working Group aimed to draw consensus around the best biomarkers for prognosis or determination of therapeutic effect (86). This project also aimed to define nomenclature regarding biomarker qualification, dividing them into four categories, exploration, demonstration, characterisation and surrogacy, linking it to terminology used in FDA guidance (Table 1.3) (86,164). There are additional hurdles to overcome, including the standardisation of collection, analysis and interpretation, with the influence of possible dilution when measured systemically, impact of exercise and activity, and challenges regarding multi-site disease all requiring addressing throughout the next few years.

Table 1.3 OARSI/FDA Working Group levels of biomarker qualification

Level of qualification	Definition
Exploration	Research and development tool, in-vitro or preclinical evidence, no consistent information linking biomarker to human clinical outcome
Demonstration	Associated with clinical outcomes, not reproducibly demonstrated in clinical trials, ‘probable valid biomarkers’
Characterisation	Reproducibly linked to clinical outcomes in more than one prospective study, ‘known valid biomarkers’
Surrogacy	Can substitute for clinical endpoint, ‘surrogate endpoint’ when agreed with regulatory bodies

OARSI: Osteoarthritis Research Society International, FDA: Food & Drugs Administration

1.4.2 Biomarkers and pathophysiological processes

Any proposed biomarker is only as informative as its ability to quantify the pathophysiological change it is supposed to represent. PTOA development follows disturbed joint homeostasis as a result of changes in joint loading triggered by pain, structural changes or alterations in the production of inflammatory mediators, growth factors, and ECM components due to a failure of injury repair and/or remodelling (Section 1.3) (94,127,139,165,166). Most molecular biomarkers demonstrate inappropriate ECM turnover and repair of the articular cartilage, bone, and synovium, often within the presence of inflammation. It is hoped that, in the future, specific biomarkers can identify an individual's primary/predominant mechanism, thus enabling personalised medicine or targeted therapeutics.

An imbalance of articular cartilage anabolism and catabolism leads to progressive failure of the physiological and mechanical properties of the tissues through the solid phase and fluid phase and has been suggested as a driver for incident and progressive disease (167,168). Cartilage-derived biomarkers can act as proxy markers to the process of cartilage degeneration and synthesis, with protein-specific fragments, ‘neoepitopes’, released during the proteolytic or generation process. As described in Section 1.3, type II collagen is the most abundant protein component, forming a fibril stabilising network with proteoglycans (88), and damage to this collagen meshwork is a key process of OA development (169). The location and process of epitope formation categorise type II collagen biomarkers and include cleavage neoepitopes (such as collagen type-2 specific neoepitope, C2M), denaturation epitopes (including Coll2-1), epitopes localised to telopeptides (e.g., carboxyl-terminal telopeptide of collagen type II, CTX-II) and synthesis propeptide protein fragment epitopes (i.e. type II collagen N-propeptide, PIIANP) (169). The breaking down process is often instigated and promoted by proteases, including MMPs and

aggrecanases, which can be identified alongside their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) (170).

Type II collagen-related biomarkers are well studied, with C2M present in the core of articular cartilage (171) reflecting cartilage degeneration and associated with knee OA incidence and progression (172,173), and CTX-II also representing collagen degeneration and proteoglycan depletion, with associations with cartilage fibrillation and remodelling, and a 6- to 8-fold risk of knee or hip OA, respectively (167,173). CTX-II, localised to the bone-cartilage interface (the 'tidemark'), has also been shown to be associated with osteophyte progression and severity (167,174,175). In addition, the balance between synthesis/anabolism (with PIIANP) and degeneration/catabolism (with CTX-II) has been postulated to differentiate between slow and fast progressors (170,176). It is likely that, despite their common origin, different type II collagen epitopes reflect different mechanisms or phases/progression of the OA pathophysiological process and, therefore, could be utilised for different purposes (for example, diagnostic v prognostic).

Other investigated biomarkers that reflect collagen or ECM turnover include aggrecan and cartilage oligomeric matrix protein (COMP). Aggrecan is a key glycoprotein contained in the ECM, binding to hyaluronic acid (HA) and other link proteins, drawing water via osmosis to form the hydrated gel that gives cartilage its mechanical strength (176). It is broken down by MMP and A Disintegrin and Metalloprotinease with Thrombospondon motifs (ADAMTS) (176). Chondroitin sulfate-846 (CS846) has been proposed as a marker of aggrecan synthesis, with associations seen with JSN (170,177). There have been several suggestions for degenerative epitopes, of which ARGS (aggrecan amino acids, 'alanine, arginine, glycine, serine' created after cleavage by the aggrecanese, ADAMTS) is the most promising (176). The non-collagenous protein COMP is possibly the most studied cartilage-related biomarker, showing associations with OA progression (178), cartilage loss on MRI (179), and increased risk of

radiographic and painful knee OA (180) over the last 30, 20, and 10 years, respectively. However, despite that, the evidence for it remains mixed (181), and it was not able to predict case status (pain or JSN progression) in the early stages of the OARSI/FDA Biomarker Consortium study and therefore, was not carried forward to the Phase Two PROGRESS OA study (50,52). At the current time, a key challenge is that biomarkers measured in the serum or urine reflect whole body burden and multiple joints, not only the single target joint – this might represent a key example of this problem.

Bone markers have also been investigated, including type I collagen biomarkers, no surprise given the subchondral bone changes that occur during OA. Similar to cartilage homeostasis, osteoclast bone resorption is balanced by osteoblasts' bone formation, and pathology occurs when these become unbalanced (58). Those biomarkers showing new bone growth include osteocalcin, alkaline phosphatase (bone isotope) and amino-terminal propeptide of type I collagen (α-CTX-I) (182). Resorptive markers include the amino- and carboxy-terminal cross-linked telopeptide of type I collagen (CTX-I, N-terminal cross-linked type I collagen, NTX-1) (182). Many of these markers are influenced at a systemic level by global skeletal turnover when measured in serum or urine (and further impacted by age, bone disease including osteoporosis, and menopausal status) therefore, it is harder to ascertain the true effect of OA (182). Associations have, however, been seen between α-CTX-I and subchondral bony turnover with subsequent osteophyte formation, JSN, and OA progression (58,175). Given that osteophytes are often the earliest radiograph change (and are associated with painful disease), and the close relationship between bone and articular cartilage, this interface is a key target for therapeutics (58,183).

In addition to the formal tissue biomarkers, other markers represent pathology, including damage to the synovium and inflammation. Biomarkers, including N-propeptide of type III collagen (PIIINP), HA,

and YKL-40, have been proposed to assess synovitis, but none of these are specific to the synovium (182). However, HA, included in the OARSI/FDA Biomarker Consortium study, could predict the case study of pain or JSN progression (OR 1.22) (50). Low-grade inflammation is a key driver for OA development, so it is no surprise to see a focus on local and systemic inflammatory markers. Local markers include HA (again), endostatin, C1M and C3M (58,184). Systemic markers of interest have included cytokines (including tumour necrosis factor-alpha, TNF-α, and interleukins, IL), c-reactive protein (CRP, synthesised by the liver), and chemokines (acting as chemoattractants). Pro-inflammatory cytokines, including TNF-α and IL-1, -6, -15, -17 and -18, have been seen to be associated with the initiation and progression of joint inflammation and cartilage, with the former being the most studied (185). An association between pain severity, independent of the radiographic grade, suggests a role for inflammation in the discrepancy between OA 'disease' and OA 'illness' (186). The complementary, anti-inflammatory cytokines, including IL-10, insulin-like growth factor (IGF)-1 and transforming growth factor (TGF)-β, have been seen to decrease proinflammatory cytokine activity, MMP production and inhibit chondrocyte apoptosis (185,187). Despite this, and the success of immunotherapy in rheumatological conditions like RA or Axial Spondyloarthropathy (AxSpA), no successful immunological agent has been identified yet, including trials with anti-TNF-α or anti-IL-1 agents for disease activity or symptomatic improvement (185,188).

A final type of molecular biomarker is under investigation. Adipokines (released by adipose tissue) are associated with both inflammatory action and disease progression (via cartilage catabolism) (187,189). The two most studied adipokines, adiponectin (a modulator of metabolic processes) and leptin (fat storage regulator) are associated with increased pain and OA progression (190,191). In addition, adipsin (complement factor D, activates complement system alternative pathway) and resistin (inhibits insulin and glucose homeostasis) are associated with synovial hypertrophy, cartilage degeneration and

incidence of bone marrow lesions (BMLs) (192,193). Given the close relationship between OA, metabolic syndrome and cardiovascular health, it is no surprise to see so many mediators of both processes exerting an effect.

From an imaging point of view, there have been advances in USS and MRI technology which have improved the ability for the detection and prognostication of PTOA. Non-invasive imaging techniques may provide insights into early joint changes or established disease by quantifying various features of the image intensity, texture, shape, and spatial relationships to detect pathological processes (194). To enhance the ability to detect subtle changes, MRI has been proposed as a more sensitive and reliable marker of early OA changes, as it can visualise the whole joint and identify structural changes or the presence of effusion. A previous review identified that adaptive cartilage morphological changes, joint fluid volumes and BMLs were present within the first year following ACL injury, resolving over time (195). In addition, advanced techniques, including contrast-enhanced, T1rho (also known as T1R and 'spin-lock'), and T2 relaxation time, can indicate macromolecular content, hydration and molecular interactions in ECM (196,197). For example, 50% depletion of proteoglycan from articular cartilage results in average T1rho increases by more than 50% (from 110 to 170 ms) (198). These compositional modalities are transitioning from descriptive evaluations of calcified tissues to the identification of key changes in soft tissue composition, such as cartilage water content increases and collagen organisation or proteoglycan density decreases, indicating tissue degradation, inflammation and oedema, all of which are closely linked to early-stage cartilage degeneration and future OA.

Using a high-risk population to test the ability of biomarkers to identify and assess early-stage and future OA as 'proof of concept' would be the first step in this direction. First, we need to understand who these populations are.

1.5 Osteoarthritis in Military Populations

Activity and occupation are key risk factors in both the initiation and ongoing development of OA, related to job-role and occupationally associated risks. Given the end-point of functional impairment and the public health nature of the occupationally-related disease, understanding and mitigating these risks is important.

1.5.1 OA in physically active populations

Jobs with a physical component have been seen to be consistently associated with a higher risk of developing OA, including farmers (199), construction workers (200), and carpenters (201). Jobs such as nursing and personal care (with high physical demand to the knee), have also been seen to increase risk (202). This occupationally associated risk is independent of sex (202), with a cumulative increased risk and 'dose-response' relationship for those in high-risk employment for longer time-periods, with a 5-10 year exposure time enough to imply a significant risk of OA (202,203).

Several specific occupational risk factors have been identified, including over 30 minutes/day of squatting (odds ratio, OR 6.9, 95% confidence interval, CI: 1.8,26.4) or kneeling (OR 3.4, 95 % CI: 1.3,9.1), climbing more than ten flights of stairs a day (OR 2.7, 95% CI: 1.2,6.1) (203), and lifting/carrying (OR 1.58, 95% CI: 1.28,1.94) (204). However, in addition to these biomechanical factors, there are other occupationally-relevant risk factors to consider, including exposure to trauma and MSKI (37). Therefore, it is no surprise to see that certain groups, such as professional or tactical athletes with exposure to these risks, having higher rates of OA (7.86/1000 person-years, PY) than age-matched general population peers (7.16/1000 PY), with a subsequent impact on their employability (2,96,135,137,205).

In the elite sports population, an OR of 1.72 (95% CI: 1.35-2.20) has been reported for subsequent OA, with hip- and knee-specific OA rates between 2-60% and 16-95%, respectively, in former elite athletes (204). Increased rates of OA have been reported in football (soccer) players (206-208) (unsurprisingly higher in those retiring on medical grounds (209)), but has also been seen in other sports, including handball (210), ice-hockey (211), tennis (212) and track-and-field events (213). Increased risk in this population is multi-factorial, including the training volume, training load and sport-specific movements (such as rapid acceleration/deceleration, cutting or pivoting), likely causing an overload of the joint's mechanical tolerance, resulting in MSKI and/or OA (214,215).

Similar findings are seen for tactical athletes (occupational groups including the armed forces, firefighters, and law enforcement staff), with studies placing the incidence of OA in this population to be nearly 3x higher than general population. Occupationally-specific risk factors included increased physical activity, greater mechanical joint loading (due to load carriage), vibration and exposure to trauma (95,216-218).

1.5.2 Military-specific risks

The risk of OA in the military population is increased, regardless of age or sex (219,220). This risk diverged further compared to the reference population (i.e., increased relative to an equivalent civilian population) with increasing age (219,220), which might be related to the cumulative exposure, as seen in other occupational groups (202,203). The risk of primary or secondary OA in military personnel is increased inline with increased occupational exposure (205); however, the predicted assumption of cumulative exposure is confounded slightly by the nature of military work. Those who are younger are generally in more junior roles, with higher risk-exposure, and senior military roles are typically more sedentary in nature. This is demonstrated by a different analysis that controlled for sex, age, military job and specific Service and showed that those in junior enlisted roles have the highest incidence rates (218).

The specific Service also confers differing levels of risk, with the UK having three Services; British Army, Royal Air Forces and Royal Navy (the latter also includes the Royal Marines). Those who perform ‘close combat’ or ‘dismounted roles’, duties which require walking long distances over undulating terrain for periods of time whilst carrying heavy weight, are typically in the Army or Marines. During recent conflicts in Afghanistan and Iraq, this load could be up to 50kg once military equipment was accounted for (such as personal protective equipment, weapon systems and ammunition, water, food, and mission-specific equipment) (221). This duty, known as ‘patrolling’, would be happening on a daily or so basis and clearly can lead to biomechanical overloading not dissimilar to that experienced by individuals who are obese. In addition, these duties increase the potential risk of incidental MSKI and combat injury. Analyses of the US military, which has a similar structure, demonstrated that service in the Army has the highest incidence rate due to the highest physical activity requirement compared to the other two Services (205,218). The Royal Navy and

Royal Air Force have slightly different trade-specific risks, including heavy fire-fighting equipment and small occupational spaces on board a ship or in an aeroplane, and this is reflected by the differing Service MSKI rates (222).

American data demonstrates the occupational impact, with OA accounting for 70% of service-limiting disability, with 95% of those due to traumatic injury (36). Due to its accelerated pathophysiological process and typical manifestation in the third or fourth decade, PTOA can have a long-lasting impact on individuals, including occupation and decades lived with disability (218). Those who develop OA have a significant symptom burden (217,223), likely to contribute to PTOA being the most common cause of injury-related medical discharge (136). Those with PTOA (up to 36% in a military cohort) are likely to be discharged earlier than idiopathic OA (224) , and knee OA accounted for 10% of medical discharges and featured as a co-morbidity in 30% (36,225). In one study, every traumatic knee injury resulted in OA (36), with several studies reflecting how young this affects those in military service (205,217). It is important to note that this risk of OA and subsequent military medical discharge exists both in times of increased combat exposure and in times of peace, suggesting that work is required to reduce risk across the spectrum of military activity (218,225,226). Early OA disability likely contributes to the failure of military personnel to return to full duty following meniscal (227), ACL (228) or other knee injuries (229).

A preceding MSKI is a strong risk factor and predictor for subsequent OA. This is seen within the Armed Forces, with those in the military more likely to damage their ACL than the general population (230). ACL injury is the leading contributing injury for PTOA and subsequent total knee arthroplasty in the US military (231), with significant levels of OA by age 20-30 (205,217). When examined for MSKI and OA, the American military literature shows a stark picture, with 1.6 million MSKI annually (232), 60% of battle injuries resulting in medical discharge are

MSKI-related (233), and 20% of non-battle injuries are MSKI (with 16% of all MSKI related to the knee) (234). Those with a knee injury are 5x more likely to develop OA (224), with OA present three-four years after an injury (36,235), with specific injuries, such as dislocation or fracture, as well as ACL rupture, particularly high risk (231,235), as is a combat-related amputation (236). International work is underway to better understand the specific risk factors to mitigate them with primary prevention (237-239). However, it is not yet clear which are the most significant to be prioritised (240), and therefore, there is also concurrent interest in secondary & tertiary prevention (241).

This picture is not quite as clear for the British Military. Despite MSKI accounting for 54% of medical discharges between 2015 and 2020 and 56% of medical downgrades between 2010 and 2020 in the UK Military, there has been little focus on the long-term consequences of this (242). The Defence Medical Services (DMS) prioritises MSKI research to understand the epidemiology, causes and mechanisms to optimise existing and develop new prevention, mitigation, and management strategies (239,243). These injuries typically occur during the initial stages of training, after strenuous activity, or as a result of trauma, and most commonly affect the lower limbs and spine (216,230,244-246). However, given the likely contribution of OA to this burden, my research focus on early recognition and effective management is designed (and determined) to fill the evidence gap. It is clear that OA rates, as determined from electronic health records (EHR), are far lower than expected, and it is not mentioned as a contributor to medical downgrade or discharge, both likely to be evidence of underreporting.

Preliminary unpublished data from UK Defence Statistics (Health), drawn from military EHR, show 4218 individuals with a read code of OA of the knee or hip, 513 of whom had their first code entered since 2014, with an average of 53 ‘first-time codes’ per year. In total, there are approximately 9,500 military personnel with any OA-related read code, within a population of nearly 150,000 people (247). Given these codes

could be primary or secondary, a follow up question was asked to identify the number of ACL injuries per year, which could act as a proxy measure of future PTOA incidence. This identified 11,144 military personnel with ACL codes, 4307 of whom had it entered since 2014. There are approximately 450 military personnel with a ‘first code’ per year, with figures decreasing slightly between 2015-2020 and then increasing from 2020-2024. These findings suggest an annual incidence of approximately 50 OA cases and 450 ACL ruptures, with the latter making the former appear very low. Therefore, there is a need for better diagnosis and identification of OA within military populations.

One way this could be achieved, with individual risk stratification, is through molecular biomarkers. In particular, the use of these biomarkers at the completion of their care pathway (which might take a year or more), might be able to identify those with ongoing inflammation or aberrant healing pathways who, therefore, are at highest risk. Specifically, they might be able to identify those at risk of PTOA in the prodromal pre-clinical stage and those with early initial established PTOA who might require different interventions.

Chapter 2 : What does this PhD want to explore?

The Biomarkers and Joint Pain in Military Osteoarthritis (BioMilOA) study aims to understand the role of circulating molecular biomarkers, both their abundance and the change in their concentration, between those with and without osteoarthritis (OA) and to investigate their predictive value for incidence of, and worsening over time, of OA, joint pain, and function.

BioMilOA is a nested study within the ArmeD SerVices TrAuma RehabilitatioN OutComE (ADVANCE) cohort study in collaboration with the University of Nottingham, funded by Versus Arthritis (grant number 21076). It seeks to understand the role of biomarkers in a large military population, laying the foundations for targeted surveillance and intervention and providing a unique opportunity to compare baseline and follow-up factors in those with painful and non-painful PTOA and idiopathic OA. Whilst the primary focus will be on those sustaining trauma, it will also provide important data on the utility of biomarkers in a general military population, which has never previously been performed.

2.1 The Armed Services Trauma Rehabilitation Outcome (ADVANCE) Study

The ADVANCE study is a 20-year longitudinal cohort study of 579 male combat casualties and 566 matched participants comparing medical and psychosocial outcomes of military personnel exposed and not exposed to significant combat-related trauma. Within this cohort, three-quarters of the injuries were blast, caused by explosions, with the remainder caused by gunshots, burns or accidents. These injuries were all severely traumatic, requiring emergency evacuation to the UK from Afghanistan for definitive treatment after initial in-country life-saving treatment. As a result of these injuries, there has been a widespread inflammatory and metabolic response, increasing the risk of post-traumatic complications, including post-traumatic OA (PTOA). Furthermore, within the trauma-exposed injured group, approximately a third sustained a traumatic lower-limb amputation, and a small sub-group sustained a local knee injury during the traumatic episode, allowing further exploration of injury pattern on subsequent OA and pain development, against both the remainder of the injured participants and the uninjured frequency-matched comparison group. The primary outcomes for this study are cardiovascular, mental health and musculoskeletal, with study visits planned at 3, 6, 10, 15, and 20 years after the initial baseline visit. This thesis will use data from the Baseline visit and Follow-Up 1 visit three years later.

2.1.1 Context

Since the increases in initial survival from the First World War, those who sustained a combat injury were more likely to have, and potentially die from, conditions which would be expected much later in life, including cardiovascular disease (248-250). This phenomenon has been seen, retrospectively, following the Second World War (250,251), Vietnam (249,252,253), the first Gulf War (254,255), and later conflicts (234,256). As well as cardiovascular, there is also a significant burden of mental health and musculoskeletal sequalae (36,233,257,258), particularly in those who survived injuries which would have been fatal in previous conflicts (259). However, the mechanism underpinning these is not clear, and so, as a result the prospective ADVANCE cohort study was initiated to investigate the long-term outcomes of combat-injury in British service personnel following the Afghanistan conflict (2003-2014) (260).

Outcomes from ADVANCE are already shaping clinical interventions and policy, including through the findings of the cardiovascular and mental health groups (261-263), implemented through advice to panels such as the Independent Medical Expert Group (264), and the impact of lower limb amputations on bone mineral density (265,266). Earlier work in this cohort has demonstrated that the amputee sub-population is a distinct population from a metabolic, musculoskeletal, and psychological perspective (138,261,263), with an amputation conferring a 4x increased risk of OA, as did sustaining a local knee injury during the traumatic episode (138). ADVANCE 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, as seen, can be translated into clinical practice to enable targeted interventions (267).

2.1.2 Participants

Potential study participants were identified from lists produced by Defence Statistics (Health), operating within the UK Ministry of Defence (MoD). Multiple other data sources were also used: the initial Notification of Casualty System; the Defence Patient Tracking System; Joint Personnel Administration (JPA); the Joint Theatre Trauma Registry (JTTR); the Defence Medical Information Capability Programme (DMICP); the Defence Medical Rehabilitation Centre (DMRC) Complex Trauma Database; and the DRMC Prosthetic database (260). The first list, n=1400, were potential exposed participants who were male, deployed to Afghanistan between 2003 and 2014, and sustained a combat injury severe enough to warrant evacuation back to the UK for treatment (Figure 2.1). The second list, n=2100, was male service personnel who were frequency matched to the initial list on age, service, rank, role, regiment and deployment (Figure 2.2). Deployment matching was performed in relation to the deployment upon which the exposed participant sustained a combat injury (260).

Eligibility criteria included male British service personnel (≥18 years), who sustained any combat-related traumatic injury (defined as requiring aeromedical evacuation from Afghanistan) between 2003-2014. 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. Other exclusion criteria included age <18 or >50, unwilling or unable to give informed consent, medical history of established cardiovascular disease or diabetes mellitus prior to index deployment or active acute infection (temperature <36 or >38, respiratory rate >20 breaths/min or heart rate >90bpm). For potential, unexposed participants, any subsequent combat injury sustained in Afghanistan excluded them from recruitment.

Participants were approached through postal invitations, email invitations or telephone calls. When necessary, contact tracing via JPA was performed, if still serving. Other methods, including electoral roll data, and advertising via social media or military charities were also used to extend visibility to potential participants (260). Further details on participant identification and recruitment can be found here (260,263).

Sample size calculations for the ADVANCE study were based on the primary composite cardiovascular disease (CVD) endpoint, the Major Adverse Cardiovascular Endpoint (MACE). Previous data showed combat-injury incurs a greater risk of CVD, hazard rate of ≥1.7 (250), therefore a sample size of at least 400 in both groups would be required to provide >80% power at a two-sided alpha of 0.05 at 20 years given the likely low event rates in the age and demography of the population (260). Initial recruitment of approximately 600 per group would allow for a natural drop-out of 10% every five years (260). This sample size should be sufficient for OA outcomes, based on a mean prevalence of knee OA of 8.7% and hip OA of 2.8% in 40-50-year-olds, and expected two-fold in those with trauma exposure, with an initial sample size calculation of 82 per group deemed adequate for two-sided alpha of 0.05 and power >95% (268-270).

Recruitment for the study commenced in March 2016 and continued until August 2020, with n=579 exposed participants and n=566 uninjured participants as a comparison population, frequency-matched for age, rank, service, deployment and job-role, recruited (260).

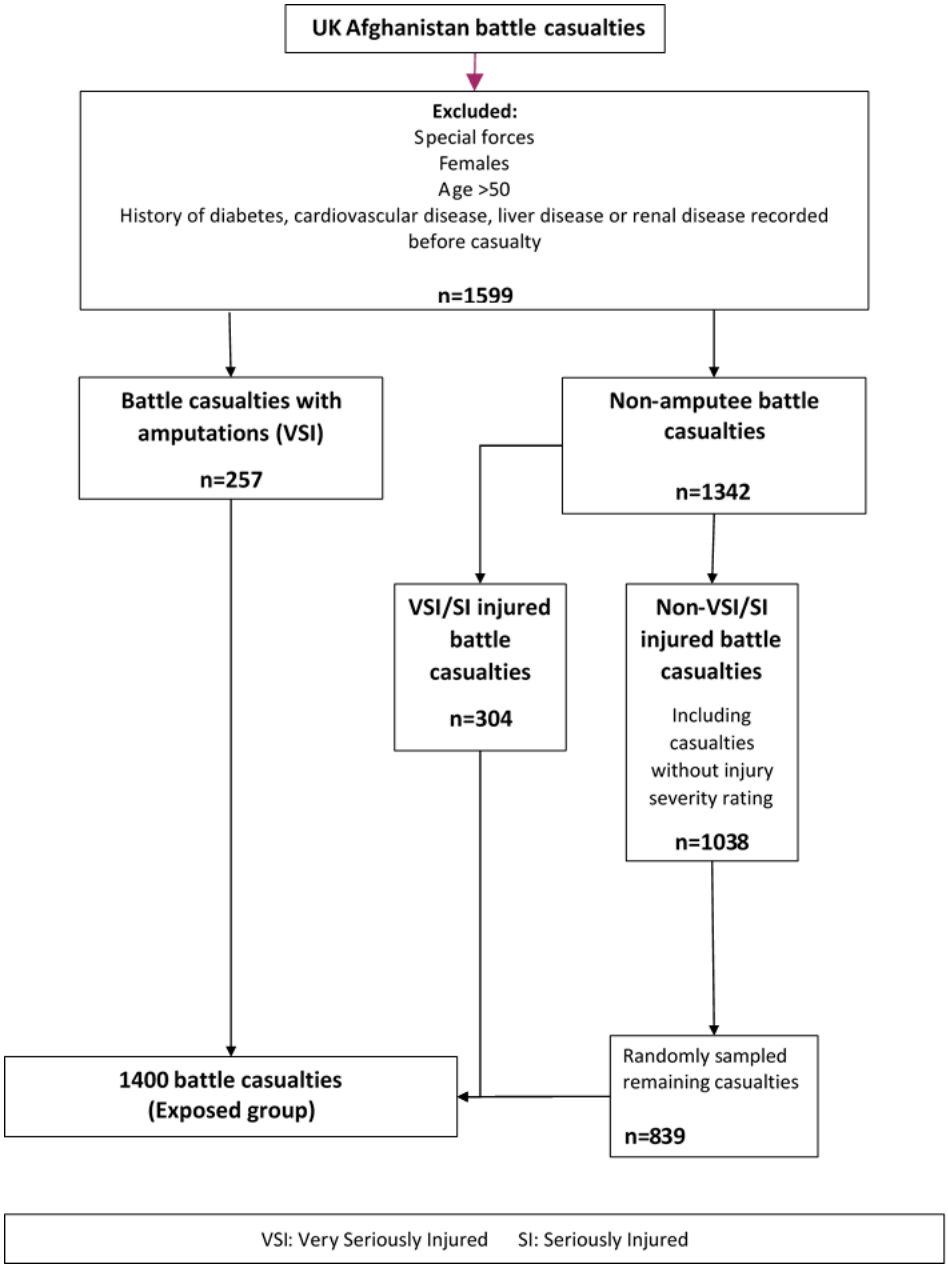


Figure 2.1 Flowchart representing the recruitment process for Exposed ADVANCE participants (reproduced from (260))

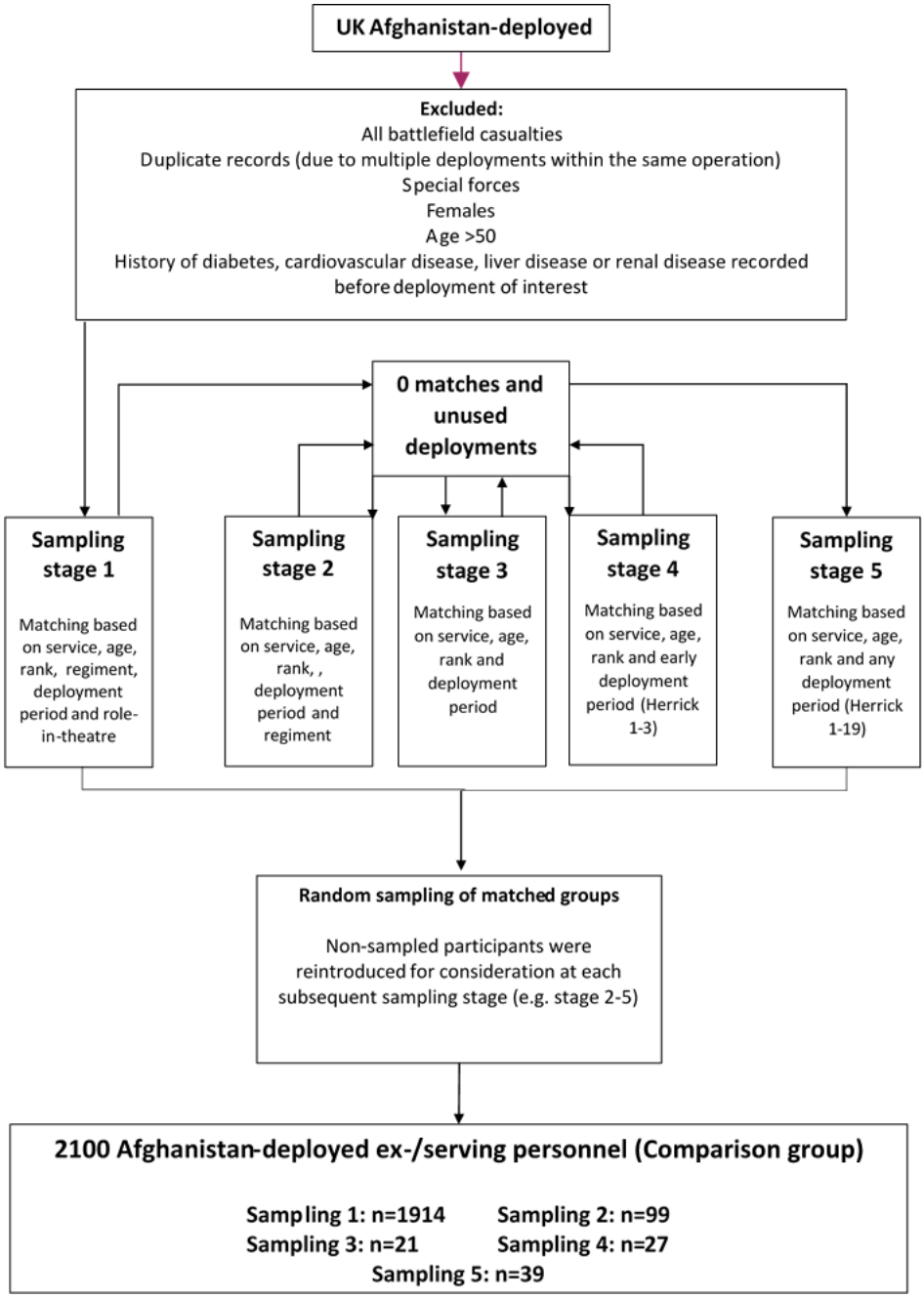


Figure 2.2 Flowchart representing the recruitment process for Unexposed ADVANCE participants (reproduced from (260))

2.1.3 Ethical approval

Favourable opinion for ADVANCE was granted by the MoD Research Ethics Committee (MODREC:357PPE12) on the 15th of January 2013. The study is conducted in line with the recommendations guiding ethical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, in June 1964 and amended at the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013.

In addition to this, upon commencement of my PhD, I sought, and was subsequently granted, approval for BioMilOA from the University of Nottingham Faculty of Medicine and Health Sciences REC (UoN FMHS 170-1122). Study participation was voluntary, with written informed consent from each participant. Informed written consent is repeated and confirmed at each study visit, which includes consent for access to NHS and MoD health records, held securely at the Academic Department of Military Rehabilitation (ADMR), DMRC Stanford Hall. This consent form includes all investigations and the potential use of anonymised data by other research teams.

2.1.4 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 relevant to the interests of the participants, and study logistics. In addition, PPI improves study accessibility, such as during outcome measure recording - especially with regard to adjustments for those living with disability, such as paper questionnaires for those struggling with using computer-based questionnaires and verbal instructions for those living with sight impairment.

My initial results (as described in Chapter Four) were discussed at the ADVANCE Participant Panel on 10th September 2024. I explained the purpose of my study and key results, followed by some formal questions and open discussion with participants. When the manuscripts associated with Chapter Five are published, they will be discussed at the next participant panel.

2.1.5 Study visits

Study visits occurred at the DMRC Headley Court (2015-2018) or Stanford Hall (2018-2020) for Baseline, and Stanford Hall only (2019-2024) for Follow-Up 1, over the period of one day (usually 0730 – 1600, overnight accommodation was available if needed). All participants were fasted and absent from caffeine and alcohol for at least 8 hours before the visit. Study data were collected and managed using Research Electronic Data Capture (REDCap), hosted at Imperial College London. REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources (271).

A trained research nurse collected a range of assessments (summarised in Figure 2.3). These data included sociodemographic (age, rank, regiment, educational and employment status) and anthropometric (height using SECA 704, UK, weight using SECA 956, UK, waist circumference using tape measure), with body mass was corrected using an appropriate formula for any missing limbs (272). Details of the medical history of the participants and immediate family and their injury (if exposed to combat trauma) were recorded, including dates, specific injuries, severity and post-injury treatment. These data were also gathered using JTTR and DMICP records to correlate results.

As the Follow-Up 1 data collection period was ongoing during my PhD programme, I was able to join the team, and consent was one of my responsibilities when collecting data and involved repeating the study aims, purposes, and study visit requirements, with all participants receiving new written versions of these when their study visit was booked. Given the co-morbidities in the Exposed group, I ensured I

provided extra time and attention for those with visual impairment or a history of traumatic brain injury to ensure that they had retained and understood the information. Other responsibilities were serum collection, functional testing and general dogsbody! To prevent any potential unbalanced power dynamic, I wore ADVANCE-branded attire, not military uniform.

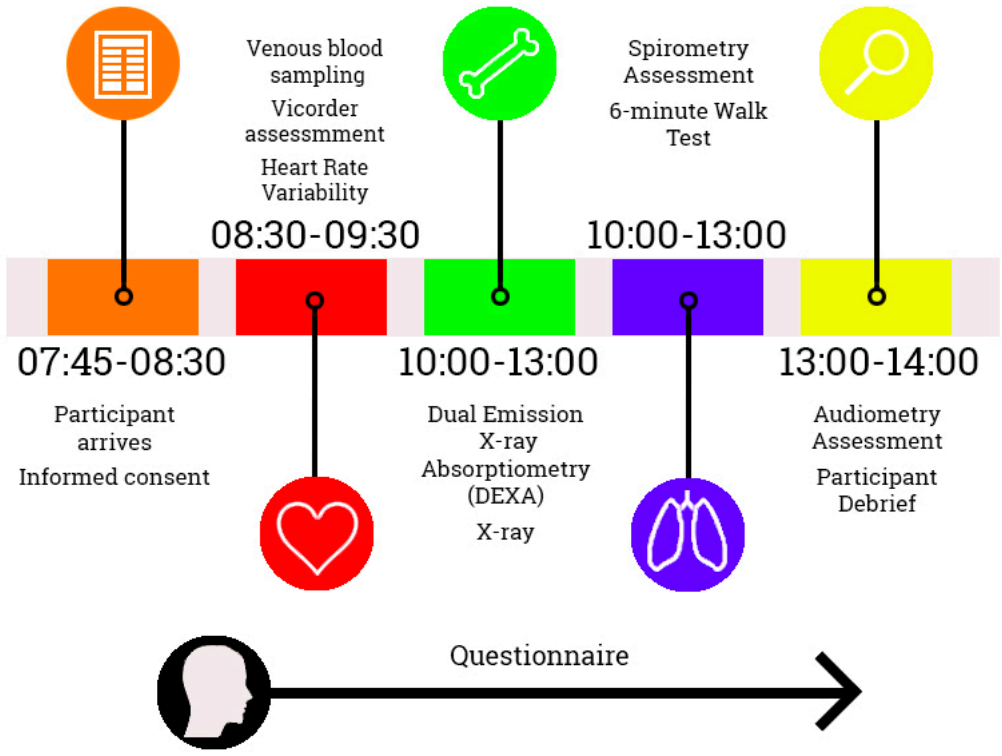


Figure 2.3 Flowchart representing the ADVANCE Study Visits (reproduced from (260))

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. Exposed participants were further classified as Exposed – No Lower Limb Amputation (Exp-NA), Exposed – Lower Limb Amputation (Exp-A), and Exposed – Knee Injured (Exp-K) during the analysis performed in Chapter Four and Five. Given the hypothesis that local trauma to a joint is likely to confer a higher acute risk of subsequent PTOA when compared to the chronic biomechanical changes occurring following lower limb amputation, in line with a previous analysis, when both Exp-A and Exp-K were present, they were coded as Exp-K (138).

2.1.5.1 Patient reported outcome measures

Patient-reported outcome measures (PROMs) were gathered, including those related to musculoskeletal injury and function; the Knee injury and OA Outcome Score (KOOS) (273), knee and hip visual analogue scales (VAS, 0-10, for impact, severity, and frequency for right and left side); and many others, including those related to amputation (pain, function, mobility), mental health; generalised anxiety disorder-7 (GAD-7) (274), patient health questionnaire -9 (PHQ-9) (275) and post-traumatic stress disorder checklist for DSM-5 (PCL-5) (276), pain (joint-specific and global), international physical activity questionnaire (IPAQ) (277), alcohol and drug use, and those related to social support, sleep and quality of life (260).

2.1.5.2 Functional assessment

To assess function, the sub-maximal exercise six-minute walk-tests (6MWT) were performed on a linear, flat 20m course, with verbal instructions before and during. These instructions included asking them to walk as far as they could between two cones, but not run, for six minutes, with regular time updates. No verbal or physical encouragement was given, with the participants self-pacing throughout. Participants could use aids and stop to rest if required, with distance recorded to the nearest 0.5m - further details on protocols used can be found here (278).

2.1.5.3 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') (279). The FDA-approved Knee OA Labelling Assistant (KOALA, Image Biopsy Lab, Vienna, Austria) with manual checking was used, which offers an accuracy of 82%, sensitivity of 78% and specificity of 88% for KL grades ≥1 (280). The aided AI KOALA tool has an inter-rater reliability of 0.86 for all KL grades, and improves reader agreement rates by 2-fold when assessing KL grade (281,282). When participants had two variables for KL grade (from both knees), the index knee score signifying more advanced rOA was selected (higher KL grade). For those with an above-knee amputation, the single variable was used. In addition, the Osteoarthritis Research Symposium International (OARSI) atlas scoring were undertaken, again using KOALA with manual checking, which grades joint space narrowing (JSN), sclerosis (Scl) and osteophytes (OP) using a 4-point scale (0 – none, 1 – mild, 2 – moderate, 3 – severe) (283).

2.2 The Biomarkers and Joint Pain in Military Osteoarthritis (BioMilOA) Study

Using data from the Baseline and Follow-Up 1 visits, the nested sub-study, BioMilOA, was established to investigate the associative and 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. My PhD programme is the delivery of the BioMilOA study.

During this programme, I have collected data during the Follow-Up 1 visits, organised the logistics and contracts between the MoD, UoN and ABL, planned and conducted the statistical analysis, and interpreted all the results. I have led the writing and submission of all associated manuscripts. In addition, I have led and performed all the systematic reviews, designed and delivered the pilot study described in Chapter Six, and have fostered international collaborations to enable me to deliver clinical implementations.

2.2.1 Hypothesis

The hypothesis of this PhD is that a panel of serum biomarkers will be able to identify those with new or progressive OA within the ADVANCE cohort.

2.2.1.1 Aims

Within this thesis, there are several pre-planned aims nested within the overall hypothesis, which will be addressed in each chapter.

Aim 1: To summarise published literature in human studies on the associations of known serum and synovial fluid biomarkers at least a year from injury to structural and symptomatic changes and underlying PTOA processes, and to describe cross-sectional knee OA imaging

features present one year or more following injury and their associations with structural, functional, or symptomatic changes.

Aim 2: To describe serum biomarker concentrations differences between those with/without trauma-exposure, knee OA or pain, with a secondary aim to describe serum biomarker concentrations differences between those with idiopathic v PTOA, painful v painfree OA, or with different injury or function.

Aim 3: To assess the predictive value of pre-determined characteristics for progression and incidence of knee rOA, pain and function (including candidate serum biomarkers). A secondary aim will be to explore how knee rOA and pain progression and incidence, and function is influenced by injury.

Based on the results of these chapters, further work was required, and so as my PhD progressed, two further aims were developed.

Aim 4: To understand the effect of the location of sampling and non-weight bearing sub-maximal exercise intervention on the concentrations of candidate serum OA biomarkers

Aim 5: To identify the functional tasks utilised to assess knee kinematics and kinetics at least one year from significant injury, and describe the differences found between the injured and uninjured knees. A secondary aim is to report any features associated with the presence of PTOA.

2.2.2 Biomarker selection

Sera underwent analysis for selected cartilage turnover biomarkers (cartilage oligomeric matrix protein, COMP, carboxyl-terminal telopeptide of collagen type II, CTX-II, N-propeptide of collagen IIA, PIIANP), pro-inflammatory cytokines (interleukin, IL-1β, IL-17α, tumour necrosis factor, TNF-α) and metabolic markers (leptin and adiponectin). The biomarkers selected were deliberately chosen to provide insights into different possible pathological mechanisms during the initial grant application in 2015, including aberrant inflammatory responses, tissue turnover and metabolic dysfunction, and it was hoped that all the biomarkers selected would also be able to validate findings made in different populations.

Each biomarker selected had differences within its type, and to allow understanding of potential different patterns between idiopathic OA and post-traumatic OA. IL-17α is released by T-helper cells (and others), acting on synoviocytes (284), influencing the release of IL-1β, which act on chrondocytes and osteoblasts/clasts (285), with TNF-α seen to influence the cartilage response to trauma (286). The biomarkers related to tissue turnover, COMP (cartilage metabolism) (173), CTX-II & PIIANP (collagen type II degradation and synthesis) (169) are some of the most researched biomarkers, offering insight into different components of tissue breakdown and production. Finally, adipokines, including leptin and adiponectin, are likely to demonstrate a link to a wider systemic influence of OA on metabolic dysfunction (190,191)

2.2.3 Biomarker assessment

Fasted blood was taken from the antecubital fossa using the Vacutainer system, centrifuged at 3500rpm for 10 minutes, with serum aliquoted and stored in cryovials in monitored freezers at -80°. Sera samples were obtained on 1121 participants during their Baseline visit (24 participants declined or sample were unable to be obtained). Frozen samples, contained in ice boxes, were transferred to Affinity Biomarker Labs (ABL, London, UK) for analysis using enzyme-linked immunosorbent assay (ELISA) or meso scale discovery (MSD), using a courier service once appropriate transfer agreements were in place between the MoD and UoN. Prior to analysis, samples were randomised to reduce the risk of bias, with randomisation aiming to minimise the effect of exposure, date of sampling and box number.

Upon receiving the biomarker analysis data from ABL, I cleaned it to ensure missing values were coded in a uniform manner. Having undergone quality assurance and control at Affinity labs, these results were reviewed, and subsequent thresholds for analysis were set. 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). This latter process enabled all data to be used to avoid excluding variables and participants.

2.2.4 Data management

All study visits were performed at DMRC, where study data will be held securely in line with requirements from the Caldicott Guardian, Data Protection Act and General Data Protection Regulations. Data and material transfer agreements were arranged between DMRC and the UoN (Defence Medical Services Contract no: 702903455), with the analysis and data generated by this PhD held securely on servers hosted and monitored by the UoN. Results from additional analysis and derived variables will be secure transferred to the ADVANCE Data Manager and hosted on secure servers at Imperial College London for use by collaborators on request. Further details can be found in the BioMilOA Data Management Plan (Version 1.0, dated January 2022).

Once the contract was in place between the MoD and UoN, I underwent the ADVANCE sample transfer process, and baseline frozen serum samples were transferred under UoN and subsequently transported to ABL for processing and sampling.

2.2.5 Statistical analysis

2.2.5.1 Pre-processing

A data analysis plan was created prior to analysis formally beginning. The stages of this plan involved data pre-processing, normalisation, exploratory, descriptive and hypothesis-driven. Data related to participant demography, medical history, PROMs, radiographs and functional tests collected at their baseline visit were requested from the ADVANCE data management team. These data had undergone quality control and assurance by the data team and, therefore, did not require substantial review. However, an element of pre-processing was required, such as standardisation in the terms used for missing data (as different variables employed different notations, such as ".", "0", or "-9"). In addition, in order to undergo analysis, a long-to-wide transformation was performed in STATA, enabling each participant to have a single row of results (particularly relevant when two variables existed for the same outcome, such as right and left knee radiograph). Data not required for each analysis was 'dropped' from the using file with 'do-files' written specifically, detailing each step, to avoid any changes or alterations to the original data.

2.2.5.2 Initial steps

All data were screened for normality visually using histograms, with parametric and non-parametric testing used accordingly. Different transformational techniques were trialled to ascertain the best ways to analyse the biomarker variables, given the significant skew of certain values (due to the natural variation within the population and the threshold setting referred to above). This was conducted with the ambition of minimising the effect of any skew and increasing the number of variables which were normally distributed (thus enabling parametric testing and the increase in statistical power that this

creates). These included logarithmic (log), log10, and standardisation to a mean of 0 and standard deviation (SD) of 1. Different approaches were adopted for different phases of the analysis plan to enable adjustment for confounders and direct comparability of biomarker effect. In summary, biomarkers were analysed in their 'raw' form, with adjusted biomarkers utilising their natural logarithm, and standardisation was undertaken to calculate odds ratios (OR).

2.2.5.3 Confounders

Throughout this thesis, unadjusted analyses were initially performed, followed by adjusted, with the confounders age, body mass, time from injury/deployment, rank (as a proxy for socio-economic status, SES (287,288)) and ethnicity adjusted for. This was performed by transforming the biomarkers using their natural logarithm and adjusted for the confounders using a regression model, with studentised residuals created and taken forward for analysis (289). 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 (290), was utilised in Chapter Four, which gives a balanced reflection of body weight and central adiposity. Time from injury/deployment was measured from the participant's index deployment. Presenting data both in its unadjusted and adjusted forms allows other studies to compare results and enables the effect of OA to be more accurately partitioned.

In order to deploy on their index deployment, the individual would have had to have been fully fit, as deemed by their military General Practitioner. However, given the likelihood of serving personnel sustaining a musculoskeletal injury prior to this, patient-reported and

EHR were reviewed to identify those with knee pathology prior to index injury/deployment, of which there were 93 participants. Thirty-seven of these had a specific injury associated with increased risk of OA (meniscal, cruciate, fracture), and the remainder reported persistent knee pain. Using the higher number as a more conservative measure, I performed a chi-squared test, which showed there was no significant difference between the two groups (52 participants in unexposed, 41 in exposed, chi2 = 1.7247, p = 0.189). I was, therefore, happy that this would not influence the rate of OA development in either group compared to the other. I subsequently performed pairwise correlations between the history of a prior knee pathology and biomarker concentration, with the vast majority of biomarkers yielding no statistically significant results. The lowest p-value, not adjusted for multiple testing, was 0.07, and therefore I felt that this was unlikely to unduly influence the results. Given the known risk factors of OA, it was my view that the other confounders selected (age, body mass, SES, ethnicity and time from injury) were more likely to influence any results, and thus were prioritised in the model.

Chapter 3 : What are biomarkers and how are they used?

As described in Chapter One, osteoarthritis (OA) is a whole-joint organ disease with many contributing mechanisms to the pathological process and subsequent clinical syndrome. Until relatively recently, diagnosis, prognosis, and management plans were made from radiographic evidence, predominantly using a scale created in the 1950s (the Kellgren-Lawrence, KL, scale), supported by patient-reported measures. The KL scale has five grades; grade 0 (none), 1 (doubtful/early), 2 (minimal), 3 (moderate) and 4 (severe), and categorises the presence and severity of osteophytes, joint space narrowing (JSN), sclerosis and deformity of bone ends (279). Given the lack of clear criteria for each group, and likely distinct tissue-specific pathophysiological processes occurring at each point, an additional scoring system was created by the Osteoarthritis Research Society International (OARSI), with a separate score (0-3) for osteophytes, JSN and sclerosis, where 0 is none and 3 is severe (283). X-ray is no longer used for diagnosis, but continues to be used for prognostication and research. JSN has been used as a research outcome for regulatory bodies, as a proxy for the thickness, integrity and health of hyaline articular cartilage (40,64).

Alternative metrics and parameters have been under investigation for clinical and research use, in particular molecular biomarkers, to diagnose diseases, stratify treatment, monitor disease progression, predict treatment response, and evaluate the effectiveness of new therapies. Biomarkers are defined as *'a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'* by the National Institute of Health (NIH) (291). Biomarkers have a wide range of potential applications, including for disease-modifying OA drug (DMOADs) trials. Specifically, they can enhance the identification and recruitment of those with high-risk progressive OA and can act as outcome measures alongside existing measures, including

JSN and pain measures (292,293). In addition, clinical use of biomarkers may also enable the characterisation of OA disease to identify the pre-radiological and pre-symptomatic phases, thus allowing the opportunity for earlier identification, such as preventative measures, and demonstrating the effect of interventions (2).

Given the known starting point of post traumatic OA (PTOA), and accelerated pathophysiology for this secondary type of OA, this condition is often a focus for biomarker research. Individuals with exposure to injury have fewer comorbidities and are younger; therefore, fewer confounding factors are present. A key question for biomarker research is that of time; how do the biomarkers change as the injury occurs, heals and is rehabilitated, with previous reviews performed which aimed to understand those biomarkers that were implicated in the acute phase (hours-day) and post-acute (days-weeks) phase following injury (166,294-296). However, little is known beyond that, despite certain rehabilitation pathways, including those for ACL injury, commonly taking 9-12 months. In real life, clinically, individuals present with recurrent or persistent joint pain with a history of an injury years before. Therefore, I defined a new term, the chronic phase of PTOA, i.e. a year or more from injury, and aimed to synthesise the evidence regarding wet and dry biomarkers at this time-period.

3.1 Biochemical and imaging biomarkers in the chronic phase of post-traumatic osteoarthritis

Ongoing inflammation and joint damage after injury can occur without symptoms or functional loss with PTOA in the asymptomatic, prodromal phase prior to radiographic and symptomatic change. The hypotheses of this systematic review are that serum and synovial fluid (SF) biomarkers and post-traumatic knee magnetic resonance imaging (MRI) features are linked to structural, patient-reported, functional or clinical knee changes, and offer insights into PTOA mechanisms.

Therefore, this systematic review aims to summarise published literature in human studies on the associations of known serum and SF biomarkers at least a year from injury to structural and symptomatic changes and underlying PTOA processes, and to describe cross-sectional knee OA imaging features present one year or more following injury and their associations with structural, functional, or symptomatic changes.

3.1.1 Methodology

A systematic review was conducted in line with preferred reporting items for systematic review and meta-analyses (PRISMA) guidance (297). Inclusion criteria included full-text studies in languages spoken by the research team, in participants with a significant knee injury aged 18-45 (to avoid confounding with skeletal immaturity or idiopathic OA), involving wet or dry biomarkers measured at least a year from injury (to ensure physiological remodelling changes have concluded) (Table 3.1).

In order to ensure PRISMA and Cochrane best practice were adhered to, each screening process (initial title/abstract and full-text), data extraction and risk of bias assessment were performed by two researchers independently with a third for arbitration when required (297,298). I led the process and was involved in each element of the review. Two project teams were developed, each focusing on wet and dry biomarkers, respectively. I co-ordinated and was involved in both teams to ensure a consistent approach, oversee progress, and perform the subsequent analysis and write-up.

Table 3.1Study selection inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Full text articles, in English, Polish, Danish, or Spanish	Laboratory based, in-vivo or animal studies
Participants aged between, inclusive of, 18 and 45 years old	Participants under 18 or over 45 years old
Significant injury one year or more previously	Significant injury sustained less than 1 year ago
Study involved a ‘wet or ‘dry’ biomarker	

The protocol was registered prospectively on PROSPERO (CRD42022371838). Medline and Embase (both via Ovid), Cochrane central register of controlled trials (CENTRAL) (via Wiley) and ClinicalTrials.gov were all searched on 8/11/22, and the World Health Organisation (WHO) international clinical trials registry platform on 9/11/22. Conference proceedings were searched on 10/11/22.

Corresponding authors of similar systematic reviews registered on PROSPERO were contacted. Subject matter experts recommended additional studies in addition to those found in searches. A hedge for human studies was used in Medline and Embase (299). No other filters or limits were used. Searches incorporated keywords and subject headings relating to knee PTOA and biomarkers (Supplementary File 1). Results were deduplicated using EndNote 20 and SR Accelerator.

Initial title and abstract screen was performed by two reviewers independently against pre-determined inclusion and exclusion criteria (Table 3.1) with a third reviewer resolving conflicts, using Rayyan (www.rayyan.ai). A second, full-text screen, was undertaken in the same manner, prior to independent data extraction using a pre-prepared data extraction form (Excel, Microsoft).

Relevant extracted data included;

- First Author, Title, Journal, Year
- Population: Number (cases/control), sex, injury type, time from injury, occupation (if mentioned)
- Biomarkers: Which used, type, when/how measured
- Molecular biomarker testing assay and company
- Imaging modality, machine settings, sequence, strength, positioning, processing
- Comparators used

The Newcastle-Ottawa Scale (NOS) was used to assess the risk of bias. This scale was chosen due to its simple, easy-to-understand scale (for both users and readers) and ability to have both a cohort and a cross-sectional version (reflecting both types of study design included in the review). The NOS assesses participant selection, case-control comparability and outcome assessment (Table 3.2) (300). Scoring for cohort studies is 0-9, with studies scoring 0-2 rated poor, 3-5 fair, 6-9 good/high and cross-sectional studies scored 0-10; unsatisfactory 0-4, satisfactory 5-6, good 7-8, and very good 9-10 points (300). Risk of bias

assessment was performed by two reviewers independently, with a third available for arbitration.

Due to significant differences in study methodology (including wet biomarkers measured, MRI protocols and scoring systems, comparators, time from injury, and statistical analysis plans), a meta-analysis of results was not possible, so all the results have been reported using the synthesis without meta-analysis (SWiM) guidelines in narrative form (301).

Table 3.2 Newcastle-Ottawa Scale categories and scoring

Category	Selection	Comparability	Exposure	Scoring
Cohort	1) Representativeness of the exposed cohort* 2) Selection of the non exposed cohort* 3) Ascertainment of exposure* 4) Demonstration that outcome of interest was not present at the start of the study*	1) Comparability of cohorts on the basis of the design or analysis**	1) Assessment of outcome* 2) Was the follow-up long enough for outcomes to occur* 3) Adequacy of follow up of cohorts*	Total: 9 Good/high: 6-9 Fair: 3-5 Poor: 0-2
Cross-sectional	1) Representative of the sample* 2) Sample size* 3) Non-respondents* 4) Ascertainment of the exposure**	1) Comparability of subjects in different outcome groups on the basis of design or analysis. Confounding factors controlled.**	1) Assessment of outcome** 2) Statistical test*	Total:10 Very good; 9-10 Good: 7-8 Satisfactory: 5-6 Unsatisfactory: 0-4

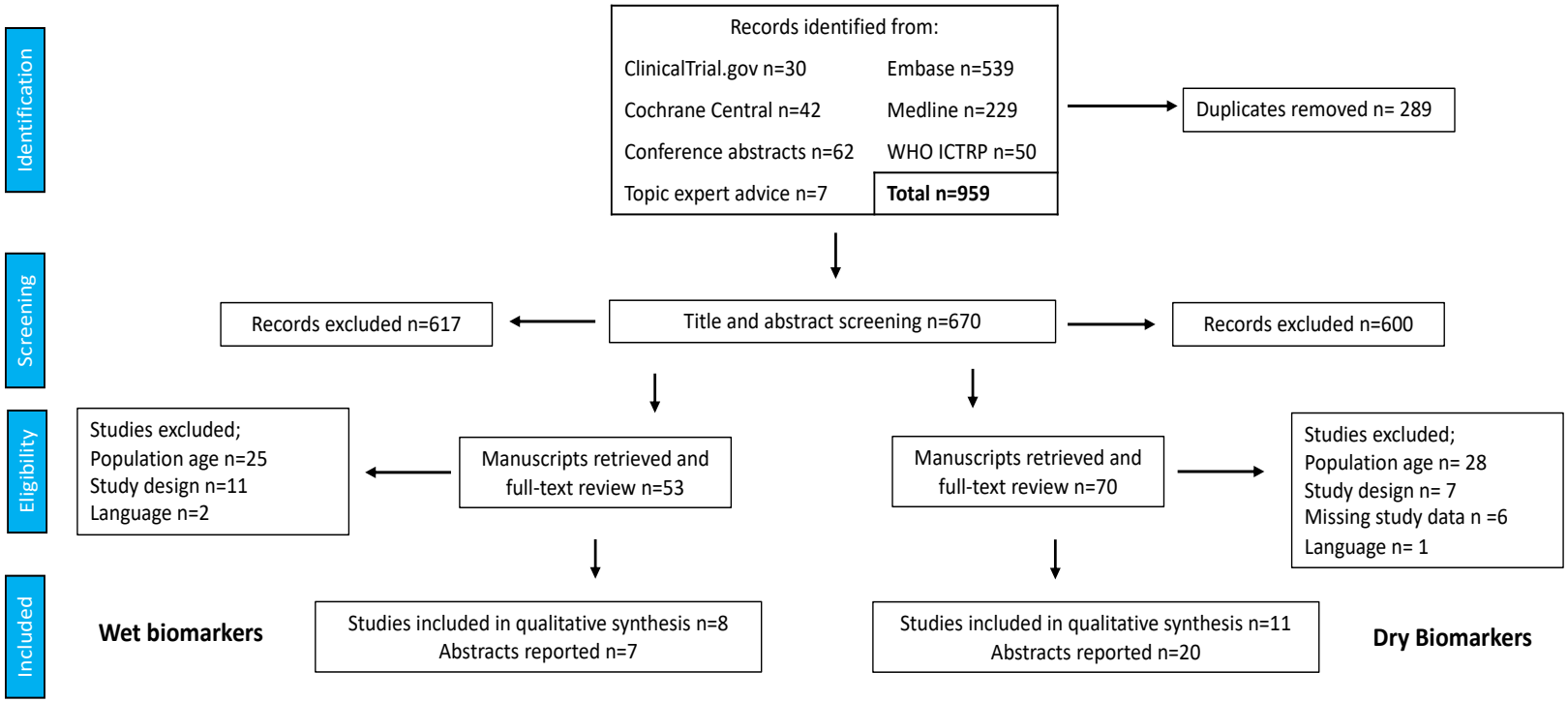


Figure 3.1 PRISMA flow chart of study

3.1.2 Results

A total of 959 studies were identified following the search, with 670 remaining after deduplication. A title/abstract screen was performed, identifying 53 wet biomarker papers and 70 involving dry biomarkers. After full text review, this was eight and eleven, respectively, with one manuscript meeting the criteria for both (302). In total, therefore, there were 18 separate papers included with a total of 1629 participants (96,302-318). Conference abstracts which met criteria numbered seven (141,298,319-324) and 20 (140,324-342) respectively, in line with best practice from the Cochrane Collaborative (298,343). The most common reasons for exclusion were time from injury to biomarker measurement and participant age. Figure 3.1 summarises this process.

3.1.2.1 Study characteristics

All wet biomarker studies measured molecular biomarkers in serum or SF, with only samples taken at least a year from injury included in this review. No studies involving plasma-, urinary-based biomarkers or metabolomics were identified. All dry biomarker studies utilised MRI, with no USS or other modalities identified.

The studies varied in terms of design and observation periods. The dry biomarker studies were all prospective cohort studies, bar one retrospective study, whereas the wet biomarker studies were cross-sectional. The Knee ACL, Nonsurgical versus Surgical (KANON) studies also used prospective methodology (302,309,310,317,318). Four studies used different treatment methods (surgical vs. non-surgical) to define their exposure and risk of PTOA (302,306,317,318).

Four molecular biomarker studies had a comparison population, only one of which matched the exposed population (305). One study comparison was half age-matched ‘within 7 years’ and the rest older

(303). One study used one reference population to compare serum and another for SF (309), and the last did not fully describe their reference population (312). Another study used the contralateral limb as a reference (304). Four imaging studies adopted a case-control methodology with healthy matched controls (311,313,315,316).

Studies ranged from a mean of one to 11 years post-injury, with some individuals 14- (305), 16- (312), and 18-years (96) from their initial traumatic injury (Figure 3.2 and 3.3). The date from injury was confounded by some studies, with two reporting the date from ACL reconstruction (ACL-R) (the latter also reported average time from injury to ACL-R) (303,304). Furthermore, the time interval from injury to ACL-R varied, with nearly all performed within six months, aside from the four studies with a late ACL-R arm (302,306,317,318). When reported, time to follow-up post-surgery ranged from one (303,307,308), two (302,306,313,315,317,318), three (311,314), four (316) or five years (302,317,318)

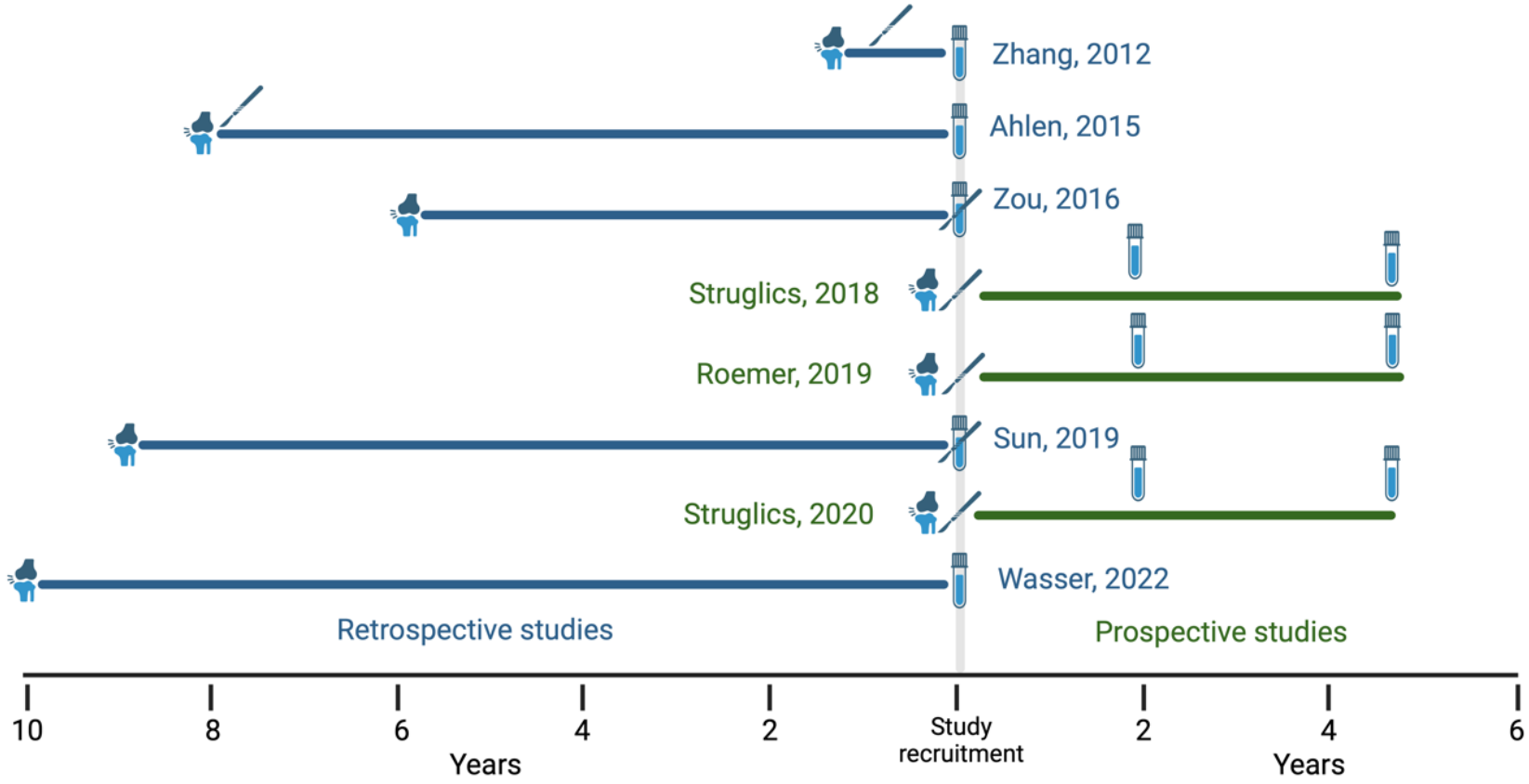


Figure 3.2 Pictorial representation of 'wet' biomarker study designs, including time from injury and surgery, sample collection and direction of study.
Blue colour represent retrospective studies, green colour represent prospective studies. Knee icon represents time of initial injury (when reported), scalpel icon represents time of surgery (when reported) and sample tubes represent data collection points. Created in BioRender.

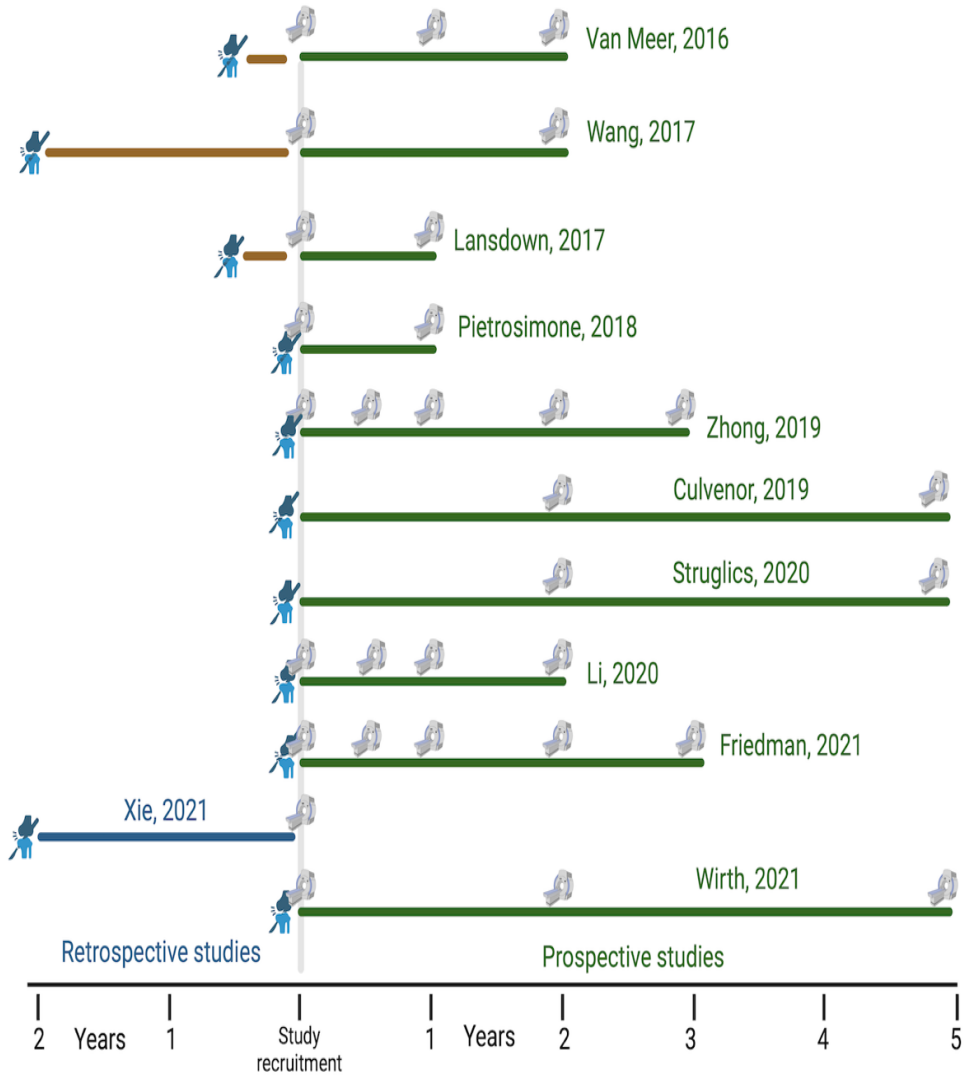


Figure 3.3 Pictorial representation of ‘dry' biomarker study designs, including time from injury and surgery, imaging and direction of study.
Blue colour represent retrospective studies, green colour represent prospective studies, brown colour represent gap between injury/surgery and study recruitment.. Knee icon represents time of initial injury (when reported), scalpel icon represents time of surgery (when reported) and MRI machine represent data collection points. Created in BioRender.

3.1.2.2 Participants

In total, there were 1424 exposed participants involved, 911 male and 411 female (one study, n=102, did not report sex (303)). Across the 18 papers, however, there were several studies which analysed the same population, including those involved in the KANON study (302,309,310,317,318), and due to significant similarities in study location, timing and design, the participants in Lansdown and Friedman (307,314), and Zhong and Li (311,313), are likely the same. Therefore, 780 separate individuals are involved in this review (450 male, 228 female, 102 unknown).

All participants were aged between early-mid 20's and early-mid 30's, with a single study reporting age as '<41' (303). Only one study reported the ethnicity of their participants (Chinese, (312)). All participants sustained a traumatic injury, with all studies bar one involving an ACL injury, with subsequent ACL-R in nearly all cases. One study in military personnel involved combat-associated injury, CAI (96). Only two studies reported the mechanisms of injury, one CAI (96), and the other reporting sports (86%) and everyday activity (14%) (312). Most imaging studies excluded concurrent joint pathology, including known OA (306-308,313-316) or meniscal and multi-ligamental injury (307,308,313-315). Two studies reported meniscal interventions (306,311).

3.1.2.3 Biomarkers

Seven studies (87.5%) measured serum biomarkers (96,302,303,305,309,310,312), six studies (75%) measured SF biomarkers (302,304,305,309,310,312), and five (62.5%) measured both (302,305,309,310,312). Two studies only measured serum (96,303) and one only measured SF (304). To assess correlation, all five studies involving paired serum and SF samples measured virtually the same panel (total exposed n=383). All studies described sample collection (including centrifugation and freezing) and laboratory techniques. Two studies reported fasted serum sample collection (305,312). Four synovial aspirations (80%) were performed without lavage (302,309,310,312); one was performed under ultrasound by an experienced radiologist (304). Two studies (40%) utilised SF from a comparison group (309,312).

Forty-one different biomarkers were sampled, of which, only five were performed by more than one study (Table 3.3). Within these studies, imaging (MRI by all, x-ray by three), patient-reported outcome measures (PROMs, four studies), clinical assessment (two studies) and histology (one study) were used as comparators (Table 3.5).

Table 3.3 Serum and synovial fluid biomarkers performed by multiple studies

Marker	Assay Company	Associations
Serum		
COMP*	R&D Systems Inc (96) AnaMar (309) BioVender (309)	Positively correlated with age, BMI, and increased in males, (309), no difference between injured and controls (96,309)
Synovial fluid		
IL-1β	Meso Scale Discovery (304) Cosmobio Co Ltd (312)	No difference between injured and controls,(304) poor discrimination for meniscal injury (312)
IL-6	Meso Scale Discovery (302,304,310) IBL America (305)	No difference between injured and controls,(304) no cross-sectional or longitudinal association with KOOS or SF-36,(302) no association with inflammatory MRI biomarkers and weak discriminatory accuracy for knee OA in combined model (310), poor discrimination for meniscal injury (305)
TNF-α	Meso Scale Discovery (304,310) IBL America	No difference between injured and controls,(304) no association with inflammatory

	(305) Cosmobio Co Ltd (312)	MRI biomarkers and weak discriminatory accuracy for knee OA in combined model,(310) poor discrimination for meniscal injury (305,312)
COMP	AnaMar Medical AB (304) R&D Systems Inc (305) BioVendor (309)	No difference between injured and controls,(304) poor correlation to meniscal injury,(305) higher in males and injured cohort and associations with multiple other molecular biomarkers (309)
Note: Two studies completed the same panel in the same population, Roemer (310) and Struglics (2020) (302), so the markers that only they share are not included in this table **Serum COMP is on the OARSI FDA Osteoarthritis Biomarker Working Group panel (86)*		

COMP: cartilage oligomeric matrix protein, BMI: Body Mass Index, IL: interleukin, KOOS: Knee injury and OA outcome Score, MRI: Magnetic Resonance Imaging, TNF: tumour necrosis factor, OA: Osteoarthritis, OARSI: Osteoarthritis Research Society International, FDA: Federal Drugs Administration

Within the nine dry biomarker studies, all studies used MRI as their primary outcome measure, with a variety of scoring systems employed, and a range of comparators. These included contralateral knee (three studies) or previous (three studies) MRI, different MRI measures (four studies), PROMs (six studies), clinical examination or molecular biomarkers (one study each) (Table 3.6).

Within the imaging studies, there was variation in methodology. MR systems with different magnetic field strengths (measured in Tesla, T) and imaging sequences were employed. Seven studies utilised 3T machines, representing 65% of all study participants (n=297). However, the KANON studies (302,317,318) used 1.5T, and Van Meer (306) utilised a combination of 1T, 1.5T, and 3T machines. Six studies reported the use of specific knee coils, including 93% of participants (n=422) (306,307,311,313-316), one a four-channel large flex coil (308), and three a circular polarised surface coil (302,317,318). Five studies reported participant position during MRI acquisition (57% of exposed participants, n=261), either neural (306), sitting and supine (316), only seated (308), or partial weight-bearing extended and flexed (307,313). 81% of studies reported detailed sequencing information, and 63% reported post-imaging processing methods. Fifty-four percent of studies reported the reliability of their scoring methodology (306-308,313,315,316), and 36% reported the experience of their reporting radiologists, either fellowship-trained (308) or with 8-ten years'

experience (306,315,317). Two studies, with 7% of participants (n=56), also imaged the contralateral knee (307,308)

3.1.2.4 Risk of bias assessment

All studies were graded for risk of bias using the NOS (cohort or cross-sectional version, depending on study design); one was unsatisfactory, 10 were satisfactory, and six were good (Table 3.4).

Author, Date	Selection	Comparability	Outcome	Overall	Rating
Zhang, 2012~	**	-	*	3	Unsatisfactory
Ahlen, 2015~	****	-	***	7	Good
Zou, 2016~	**	**	***	7	Good
Van Meer, 2016^	***	-	**	5	Satisfactory
Wang, 2017^	***	**	***	8	Good
Lansdown, 2017^	**	-	**	4	Satisfactory
Pietrosimone, 2018^	***	-	**	5	Satisfactory
Struglics, 2018~	***	-	***	6	Satisfactory
Culvenor, 2019^	***	-	***	6	Good
Roemer, 2019^	**	-	***	5	Satisfactory
Sun, 2019~	**	-	***	5	Satisfactory
Zhong, 2019^	*	**	**	5	Satisfactory
Struglics, 2020^	**	-	***	5	Satisfactory
Li, 2020^	**	**	**	6	Good
Friedman, 2021^	**	-	**	4	Satisfactory
Wirth, 2021^	***	-	***	6	Good
Xie, 2021~	***	**	***	8	Good
Wasser, 2022~	***	**	***	8	Good

~Cross-sectional study or ^cohort study NOS tool used. Cohort studies is 0-9; 0-2 poor, 3-5 fair, 6-9 good/high and cross-sectional studies 0-10; 0-4 unsatisfactory, 5-6 satisfactory, 7-8 good, 9-10 very good

Table 3.4 Study characteristics of included studies involving serum and synovial fluid biomarkers

Author, year	n= case / control Age, mean (SD) Sex, M:F	Type (s/sf)	Time from injury (Yrs) mean (SD)	Markers measured	Imaging	PROMs	Clinical	Surgical/ Histology
Zhang, 2012 (303)	n= 102 / 60 <41YO sex *NR*	s	1 post ACL-R	U24, U38, U48, U49	MRI (WORMS)	-	-	-
Ahlen, 2015 (304)	n= 11 / 0 26 (range 18 - 40) 6M:5F	sf	8 post ACL-R (2-48m from inj to ACL-R)	IL-1b, IL-6, TNF-a, sGAG, ARGS-aggrecan, COMP	MRI XR (Fairbank)	TAS, KOOS, Lysholm	Single-leg hop, pivot-shift, ROM, Lachman	-
Zou, 2016 (305)	n = 61 / 65 30.5 (6) / 31.1 (6) 10M:51F / 9M:56F	s sf	6 (range 1-14)	sGherlin, sfGherlin, (IL-6, TNF-a, COMP, CTX-II)*	MRI (signal intensity)	IKDC, Lysholm	-	Noyes scale, Mankin score
Struglics, 2018 (309)	n = 121 / 50 (25sf / 25s) 26 (5) / 30 (12) sf & 31 (10) s 91M:30F / 16M:9F sf & 13M:12F s	s sf	1 (n=64) 2 (n=121) 5 (n=121)	sCOMP, sfCOMP (Two immunoassays used, AnaMar (COMP-Ana) and BioVender (COMP-Bio)	MRI XR		-	-
Roemer, 2019 (310)	n = 113 / 0 26 (5) 85M:28F	s sf	2 5	sIL-6/8/10/12p70, sTNF-a, sIFN-y, sfIL6/8/10, sfTNF-a, sfIFN-y	MRI		-	-
Sun, 2019 (312)	n = 72 / 70 30 (6) / 30 (5) 40M:32F / 36F:24M**	s sf	9 (range 6-16)	sPACAP, sfPACAP, (IL-1b, TNF-a)*	MRI	VAS, IKDC, Lysholm	-	Mankin score
Struglics, 2020 (302)	n = 116 / 0 28 (5) 86M:30F	s sf	2	sIL-6/8/10/12p70, sTNF, sIFN-g116, sfIL6/8/10, sfTNF,	MRI (ACLOAS)		-	-
Wasser, 2022 (96)	n = 38 / 0 37 (7) 38M:0F	s	11(7)	CTX-1, HA, C2C, PIIANP, NTX-1, CCL-2/4/5/11, CXCL, COMP, INF-a, IL-1a/7, SDF-1, TIMP-1, TNF-a, MMP-2/3/7/8/9/12/13	MRI XR (K-L, OC)	Pain severity, KOOS, VR-36, SF-8a	15m gait assessment	-

*SD: Standard Deviation, M: Male, F: Female, PROMs: Patient Reported Outcome Measures, s: serum, sf: synovial fluid, YO: Year Old, NR: Non Reported, ACL-R: Anterior Cruciate Ligament Reconstruction, OA: Osteoarthritis, MRI: Magnetic Resonance Imaging, WORMS: Whole Organ MRI score, XR: X-ray, TAS: Tegner activity scale, KOOS: knee injury and OA outcome score, IKDC: international knee documentation committee, ROM: Range of movement, VR-36: Veterans-RAND, SF-8: Short Form 8, IL: interleukin, sGAG: sulphated glycosaminoglycans, COMP: cartilage oligomeric matrix protein, TNF: tumour necrosis factor, CTX: c-terminal telopeptide, IFN: interferon, PACAP: pituitary adenylate cyclase activating polypeptide, HA: hyaluronic acid, C2C: Cleavage of Type II collagen, NTX: N-telopeptide of Type 1 Collagen, PIIANP: N-Propeptide of Collagen IIA, TIMP: Tissue inhibitor matrix metalloproteinase, SDF: Stromal cell-derived factor, MMP: Matrix metalloproteinase, CCL: Chemokine (C-C Motif) ligand, CXCL: Chemokine (C-X-C Motif) Ligand, K-L: Kellgren-Lawrence, OC: Outerbridge *It is not reported if these markers were in serum as well as synovial fluid **The control population is described ambiguously*

Table 3.5 Summary of included study characteristics for imaging biomarkers

Author, year	Exposed	Controls	Age (yrs)*	Study period**	Strength	Scoring	Comparators
Van Meer, 2016	n=143 94M:49F	No	25.2 (21.4-32.6)	B/L: <6mo FU: 2 yrs	1T, 1.5T, 3T	MOAKS	TAS, clinical exam, B/L MRI
Wang, 2017	n=28 17M:11F	n=9 4M:5F	29.8 (SD 6.3)	B/L: 2-3yrs FU: 2 yrs	3T	T2	ICRS, B/L MRI
Lansdown, 2017	n=38 21M:17F	Contra- lateral knee	29.0 (SD 8.0)	B/L: <6mo FU: 1yr	3T	SSM	SSD, B/L MRI
Pietrosimone, 2018	n=18 8M:10F	Contra- lateral knee	22.4 (SD 4.2)	B/L: <2wks FU: 1yr	3T	T1rho	KOOS
Zhong, 2019	n=30 15M:15F	n=13 5M:8F	32.0 (SD 8)	B/L: NS FU: 6mo, 1,2,3yrs	3T	SSM, WORMS	T1rho, T2, KOOS, previous MRI
Culvenor, 2019	n=117 85M:32F	No	28.2 (SD 4.9)	B/L: <4wks FU: 2,5yrs	1.5T	Cartilage thickness (PF)	Previous MRI
Struglics, 2020	n=116 86M:30F	No	28.2 (SD 4.9)	B/L: <4wks FU: 2,5yrs	1.5T	ACLOAS	KOOS, SF-36, molecular biomarkers
Li, 2020	n=34 17M:17F	N=9 8M:5F	30.7 (SD 8)	B/L: <3mo FU: 6mo, 1,2,3yrs	3T	TT, TR	T1rho, T2, WORMS, previous MRI
Friedman, 2021	n=35 19M:16F	No	31.0 (SD 7.6)	B/L: NS FU: 6mo, 1,2,3yr	3T	T1rho, WORMS	MARS, KOOS, previous MRI
Xie, 2021	n=114 108M:6F	n=43 38M:5F	26.2 (SD 3.8)	2yrs (retrospective)	3T	Radiomics modelling	T2
Wirth, 2021	n=117 85M:32F	No	28.2 (SD 4.9)	B/L: <4wks FU: 2,5yrs	1.5T	Cartilage thickness (FT)	Previous MRI

M: Male, F: Female, Mo: Month, yrs: Years, wks: Weeks, T: Telsa, Con: Contralateral, B/L: Baseline, FU: Follow up, MRI: Magnetic Resonance Imaging, OA: Osteoarthritis, MOAKS: MRI OA Score, TAS: Tegner Activity Scale, ICRS: International Cartilage Repair Society, SSM: Statistical Shape Modelling, SSD: Side-to-Side Difference, T2: Transverse relaxation time, T1rho: spin-lattice relaxation time constant in rotating frame, WORMS: Whole-Organ MRI Score, KOOS: Knee injury and OA Outcome Score, SF-:36: Short Form 36 questions, TT: Tibial translation, TR: Tibial Rotation, MARS: Marx Activity Rating Scale

** age of injured participants only, reported in Mean (SD) with exception of Van Meer 2016 which is reported as Median (Range)*

*** Baseline signifies time from injury to recruitment, follow up reports time from recruitment*

3.1.3 Findings

Three wet biomarker studies focus on one marker, including ghrelin (305), COMP (309), and pituitary adenylate cyclase-activating polypeptide (PACAP) (312), with others assessing a panel of markers (Table 3.5) (96,302,303,310). Three studies reported values in relevant units (96,305,309), two used log10 to calculate associations (302,310). Most studies used either multiplex or enzyme-linked immunosorbent assay (ELISA), and a single study used reverse transcription and preamplification prior to polymerase chain reaction (PCR) (303). Cross-sectional associations are described below, with biomarkers classified by type (serum or SF) and primary mechanism (catabolic, anabolic, inflammatory).

3.1.3.1 Serum biomarkers

The total number of serum biomarkers measured across all studies was 38. Serum biomarkers measured either anabolic, catabolic or pro-inflammatory processes (Table 3.5) (96,302,305,309,310,312). In addition, one study measured microRNA (miRNA) and small nucleolar RNA (snoRNA) (303).

3.1.3.1.1 Catabolic biomarkers

Serum biomarkers included in this review associated primarily with catabolism were HA (96), cleavage of type II collagen (C2C) (96), TIMP-1 (96), stromal cell-derived factor (SDF)-1 (96), PIIANP (96), ghrelin (305) and PACAP (312). In a study with CAI, HA levels were 73% lower and C2C was 44% higher in the group with radiographic OA compared to those without, with wide variability in time from injury and minimal matching between groups (96). No other catabolic biomarkers demonstrated any relationships to dependent variables.

3.1.3.1.2 Anabolic biomarkers

Biomarkers related to anabolism included NTX-1, COMP (measured by three different assays, Table 3.3) (96,309), MMP-2/3/7/8/9/12/13 and CTX-1, all measured in the same study (96). In those with radiographic OA following CAI, NTX-1 was 49% lower than those without (96), with COMP showing no differences (96). In another study, COMP did have a relationship with age, sex and other biomarkers, but not with injury, however, multiple imputation was used, possibly masking the associations at lower detection levels (309). No other anabolic biomarkers demonstrated a relationship to dependent variables.

3.1.3.1.3 Inflammatory biomarkers

Inflammatory serum biomarkers included IL-6 (302,310), IL-8 (302,310), IL-10 (302,310), IL-12p70 (302,310), interferon (IFN)-γ (310), IFN-g116 (302), TNF (302) and TNF-α (96,310), with one study also measuring chemokine (C-C Motif) ligand (CCL)-2/4/5/11, chemokine (C-X-C Motif) ligand (CXCL), IFN-α, IL-1α, and IL-7 (96). IL-7 had a 180% higher concentration in CAI individuals with radiographic OA compared to those without (96), with the same caveats as previously (minimal matching, wide variation in time from injury). TNF and IL-10 demonstrated a relationship to worsening KOOS scores (TNF to KOOS-pain, QoL and KOOS4, and IL-10 to QoL) in adjusted multivariable and unadjusted univariable linear regression, though this study did utilise multiple imputations and did not adjust for treatment (surgical vs. non-surgical) (302). No other serum inflammatory biomarkers demonstrated a relationship with the dependent variables.

3.1.3.1.4 Non-coding RNA

One study measured non-coding RNA, using miRNA and snoRNA (303). Serum snoRNA U38 concentrations were higher in those with significant cartilage degeneration (WORMS score ≥4), though this study was limited by an unclear methodology, significant results were only found on sub-group analysis, lack of correction for multiple testing and undetectable levels of snoRNA U38 in the control group (303). Neither miRNAs nor the other snoRNAs showed any significant associations.

3.1.3.2 Synovial fluid markers

Five studies collected SF in addition to serum (302,305,309,310,312), while the final study (304) collected only SF to measure the local effect of biomarkers. Markers of anabolism, catabolism and inflammation were measured (Table 3.5). The total number of SF biomarkers measured was 13.

3.1.3.2.1 Catabolic biomarkers

SF biomarkers related to catabolism were ARGS-aggrecan (304), sulphated glycoaminoglycans (sGAG) (304), ghrelin (305), and PACAP (312). SF ghrelin and PACAP were both seen to be negatively correlated to histological severity and positively correlated to PROMs related to pain and function (305,312), although Zou (305) had a wide range of time from injury, no demographic data and no control samples, and Sun (312) had ambiguity regarding their control participants. No other catabolic biomarkers demonstrated a relationship to the dependent variables.

3.1.3.2.2 Anabolic biomarkers

The SF biomarkers related to anabolism were CTX-II (305) and COMP (304,305,309). CTX-II was seen to have an area under the curve (AUC) of >0.70 for meniscal injury, however, this study was missing participant demographic data and control group SF, with a wide variety of time from injury (305). Three studies examined COMP. One showed that SF COMP showed no association to injury in the smallest study population with extensive variation in age and time from injury (304), in another COMP provided no predictive value for meniscal injury (305), and a third study demonstrated significantly higher concentrations of SF COMP in the injured cohort and was associated with other molecular

biomarkers (including ARGS-aggrecan, NTX-1 and CTX-II), however, this study did not have SF for the entire population (309).

3.1.3.2.3 Inflammatory biomarkers

Pro-inflammatory SF biomarkers included IL-1ß (304,312), IL-6 (304,305), IL-8 (302,310), IL-10 (302,310), TNF (302), TNF-α (304,305,310,312), and IFN-γ (310), with more homogeneity across studies studying the same markers. IL-8 showed weak associations to MRI-related inflammation (specifically, grade 2/3 effusion-synovitis on WORMS) in an unadjusted model, in a study population missing some samples and requiring multiple imputation (310). No other SF inflammatory biomarkers demonstrated a relationship to the dependent variables.

3.1.3.3 Imaging biomarkers

Ninety-percent of studies divided the knee into different regions, between three (306) and 20 (314), with most adopting variations of anatomical location (medial, lateral femur and tibia, and patella) (306,308,311,313-318) (Table 3.6), with different scoring methods used, including semi-quantitative and quantitative.

Semi-quantitative scoring systems employed included the WORMS, MOAKS, and ACL OA Score (ACLOAS) (Table 3.6). WORMS is a multi-feature score comprising of 14 elements, including cartilage morphology, subarticular and articular bone, ligaments, meniscus and synovitis (160). MOAKS developed later, refines meniscus, cartilage and BML component scoring (163). ACLOAS is a system specifically developed following ACL injury and includes baseline joint damage, ligament and graft characteristics and other incident features (344).

Eighteen-percent of studies performed quantitative analysis of cartilage thickness in different regions (patellofemoral, PF, and tibiofemoral, TF) two and five years after ACL injury (317,318). Lansdown (307) reported bone morphology, including of the contralateral knee, to determine the position of the tibia to the femur (side-to-side difference, SSD), semiautomatic segmentation and shape of bony features (statistical shape modelling, SSM) and kinematics using multiple weight-bearing positions. Zhong (311) also used SSM to compare the bone shape and Li (313) bone position. Xie (315) developed quantitative radiomics models to describe cartilage and subchondral bone characteristics. Quantitative cartilage composition measurements were employed in 55% of studies, with two studies using T1rho values (308,314), two T2 values (315,316), and two both (311,313).

3.1.3.3.1 Bone position

Using the anatomical position of the tibia (translation, TT, and rotation, TR), T1rho & T2 and WORMS, Li demonstrated that ACL-R is unable to fully restore joint position, with significantly increased anterior TT and internal TR in the injured knee (313). TT and TR changes were also seen in the contralateral knee throughout the study period, suggesting significant biomechanical adaption. TT and internal TR increased from 1 to 2 years and were associated with significantly longer T1rho/T2 relaxation time at one year in the medial tibial (MT) region, and MT and medial femoral condyle (MFC) region at two years, suggesting a relationship between TF position and cartilage composition. There was no cross-sectional correlation seen between TT/TR and WORMS. This study had a 33% attrition rate in the injured group, and the follow-up period varied for injured (2 years) and control participants (3 years), implicating potential selection bias.

3.1.3.3.2 Bone shape

Using SSD to compare the injured to non-injured knee, Lansdown demonstrated that SSM bone shape changes were associated with abnormal knee kinematics a year after ACL-R (307). These included increased sphericity and height of the MFC in extension and MFC height in flexion, and the lateral tibial plateau (LTP) length, medial tibial plateau (MTP) height and slope. They suggest that bone shape and altered biomechanics could contribute to OA development. There were multiple significant confounders (including age, muscle function, rehabilitation, and surgical techniques) and a 30% attrition, potentially representing bias. Lansdown suggests their findings are correlation, not causation, but also demonstrate that ACL-R does not fully restore pre-injury joint anatomy.

Zhong also employed SSM alongside T1rho, T2 relaxation, and WORMS 1, 2 and 3 years after ACL-R (311). The tibial plateau area and posterior tibial slope increased between injured and controls by one year, with the former increasing by three years, possibly representing early joint degeneration. There was a significant correlation between trochlear inclination and MFC height to the KOOS pain subscale at three years, and early bone changes correlated to T1rho and T2 relaxation times. This study had significant variation in time between ACL injury and ACL-R and contained no details on recruitment or attrition.

3.1.3.3.3 Cartilage morphology

Culvenor demonstrated significantly greater loss of PF cartilage in the early ACLR-R group after five years, most prominent in the trochlear region, compared to the other two groups, with the majority of this occurring in the first two years (318). Wirth did not find a significant difference in the TF cartilage at five years but noted thickening of this region in the first two years (317). This suggests that the PF region may be more susceptible to cartilage loss. Neither study analysed features on the two-year MRI for their predictive value nor compared them to other measures, such as function or symptoms.

3.1.3.3.4 Meniscal features

One study performed a post-hoc analysis to identify the impact of chondral and meniscal injury (308). In participants with a lateral meniscal injury (the most common subset), associations were seen between increased T1rho relaxation time in lateral femoral condyle (LFC) cartilage and worse KOOS outcomes, although as a sub-analysis in an exploratory study, this was not powered.

3.1.3.3.5 Inflammation

Struglics was the only study to measure MRI markers of inflammation using ACLOAS (302). At two years, 43% of individuals had MRI-defined inflammation, of which 8% was moderate/severe effusion-synovitis, and those with effusion-synovitis present had worse KOOS subscale and SF-36 PCS scores. No longitudinal comparisons were performed, nor were correlations to cartilage composition evaluated.

3.1.3.3.6 Compositional

Using T1rho relaxation times between the injured and contralateral knees and their relationship to KOOS, Pietrosimone demonstrated that indicators of reduction in articular cartilage proteoglycan density were associated with worse patient outcomes a year from injury (308). The increase in interlimb T1rho mean relaxation time at the LFC and the MFC was correlated with worse KOOS subscores. Specifically, the Posterior-LFC correlated with KOOS Pain, and KOOS ADL, while the Central-LFC, Posterior-LFC and Medial-MFC correlated with KOOS Sport and KOOS QoL. They suggest that this shows a link between decreased proteoglycan density and patient-reported knee symptoms in patients after ACL-R. This study did not fully address confounders and, being a cross-sectional study, couldn’t assess progression or causation.

Friedman reported WORMS, T1rho and PROMS over three years, demonstrating that 46% (16/35) participants had cartilage degeneration, most frequently in the medial compartment (12/16), but also lateral (7/16) and patella (7/16) regions by the end of the study period (314). Three-year activity Marx scores positively correlated with medial femoral (MF) and MT cartilage changes, and KOOS QoL scores were inversely correlated with MT changes at three years. No relationship was seen between WORMS to T1rho or PROMs at three years, with the authors suggesting that semi-quantitative scoring alone

may not be a good predictive tool. The definition of cartilage degeneration was a 14.3% change in T1rho score, based on a population with more advanced OA (n=10) (345). This might not apply to a post-traumatic study population, nor were different surgical procedures or injuries controlled for, with no details on attrition.

Wang compared longitudinal T2 values at 2-3- and 4-5-years post ACL-R across six sub-regions to a control population (316). They demonstrated higher T2 values in the deep layer of the MFC region at the first time point, followed by lower T2 values in the deep layer of the lateral tibia (LT) two years later in their ACL-R group compared to their control group. They concluded that the MFC results were due to early degenerative cartilage changes post-injury, and the LT values suggested ineffective cartilage repair with poor mechanical properties. In the control group, no follow-up MRI scans were conducted, limiting relative comparisons over time, and no comparisons were made to patient-related or functional outcomes.

3.1.3.3.7 Combined MRI features

Using MOAKS, Van Meer showed that 40% of participants had cartilage defects and osteophyte progression over two years (306). BMLs were positively associated with the progression of osteophytes and cartilage defect a year later, with an odds ratio (OR) of 5.19 (95% confidence interval, 95% CI: 1.56,17.25). On the other hand, joint effusion was associated with a progression of osteophytes alone (OR 4.19; 95% CI: 1.05,16.72). The authors state that their findings are hindered by the variety of MRI equipment used and MOAKS's inability to detect subtle joint abnormalities (306).

Xie developed a radiomics model for distinguishing individuals at risk of PTOA two years from injury using T2 mapping of cartilage and subchondral bone, with a training (n=110, n=80 injured, n=30 control)

and testing cohort (n=47, n=34 injured n=13 control) (315). The model, utilising 13 (out of 1116) features based on compositional cartilage and subchondral bone markers, was able to differentiate well between post-ACL-R knees and controls. Each radiomic model reported acceptable or good AUC values, in particular for a model using combined cartilage regions (AUC 0.982) (315), This cross-sectional study had one of the largest study populations (n=114); however, it consisted predominantly of males (92%) and did not control for other injuries.

3.1.4 Discussion

These systematic reviews synthesise the current evidence regarding molecular and imaging biomarkers in the chronic phase of PTOA, using 18 studies involving 780 exposed participants at least one year from injury. Across all studies, 51 serum or SF biomarkers were studied, with 11 individual biomarkers related to catabolism, anabolism and inflammation seen to be associated with radiological changes (osteoarthritic, cartilage and inflammatory) or PROMs (pain, function, quality of life) (Figure 3.4), with MRI features also demonstrating significant structural differences in terms of tibial position post-ACL-R and MFC bone shape, plus bone and cartilage compositional changes, BMLs and effusion, and their respective associations with pain, function and radiological changes (Figure 3.5). These biomarkers may offer insight into a future biomarker panel. However, the strength of evidence is low due to study methodological weaknesses and challenges of study comparability.

Classifying biomarkers based on their ability to monitor pathophysiological changes is fundamental for comprehending their value and utility in monitoring diverse processes in PTOA development (346). Some of these mechanisms were explored in Section 1.3, and involve a failure of appropriate repair, remodelling, and adaptation, in the presence of inflammation and metabolic dysfunction, leading to early structural change and molecular abnormalities as identified in this review.

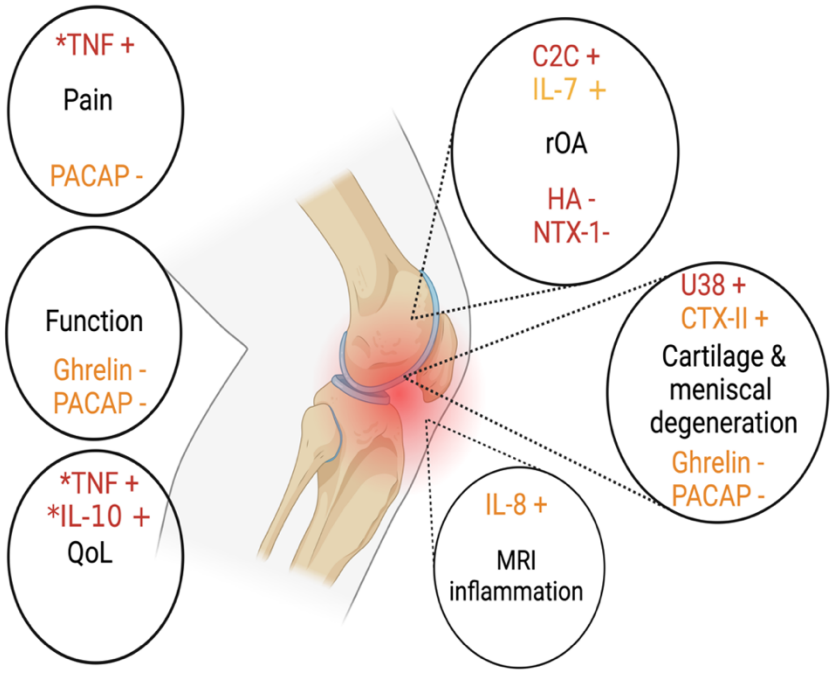


Figure 3.4 Associations between serum (red) and synovial fluid (orange) biomarkers to radiological and patient-reported outcome measures for post-traumatic osteoarthritis of the knee

*TNF: Tumour necrosis factor, PACAP: Pituitary adenylate cyclase activating polypeptide, C2C: Cleavage of Type II collagen, IL: Interleukin, NTX: N-telopeptide of type 1 collagen, HA: Hyaluronic acid, CTX: Type II collagen cross-linked C-telopeptide, rOA: Radiological osteoarthritis, MRI: Magnetic Resonance Imaging, QoL: Quality of life. + positive correlation – negative correlation *biomarker log^{10} transformed. Created in BioRender.*

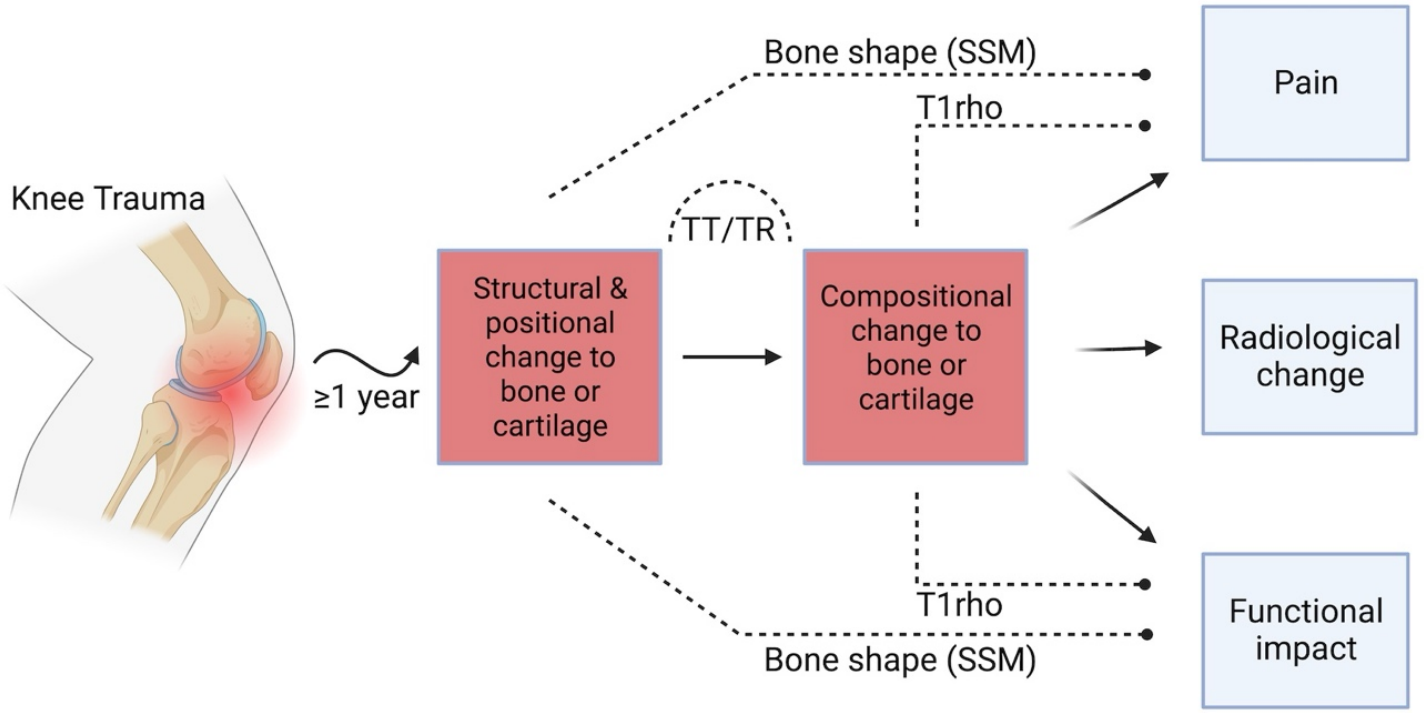


Figure 3.5 The proposed pathophysiological mechanism of post-traumatic osteoarthritis with key MRI features and their associations a year or more after a significant injury.
MRI: Magnetic Resonance Imaging, SSM: Statistical Shape Modelling, TT: Tibial translation, TR: Tibial rotation, T1rho represents cartilage proteoglycan density. Created in BioRender

The majority of markers studied in this review were associated with pro-inflammatory processes (45%), with fewer measuring catabolic (24%) and anabolic (21%) activities (Figure 3.6). The imbalance between the latter two processes likely leads to ineffective tissue repair or incomplete remodelling in a pro-inflammatory environment (21,94). Equally, lowering pro-inflammatory mechanisms may decrease cartilage repair and remodelling, accelerating cartilage deterioration, which may be observed in OA patients receiving steroid-based anti-inflammatory therapies. Further partitioning of outcome measures to monitor specific pathophysiological mechanisms, such as cartilage degeneration and development (e.g. COMP), osteophyte development (e.g. HA), or inflammation (e.g. IL-6) allows the understanding of responses to targeted mechanism-specific interventions. This is relevant for DMOAD development and would allow heterogeneous study populations to be classified based on predominant pathophysiological pathways or likelihood of rapid progression, as well as overcoming some of the challenges associated with outcome measures (34,347-349).

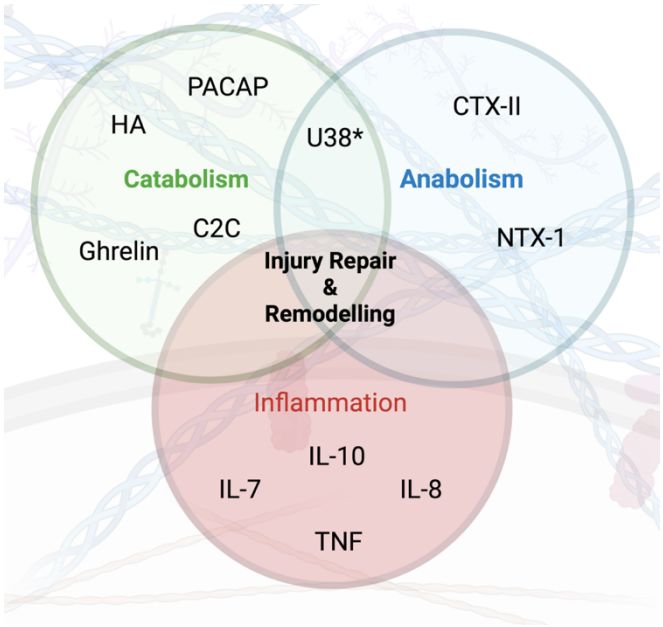


Figure 3.6 Summary of the main action of each biomarker of significance and their interaction during injury repair and remodelling.
*TNF: Tumour necrosis factor, PACAP: Pituitary adenylate cyclase activating polypeptide, C2C: Cleavage of Type II collagen, IL: Interleukin, NTX: N-telopeptide of type 1 collagen, HA: Hyaluronic acid, CTX: Type II collagen cross-linked C-telopeptide. *action of non-coding RNA U38 is uncertain. Created in BioRender*

Therefore, understanding pathway relationships is important (Figures 3.4-3.6) (346). Those associated with catabolism demonstrated some relationship with imaging, histology, and PROMs. Serum HA was lower, and C2C higher, in those with radiographic evidence of PTOA (96), with SF PACAP and ghrelin both positively associated with Lysholm and IKDC scores and negatively associated with VAS, MRI and histology (305,312). Anabolic markers were also associated with imaging and structural change, with NTX-1 levels lower in those with radiographic OA and CTX-II consistently having an AUC of >0.70 for meniscal injury on MRI, exceeding the threshold for a clinically useful diagnostic test (305,350). Many studies included COMP, which did not reveal a strong association with any dependent variable, and in fact, one study (309) characterised their negative serum COMP result as a "somewhat disheartening outcome" given the extensive use of the biomarker (351). Variations of different ELISA kits and protocols for well-established markers may explain variations in the sensitivity, specificity, and accuracy of those findings, making it challenging to directly compare data from different studies and hindering the ability to establish universal cutoffs for abnormal cartilage turnover.

Inflammation is felt to be a key contributor to PTOA (352) and plays a role in direct response to injury, joint remodelling and adaptation in later stages. Across the pro-inflammatory biomarkers, relationships were seen with IL-7 to radiographic OA (96), IL-8 with effusion-synovitis (310) and TNF with increased quality of life (302). Effusion-synovitis has been seen acutely and chronically to demonstrate worse OA outcomes in those with traumatic joint injuries (2,126), and in a previous review, Khella reported SF TNF-α and IL-6 as ‘causal factors’ and IL-1β and IL-17 as ‘credible factors’ for PTOA progression (294). This systematic review does not draw the same conclusion, suggesting further work is required to understand the interactions between tissue turnover and inflammation fully. The discrepancy in conclusions might be due to Khella’s classification for the chronic phase (‘1.5 months to years’), whereas this systematic review employed a more rigorous ‘one year or

greater' (although even this definition might require further nuance, given the likely difference between individuals who are one or 11 years from injury and might also be influenced by injury type and/or management).

MRI studies also gave an indication into the role of inflammation, with the presence of effusion seen to increase osteophyte progression (306) and decline of PROMs (302), in support of previous findings describing the long-term impact of effusion, especially when associated with haemarthrosis, in the acute phase (126). There was, however, poor concordance between imaging and molecular inflammatory biomarkers—further work is required to understand the value of imaging to identify and classify inflammation (302).

The type of sample is relevant (Figure 3.4). Serum biomarkers are well-studied, often due to ease of measurement, however, similar to previous reviews (166,346), there is currently no strong evidence to suggest any single serum biomarker can be used individually for diagnosis, prognosis, or to measure the impact of an intervention. SF samples seemed to have more value as potential biomarkers with associations with injury, structural and patient-reported outcomes; however, not all studies had appropriate control samples, and therefore, it is not fully clear how SF differs in those with PTOA. The correlation between paired serum and SF samples was consistently weak, possibly indicating variations in systemic and local concentrations due to some biomarkers being produced locally within the joint tissues, with subsequent systemic dilution, and others released into the circulation before diffusing into the SF, as well as differences in pathophysiological mechanisms (21,353). Additionally, the rapid and fluctuating proinflammatory expansion of SF volume may decrease any locally-produced biomarker concentration, further reducing correlations between synovial and serum spaces. Another uncontrolled confounding variable for inflammation and cartilage/bone metabolism, is the effect of ACL-R-related trauma and subsequent rehabilitation on the joint

remodelling response and associated biomarkers, as demonstrated in the KANON study (310). These patients may present a higher risk of PTOA, although surgery also has the potential to reduce long-term joint instability.

Time from injury will likely influence biomarker concentration, depending on its source and role in ongoing joint pathology. This has a clinical relevance too, given that some care pathways, like ACL, can take a year to complete and individuals can also present years down the line after an injury with a painful joint. All included studies had differing times from injury (Figure 3.2 and 3.3), and this remains an important unanswered question requiring further attention, as do relative changes in biomarker concentration over time. Longitudinal studies, such as those cited in this review (96,302,310) and elsewhere, offer an opportunity for this. However, several studies in this review demonstrated widespread structural joint changes from a year post-injury (306,307,311,313,315). These bone features, identified with SSM and radiomics, including position, shape, and BMLs, have been previously associated with PTOA (354-356). Significantly, these features were associated with abnormal knee biomechanics (307), pain (311), radiological changes (306) and cartilage degeneration (313). The medial femoral region appears to be particularly susceptible to early changes in our review (307,308,311,313,314,316), also seen elsewhere (345), and could potentially act as a sentinel region in early OA. In addition, changes in cartilage morphology, examined in two studies, were consistent with rates of radiographic OA seen in the main KANON analysis (PF 19%, TF 12%) (317,318,357).

Changes to joint position post-ACL-R do not seem to restore anatomical position (311,313), and bone shape, such as condylar height and length, can influence the integrity and quality of local articular cartilage (355). Cartilage ECM degeneration with decreased proteoglycan density is linked to the subsequent development of OA and can be investigated using advanced techniques, including T1rho

and T2 (308,311,313,314,316). Significant compositional changes were seen adjacent to the femoral and tibial condyles (308,314) and also at different depths of cartilage (316). Three studies demonstrated a relationship between joint structure and cartilage composition (311,313,316), with cartilage degeneration associated with worsening pain and function (308,311,314), as seen elsewhere (358). Based on these results, it is plausible to argue that PTOA and early idiopathic OA have distinct initial pathophysiological mechanisms. Initial structural changes likely influence cartilage and subchondral compositions during the development of PTOA (Figure 3.5), as opposed to progressive deterioration and loss of articular cartilage contributing to structural changes in the latter (21), before both processes share common mechanisms. This suggested initial diversion of mechanism has been proposed previously (345,355) and may influence tissue-specific biomarker detection in PTOA compared to idiopathic OA.

These findings suggest that timely and prospective whole-joint assessment may lead to advancements in diagnostic and prognostic tools and, consequently, the advantages of MRI over radiography. These advantages are compounded by the use of semi-quantitative methods, such as the scoring systems outlined earlier, and the use of quantitative methods to explore specific tissues in more depth. Whole-joint assessment offers the potential to identify radiographically occult injuries that may contribute to ongoing symptoms or functional impairment and provide the possibility of improved phenotyping, leading to disease stratification and personalised interventions. Another modality which provides comprehensive joint assessment is USS, and it was surprising that no USS studies were found as this modality is more accessible and cheaper than MRI, given evidence demonstrating its reliability and validity (194,359).

A whole joint assessment should include a molecular assessment, however, there was too much heterogeneity to understand what this should look like. Methodological differences prevented direct

comparison with differing biomarkers, time points, assays, and study quality. There was consensus around common biomarkers, such as those shortlisted by the FDA/OARSI initiative (33,86), although only one of the five biomarkers measured by multiple studies (serum COMP, Table 3.3) is on that list, highlighting the need for a unified approach as evidence is gathered regarding nascent biomarkers in differing populations. Likewise, standardisation of MR techniques is required, including strength and reporting. Most, but not all, studies used 3T machines, which offer better image quality, higher scan efficiency and the ability to detect small lesions for musculoskeletal imaging than 1.5T (360). However, multiple semi-quantitative systems were in use, including MOAKS, ACLOAS and WORMS, preventing direct comparisons, and consensus should be drawn regarding this, especially in relation to time from injury, as WORMS has limitations in the acute setting, and bony changes, given the risk of MRI underreporting them (194,361,362).

In this systematic review, all bar one study focussed on ACL injuries, with most undergoing surgical reconstruction, proving homogeneity in pathology, however, studies did not account for surgical techniques, and rehabilitation programmes, which could have affected the variability of the findings. Current evidence suggests that concurrent meniscal injury can significantly worsen outcomes and increase the development of PTOA (363,364), with two studies analysed this, five excluding participants with meniscal injury, and the others not taking account of it. With the two studies, one demonstrated worse KOOS outcomes and increased T1rho relaxation time in the presence of meniscal pathology (308). Given the high prevalence of combined injuries, the relevance of the results of studies excluding meniscal injuries to the real world is reduced (363). This is an area which requires further exploration, to see if there are other associations to be found with differing aetiologies, especially injury types, single versus many structural injuries, and the mechanistic pathways. The single study with different pathology, combat-related traumatic amputation (96), had the most significant

changes in serum biomarkers. It is possible that this could be related to the systemic response following trauma, and the influence of this on PTOA and biomarker concentrations should be explored (2), and will be over the next two chapters.

Limitations of this review include the number, and variable quality, of the studies included and despite sporting injuries being more common in females (104), the literature reviewed was heavily biased toward males. Whilst most studies were satisfactory or good for RoB assessment, individual study limitations weaken the overall results, including small study populations, no appropriately controlled comparison population, and a significant risk of unrecognised bias with study methodology, lack of power and validation in other populations. In addition, studies were exploratory, without set hypotheses or powered sample sizes, and did not all control for confounders such as injury type or treatment, with high attrition rates. There was also a considerable variation in time from injury to surgery and follow-up. Significant differences in study methodology prevent too much generalisability and direct comparison between studies. There were no studies measuring plasma, urine biomarkers or non-MRI modalities. Only five biomarkers were performed by multiple studies, all performed by different laboratories, minimising comparability (Table 3.3). Further limitations apply between studies, such as the variation in reporting methods (such as MRI-scoring, ACLOAS or WORMS) or variation in criteria applied (such as different KL classifications, ≥1 v ≥2, employed in different studies). Strengths of this study include the range of databases searched and the inclusion of abstracts that meet inclusion/exclusion criteria to demonstrate ongoing work in progress and mitigate potential publication bias (298). There was insufficient homogeneity to permit direct comparison and meta-analysis, however SWiM guidance provides a reliable and comparable method to report findings. A further challenge was the lack of clarity from the authors regarding the purpose of the biomarkers under investigation. Based on study discussions / interpretations, it was possible to infer that most were interested in the

predictive or prognostic use, however, the studies were not designed appropriately, nor was the required discrimination or predictive statistics (such as c-statistic, AUROC or r2 value) stated. As a result, frustratingly, I was unable to present the data inline with the BIPED(S) classification (155), nor utilise specialist review methods for prognostic studies (365,366).

In conclusion, whilst the use of molecular and imaging biomarkers have the potential to offer insight into the development, progression, and impact of therapy, at present, this review of biomarkers implicated in the chronic phase of PTOA demonstrates that better evidence is required to achieve that. Across all studies, there was very little methodological overlap, either in outcome measures assessed (such as molecular biomarker panel or comparators), scoring systems employed (such as radiographic or MRI scoring), participant time from injury or clinical pathways, or statistical analysis plans, nor validation of findings in other populations. This review did not identify any studies using metabolomics, which may offer another route for PTOA biomarker identification in the future (296,322). The heterogeneity of the included studies has not only precluded a meta-analysis but also prevented firm conclusions from being drawn about future avenues of research, with evidence on mechanistic pathways from different injury patterns and early structural/compositional changes offering hypotheses for future work.

The variability across all studies in this review demonstrates the difficulty of synthesising evidence and drawing firm conclusions, highlighting the need for a standardised approach in study design and outcome measures. In both fields, initiatives have begun to improve this, such as the FDA/OARSI biomarker consortium (86). This consortium highlights the importance of whole-joint assessment, given the interaction of different joint components, especially when developing diagnostic, prognostic, and predictive tools. An internationally agreed consensus is required to create recommended

guidelines for PTOA research, including standardisation of the biomarker panel assessment, performing ‘time from injury’ sub-analysis, and collection of the same outcome measures to enable future direct study comparisons and meta-analysis.

Further evidence is required in the chronic phase of PTOA to understand the utility of biomarkers. This thesis aims to help fill this research gap.

Chapter 4 : What do biomarkers reflect in the ADVANCE cohort?

In this first experimental chapter, I will undertake cross sectional analysis of a panel of candidate serum biomarkers with knee radiographic osteoarthritis (rOA), knee pain and function. It is hoped that this analysis will be able to differentiate between those with and without the presence of those outcomes. I also hope to understand the molecular difference between post-traumatic OA (PTOA), sustained in those with a combat injury, and idiopathic OA (iOA), sustained in the unexposed group.

4.1 Background

As a recap, OA is a progressive deterioration of the 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 (21,83). The annual medical cost of OA in the US is estimated at $72 billion (67), with an OA-related two-fold indirect cost of lost workplace productivity (367), and rising incidence; the approximate 600 million global cases of OA in 2020 are expected to double by 2050 (26). Therefore, successful interventions to improve this cost and burden are a priority. A key research challenge impeding progress is population heterogeneity, with biomarkers offering an opportunity for phenotyping, thus enabling personalised treatment and improved drug trial recruitment (2,32).

Identifying OA in the pre-clinical phase is vital to enable appropriate interventions and guide drug discovery, with molecular and imaging biomarkers employed as proxy measures to identify pathophysiological mechanisms related to extracellular matrix (ECM), inflammation or metabolic dysregulation (5,52,347). As outlined in Chapters One and Three, cartilage-derived biomarkers, such as cartilage oligomeric matrix protein (COMP) and C-terminal cross-linked telopeptide of type II

collagen (CTX-II), are associated with OA progression and cartilage loss (173) (Section 1.4.2). 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 (368) (Section 1.4.2). Identification of differences in pathological mechanisms or clinical presentation will allow individual phenotypes to be created, enabling targeted trial recruitment (e.g. for 'rapid progressing phenotypes') or clinical interventions (e.g. for analgesia in a 'painful phenotype') (Section 1.1.3).

PTOA is a widely used paradigm for biomarkers studies as it commonly presents in younger individuals with fewer co-morbidities after a clear initiating event (2,369). As the field is growing, it is important to understand if there are any aetiological, pathological or molecular differences between those with iOA and PTOA. Certain populations, including tactical and professional athletes, are at higher risk of OA due to occupational factors, as described in Section 1.5.2, including trauma (2,36,207,217,218). Therefore, these high-risk populations are useful to study as they are more likely to yield positive findings which can be extrapolated to other populations and generate hypotheses to study. Using the Baseline Armed Services Trauma Rehabilitation Outcome (ADVANCE) cohort study visit data, I will perform a cross-sectional analysis between serum biomarkers and knee rOA, knee pain and function. My hypothesis for this first analysis 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. The analysis has been formulated accordingly, with secondary research questions examining any differences between those with rOA between the exposure groups and, therefore, aetiology (idiopathic v post-traumatic), any differences between those with rOA who experience pain compared to those who

don’t, the relationship between biomarkers and physical function, and any molecular differences between specific radiological features of OA.

4.2 Methods

This analysis is a cross-sectional analysis at the inception of a longitudinal cohort study. The exposure (combat-trauma) occurred 8 years prior to this Baseline visit. The outcomes of interest are rOA, knee pain and physical function.

4.2.1 Radiographic OA

Radiological OA was categorised as a KL score of ≥1 for the primary analysis, with participants classified as rOA+ or rOA-. This threshold was deliberately chosen, as it would not be expected in a population of this age and is the strongest predictor of confirmed OA (32). The ambition of this PhD was to provide the ability to identify OA in the early stage, prior to confirmed clinical and radiological disease – if a threshold of KL≥2 were adopted, this would be, by definition, diagnosed OA, and indicative of a late stage of established disease.

A sensitivity analysis was subsequently performed, using KL≥2, to ascertain if there was a notable difference in the molecular picture between ‘early’ and ‘confirmed’ rOA. In addition, analyses were performed using the presence of any individual measures of the OARSI atlas (JSN, Scl, OP) to ascertain if any specific biomarkers offered improved identification of different pathological mechanisms in the cartilage, surface or subchondral bone matrix.

4.2.2 Pain

The KOOS is the key PROM of interest for the primary and secondary analyses in this chapter. The KOOS is 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 (273,370). Due to an error during data entry, only the KOOS Pain and

KOOS Symptoms sub-scores were correctly recorded and therefore, these are the only two KOOS sub-scales available following the baseline study visit.

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 (interquartile range, IQR: 17-35) with an anterior cruciate ligament reconstruction (89,371). Trying to achieve consensus on a 'score' to grade pain as significant is a challenge, and the wealth of literature published on the matter is a testament to that (372-375). I felt it was important not to create a new categorisation but to utilise a previously adopted one to allow comparisons with other study populations. This cut-off was first developed in a population older than ours, but significantly, many years after their index injury/trauma (meniscectomy), which I felt was important, and then validated in a younger population far closer to ours in age.

4.2.3 Function

Function was assessed using the six-minute walk-test (6MWT), specifically, the distance walked (6MWD) as outlined in Section 2.1.5.2.

4.2.4 Statistical methods

Initial descriptive analysis was performed to understand the cohort of interest, the presence and amount of rOA, pain, and functional ability. These analyses were performed to describe outcomes per exposure status, specifically the presence and quantity of rOA. Parametric data is presented as mean (SD), and non-parametric data as median (IQR). Following this, hypothesis-driven univariate analysis was performed, depending on normality and groups (Mann-Whitney-U and Student's t, or Kruskal-Wallis and analysis of variance, ANOVA).

As described in Section 2.2.5.3, unadjusted analyses were initially performed, followed by adjusted, for age, body mass, time from injury/deployment, rank (as a proxy for socio-economic status, SES (287,288)) and ethnicity. When the adjusted analyses were significant, OR were calculated using binary or multinominal logistic regression models containing the same confounders (depending on the number of outcome groups, either two, three or four, see Section 4.2.4.1 and 4.2.4.2), using standardised biomarkers units (mean = 0, SD = 1), reported with 95% confidence intervals (95% CI). This allows comparison of the relative effects between biomarkers with different units and ranges of reporting scale.

Finally, after OR were calculated, correlation analysis (using Spearman's or Pearson's, accordingly) was undertaken between the adjusted biomarkers and the rOA, pain, and 6MWT distance (6MWD). Correlation analysis was also performed between the KOOS Pain and Symptoms sub-scale to the 6MWD to understand the relationship between these subjective and objective outcome measures, with a sensitivity analysis further performed in those with KL≥1. As all hypotheses and statistical tests were pre-planned, after discussion with the ADVANCE Study specialist statistician, adjustment for multiple testing was not required (376). Significance was set at 0.05. Analyses

were performed in Stata 18 (StataCorp LLC, Texas) and GraphPad Prism 10 (Dotmatics, Boston).

4.2.4.1 Primary analysis:

Do serum biomarker concentrations differ between those with/without trauma-exposure, knee OA 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-)

4.2.4.2 Secondary analyses:

Do serum biomarker concentrations differ between those with idiopathic v PTOA, painful v painfree OA, with different injury or pathological patterns or functional levels?

- 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 or rOA, 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: injured non-amputation (Exp-NA) vs injured amputation (Exp-A) vs knee injury (Exp-K)
- Pathological mechanisms: OARSI joint space narrowing (JSN), sclerosis (Scl) and osteophytes (OP)
- Functional level: Six-minute walk test distance (6MWD)

4.3 Results

A total of 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-). Within the Exp+ group, n=161 sustained a traumatic amputation (28%); number of amputations 1 n=85, 2 n=65 and 3 n=12, respectively. Demographic data for all participants can be found in Table 4.1 (overleaf), with additional data on ethnicity and injury type found in Tables 4.2 and 4.3. Complete biomarker data were available for 1118 participants, and radiographic data for 1074, which are presented, alongside KOOS Pain, Symptoms and 6MWD, in Table 4.4. Participant refusal, sampling or analysis errors, and individuals with amputations account for all missing values.

4.3.1 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 ($p=0.03$) (Figure 4.1a), remaining after adjustment for age, SES, ethnicity and time from injury ($p=0.02$). No other biomarkers were different.

4.3.2 Radiographic OA change

In total, 955 participants had paired radiographic and serum data (rOA+ n=210, rOA- n=745). Those with rOA were older, had a higher BMI, and reported 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 4.5). After adjustment for age, body mass, ethnicity, SES, and time from injury, no biomarker remained significant in the primary radiographic analysis. For the sensitivity analysis, those with KL0/1 (n=868) were compared to KL≥2 (n=86), which showed that leptin ($p<0.001$) and COMP ($p=0.011$) were significantly higher in the KL≥2 group (Table 4.6). After adjustment, only leptin remained significantly different ($p=0.028$).

Table 4.1 Demographic data for all participants, stratified by exposure (Exp-/Exp+) and amputation status

	Total	**Unexposed**	**Exposed (Exp+)**		
		(Exp-)	All	No Amputation	Amputation
	N=1,145	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 circum – abdominal circumference, cms – centimetres, NCO – non-commissioned officer, NISS – New Injury Severity Scale

Table 4.2 Ethnicity of all participants recruited into the ADVANCE study

Ethnicity group	N (%)	Ethnicity group	N (%)
Asian/Asian British (Bangladeshi)	1 (0.09%)	**Mixed (White and Asian)**	4 (0.35%)
Asian/Asian British (Indian)	6 (0.52%)	**Mixed (White and Black Asian)**	5 (0.44%)
Asian/Asian British (Pakistani)	1 (0.09%)	**Mixed (White and Black Caribbean)**	9 (0.79%)
Asian/Asian British (other)	33 (2.88%)	**Mixed (Other)**	3 (0.26%)
Black/Black British (African)	12 (1.05%)	**White (British)**	1008 (88.11%)
Black/Black British (Caribbean)	13 (1.14%)	**White (Irish)**	12 (1.05%)
Black/Black British (Other)	21 (1.84%)	**White (Other)**	16 (1.4%)

One participant did not complete the ethnicity questionnaire

Table 4.3 Category of combat-related traumatic injury sustained by ADVANCE participants in the exposed group

Injury type	N (%)	Injury type	N (%)
Blast	435 (75.1%)	**Non-blast (accident, gunshot, burns)**	144 (24.9%)

Table 4.4 Biomarker, radiographic, patient-reported and functional outcomes for all participants, stratified by exposure (Exp-/Exp+) and amputation status

	Total	**Unexposed (Exp-)**	**Exposed (Exp+)**		**p-value** Unadj. Adj.
			No Amputation	Amputation	
	N=1,118	N=553	N=409	N=156	
IL1β (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~
TNFα (ng/l) Mean (SD)	1.9 (± 0.6)	1.9 (± 0.6)	1.9 (± 0.5)	1.9 (± 0.4)	0.818^ 0.736^
IL17α (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~
CTXII (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 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 Sympt Median (IQR)	81 (65-95)	85 (70-95)	75 (60-90)	85 (70-95)	<0.001
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 matrix protein, PIIANP – N-propeptide of collagen IIA, Adipo – Adiponectin, KL – Kellgren-Lawrence, KOOS – Knee Injury and Osteoarthritis Outcome Score, Sympt: Symptoms, 6MWT – Six-minute walk-test distance, 95% CI – 95% confidence interval, Unadj. – unadjusted, Adj. – Adjusted ^three group oneway analysis of variance ~three group Kruskal-Wallis, dichotomised by exposure and amputation status

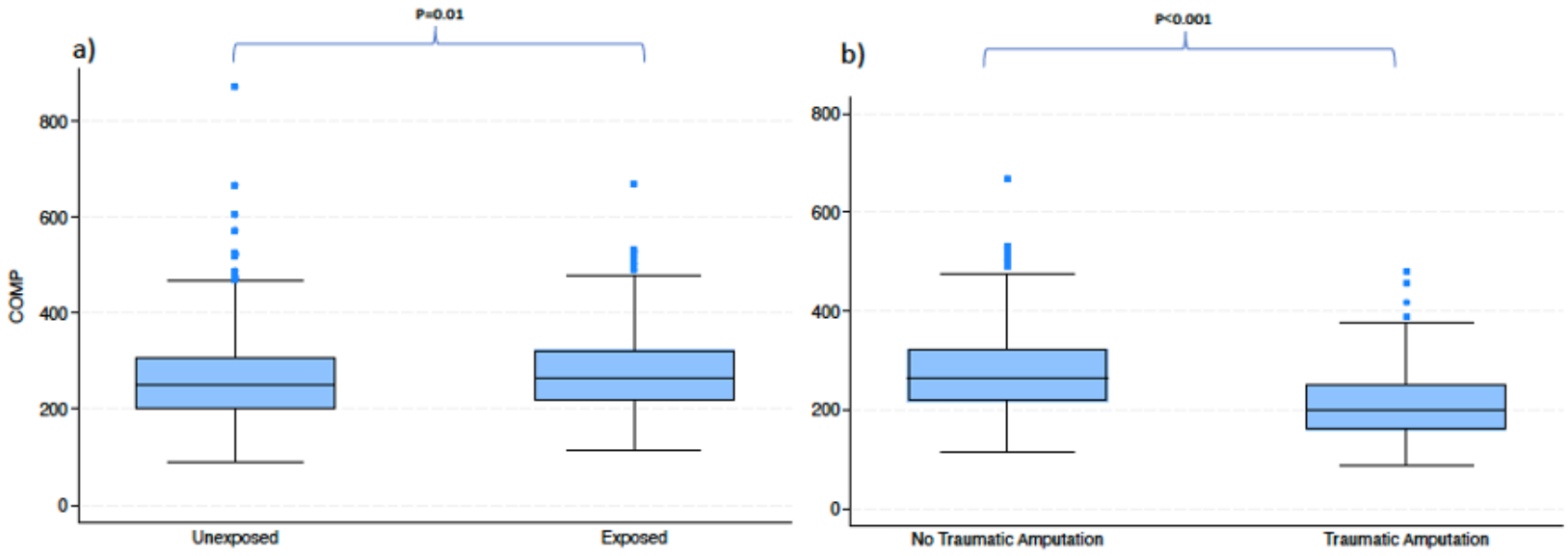


Figure 4.1 Differences in concentrations of cartilage oligomeric matrix protein (COMP) between those unexposed and exposed to combat trauma (a) and within those exposed to combat trauma, those without and with a traumatic amputation (b)
Values in (ug/l), shown with median (IQR), test used Student's t test

Table 4.5 Demographic and outcome differences between those with and without knee radiographic osteoarthritis changes (rOA-/rOA+), further stratified by exposure status (Exp-/Exp+)

	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^
IL1β (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~
TNFα (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^
IL17α (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~
CTXII (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 matrix 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 one way analysis of variance ~four-group Kruskal-Wallis, dichotomised by presence of radiographic osteoarthritis and exposure status

An additional analysis was performed, comparing each OARSI atlas measure in isolation (JSN, Scl, OP) to determine if any specific biomarkers were related to specific pathological changes (Table 4.6). For those with JSN≥1 (n=358), both leptin ($p<0.001$) and COMP ($p=0.005$) were significantly higher compared to JSN0 (n=596), with leptin remaining significant after adjustment ($p=0.004$). For those with Scl≥1 (n=85), IL-17α ($p=0.029$) and leptin ($p<0.001$) were significantly higher, and adiponectin ($p=0.02$) and PIIANP ($p=0.012$) significantly lower, than those with Scl0 (n=869). Both leptin and adiponectin remained significant after adjustment. Finally, in those with OP≥1 (n=180), leptin ($p=0.006$) and COMP ($p<0.001$) were significantly higher, and PIIANP ($p=0.008$) significantly lower, than those with OP 0 (n=774). No biomarker remained significant after adjustment.

4.3.3 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.

Table 4.6 Sensitivity analysis of radiographic osteoarthritis and serum biomarker concentrations for Kellgren-Lawrence ≥2 and individual measures from the Osteoarthritis Research Society International (OARSI) atlas

	Kellgren-Lawrence (KL≥2)			**Osteophytes (OP)**		
	KL 0/1 N=868	KL≥2 N=86	p-value	OP 0 N=774	OP ≥1 N=180	p-value
IL-1β (ng/l)	0.02 (0.02-0.06)	0.02 (0.02-0.06)	0.57	0.02 (0.02-0.06)	0.02 (0.02-0.05)	0.37
TNF-α (ng/l)	1.94 (±0.62)	1.95 (±0.47)	0.88	1.94 (±0.64)	1.93 (±0.45)	0.78
IL-17α (ng/l)	1.28 (0.97-1.81)	1.32 (0.99-1.86)	0.62	1.28 (0.96-1.78)	1.30 (1.00-1.91)	0.30
CTX-II (ug/l)	0.20 (0.05-0.62)	0.29 (0.05-0.72)	0.47	0.21 (0.05-0.63)	0.20 (0.05-0.67)	0.45
Leptin (ug/l)	5.46 (2.91-8.87)	7.09 (4.81-10.12)	<0.001	5.38 (2.91-8.85)	6.24 (3.73-10.12)	0.006
COMP (ug/l)	270.19 (±87.86)	295.31 (±84.57)	0.011	267.38 (±86.09)	294.27 (±92.05)	<0.001
Adipo (mg/l)	6.43 (±4.51)	5.67 ± (4.86)	0.14	6.45 (±4.40)	5.97 (±5.14)	0.20
PIIANP (ug/l)	110.50 (75.30-163.95)	95.60 (65.30-156.90)	0.16	112.80 (77.00-166.90)	96.80 (65.45-149.75)	0.008
	Joint Space Narrowing (JSN)			**Sclerosis (Scl)**		
	JSN 0 N=596	JSN ≥1 N=358	p-value	Scl 0 N=869	Scl ≥1 N=85	p-value
IL-1β (ng/l)	0.02 (0.02-0.06)	0.02 (0.02-0.06)	0.80	0.02 (0.02-0.06)	0.02 (0.02-0.06)	0.87
TNF-α (ng/l)	1.93 (±0.59)	1.97 (±0.63)	0.31	1.94 (±0.62)	1.93 (±0.47)	0.87
IL-17α (ng/l)	1.27 (0.97-1.77)	1.33 (0.98-1.88)	0.15	1.27 (0.96-1.78)	1.42 (1.09-1.94)	0.029
CTX-II (ug/l)	0.20 (0.05-0.62)	0.21 (0.05-0.64)	0.63	0.20 (0.05-0.62)	0.36 (0.05-0.74)	0.15
Leptin (ug/l)	5.11 (2.56-8.37)	6.57 (3.59-9.79)	<0.001	5.50 (2.90-8.85)	7.03 (4.25-11.05)	<0.001
COMP (ug/l)	266.24 (±85.40)	282.81 (±90.90)	0.005	272.05 (±88.29)	276.59 (±83.28)	0.65
Adipo (mg/l)	6.47 (±4.45)	6.17 (±4.70)	0.33	6.47 (±4.69)	5.27 (±2.41)	0.020
PIIANP (ug/l)	113.75 (74.70-168.35)	102.70 (73.70-156.90)	0.11	111.40 (74.90-164.50)	90.90 (64.80-146.20)	0.012

IL: Interleukin, TNF: Tumour Necrosis Factor, CTX-II: C-terminal cross-linked telopeptide of type II collagen, COMP: cartilage oligomeric matrix protein, PIIANP: N-propeptide of collagen IIA, Results are either median (interquartile range) or mean (standard deviation)

4.3.4 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 4.7, Figure 4.2). Both remained significant after adjustment, 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).

4.3.5 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 4.7). 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, 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.

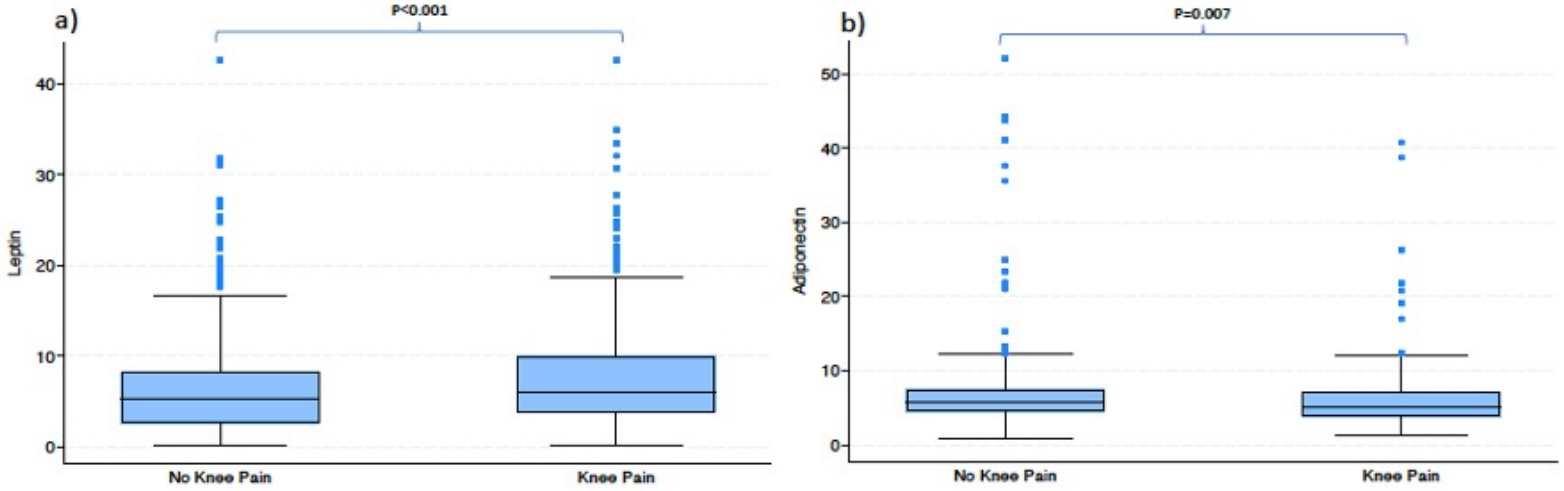


Figure 4.2 Differences in leptin (a) and adiponectin (b) between those not reporting and reporting knee pain
Leptin values in ug/l, adiponectin mg/l, shown with median (IQR), test used Student's t and Mann-Whitney-U

Table 4.7 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+)

	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^
IL1β (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~
TNFα (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^
IL17α (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~
CTXII (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 matrix 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 oneway analysis of variance ~four-group Kruskal-Wallis, dichotomised by the presence of pain and radiographic osteoarthritis

4.3.6 Associations between biomarkers 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 (Figure 4.3).

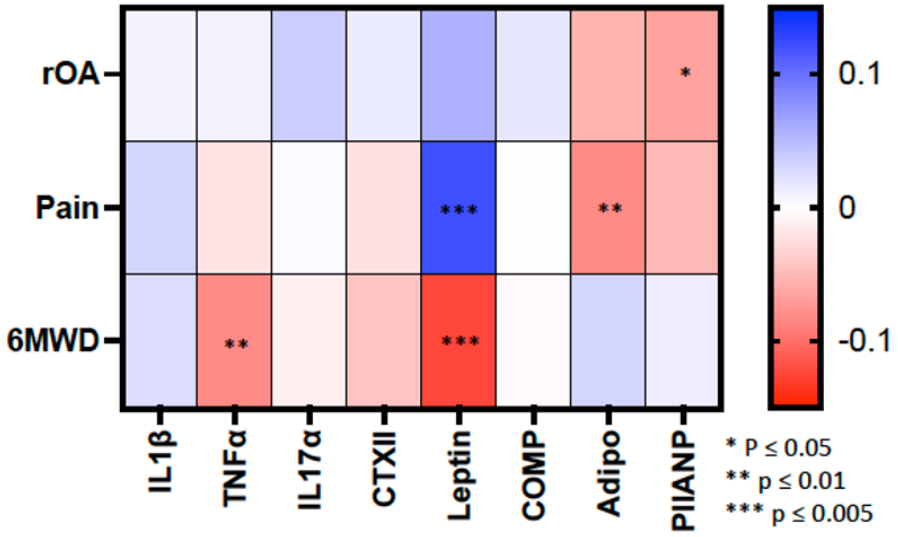


Figure 4.3 Heatmap demonstrating the correlations between the panel of fully adjusted biomarkers and the presence of early radiographic osteoarthritis change, the presence of knee pain and the distance achieved on the six-minute walk test
Spearman or Pearson's correlation, depending on normality

In addition, associations were assessed between the adjusted biomarkers and each individual metric of the OARSI atlas (Figure 4.4), revealing that leptin was positively correlated with JSN (0.09, p=0.008) and Scl (0.08, p=0.014) and adiponectin was negatively correlated with OP (-0.07, p=0.043).

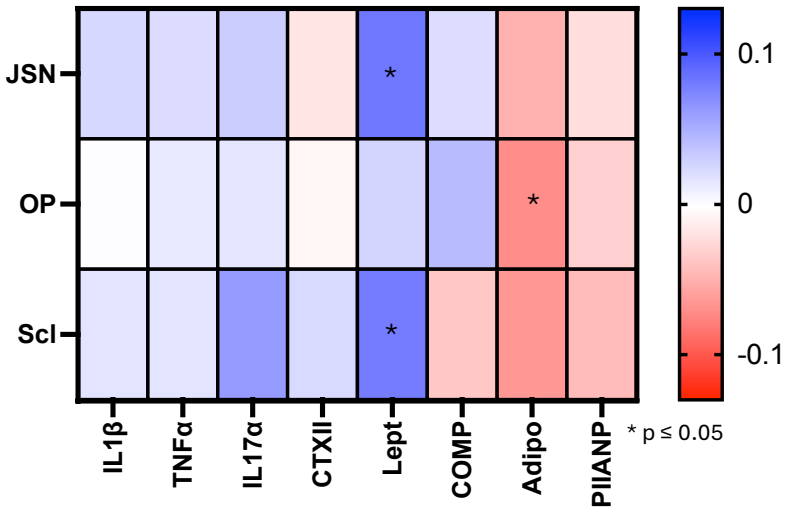
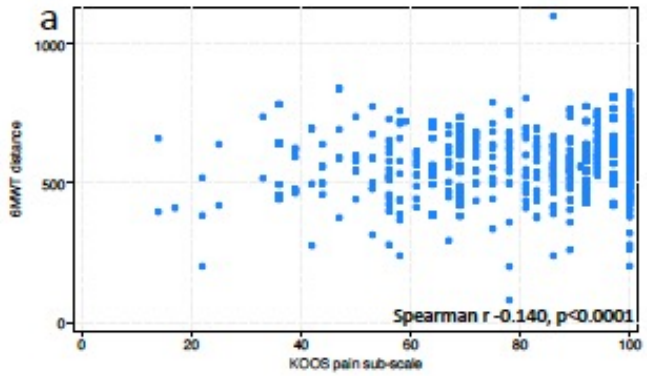


Figure 4.4 Heatmap demonstrating the correlations between the panel of molecular biomarkers and each individual component of the Osteoarthritis Research Society International atlas
Spearman or Pearson's correlation, depending on normality

Finally, the correlation analysis was performed between KOOS Pain and Symptoms sub-scales and 6MWD for 1067 participants for whom both KOOS and 6MWT data existed. Spearman's rank correlation showed that KOOS pain and 6MWD had a coefficient of -0.140 (95% CI -0.200,-0.079), $p<0.0001$, and KOOS symptoms and 6MWD had a coefficient of -0.084 (95% CI -0.145,-0.023), $p=0.005$ (Figure 4.5). In the 256 participants with a K-L≥1, correlation coefficients with 6MWD were 0.188 (0.061,0.308), $p=0.0003$ and 0.124 (95% -0.004,0.247), $p=0.052$, for KOOS pain and symptoms, respectively.



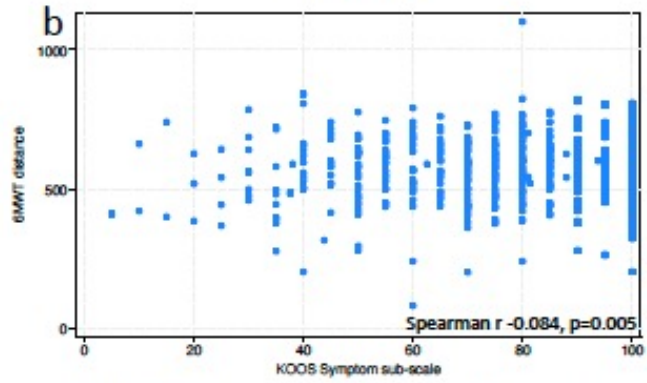


Figure 4.5 Scatter plots demonstrating the correlation between six-minute walk test distance and the Knee Osteoarthritis Outcome Score Pain (a) and Symptom (b) subscale in 1067 participants

4.3.7 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 (Figure 4.1b).

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), $p<0.001$ (Figure 4.6).

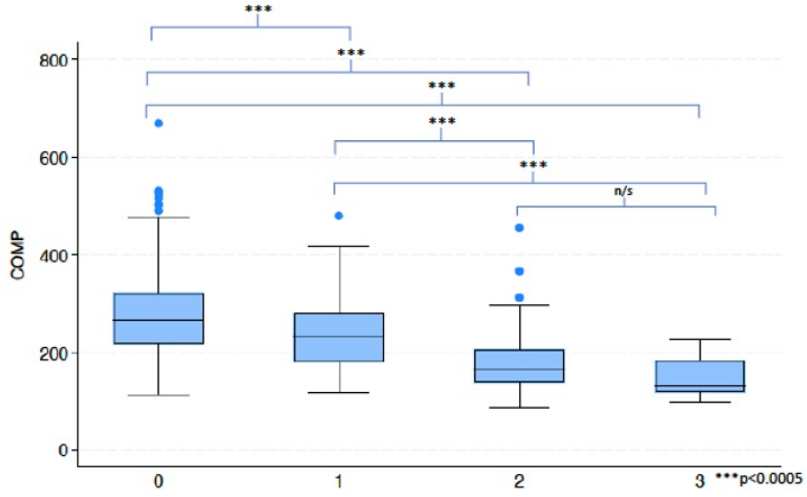


Figure 4.6 Differences in levels of cartilage oligomeric protein stratified by quantity of traumatic limb amputations
Values in (ug/l), shown with median (IQR), test used oneway analysis of variance

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 (Table 4.8). Only IL-17α remained significant after adjustment for age, body mass, SES, ethnicity and time from injury (p=0.02).

Table 4.8 Differences in serum biomarkers in those with a traumatic amputation stratified by the presence of radiographic osteoarthritis

Amputees	**Total**	**No rOA**	**rOA**	**p-value**
	N=94	N=54	N=41	
IL1β (ng/l) Median (IQR)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.0)	0.350”
TNFα (ng/l) Mean (SD)	1.9 (± 0.4)	1.9 (± 0.4)	1.8 (± 0.5)	0.422#
IL17α (ng/l) Median (IQR)	1.3 (0.9-1.9)	1.5 (1.1-2.0)	1.1 (0.8-1.6)	0.028”
CTXII (ug/l) Median (IQR)	0.1 (0.1-0.6)	0.1 (0.1-0.3)	0.4 (0.1-1.1)	0.009”
Leptin (ug/l) Median (IQR)	6.8 (3.5-12.0)	6.8 (3.5-12.9)	7.0 (3.6-9.5)	0.926”
COMP (ug/l) Mean (SD)	233.9 (± 76.7)	241.1 (± 79.0)	224.1 (± 73.3)	0.291#
Adipo (mg/l) Mean (SD)	6.2 (± 4.7)	6.5 (± 5.5)	5.8 (± 3.3)	0.447#
PIIANP (ug/l) Median (IQR)	104.2 (71.4-138.2)	104.0 (65.2-135.6)	104.8 (74.4-154.2)	0.723”

IL – Interleukin, TNF – Tumour Necrosis Factor, CTX-II - C-terminal cross-linked telopeptide of type II collagen, COMP - cartilage oligomeric matrix protein, PIIANP - N-propeptide of collagen IIA, rOA – radiographic osteoarthritis, KL - Kellgren-Lawrence
#Student’s t “Mann-Whitney-U

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

4.3.8 Specific knee injury

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

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

Two biomarkers were significant on unadjusted analysis (COMP, p<0.001 and leptin, p=0.02) (Table 4.9). COMP was significantly lower in Exp-A (p<0.001) and Exp-K (p=0.03) v reference, which remained significant after adjustment (Exp-A p<0.001, Exp-K p=0.05). Those with Exp-K had significantly higher leptin than reference (p=0.003) and Exp-A (p=0.02), which after adjustment, remained significant for Exp-K v reference (p=0.003) and Exp-A (p=0.009). COMP had a non-significant OR, and leptin an OR of 1.33 (95% CI: 1.09,1.63) for rOA after Exp-K.

Table 4.9 Differences in serum biomarkers in exposed participants, stratified by injury type (non-amputation, amputation, knee injury)

	Total	**Exposed - Non-amputee**	**Exposed - Amputee**	**Exposed- Knee Injury**	**p-value**
	N=565	N=389	N=141	N=35	
IL1β (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)	0.350~
TNFα (ng/l) Mean (SD)	1.9 (0.5)	1.9 (0.6)	1.9 (0.4)	1.9 (0.4)	0.422^
IL17α (ng/l) Median (IQR)	1.3 (1.0-1.8)	1.3 (1.0-1.8)	1.3 (1.0-1.9)	1.2 (1.0-1.7)	0.028~
CTXII (ug/l) Median (IQR)	0.2 (0.1-0.6)	0.2 (0.1-0.6)	0.1 (0.1-0.7)	0.1 (0.1-0.4)	0.009~
Leptin (ug/l) Median (IQR)	5.8 (3.2-9.9)	5.7 (3.2-9.4)	6.1 (3.2-11.7)	8.7 (4.6-12.9)	0.926~
COMP (ug/l) Mean (SD)	260.1 (88.3)	280.3 (86.2)	208.6 (76.2)	243.3 (69.0)	0.291^
Adipo (mg/l) Mean (SD)	6.5 (4.9)	6.6 (5.2)	6.3 (4.4)	5.3 (2.3)	0.447^
PIIANP (ug/l) Median (IQR)	109.1 (72.9-160.7)	111.4 (74.9-168.5)	107.1 (71.5-155.5)	92.6 (68.1-123.4)	0.723~

IL – Interleukin, TNF – Tumour Necrosis Factor, CTX-II - C-terminal cross-linked telopeptide of type II collagen, COMP - cartilage oligomeric matrix protein, PIIANP - N-propeptide of collagen IIA, rOA – radiographic osteoarthritis, KL - Kellgren-Lawrence
^oneway analysis of variance ~Kruskal-Wallis

4.4 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 my knowledge, this is the first description of such finding.

Remarkably, there were no differences between those with trauma-exposed rOA (PTOA) v non-trauma-exposed rOA (iOA) in opposition to my initial hypothesis. Increased leptin and decreased adiponectin were associated with an increased risk of knee pain of 22% and 17%, and painful rOA 46% and 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.

4.4.1 Injury and implications for pathophysiology

Understanding the molecular picture following trauma is important, given the frequency and severity similarities of polytrauma following road traffic accidents (377). 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 mainly expressed by cartilage (378), is associated with OA (173) and other conditions including liver, lung, and skin fibrosis (378,379). 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 (263). 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 number of amputated limbs. This is a notable finding, providing evidence for the importance of cartilage volume and health in COMP synthesis, 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, vs n=161 in this study) (96).

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 (PTOA) compared to the unexposed (idiopathic OA), predicated on the traumatic-dominant mechanism displaying a significantly different molecular pattern to an idiopathic-dominant (albeit accelerated) pattern. This was what was expected from the findings of the previous chapter, but 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 (123,125) and animal models postulating idiopathic and PTOA share a common ‘mechanoinflammation’ mechanism (380). It might be that the differences are only relevant in the pre-clinical pre-radiological phase, and by the time radiographic change is evident, there is a common process. This finding, if validated in other populations, would enable results from prognostic or even interventional PTOA studies to be extrapolated across all OA research fields thereby benefitting more patients, and is perhaps one of the most significant findings of this analysis. This concept is gaining momentum with other international clinical research teams (23), not only those working in animal models (380). Further work is planned to analyse this cohort's proteome, which would potentially allow internal validation of this finding using another method.

4.4.2 Implications for future research

The next area was the differences in serum biomarkers between those with and without rOA. KL≥1 was selected purposefully as the primary criteria as the strongest predictor of diagnosed OA (32), 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 of which are independent risk factors for OA development and progression (2,21,37)). When the sensitivity analysis was performed with KL≥2, only leptin was statistically significant, with higher concentrations in the KL≥2 group.

The minimal significant results might reflect their fluid type or the time from injury, as the evidence is poor regarding the utility of serum biomarkers over a year from injury. As reported in Chapter Three (Section 3.2.4), of the 38 serum biomarkers measured, only three of them (cleavage of type II collagen, hyaluronic acid, N-telopeptide of type I collagen) had a relationship to rOA, and one (TNF) to pain (also, similar to this study, measured by KOOS) (5). 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 (5,52,381). In addition, CTX-II, selected by the Foundation for the National Institutes of Health (FNIH) OA Biomarkers Consortium as a candidate marker (52) was measured using the serum, not the urinary form, which might also explain the results. This study will provide a significant addition to the medical canon regarding chronic phase biomarkers, and while these results may be disappointing at this stage, they will hopefully shape future research avenues and develop as the cohort progresses.

4.4.3 Implications for clinical care

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 in the weak range (r= -0.11-0.12). When this was undertaken for the individual measures of the OARSI atlas, leptin was positively correlated with JSN (0.09) and Scl (0.08), and adiponectin negatively to OP (-0.07), which were each negligible. 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 (5,381). Also of note, a weak correlation between two commonly used and well-validated outcome measures in OA was identified (KOOS and 6MWT). Further to the well-known discordance between OA radiographic change (indicative of the 'disease') and clinical presentation ('illness'), these findings accentuate a disparity between patients' perception of their illness/symptoms and their objective functional outcomes. They highlight the importance of carefully considering which outcome measures to collect across the spectrum of patient-reported, clinician-measured, imaging, and molecular, to be specific to the population and research question of interest (382). These results will act as a reference point for the planned analysis using longitudinal data gathered during the first follow up visit which will allow the analysis for the predictive of these specific markers on changes in pain and functional status.

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 (374,383). 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 (117). Adiponectin has been seen to have an anti-inflammatory effect and, in addition, analgesic properties through inhibition of p-p38 MAPK signalling (384). These findings are consistent with the understanding that adipokines can influence pain via serotonin, inflammatory and metabolic pathways (119,384); therefore, potentially, they could be used to identify and categorise a painful OA phenotype (385). The increased leptin levels in those with a local knee injury suggest that this biomarker may offer some increased specificity for the knee joint, however, this relationship needs to be explored further, with correlations also seen between leptin to KL≥2, JSN and Scl, and adiponectin to OP. It is important to note that these differences and associations, whilst statistically significant, are small and therefore of uncertain clinical significance but do generate hypotheses for further analysis. Specifically, the available proteomic data offers the opportunity to undertake a hypothesis-driven assessment of the painful v painfree groups to determine if there are any differences in potential mechanisms using a pathway analysis.

4.4.4 Strengths and Limitations

The key strength of this study is its design; large numbers (n=1145), and the 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 (123). 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 are likely to be comparable with other military populations, including the US (96), and therefore 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.

4.5 Conclusion

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. Further work is planned, and will be presented in the next chapter of this thesis, to understand their prospective value in predicting the incidence or progression of rOA, and changes in pain and functional status within this cohort.

Chapter 5 : Can biomarkers predict future pathology within the ADVANCE cohort?

In the previous chapter, I reported the cross-sectional results of the associations between a panel of candidate serum osteoarthritis (OA) biomarkers and the outcomes of knee radiographic OA (rOA), pain and function. Overall, this analysis showed there were no associations to rOA, with the exception of interleukin (IL)-17 in those with a traumatic lower-limb amputation and higher leptin concentrations in those with established OA. Excitingly, there were no molecular differences between those with OA in the trauma-exposed (post-traumatic OA, PTOA) and unexposed (idiopathic, iOA) groups. However, of interest, there were associations between leptin and adiponectin for knee pain and painful radiographic OA, which potentially might enable phenotyping in the future. In the sub-group with a specific knee injury at the time of trauma, cartilage oligomeric matrix protein (COMP) was significantly lower and leptin significantly higher than the comparison group. This analysis was performed at a median 8 years from index deployment and provides a snapshot of a single point of time. This next chapter will build upon this work using the data collected during the subsequent ADVANCE study visit, three years later, the Follow-Up 1 visit. The aim of this chapter is to describe the predictive value of the serum biomarker panel and other measures for the incidence and progression of knee rOA, pain and function, to understand if they can be used in the clinical environment for early identification or prognostication for those at risk of OA.

5.1 Background

As discussed in Chapter One (Section 1.2), OA of the knee is extremely common, with prevalence rates as high as 64.5-78.6% for males and females, and extremely disabling, accounting for 85% of OA disease burden and approximately three times as many healthcare consultations as hip or hand OA (1,68,70,386). The risk of knee OA incidence typically increases between 50- and 75-years old, with previous knee injury, female sex, obesity, mechanical factors, manual work, high-impact sports, occupation, and genetics instigating different mechanistic pathways which can increase an individual's risk for knee OA (2,68,386). Current management of established knee OA is focussed on symptomatic improvement, with pain often the most disabling symptom and end-stage OA often requiring surgical interventions (27,68). OA is diagnosed using patient-reported and clinical examination features; however, there is a new focus on identifying early-stage knee OA (387), particularly recognising the factors predicting OA development to enable earlier intervention (37,388). Radiographic or magnetic resonance imaging (MRI) measures of structural OA are often only moderately correlated to pain (389,390), despite structural OA being more prevalent than symptomatic OA (70). This discrepancy, articulated in Chapter One as the 'disease of OA' vs the 'illness of OA' (Section 1.1.4), is most divergent in early OA, with the radiographic changes and symptoms marrying closer in more severe and end-stage OA. However, the relationship between symptomatic and rOA remains important; 75% of those with incident symptomatic OA have pre-existing rOA (391), or could develop rOA in as little as 2-years (392).

Evidence from large cohort studies (e.g., UK Biobank) suggests a knee injury is associated with expedited OA progression for a short period of five years before stabilising (123), suggesting a brief window for potential optimal intervention during this time of heightened risk. Studies performed in athletes showed increased OA rates, with one

study in ex-professional footballers demonstrating that this increased OA risk subsequently plateaus (207,211,214,215). With Armed Forces populations, studies have demonstrated OA between 2 and 8 years following combat and musculoskeletal injury (36,235), with those within the military more likely to sustain significant injuries leading to OA (such as anterior cruciate ligament) as well as exposure to ongoing risks such as micro-trauma, vibration and repetitive movements (2,230,235). In addition, military personnel with lower limb loss are known to have greater knee OA prevalence than controls (393). Computational biomechanics studies demonstrate that increased joint contact forces through the medial compartment of the intact side limb, on which people with lower limb loss preferentially rely, drive this elevated risk (139,394). As a result, following initial trauma, an altered mechanical environment may mean that people with limb loss have a different disease trajectory from those who sustained other combat trauma.

The Armed Services Trauma Rehabilitation Outcomes (ADVANCE) study is a longitudinal cohort study investigating physical and psychosocial outcomes of exposure to combat trauma following the Afghanistan war (260). Earlier work in this cohort has shown that those sustaining combat-injury had 2-times greater odds for having knee rOA 8-years post-injury, with those sustaining a lower-limb loss having a 4x risk (138). In addition, as discussed in the last chapter, the inability of molecular biomarkers to differentiate between those developing OA in the comparison group (iOA) and those exposed to combat-trauma (PTOA), suggests that by this point, both processes share a common pathomechanistic pathway (Section 4.4.1), and therefore, likely similar disease trajectories. The completion of the first Follow-Up (FU1) visit of the ADVANCE study offers the opportunity to compare two-wave trends for rOA and knee pain incidence and progression and explore the ability to predict the progression of knee rOA, pain and functional levels of participants.

This chapter aims to assess the predictive value of pre-determined characteristics for progression and incidence of knee rOA, pain and function, including the panel of candidate serum biomarkers. In addition, this chapter will also investigate the relationship between the risk of progression and incidence of knee rOA and knee pain to injury status, hypothesising that there will be (i) no increased risk of progression and incidence for those exposed to combat trauma compared to a control group, but (ii) an increased risk of progression and incidence for those with lower limb loss compared to a control group.

5.2 Methods

The ADVANCE study is a longitudinal cohort study monitoring the long-term physical and psychosocial outcomes associated with exposure to severe combat injury in UK military personnel who served in the Afghanistan war. Details are found in Chapter Two (Section 2.1)

5.2.1 Groups

Participants were divided into Unexposed and Exposed groups, and as described previously (Section 2.1.5), the Exposed group was further subdivided into Exposed—no Lower Limb Amputation (Exp-NA), Exposed—Lower Limb Amputation (Exp-A), and Exposed – Knee Injured (Exp-K), with the Exp-K group contained participants with and without lower limb loss who sustained a knee-specific combat injury.

5.2.2 Study visits

Baseline data was collected at the UK Defence Medical Rehabilitation Centre (DMRC) Headley Court (2015-2018) and Stanford Hall (2018-2020), with Follow-up data collected at Stanford Hall (2019-2024).

Like Baseline, each Follow-up study visit required one day of comprehensive, nurse-led physical and psychosocial assessment, described comprehensively in Section 2.1.5. In brief, these included demographic details, medical history of combat-injuries (including severity, using the New Injury Severity Scale; NISS), patient-reported outcome measures (including the Knee injury and OA Outcome Score, KOOS, the Knee and Hip Joint Pain scale, KHJP and the International Physical Activity Questionnaire, IPAQ), the functional six-minute walk-test (6MWT), knee radiographs and serum collection (273,278,395).

5.2.2.1 Self-reported Questionnaires

5.2.2.1.1 KOOS

The KOOS is a widely used, reliable, and valid patient-reported outcome measure responsive to change, with five independent subscales, scored 0-100 (100 denotes no knee problems) (273,370,396). The Pain subscale was used for this analysis with a threshold of ≤86.1 to determine the presence of pain (89,371) and a minimum clinically important difference (MCID) of 12.4 (397), utilised throughout. Specifically, knee pain progression was defined as a decrease in intra-knee KOOS Pain score at Follow-up of ≥12.4 than the score recorded at Baseline. Incidence of knee pain was defined as a participant documenting a KOOS Pain score ≤86.1 at Follow-up after reporting a KOOS Pain score of >86.1 in the same knee at Baseline. Similar to the cross-sectional analysis detailed in the last chapter, I felt it was important to use values that had been determined previously within the literature rather than creating arbitrary new cut-offs.

5.2.2.1.2 KHJP

The Knee and Hip Joint Pain (KHJP) score comprises a pain scale for severity, frequency, and impact for each knee and hip joint, scored from 0 (e.g., no pain) to 10 (e.g., worst pain imaginable). Only knee scores were considered in this analysis, Knee Pain Severity, Knee Pain Frequency and Knee Pain Impact.

5.2.2.1.3 IPAQ

The international physical activity questionnaire (IPAQ) was developed in the late 1990's, with reliability and validity studies performed in 2000, to enable robust self-report physical activity measurement (398). It has become the most widely used physical activity questionnaire, especially in the short form, and records the amount and intensity of physical

activity over the last seven days (399). During this analysis, the 'total exercise minutes per week' over the last week is used as a self-reported measure of physical activity, and therefore, function.

5.2.2.2 Six-minute walk-test

The sub-maximal six-minute walk-test (6MWT) is widely used as a measure of physical activity and daily function (278,400). During this analysis, the total distance walked at Baseline was used as a predictor for knee rOA and pain at Follow-up, and total distance walked at Follow-Up as an outcome. In addition, a reduction in physical function was analysed using a minimal detectable change (MDC) of 81.25m, a threshold established in a study of 180 US military service personnel, with an intraclass correlation coefficient of 0.93 (401).

5.2.2.3 Radiographic assessment

Semi-flexed posterior-anterior radiographs were taken and reported as previously described in Chapter Two (Section 2.1.5.3). Progression was defined as an increase in KL by ≥1 at Follow-up in a knee with knee rOA (KL≥1) at Baseline. Knees with KL4 at Baseline were excluded from the Progression analysis due to a ceiling effect (4 knees). Incidence was defined as the presence of knee rOA (KL≥1) at Follow-up in a knee that was KL0 at Baseline.

5.2.2.4 Biomarker analysis

The sampling and analysis of the biomarkers are described in a previous chapter (Section 2.2.3). In brief, fasted blood was collected at Baseline visit, centrifuged (3500rpm for 10 minutes), aliquoted and frozen at – 80°C before transfer to Affinity Biomarker Labs (London, UK). Using enzyme-linked immunosorbent assay (ELISA) or meso scale discovery (MSD), samples were analysed for metabolic (adiponectin, leptin), inflammatory (IL-1β, IL-17α, tumour necrosis factor-alpha, TNF-α) and extracellular matrix (ECM) turnover (cartilage

oligomeric matrix protein, COMP, N-propeptide of collagen IIA, PIIANP, and C-terminal cross-linked telopeptide of type II collagen, CTX-II) biomarkers, on plates containing kit and quality control samples. Individual biomarker intra- and inter-variability coefficient of variation and quantification concentration thresholds can be found in Chapter Two (Section 2.2.3).

5.2.3 Statistical analysis

One Unexposed participant was excluded due to non-combat trauma lower-limb loss following matched deployment, and a further 92 participants were excluded because they did not attend Follow-Up 1 data collection (attrition rate 8.04%). Radiographic data was available at Baseline and Follow-Up for 1905 knees (n=974 participants) (Figure 5.1). Pain data was available for 1796 knees (n=909 participants). Knee and participant numbers do not match due to retaining of participants with single knee data due to invalid questionnaires, radiographs, or limb-loss.

The rates of knee rOA and pain progression and incidence were assessed for each knee. A mixed-effects Poisson regression model was used to assess the risk of progression and incidence of knee rOA and pain in Unexposed vs. Exposed, and Unexposed vs. Exp-NA/Exp-A/Exp-K groups. The models were adjusted for age at Baseline, time between Baseline and Follow-Up, and socioeconomic status (SES) defined using military rank as a proxy for National Statistics socioeconomic classification (287,402). A sensitivity analysis was carried out to test for potential confounding resulting from the inclusion of an individual's left and right knee in the same analysis, as they are not considered truly independent. Regression models were run for all knees, then separately for left and right knees, and results were compared.

As reported in Chapter Two (Section 2.2.5.3), knee injuries before index deployment identified 93 individuals, 37 with a specific pathology known to increase the risk of OA (fracture, cruciate, meniscal), and the rest with chronic knee pain. A chi-squared test revealed no differences between the unexposed and exposed groups (p=0.189), negating any effect of prior injury in this study.

The presence of knee rOA or pain at Baseline was used to dichotomise individuals for predictive modelling due to the previous similarities in molecular patterns between iOA and PTOA. Twenty potential predictor variables were selected, based on known risk factors or earlier work: age, BMI, SES, NISS, time from injury, KOOS Pain & Symptom, knee pain impact, frequency, and severity, JSN, 6MWT distance (6MWD) and serum biomarkers. JSN was selected given its use as an endpoint in clinical trials (39).

The worst scoring variable was selected as the index variable, which was the highest KL or JSN grade, lowest KOOS Pain score, or highest Knee Pain Severity, Frequency and Impact score, respectively, when two were available. For the lower-limb loss subgroup, the single variable was used (when available). In this analysis, BMI was chosen as a predictor, rather than a body shape index (ABSI) used in the previous chapter, given its widespread use in other studies to improve reproducibility and enable possible future meta-analyses.

Predictor variables were combined into three models and assessed for their ability to predict knee rOA and pain progression and incidence at Follow-up;

- Model 1: Demographic (including injury-related and functional; age, BMI, NISS, time from injury, 6MWD)
- Model 2: Joint-specific (JSN, KOOS and knee pain scores), and
- Model 3: Molecular (serum biomarkers).

A similar methodology was applied to the prediction of function, using the subjective IPAQ measure (Total minutes of activity per week at Follow-up) and objective 6WMT (both distance at Follow-up and reduction of ≥81.25m between Baseline and Follow-up) as outcome measures.

After discussion with the ADVANCE study statistician, to avoid overfitting, Models 1, 2, and 3, which all provide different data for potential prediction, were kept separate. Predictors which demonstrated value are combined, when possible, to form an optimised Model 4.

Initially, Spearman's correlations were performed between the outcome and predictor variables to visualise patterns in the data. Subsequently, multivariable logistic regression was performed with each model, with Nagelkerke's R2, area under the receiver operator curve (AUROC) with 95% confidence intervals (95% CI) and standard error (SE), and Akaike and Bayesian information criteria (AIC, BIC) reported. These measures reflect different metrics which record the 'goodness-of-fit' for a model, and therefore give an insight into its value to predict an outcome. As a result of the continuous nature of the Follow-up IPAQ total minutes/week and 6WMD variables, linear regression was used employing the same models, with an optimised model created using any predictor variables with a p-value <0.05 and t-value <-2 or >2.

Due to the significant physical and pathomechanistic differences between those with and without a traumatic-amputation, analyses were performed in the cohort with those with lower-limb loss excluded, followed by a lower-limb loss sub-group analysis. As the Exp-A group has such smaller numbers, preventing a similar approach outlined above, a least absolute shrinkage and selection operator (LASSO) variable selection model was performed to identify significant predictor variables, with Pain incidence, Follow-up IPAQ score and Follow-up 6MWD resulting in predictive models.

5.3 Results

1052 participants attended Baseline and Follow-Up data collections with a mean interval of 40.2 months (standard deviation, SD: 6.8 months). The retention rate between Baseline and Follow-Up was 92.0%; 93% (526/565) of the Unexposed and 91% (526/579) of the Exposed groups (140 of whom had lower-limb loss, from 161 recruited at Baseline). At Follow-Up, mean age was 38.2±5.4 years old, height was 179.2±6.7cm, and adjusted weight was 90.9±14.1kg (Table 5.1). KL, JSN and KOOS Pain scores at Baseline and Follow-up are reported in Table 5.2, Figure 5.2 and 5.3, with Baseline serum biomarker results re-reported in Table 5.3. Correlation results are summarised in Figure 5.4, with the regression results reported in text and figures.

Table 5.1 Participant demographics at Follow-up for participants who attended Baseline and Follow-up data collection.

	Unexposed (n=526)	**All Exposed (n=526)**	**Exp-NA (n=371)**	**Exp-A (n=122)**	**Exp-K (n=33)**
Age (years)	38.3 (5.4)	38.0 (5.3)	38.3 (5.5)	37 (4) 4.8)	37.6 (5.7)
Time since baseline (yrs)	3.4 (0.6)	3.3 (0.6)	3.3 (0.5)	3.5 (0.6)	3.3 (0.5)
Previous knee injury (%)	27 (5.1)	10 (1.9)	9 (2.4)	1 (0.8)	0 (0.0)
Cause of injury					
Blast		360 (68.4)	218 (58.8)	118 (96.7)	24 (72.7)
Gunshot	-	124 (23.6)	112 (30.2)	4 (3.3)	8 (24.2)
Other		2 (0.4)	1 (0.3)	0 (0.0)	1 (3.0)
		40 (7.6)	40 (10.8)	0 (0.0)	0 (0.0)
Height (cm)	180.0 (6.3)	179.5 (6.9)	179.1 (6.7)	180.7 (7.6)	179.2 (6.8)
Mass* (kg)	90.3 (13.0)	91.5 (15.1)	90.8 (14.9)	93.2 (15.6)	92.5 (15.5)
BMI* (kg/m^2)	28.2 (3.7)	28.5 (4.1)	28.3 (3.9)	30.0 (4.5)	28.9 (4.6)
Race (White)	470 (89.4)	470 (89.4)	331 (89.2)	107 (87.7)	32 (97.0)
NISS (median, IQR)	-	12 (5-22)	9 (4-17)	22 (14-34)	13 (12-22)
NC-SEC					
Officer rank	75 (14.3)	58 (11.0)	44 (11.9)	12 (9.8)	2 (6.1)
Senior-rank	142 (27.0)	100 (19.0)	79 (21.3)	16 (13.1)	5 (15.2)
Junior rank	309 (58.8)	368 (70.0)	248 (66.9)	94 (77.1)	26 (78.8)
Still serving in military (yes)	355 (67.9)	109 (20.8)	104 (28.2)	3 (2.5)	2 (6.1)

**Mass and BMI are both adjusted for limb loss. Unexposed participants did not sustain a combat trauma, Exposed participants did, who are further divided by injury exposedlocal knee injury, lower-limb amputation, or a combat injury which was neither of these*
Age at follow-up, time since baseline, time between injury and assessment, height, mass, and BMI are reported as mean (SD). The remaining variables are reported as count (percentage). Cm: centimetres, kg: kilograms, m^2: metres squared, Exp-NA: Exposed, non-amputation, Exp-A: Exposed amputation, Exp-K: Exposed Knee injury, NISS: New injury severity score, NC-SEC: National statistics socio-economic classification

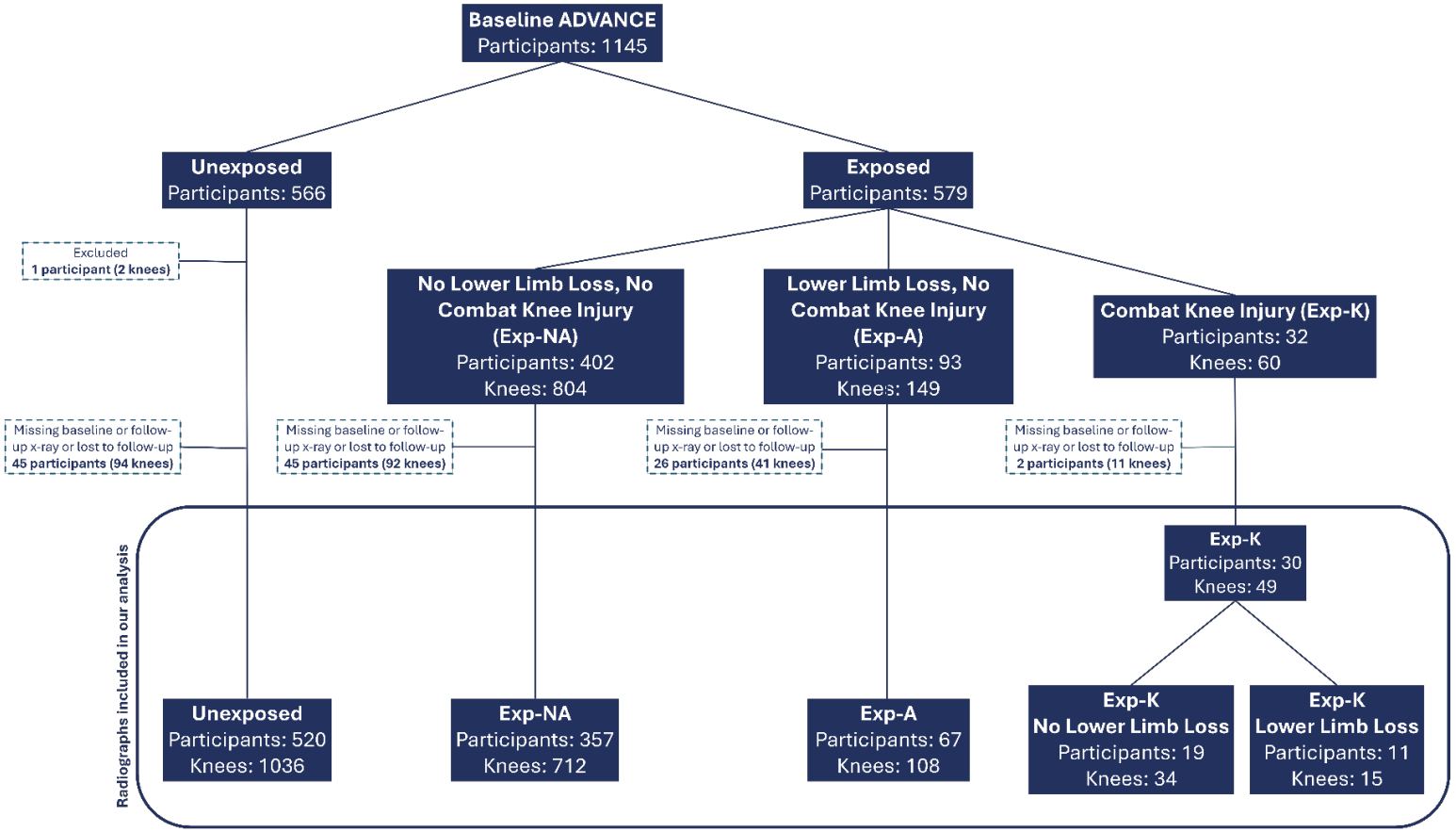


Figure 5.1 Flow diagram demonstrating participant and knee inclusion for radiographic knee osteoarthritis progression or incidence analysis
.N.B. 1 participant does not equate to 2 knees because a single knee radiograph can be lost or a single limb can be amputated at a level above the knee, without losing a participant. For inclusion in the analysis, successfully scored baseline and follow-up radiographs were required.

Table 5.2 Participant knee radiographic osteoarthritis outcomes and pain at Baseline and Follow-up

	Radiographic Knee Osteoarthritis									
	Unexposed (n=1036)		**Exposed (n=869)**		**Exp-NA (n=712)**		**Exp-A (n=108)**		**Exp-K (n=49)**	
	BL	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**
KL										
0	922 (89.0)	851 (82.1)	693 (79.8)	654 (75.3)	587 (82.4)	558 (78.4)	72 (66.7)	64 (59.3)	34 (69.4)	32 (65.3)
1	75 (7.2)	128 (12.4)	109 (12.5)	121 (13.9)	82 (11.5)	94 (13.2)	23 (21.3)	23 (21.3)	4 (8.2)	4 (8.2)
2	29 (2.8)	46 (4.4)	51 (5.9)	74 (8.5)	33 (4.6)	48 (6.7)	10 (9.3)	18 (16.7)	8 (16.3)	8 (16.3)
3	8 (0.8)	6 (0.6)	14 (1.6)	16 (1.8)	9 (1.3)	9 (1.3)	3 (2.8)	3 (2.8)	2 (4.1)	4 (8.2)
4	2 (0.2)	5 (0.5)	2 (0.2)	4 (0.5)	1 (0.1)	3 (0.4)	0 (0.0)	0 (0.0)	1 (2.0)	1 (2.0)
JSN										
0	777 (75.0)	732 (70.7)	596 (68.6)	579 (66.6)	493 (69.2)	482 (67.7)	71 (65.7)	66 (61.1)	32 (65.3)	31 (63.3)
1	249 (24.0)	293 (28.3)	251 (28.9)	263 (30.3)	209 (29.4)	217 (30.5)	31 (28.7)	35 (32.4)	11 (22.5)	11 (22.5)
2	8 (0.8)	6 (0.6)	20 (2.3)	23 (2.7)	9 (1.3)	10 (1.4)	6 (5.6)	7 (6.5)	5 (10.2)	6 (12.2)
3	2 (0.2)	5 (0.5)	2 (0.2)	4 (0.5)	1 (0.1)	3 (0.4)	0 (0.0)	0 (0.0)	1 (2.0)	1 (2.0)
	Knee Injury and Osteoarthritis Outcome Score (KOOS)									
	Unexposed (n=966)		**Exposed (n=830)**		**Exp-NA (n=669)**		**Exp-A (n=106)**		**Exp-K (n=55)**	
	BL	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**
KOOS Pain	100 (89-100)	100 (89-100)	97 (81-100)	97 (81-100)	97 (83-100)	100 (81-100)	97 (83-100)	97 (86-100)	86 (67-100)	86 (58-100)

BL: Baseline, FU: Follow-up, Exp-NA: Exposed, non-amputation, Exp-A: Exposed amputation, Exp-K: Exposed Knee injury, KL: Kellgren-Lawrence, JSN: Joint space narrowing, KOOS: Knee injury and osteoarthritis outcome score

Table 5.3 Baseline serum candidate osteoarthritis biomarker concentrations

	Total	**Unexposed**	**Exposed**	**Exp-NA**	**Exp-A**	**Exp-K**
	N=1,118	**N=553**	**N=565**	**N=389**	**N=141**	**N=35**
IL-1β (ng/l)	0.02	0.02	0.02	0.02	0.02	0.02
Median (IQR)	(0.02-0.06)	(0.02-0.06)	(0.02-0.06)	(0.02-0.06)	(0.02-0.06)	(0.02-0.06)
TNFα (ng/l)	1.94	1.94	1.93	1.94	1.92	1.93
Mean (SD)	(0.58)	(0.64)	(0.52)	(0.56)	(0.41)	(0.42)
IL-17α (ng/l)	1.30	1.27	1.33	1.32	1.33	1.23
Median (IQR)	(0.97-1.82)	(0.96-1.82)	(0.98-1.82)	(0.99-1.77)	(0.96-1.91)	(0.99-1.73)
CTX-II (ug/l)	0.20	0.22	0.18	0.20	0.15	0.05
Median (IQR)	(0.05-0.64)	(0.05-0.67)	(0.05-0.62)	(0.05-0.62)	(0.05-0.70)	(0.05-0.37)
Leptin (ug/l)	5.65	5.51	5.80	5.68	6.09	8.68
Median (IQR)	(3.04-9.29)	(2.97-8.75)	(3.23-9.93)	(3.16-9.37)	(3.16-11.67)	(4.57-12.86)
COMP (ug/l)	263.56	267.07	260.11	280.31	208.57	243.25
Mean (SD)	(88.53)	(88.71)	(88.30)	(86.15)	(76.20)	(69.00)
Adipo (mg/l)	6.33	6.21	6.46	6.62	6.29	5.32
Mean (SD)	(4.49)	(4.01)	(4.91)	(5.25)	(4.36)	(2.33)
PIIANP (ug/l)	109.10	109.20	109.10	111.40	107.10	92.60
Median (IQR)	(73.90-160.10)	(74.60-158.30)	(72.90-160.70)	(74.90-168.50)	(71.50-155.50)	(68.10-123.40)

IL: Interleukin, TNF: Tumour Necrosis Factor, CTX-II: C-terminal cross-linked telopeptide of type II collagen, COMP: cartilage oligomeric matrix protein, PIIANP: N-propeptide of collagen IIA, Adipo: Adiponectin. Exp-NA: Exposed - No lower limb amputation, Exp-A: Exposed – Lower Limb Amputation, Exp-K: Exposed – Knee Injured. At baseline, 1118 of 1145 had serum collected, 553/565 unexposed and 565/579 exposed. The 27 participants without samples were due to participant refusal, unable to obtain sample or laboratory error.

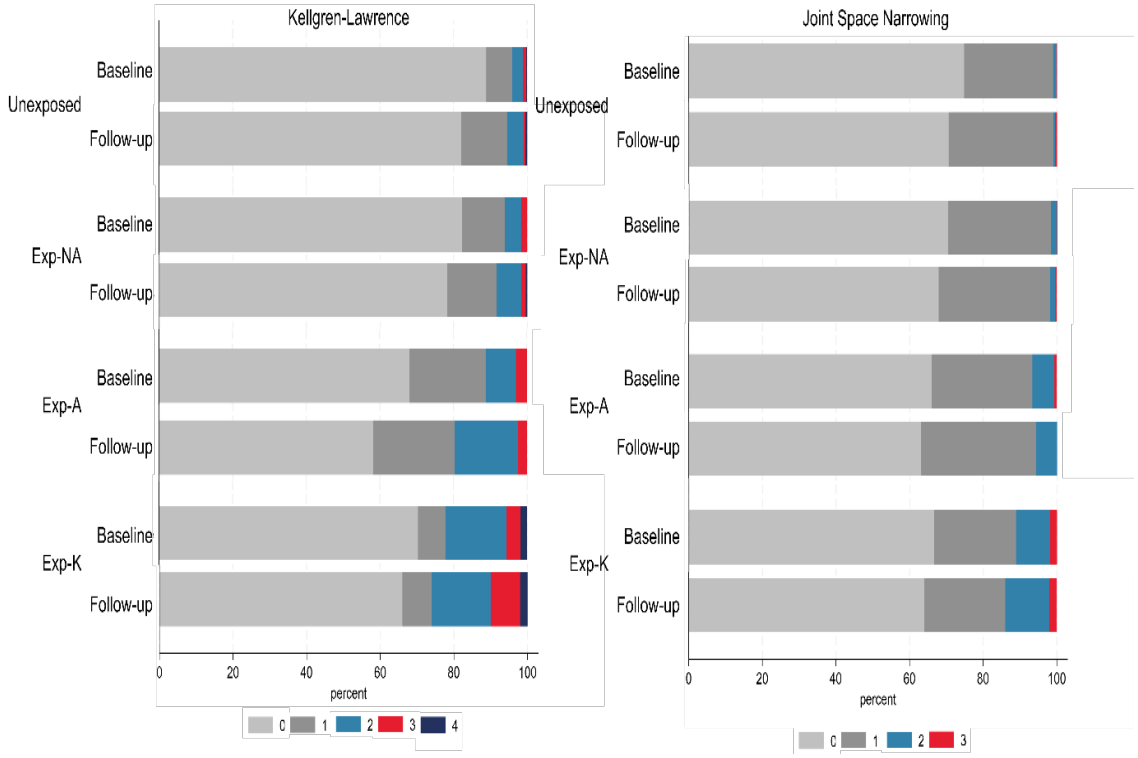


Figure 5.2 Radiographic knee osteoarthritis score for participants at Baseline and Follow-up.
Exp-NA: Exposed - No lower limb amputation, Exp-A: Exposed – Lower Limb Amputation, Exp-K: Exposed – Knee Injured.

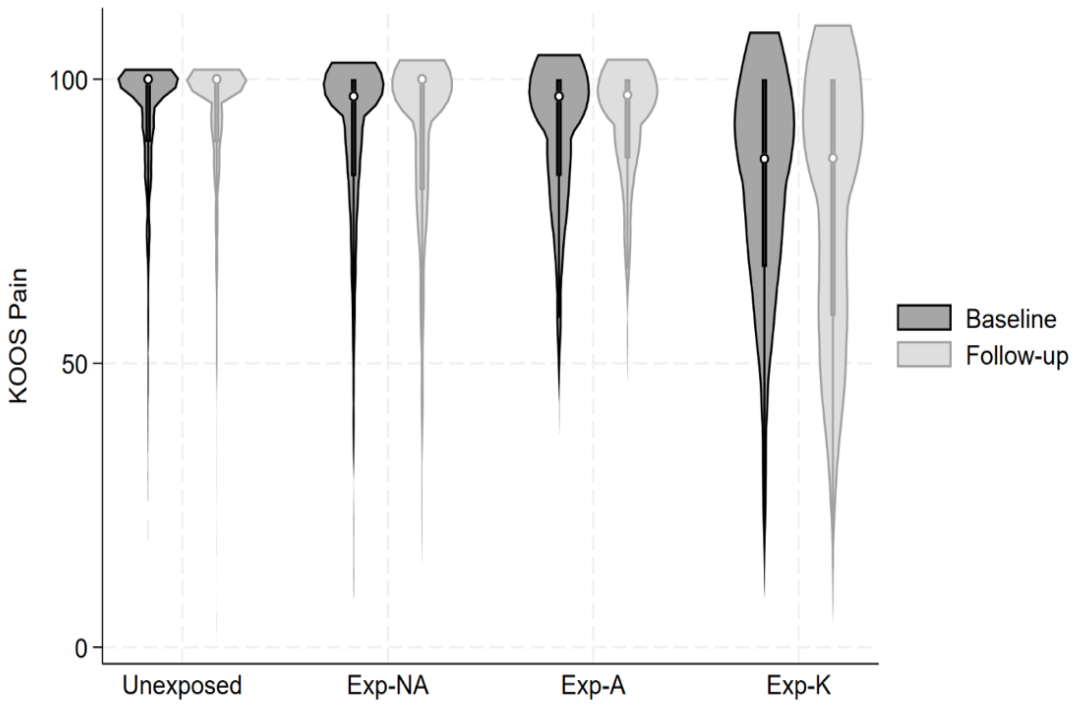


Figure 5.3 Violin plot of KOOS Pain scores for participants at Baseline and Follow-up.
KOOS: Knee injury and osteoarthritis outcome score, Exp-NA: Exposed - No lower limb amputation, Exp-A: Exposed – Lower Limb Amputation, Exp-K: Exposed – Knee Injured.

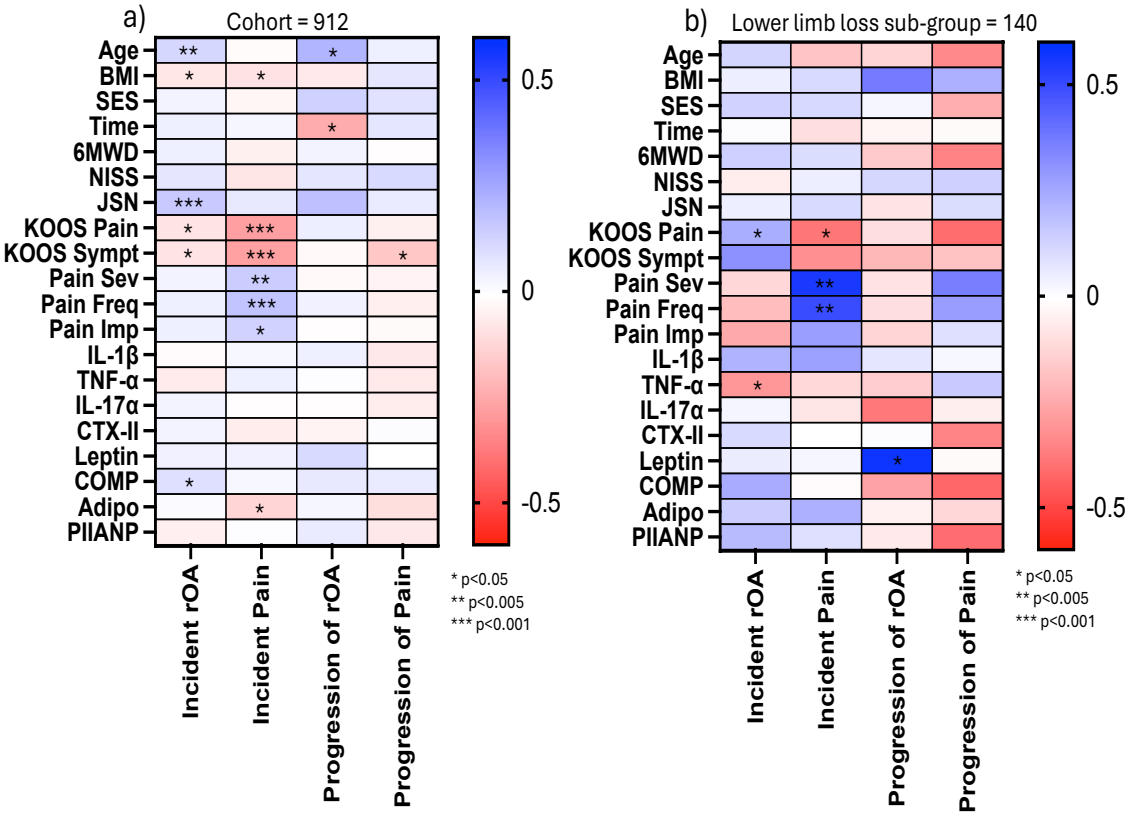


Figure 5.4 Correlations between potential predictor variables and incidence or progression of radiographic OA and KOOS pain in the whole cohort excluding those with lower-limb loss (a) and lower-limb loss sub-group (b)

*JSN: Joint space narrowing, KOOS: Knee injury and osteoarthritis outcome score, Sympt: Symptom, Sev: Severity, Freq: Frequency, Imp: Impact, rOA: Radiographic Osteoarthritis, BMI: Body mass index, SES: Socioeconomic status, 6WMD: Six minute walk distance, NISS: New injury severity score, IL: Interleukin, TNF: Tumour necrosis factor, CTX-II: C-terminal cross-linked telopeptide of type II collagen, COMP: Cartilage oligomeric matrix protein, PIIANP: N-propeptide of collagen IIA. Test used: Spearman's correlation. Not all participants had complete data for all variables, correlations were performed per variable with complete data to avoid unnecessary data exclusion. *p<0.05 **p<0.005 ***p<0.001*

5.3.1 Progression

5.3.1.1 Radiographs

Out of 1,905 knees (Figure 5.1), 286 (15%) had KL≥1 at Baseline; 46 (16.1%) had progression at Follow-Up. There was no increased rOA progression risk in the Exposed compared to the Unexposed group (relative risk, RR 1.06, 95% confidence interval, CI: 0.62,1.82, Table 5.4). Subgroup analysis revealed no significantly different risk of rOA progression in the Exp-A (RR 1.31, 95% CI: 0.58,2.92) and Exp-K (RR 2.20, 95% CI: 0.83,5.79) groups (Table 5.4). Sensitivity analysis showed no difference for results reporting left knee only, right knee only, or both knees together.

Predictive analysis demonstrated that Model 1 had an AUROC 0.80 (95% CI: 0.67,0.92); Model 2, 0.72 (95% CI: 0.61,0.83); and Model 3, 0.66 (95% CI: 0.55,0.77) for predicting rOA progression. The highest R^2 was 0.22 for Model 1 (Figure 5.5).

Table 5.4 Incidence rate ratio (IRR) for radiographic knee OA progression (rOA) and KOOS Pain score progression between Baseline and Follow-up.

rOA progression			
Group	**Unadjusted**	**Adjusted**	
	IRR (95% CI)	**IRR (95% CI)**	***p* value**
Exposure status			
Unexp (n=112)	1 (ref)	1 (ref)	
Exp (n=174)	0.92 (0.53-1.58)	1.06 (0.62-1.82)	0.840
Injury status			
Unexp (n=112)	1 (ref)	1 (ref)	
Exp-NA (n=124)	0.76 (0.41-1.40)	0.88 (0.48-1.60)	0.263
Exp-A (n=36)	1.15 (0.52-2.52)	1.31 (0.58-2.92)	
Exp-K (n=14)	1.68 (0.70-4.06)	2.20 (0.83-5.79)	
KOOS Pain progression			
Group	**Unadjusted**	**Adjusted**	
	IRR (95% CI)	**IRR (95% CI)**	***p* value**
Exposure status			
Unexp (n= 236)	1 (ref)	1 (ref)	
Exp (n= 284)	1.35 (0.78-2.34)	1.43 (0.83-2.46)	0.196
Injury status			
Unexp (n= 236)	1 (ref)	1 (ref)	
Exp-NA (n= 218)	1.29 (0.71-2.35)	1.37 (0.76-2.48)	0.137
Exp-A (n= 36)	0.83 (0.20-3.46)	0.87 (0.21-3.61)	
Exp-K (n= 30)	2.42 (1.05-5.59)	2.52 (1.08-5.86)	

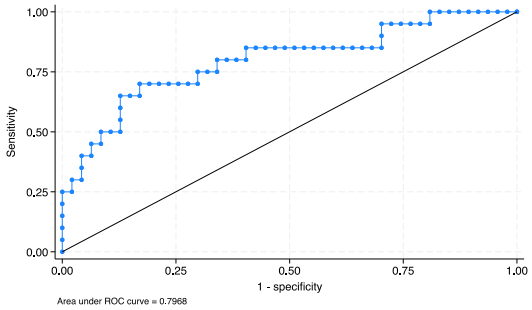
Analyses adjusted for baseline age, baseline and follow-up interval & socioeconomic status. rOA: radiographic osteoarthritis, KOOS: knee injury and OA outcome score, IRR: incidence rate ratio, CI: confidence interval, Unexp: unexposed, Exp: exposed, Exp-NA: Exposed, no lower-limb amputation, Exp-A: Exposed, lower-limb amputation, Exp-K: Exposed - knee injured

Model 1 : Demographic / Injury / Functional

Logistic regression — Number of obs = 67; LR chi2(6) = 17.84; Prob > chi2 = 0.0066; Log likelihood = −31.921867; Pseudo R2 = 0.2184

anyprogOA	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
samplingage	1.100084	.0741909	1.41	0.157	.9638729	1.255544
crf_bmiadj	1.145876	.0999527	1.56	0.119	.9658041	1.359523
rank	1.182792	.6687746	0.30	0.767	.3905005	3.582573
last_apt_years	.7158524	.0995933	−2.40	0.016	.5450041	.9402585
niss_2008	1.002188	.0314249	0.07	0.944	.9424509	1.065712
crf_6mwt6	1.004528	.0033421	1.36	0.174	.997999	1.0111
_cons	.0006046	.0022789	−1.97	0.049	3.74e−07	.9767241

Note: _cons estimates baseline odds.

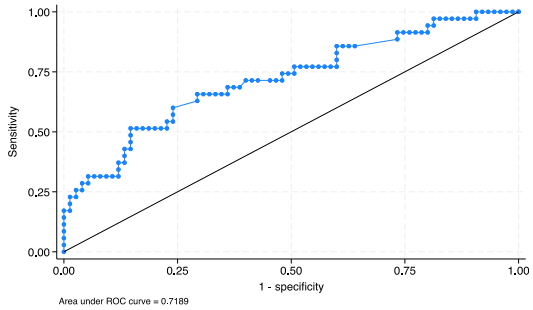


Model 2: Joint specific variables

Logistic regression — Number of obs = 110; LR chi2(6) = 18.55; Prob > chi2 = 0.0050; Log likelihood = −59.527037; Pseudo R2 = 0.1348

anyprogOA	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
max_JSN	2.348333	.8677362	2.31	0.021	1.138232	4.844941
min_KOOS_pain	1.034895	.0211997	1.67	0.094	.9941669	1.077291
min_KOOS_sympt	.9824974	.0164229	−1.06	0.291	.9508306	1.015219
min_kneepain_sev	.6401812	.253346	−1.13	0.260	.2947458	1.390459
min_kneepain_freq	2.730215	1.121428	2.45	0.014	1.220586	6.106963
min_kneepain_imp	.5910222	.2518296	−1.23	0.217	.2563977	1.362365
_cons	.0431893	.0567619	−2.39	0.017	.0032861	.5676429

Note: _cons estimates baseline odds.

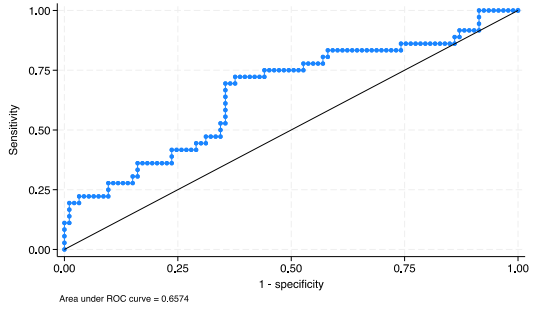


Model 3: Molecular biomarkers

Logistic regression — Number of obs = 129; LR chi2(8) = 10.79; Prob > chi2 = 0.2142; Log likelihood = −70.984654; Pseudo R2 = 0.0706

anyprogOA	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
il1b	15.22712	21.81761	1.90	0.057	.9183433	252.4819
il17	.9381057	.1523202	−0.39	0.694	.6824049	1.289619
tnf	.8285932	.378522	−0.41	0.681	.3384475	2.028577
lept	1.050791	.027423	1.90	0.058	.9983943	1.105937
adipo	.9952508	.0488738	−0.10	0.923	.9039253	1.095803
ctxii	.4750698	.1980916	−1.78	0.074	.2098113	1.075687
comp	1.000656	.0021923	0.30	0.765	.9963683	1.004962
piianp	1.003623	.0019685	1.84	0.065	.9997724	1.007489
_cons	.246308	.2842679	−1.21	0.225	.0256504	2.365176

Note: _cons estimates baseline odds.



	Model 1	Model 2	Model 3
R2	0.2184	0.1348	0.0706
P	0.0066	0.005	0.2146
AUROC	0.7968 (0.6712,0.9225)	0.7189 (0.6127,0.8250)	0.6574 (0.54842,0.76639)
SE	0.0641	0.0541	0.0556
AIC	77.8437	133.0541	159.9693
BIC	93.2766	151.9574	185.7076

Figure 5.5 Multivariate logistic regression for the identification of knee radiographic osteoarthritis progression

R2: Nagelkerke's R2, AUROC: area under the receiver operator curve (with 95% confidence intervals), SE: standard error, AIC: Akaike information criteria, BIC: Bayesian information criteria

5.3.1.2 KOOS Pain

Out of 1,796 knees, 520 (29%) had a KOOS Pain score ≤86.2 at Baseline; 61 (11.7%) showed Pain progression at Follow-Up. The risk for Pain progression was not different for the Exposed group compared to the Unexposed group (RR 1.43, 95% CI: 0.83,2.46), or for the Exp-NA or Exp-A groups compared to the Unexposed group (RR 1.37, 95% CI: 0.76,2.48; RR 0.87, 95% CI: 0.21m,3.61), but was 2.52 (95% CI: 1.08,5.86) times higher for the Exp-K group compared to the Unexposed group (Table 5.4).

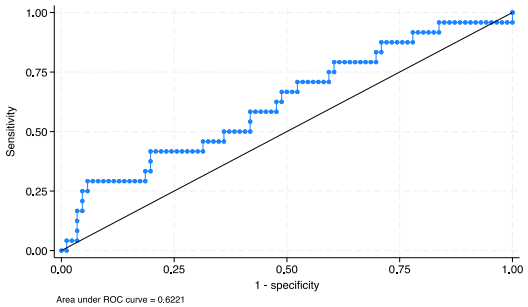
Predictive Model 1 had an AUROC 0.62 (95% CI: 0.50,0.75); Model 2, 0.70 (95% CI: 0.58,0.76); and Model 3, 0.62 (95% CI: 0.52,0.71) for the prediction of Pain progression. Model 2 had the highest R^2, 0.07 (Figure 5.6).

Model 1 : Demographic / Injury / Functional

Logistic regression — Number of obs = 110; LR chi2(6) = 4.21; Prob > chi2 = 0.6478; Log likelihood = -55.598717; Pseudo R2 = 0.0365

progression_koosp	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
samplingage	1.019279	.0572216	0.34	0.734	.9130765	1.137834
crf_bmiadj	.9782025	.0611913	-0.35	0.725	.8653304	1.105797
rank	1.290747	.5790174	0.57	0.569	.5357968	3.109442
last_apt_years	1.053119	.1150964	0.47	0.636	.8500584	1.304686
niss_2008	1.043603	.0252147	1.77	0.077	.9953352	1.094212
crf_6mwt6	1.000436	.0018732	0.23	0.816	.9967716	1.004115
_cons	.0652256	.1669707	-1.07	0.286	.0004319	9.849309

Note: _cons estimates baseline odds.

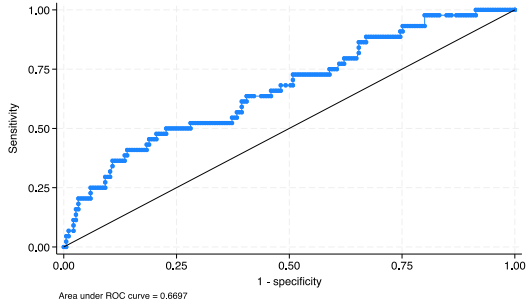


Model 2: Joint specific variables

Logistic regression — Number of obs = 229; LR chi2(6) = 15.14; Prob > chi2 = 0.0192; Log likelihood = -104.48399; Pseudo R2 = 0.0675

progression_koosp	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
max_JSN	.9277282	.2620348	-0.27	0.791	.5333358	1.613767
min_KOOS_pain	1.030606	.0171346	1.81	0.070	.9975643	1.064743
min_KOOS_sympt	.9523171	.0143465	-3.24	0.001	.9246095	.980855
min_kneepain_sev	.9978972	.2118504	-0.01	0.992	.6582312	1.512841
min_kneepain_freq	.8721209	.1539439	-0.78	0.438	.6170571	1.232617
min_kneepain_imp	1.022591	.1673883	0.14	0.891	.7419394	1.409404
_cons	.6417675	.5681548	-0.50	0.616	.1131909	3.63868

Note: _cons estimates baseline odds.

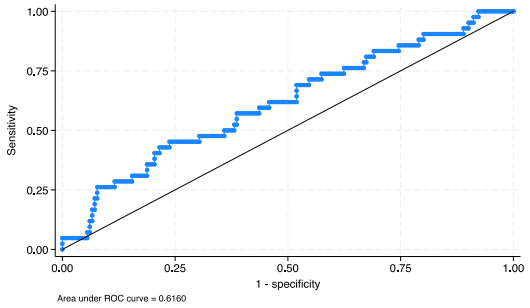


Model 3: Molecular biomarkers

Logistic regression — Number of obs = 223; LR chi2(8) = 6.52; Prob > chi2 = 0.5895; Log likelihood = -104.6304; Pseudo R2 = 0.0302

progression_koosp	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
il1b	.0509523	.1842063	-0.82	0.410	.0000426	60.88146
il17	.8169726	.155726	-1.06	0.289	.5622857	1.18702
tnf	.6668958	.2597505	-1.04	0.298	.3108293	1.43085
lept	.9960385	.0284424	-0.14	0.889	.9418236	1.053374
adipo	.9374134	.0559264	-1.08	0.279	.8339657	1.053693
ctxii	.950923	.2956123	-0.16	0.871	.517051	1.748869
comp	1.00228	.0019649	1.16	0.245	.998436	1.006138
piianp	1.00044	.0014387	0.31	0.760	.9976237	1.003263
_cons	.6045279	.6213037	-0.49	0.624	.080648	4.531468

Note: _cons estimates baseline odds.



	Model 1	Model 2	Model 3
R2	0.0365	0.0675	0.0302
P	0.6478	0.0192	0.5895
AUROC	0.6221 (0.4954,0.7536)	0.6697 (0.5795,0.7600)	0.616 (0.5191,0.71297)
SE	0.067	0.046	0.095
AIC	125.1974	222.9680	227.2608
BIC	144.1008	247.0040	257.9253

Figure 5.6 Multivariate logistic regression for the identification of knee pain progression

R2: Nagelkerke's R2, AUROC: area under the receiver operator curve (with 95% confidence intervals), SE: standard error, AIC: Akaike information criteria, BIC: Bayesian information criteria

5.3.2 Incidence

5.3.2.1 Radiographs

At Baseline 1615 knees were KL0, of which 189 (11.7%) had KL≥1 at Follow-Up. Knee rOA RR was not different for the Exposed group compared to the Unexposed group (RR 1.11, 95% CI: 0.83,1.48, *p*=0.506), but the Exp-A group had a 2.06 (95% CI:1.22,3.46, *p*=0.007) times higher risk than the Unexposed group (Table 5.5).

Table 5.5 Incidence Rate Ratios for incidence of radiographic knee OA and KOOS Pain score between Baseline and Follow-up.

rOA incidence			
Group	**Unadjusted**	**Adjusted**	
	IRR (95% CI)	**IRR (95% CI)**	***p* value**
Exposure status			
Unexp (n= 922)	1 (ref)	1 (ref)	
Exp (n= 693)	1.11 (0.83-1.49)	1.11 (0.83-1.48)	0.50
Injury status			
Unexp (n= 922)	1 (ref)	1 (ref)	
Exp-NA (n= 587)	0.99 (0.72-1.36)	0.99 (0.73-1.36)	**0.014**
Exp-A (n= 72)	2.11 (1.25-3.55)	2.06 (1.22-3.46)	
Exp-K (n= 34)	1.06 (0.39-2.89)	1.05 (0.38-2.85)	
KOOS Pain Incidence			
Group	**Unadjusted**	**Adjusted**	
	IRR (95% CI)	**IRR (95% CI)**	***p* value**
Exposure status			
Unexp (n= 730)	1 (ref)	1 (ref)	
Exp (n= 548)	1.49 (1.08-2.04)	1.44 (1.05-1.98)	**0.024**
Injury status			
Unexp (n= 730)	1 (ref)	1 (ref)	
Exp-NA (n= 451)	1.53 (1.10-2.13)	1.48 (1.07-2.06)	0.104
Exp-A (n= 72)	1.11 (0.54-2.32)	1.09 (0.52-2.27)	
Exp-K (n= 25)	1.77 (0.75-4.19)	1.64 (0.69-3.90)	

Adjusted Models were adjusted for baseline age, interval between baseline and follow-up data collection, and socioeconomic status. rOA: radiographic osteoarthritis, KOOS: Knee injury and osteoarthritis outcome score, IRR: incidence rate ratio, CI: Confidence interval, Unexp: Unexposed, Exp: Exposed Exp-NA: Exposed, no lower-limb amputation, Exp-A: Exposed, lower-limb amputation, Exp-K: Exposed, knee injured

Model 1 had an AUROC 0.66 (95% CI: 0.57,0.74); Model 2, 0.63 (95% CI: 0.57,0.68); and Model 3, 0.58 (95% CI: 0.53,0.64) for the prediction of rOA incidence. Model 1 had the highest R^2, 0.06 (Figure 5.7).

Model 1 : Demographic / Injury / Functional

Logistic regression — Number of obs = 307; LR chi2(6) = 15.87; Prob > chi2 = 0.0145; Log likelihood = −134.85539; Pseudo R2 = 0.0556

anynewOA	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
samplingage	1.082351	.0348288	2.46	0.014	1.016196	1.152813
crf_bmiadj	1.083849	.0510011	1.71	0.087	.9883601	1.188564
rank	.9249363	.2225702	−0.32	0.746	.5771447	1.48231
last_apt_years	1.13295	.0813553	1.74	0.082	.9842091	1.30417
niss_2008	1.018568	.0133158	1.41	0.159	.992801	1.045004
crf_6mwt6	1.00149	.0014312	1.04	0.297	.9986888	1.004299
_cons	.0003209	.0006463	−3.99	0.000	6.19e−06	.016626

Note: _cons estimates baseline odds.

Model 2: Joint specific variables

Logistic regression — Number of obs = 635; LR chi2(6) = 17.11; Prob > chi2 = 0.0089; Log likelihood = −300.70179; Pseudo R2 = 0.0277

anynewOA	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
max_JSN	1.964812	.3898883	3.40	0.001	1.331719	2.898875
min_KOOS_pain	.9952265	.0100149	−0.48	0.634	.9757898	1.01505
min_KOOS_sympt	.9974272	.0086069	−0.30	0.765	.9806999	1.01444
min_kneepain_sev	.9215482	.1231093	−0.61	0.541	.7092613	1.197374
min_kneepain_freq	1.040196	.1217891	0.34	0.736	.8269019	1.308508
min_kneepain_imp	1.070427	.0957792	0.76	0.447	.8982422	1.275617
_cons	.3187899	.1921315	−1.90	0.058	.0978349	1.03876

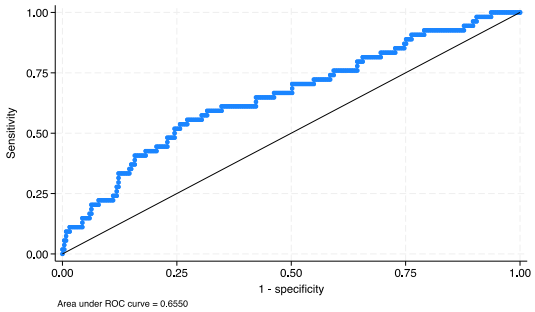
Note: _cons estimates baseline odds.

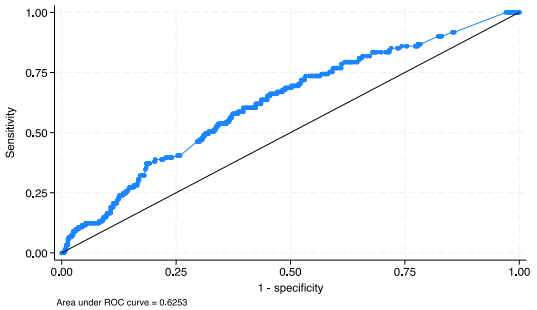
Model 3: Molecular biomarkers

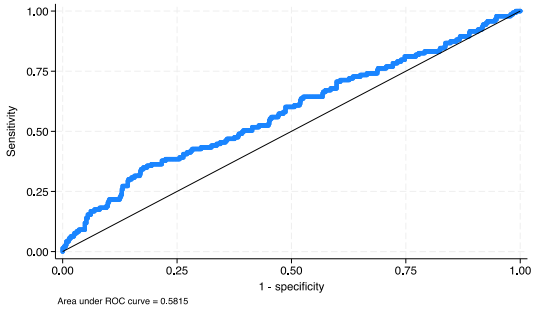
Logistic regression — Number of obs = 761; LR chi2(8) = 13.75; Prob > chi2 = 0.0885; Log likelihood = −360.82316; Pseudo R2 = 0.0187

anynewOA	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
il1b	1.784353	1.418617	0.73	0.466	.3756197	8.476432
il17	1.008608	.025486	0.34	0.734	.959873	1.059817
tnf	.7451648	.1412063	−1.55	0.121	.5139874	1.080319
lept	1.019839	.0171236	1.17	0.242	.9868235	1.053959
adipo	.9979716	.0207391	−0.10	0.922	.9581404	1.039459
ctxii	1.182122	.16174	1.22	0.221	.9040649	1.5457
comp	1.002451	.0010508	2.33	0.020	1.000393	1.004512
piianp	.9997869	.0007164	−0.30	0.766	.9983837	1.001192
_cons	.16497	.0810122	−3.67	0.000	.0630091	.4319234

Note: _cons estimates baseline odds.







	Model 1	Model 2	Model 3
R2	0.0556	0.0277	0.0187
p	0.0145	0.0089	0.0885
AUROC	0.655 (0.5714,0.7387)	0.6253 (0.5701,0.6805)	0.5815 (0.52673,0.63621)
SE	0.0427	0.0282	0.0279
AIC	283.7108	615.4036	739.6463
BIC	309.7987	646.579	781.358

Figure 5.7 Multivariate logistic regression for the identification of knee radiographic osteoarthritis incidence

R2: Nagelkerke's R2, AUROC: area under the receiver operator curve (with 95% confidence intervals), SE: standard error, AIC: Akaike information criteria, BIC: Bayesian information criteria

5.3.2.2 KOOS Pain

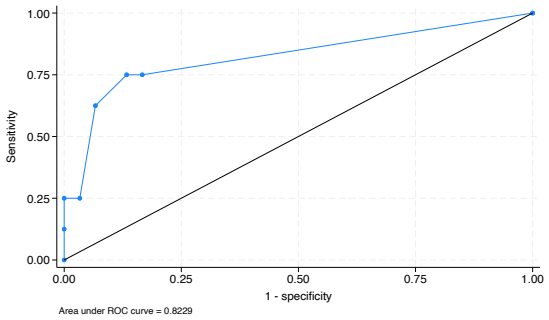
At baseline ,1278 knees had a KOOS Pain score >86.2, of which 181 (14.2%) reported new pain (≤86.1) at Follow-Up. The RR for Pain incidence was 1.44x (95% CI:1.05-1.98, *p*=0.024) higher for the Exposed group compared to the Unexposed group, with the Exp-NA group having a 1.48x (95% CI:1.07-2.06, *p*=0.018) higher risk for Pain incidence than the Unexposed group (Table 5.5).

Model 1 had an AUROC 0.69 (95% CI: 0.61,0.76); Model 2, 0.70 (95% CI: 0.64,0.76); and Model 3, 0.60 (95% CI: 0.54,0.65) for the prediction of knee Pain incidence. Model 1 and 2 both had the highest R^2 (0.08) (Figure 5.9). Within the Exp-A sub-group, knee pain frequency and severity were selected by LASSO, creating a model with AUROC 0.83 (95% CI: 0.64,1.00), R^2 0.33 (Figure 5.8).

```
Logistic regression                               Number of obs =     38
                                                  LR chi2(2)    =  12.76
                                                  Prob > chi2   = 0.0017
Log likelihood = -13.178289                       Pseudo R2     = 0.3262
```

incidence_koosp	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
min_kneepain_freq	1.798762	1.057271	1.00	0.318	.5684042	5.692332
min_kneepain_sev	3.352639	2.28036	1.78	0.075	.8839467	12.71591
_cons	.078009	.0545749	-3.65	0.000	.0197992	.3073568

Note: _cons estimates baseline odds.



R2	0.3362
P	0.0017
AUROC	0.8229 (0.6405,1.0000)
SE	0.0931
AIC	32.35658
BIC	37.26734

Figure 5.8 Multivariate logistic regression for variables selected by least absolute shrinkage and selection operator to assess the ability to identify incidence of knee pain in those with lower-limb loss
R2: Nagelkerke's R2, AUROC: area under the receiver operator curve (with 95% confidence intervals, SE: standard error, AIC: Akaike information criteria, BIC: Bayesian information criteria

Model 1 : Demographic / Injury / Functional

Logistic regression — Number of obs = 240; LR chi2(6) = 22.45; Prob > chi2 = 0.0010; Log likelihood = -126.9301; Pseudo R2 = 0.0813

incidence_koosp	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
samplingage	.9903028	.0344088	-0.28	0.779	.9251078	1.060092
crf_bmiadj	1.187471	.0561391	3.63	0.000	1.082384	1.30276
rank	.7948826	.2063977	-0.88	0.377	.477839	1.322283
last_apt_years	1.071361	.0745208	0.99	0.322	.934821	1.227843
niss_2008	.9781424	.0151201	-1.43	0.153	.948952	1.008231
crf_6mwt6	.9984059	.0014713	-1.08	0.279	.9955263	1.001294
_cons	.009544	.0170779	-2.60	0.009	.0002862	.3183183

Note: _cons estimates baseline odds.

Model 2: Joint specific variables

Logistic regression — Number of obs = 462; LR chi2(6) = 39.75; Prob > chi2 = 0.0000; Log likelihood = -231.35251; Pseudo R2 = 0.0791

incidence_koosp	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
max_JSN	1.417294	.2848729	1.74	0.083	.9558075	2.101598
min_KOOS_pain	.9960041	.0112927	-0.35	0.724	.9741149	1.018385
min_KOOS_sympt	.975075	.0091755	-2.68	0.007	.9572562	.9932254
min_kneepain_sev	.8731215	.1904533	-0.62	0.534	.5693808	1.338895
min_kneepain_freq	1.545629	.3291622	2.04	0.041	1.018194	2.34628
min_kneepain_imp	.8855295	.1447701	-0.74	0.457	.6427543	1.220003
_cons	2.510613	1.696919	1.36	0.173	.6675041	9.442904

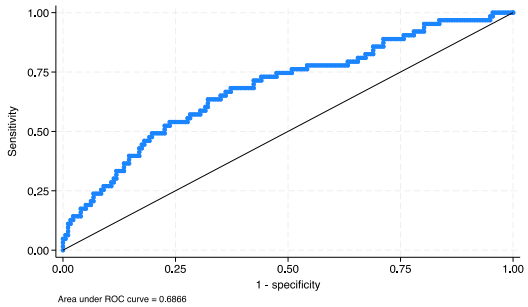
Note: _cons estimates baseline odds.

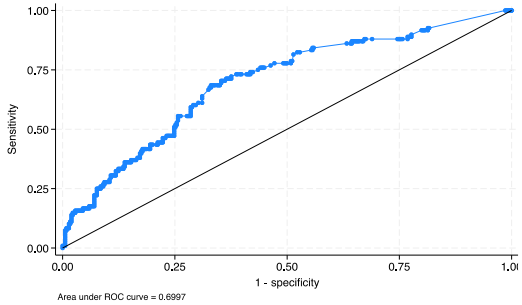
Model 3: Molecular biomarkers

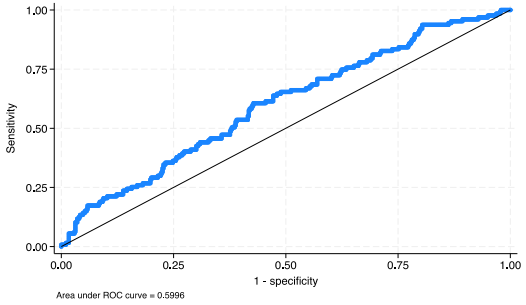
Logistic regression — Number of obs = 606; LR chi2(8) = 13.27; Prob > chi2 = 0.1029; Log likelihood = -304.47839; Pseudo R2 = 0.0213

incidence_koosp	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
il1b	1.964361	1.910796	0.69	0.488	.2918976	13.21941
il17	.9736797	.0467913	-0.56	0.579	.8861569	1.069847
tnf	1.10675	.1921865	0.58	0.559	.7874788	1.555465
lept	1.017004	.0186351	0.92	0.357	.9811283	1.054192
adipo	.9235565	.0321432	-2.28	0.022	.8626576	.9887545
ctxii	.6784381	.1324955	-1.99	0.047	.4626738	.9948223
comp	1.000002	.0011766	0.00	0.999	.9976981	1.00231
piianp	1.000208	.0007676	0.27	0.786	.998705	1.001714
_cons	.3667942	.1996743	-1.84	0.065	.1261967	1.066097

Note: _cons estimates baseline odds.







	Model 1	Model 2	Model 3
R2	0	0.0791	0.0213
P	0.001	0	0.1029
AUROC	0.6866 (0.6084,0.7648)	0.6997 (0.6429,0.7565)	0.5996 (0.5445,0.6547)
SE	0.0399	0.029	0.0281
AIC	267.8600	476.7050	626.9568
BIC	292.2250	505.6540	666.6187

Figure 5.9 Multivariate logistic regression for the identification of knee pain incidence

R2: Nagelkerke's R2, AUROC: area under the receiver operator curve (with 95% confidence intervals), SE: standard error, AIC: Akaike information criteria, BIC: Bayesian information criteria

5.3.3 Function

At Follow-Up, 1016 participants performed a 6MWT (524 Unexposed and 492 Exposed; Exp-NA n=355, Exp-A n=104, Exp-K n=33), and 999 completed the IPAQ (509 Unexposed and 490 Exposed; Exp-NA n=342, Exp-A n=121, Exp-K n=27) (Table 5.6). In both visits, the Unexposed group walked further than the Exposed group, which increased in the second visit (both $p<0.001$). Likewise, across both visits, the Exp-NA walked further than Exp-K, with Exp-A walking the least distance (all $p<0.001$). There were no significant differences in IPAQ score between Unexposed and Exposed or Exp-NA, Exp-A and Exp- K at both visits.

The initial correlation analysis is demonstrated in Figure 5.10. Following this, linear regression demonstrated that Model 1 had the highest R2 for Follow-up IPAQ scores (0.02), and Follow up 6MWD (0.44) (Figure 5.11 and 5.12). For the prediction of functional decline, Model 1 had an AUROC 0.60 (95% CI: 0.53,0.67); Model 2, 0.54 (95% CI: 0.49,0.59); and Model 3, 0.56 (95% CI: 0.51,0.61). Model 1 had the highest R^2 (0.02) (Figure 5.13). In those with a lower-limb loss, LASSO selected PIIANP as a predictor for Follow-up IPAQ, creating a linear model with an R^2 of 0.002, and ten predictors for Follow-up 6WMD, creating a linear model with an R^2 of 0.57 (Figure 5.14). No potential predictors were selected for functional decline by LASSO.

The best performing predictors based on t-value (<-2 or >2) and p-value (<0.05) were combined into optimised models. Only variables predictive of Follow-up 6MWD met these criteria. In the whole cohort, SES, Baseline 6MWD, KOOS Pain, leptin, created a model with an R^2 of 0.36, and in the lower-limb loss sub-group, time from injury, injury severity, body mass and Baseline 6MWD, created a model with an R^2 of 0.58 (Figure 5.15).

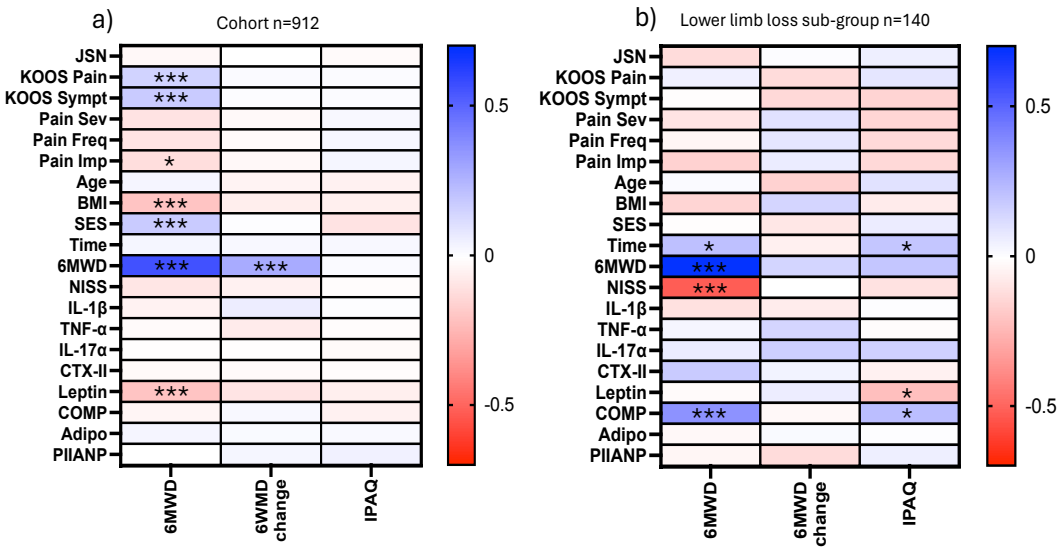


Figure 5.10 Correlations between potential predictor variables and functional outcomes in the whole cohort excluding those with lower-limb loss (a) and lower-limb loss sub-group (b)

*6MWD: Six-minute walk-test distance, IPAQ: international physical activity questionnaire, JSN: Joint space narrowing, KOOS: Knee injury and osteoarthritis outcome score, Sympt: Symptom, Sev: Severity, Freq: Frequency, Imp: Impact, BMI: Body mass index, SES: Socioeconomic status, 6WMD: Six minute walk distance, NISS: New injury severity score, IL: Interleukin, TNF: Tumour necrosis factor, CTX-II: C-terminal cross-linked telopeptide of type II collagen, COMP: Cartilage oligomeric matrix protein, PIIANP: N-propeptide of collagen IIA. Test used: Spearman's correlation. Not all participants had complete data for all variables, correlations were performed per variable with complete data to avoid unnecessary data exclusion. *p<0.05 **p<0.005 ***p<0.001*

Table 5.6 Functional outcomes of six-minute walk-test distance and international physical activity questionnaire (total minutes per week) for Baseline and Follow-up

	Total	Unexp	Exp	p-value	Exp-NA	Exp-A	Exp-K	p-value
Baseline 6MWD (m)	598 (117)	630 (96)	566 (127)	<0.001	594 (118)	489 (119)	527 (134)	<0.001
Follow-up 6WMD (m)	626 (105)	652 (86)	597 (115)	<0.001	625 (99)	519 (116)	548 (142)	<0.001
Decrease ≥81.25m	240 (21.0%)	98 (17.3%)	142 (24.5%)	0.003	79 (19.8%)	56 (38.4%)	7 (20.0%)	<0.001
Baseline IPAQ (mins)	1762 (880-3040)	1888 (930-3060)	1675 (802-3015)	0.17	1590 (800-2790)	1886 (725-3720)	1650 (1045-2880)	0.38
Follow-up IPAQ (mins)	1270 (630-2345)	1280 (680-2340)	1264 (550-2400)	0.58	1358 (630-2400)	1170 (540-2640)	930 (300-1940)	0.26

6MWD: Six-minute walk-test distance, IPAQ: International physical activity questionnaire, m: Metres, mins: Minutes, Unexp: Unexposed, Exp: Exposed, Exp-NA: Exposed, Exp-NA: Exposed, no lower-limb amputation, Exp-A: Exposed, lower-limb amputation, Exp-K: Exposed, knee injured

Model 1 : Demographic / Injury / Functional

Source	SS	df	MS	Number of obs	=	350
				F(6, 343)	=	1.07
Model	14238720.1	6	2373120.02	Prob > F	=	0.3794
Residual	759908456	343	2215476.55	R-squared	=	0.0184
				Adj R-squared	=	0.0012
Total	774147176	349	2218186.75	Root MSE	=	1488.4

pq_fu1_ipaq_~w	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
samplingage	10.68735	17.65227	0.61	0.545	-24.03297	45.40767
crf_bmiadj	-17.14108	22.70091	-0.76	0.451	-61.7916	27.50945
rank	-225.6696	128.7651	-1.75	0.081	-478.9383	27.59913
last_apt_years	59.48479	36.44003	1.63	0.104	-12.18925	131.1588
niss_2008	-.0479218	7.663031	-0.01	0.995	-15.12037	15.02453
crf_6mwt6	.3445553	.693789	0.50	0.620	-1.020061	1.709172
_cons	1516.189	879.5341	1.72	0.086	-213.7706	3246.148

Model 2: Joint specific variables

Source	SS	df	MS	Number of obs	=	694
				F(6, 687)	=	0.93
Model	11356247.5	6	1892707.92	Prob > F	=	0.4733
Residual	1.3996e+09	687	2037249.31	R-squared	=	0.0080
				Adj R-squared	=	-0.0006
Total	1.4109e+09	693	2035997.87	Root MSE	=	1427.3

pq_fu1_ipaq_exe~w	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
max_JSN	-79.47334	98.09262	-0.81	0.418	-272.0707	113.124
min_KOOS_pain	5.257143	5.290365	0.99	0.321	-5.130082	15.64437
min_KOOS_sympt	-.4183135	4.441295	-0.09	0.925	-9.138455	8.301828
min_kneepain_sev	33.00521	73.94918	0.45	0.656	-112.1883	178.1987
min_kneepain_freq	27.30968	66.11689	0.41	0.680	-102.5057	157.1251
min_kneepain_imp	27.14531	56.65881	0.48	0.632	-84.0999	138.3905
_cons	1251.06	335.0026	3.73	0.000	593.3077	1908.811

Model 3: Molecular biomarkers

Source	SS	df	MS	Number of obs	=	846
				F(8, 837)	=	1.12
Model	18242465	8	2280308.13	Prob > F	=	0.3493
Residual	1.7092e+09	837	2042046.27	R-squared	=	0.0106
				Adj R-squared	=	0.0011
Total	1.7274e+09	845	2044302.01	Root MSE	=	1429

pq_fu1_ipa~w	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
il1b	-167.8014	428.8053	-0.39	0.696	-1009.461	673.8587
il17	37.56493	15.29065	2.46	0.014	7.552417	67.57744
tnf	-76.94965	81.70341	-0.94	0.347	-237.3173	83.41799
lept	5.580132	8.919696	0.63	0.532	-11.92747	23.08773
adipo	-2.745649	10.7786	-0.25	0.799	-23.9019	18.4106
ctxii	2.88088	78.77136	0.04	0.971	-151.7317	157.4935
comp	-.1952083	.5917656	-0.33	0.742	-1.356727	.9663105
piianp	.5284264	.3768929	1.40	0.161	-.2113399	1.268193
_cons	1774.843	243.6689	7.28	0.000	1296.569	2253.117

R2	0.0184	0.0080	0.0106
P	0.3794	0.4733	0.3493
AIC	6114.028	12058.27	14701.72
BIC	6141.034	12090.06	14744.39

Figure 5.11 Multivariate linear regression for the identification international physical activity questionnaire total activity level at follow-up
R2: Nagelkerke's R2, AIC: Akaike information criteria, BIC: Bayesian information criteria

Model 1 : Demographic / Injury / Functional

Source	SS	df	MS	Number of obs	=	370
				F(6, 363)	=	47.88
Model	1624494.02	6	270749.003	Prob > F	=	0.0000
Residual	2052713.05	363	5654.8569	R-squared	=	0.4418
				Adj R-squared	=	0.4325
Total	3677207.07	369	9965.33081	Root MSE	=	75.199

crf_fu1_6min~e	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
samplingage	-1.507492	.8453795	-1.78	0.075	-3.169948	.1549642
crf_bmiadj	-2.137786	1.107911	-1.93	0.054	-4.316517	.0409443
rank	19.02639	6.309763	3.02	0.003	6.61811	31.43467
last_apt_years	-3.631241	1.788341	-2.03	0.043	-7.148051	-.1144309
niss_2008	-.3995524	.3737382	-1.07	0.286	-1.134516	.3354116
crf_6mwt6	.5497837	.036146	15.21	0.000	.4787019	.6208656
_cons	404.1258	44.76165	9.03	0.000	316.1011	492.1505

Model 2: Joint specific variables

Source	SS	df	MS	Number of obs	=	725
				F(6, 718)	=	4.74
Model	239702.517	6	39950.4195	Prob > F	=	0.0001
Residual	6047769.52	718	8423.07732	R-squared	=	0.0381
				Adj R-squared	=	0.0301
Total	6287472.03	724	8684.35364	Root MSE	=	91.777

crf_fu1_6min_di~e	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
max_JSN	-1.621307	6.099635	-0.27	0.790	-13.59656	10.35394
min_KOOS_pain	.7072574	.3317009	2.13	0.033	.0560379	1.358477
min_KOOS_sympt	.2499572	.2804588	0.89	0.373	-.30066	.8005744
min_kneepain_sev	.6208726	4.738407	0.13	0.896	-8.681916	9.923661
min_kneepain_freq	-.9444821	4.195417	-0.23	0.822	-9.181234	7.292269
min_kneepain_imp	-.1152528	3.420882	-0.03	0.973	-6.83138	6.600874
_cons	558.1571	20.51974	27.20	0.000	517.8713	598.443

Model 3: Molecular biomarkers

Source	SS	df	MS	Number of obs	=	877
				F(8, 868)	=	4.46
Model	305645.32	8	38205.6649	Prob > F	=	0.0000
Residual	7443181.95	868	8575.09441	R-squared	=	0.0394
				Adj R-squared	=	0.0306
Total	7748827.27	876	8845.69323	Root MSE	=	92.602

crf_fu1_6m~e	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
il1b	-19.47211	27.6159	-0.71	0.481	-73.67386	34.72964
il17	.9508652	.9945284	0.96	0.339	-1.001096	2.902827
tnf	-.4321795	5.259179	-0.08	0.935	-10.75437	9.890015
lept	-3.166063	.556628	-5.69	0.000	-4.258557	-2.073569
adipo	.2598809	.7107083	0.37	0.715	-1.135027	1.654789
ctxii	2.910034	5.032541	0.58	0.563	-6.967338	12.78741
comp	.0057133	.0365441	0.16	0.876	-.0660119	.0774385
piianp	-.0014508	.0239727	-0.06	0.952	-.048502	.0456005
_cons	657.91	15.62324	42.11	0.000	627.2463	688.5738

R2	0.4418	0.0381	0.0394
P	0.0000	0.0001	0.0000
AIC	4253.847	8617.506	10440.36
BIC	4281.242	8649.61	10483.41

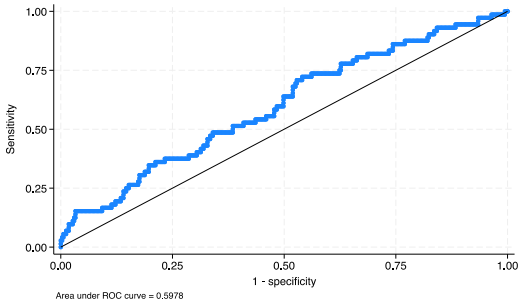
Figure 5.12 Multivariate linear regression for the identification of six-minute walk-test distance at follow-up
R2: Nagelkerke's R2, AIC: Akaike information criteria, BIC: Bayesian information criteria

Model 1 : Demographic / Injury / Functional

Logistic regression — Number of obs = 407; LR chi2(6) = 7.88; Prob > chi2 = 0.2468; Log likelihood = -185.99176; Pseudo R2 = 0.0208

SixMWTchange81	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
samplingage	.9767243	.0292344	-0.79	0.431	.9210742	1.035737
crf_bmiadj	1.010673	.0379181	0.28	0.777	.9390212	1.087791
rank	.7094517	.1748909	-1.39	0.164	.4376112	1.150158
last_apt_years	1.087544	.0661034	1.38	0.167	.9654033	1.225137
niss_2008	1.000989	.0126051	0.08	0.937	.9765863	1.026002
crf_6mwt6	1.00142	.0011983	1.19	0.236	.9990742	1.003771
_cons	.0900828	.1354545	-1.60	0.109	.0047285	1.716189

Note: _cons estimates baseline odds.

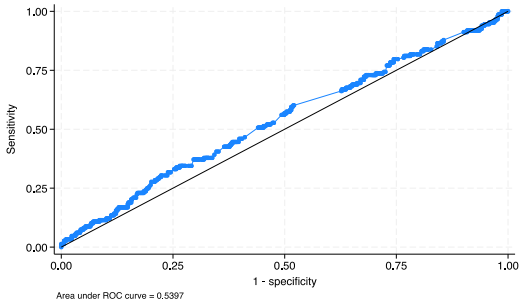


Model 2: Joint specific variables

Logistic regression — Number of obs = 793; LR chi2(6) = 3.77; Prob > chi2 = 0.7081; Log likelihood = -379.79014; Pseudo R2 = 0.0049

SixMWTchange81	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
max_JSN	.8884793	.1484959	-0.71	0.479	.6402975	1.232857
min_KOOS_pain	.9999794	.0088295	-0.00	0.998	.9828227	1.017436
min_KOOS_sympt	.9969465	.0074447	-0.41	0.682	.9824614	1.011645
min_kneepain_sev	.9564813	.1225142	-0.35	0.728	.7441276	1.229435
min_kneepain_freq	.9130916	.1077729	-0.77	0.441	.7245133	1.150754
min_kneepain_imp	1.146514	.0902052	1.74	0.082	.9826725	1.337674
_cons	.3032202	.1639437	-2.21	0.027	.1050835	.874947

Note: _cons estimates baseline odds.

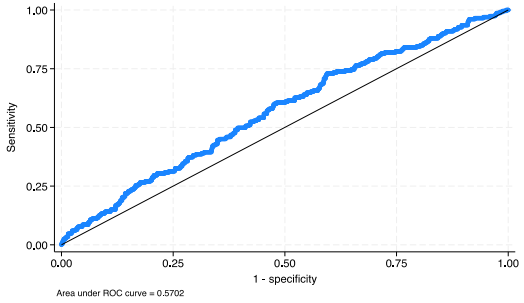


Model 3: Molecular biomarkers

Logistic regression — Number of obs = 961; LR chi2(8) = 8.41; Prob > chi2 = 0.3946; Log likelihood = -456.32887; Pseudo R2 = 0.0091

SixMWTchange81	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
il1b	2.657466	1.735063	1.50	0.134	.7391236	9.554726
il17	1.001456	.0296891	0.05	0.961	.9449248	1.06137
tnf	.8184443	.1314358	-1.25	0.212	.5974379	1.121206
lept	.9708352	.0164893	-1.74	0.081	.9390489	1.003698
adipo	.9988343	.0193195	-0.06	0.952	.9616776	1.037427
ctxii	.9194102	.1278769	-0.60	0.546	.7000342	1.207534
comp	.9996914	.0009986	-0.31	0.757	.997736	1.001651
piianp	.9999401	.0006622	-0.09	0.928	.9986431	1.001239
_cons	.439692	.1915156	-1.89	0.059	.1872375	1.032534

Note: _cons estimates baseline odds.



	Model 1	Model 2	Model 3
R2	0.0208	0.0049	0.0091
P	0.2468	0.7081	0.3946
AUROC	0.5978 (0.5252,0.6705)	0.5397 (0.48721,0.5923)	0.5606 (0.5148,0.6065)
SE	0.0371	0.0290	0.0234
AIC	385.9835	773.5803	930.6577
BIC	414.0452	806.311	974.4695

Figure 5.13 Multivariate logistic regression for the identification of functional decline between Baseline and Follow-up

R2: Nagelkerke's R2, AUROC: area under the receiver operator curve (with 95% confidence intervals), SE: standard error, AIC: Akaike information criteria, BIC: Bayesian information criteria

a) Follow-up IPAQ total mins/week

Source	SS	df	MS		
Model	10516577.8	1	10516577.8	Number of obs	128
Residual	374274009	126	2970428.64	F(1, 126)	3.54
				Prob > F	0.0622
Total	384790587	127	3029847.14	R-squared	0.0273
				Adj R-squared	0.0196
				Root MSE	1723.5

pq_fu1_ipa~w	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
piianp	1.968676	1.046277	1.88	0.062	-.101875	4.039228
_cons	1566.567	209.8752	7.46	0.000	1151.23	1981.903

b) Follow-up 6MWT Distance

Source	SS	df	MS		
Model	523522.945	10	52352.2945	Number of obs	80
Residual	392031.005	69	5681.60876	F(10, 69)	9.21
				Prob > F	0.0000
Total	915553.95	79	11589.2905	R-squared	0.5718
				Adj R-squared	0.5098
				Root MSE	75.376

crf_fu1_6min_d~e	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
samplingage	-.6332444	1.823474	-0.35	0.729	-4.270975	3.004486
last_apt_years	11.45795	4.297424	2.67	0.010	2.884826	20.03108
niss_2008	-3.239535	.9771479	-3.32	0.001	-5.188891	-1.290178
crf_6mwt6	.4592098	.0984838	4.66	0.000	.26274	.6556795
crf_bmiadj	-8.323286	2.633443	-3.16	0.002	-13.57686	-3.069712
min_KOOS_sympt	.6962054	.5396152	1.29	0.201	-.3802975	1.772708
min_kneepain_sev	-4.355426	5.658187	-0.77	0.444	-15.6432	6.932348
il1b	356.1441	261.8434	1.36	0.178	-166.2192	878.5075
ctxii	15.46923	9.083289	1.70	0.093	-2.651432	33.5899
comp	.1892295	.1365301	1.39	0.170	-.0831406	.4615997
_cons	417.0552	132.5005	3.15	0.002	152.724	681.3863

R2	0.0273	0.5718
P	0.0622	0.0000
AIC	2272.972	928.7957
BIC	2278.676	954.998

Figure 5.14 Multivariate linear regression for the identification of total minutes of activity (a) or six-minute walk-test distance (b) at follow-up in with those with lower-limb loss

R2: Nagelkerke's R2, AIC: Akaike information criteria, BIC: Bayesian information criteria

a)

Source	SS	df	MS		
Model	2767787.19	4	691946.798	Number of obs	865
Residual	4818350.25	860	5602.73285	F(4, 860)	123.50
				Prob > F	0.0000
Total	7586137.44	864	8780.25167	R-squared	0.3648
				Adj R-squared	0.3619
				Root MSE	74.851

crf_fu1_6mi~e	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
rank	10.45164	3.568311	2.93	0.003	3.448022	17.45526
crf_6mwt6	.4773928	.0251662	18.97	0.000	.4279984	.5267872
min_KOOS_pain	.484032	.1494254	3.24	0.001	.1907509	.7773131
lept	-1.56711	.4485	-3.49	0.000	-2.447393	-.6868271
_cons	298.296	19.58244	15.23	0.000	259.861	336.731

R2	0.3648
P	0.0000
AIC	9925.573
BIC	9949.386

b)

Source	SS	df	MS		
Model	906954.6	4	226738.65	Number of obs	114
Residual	651288.523	109	5975.12406	F(4, 109)	37.95
				Prob > F	0.0000
Total	1558243.12	113	13789.7621	R-squared	0.5820
				Adj R-squared	0.5667
				Root MSE	77.299

crf_fu1_6min~e	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
last_apt_years	10.10384	3.356857	3.01	0.003	3.450664	16.75703
niss_2008	-2.268767	.7500108	-3.02	0.003	-3.755264	-.7822699
crf_6mwt6	.5606577	.0736673	7.61	0.000	.4146515	.7066638
crf_bmiadj	-4.337048	1.825479	-2.38	0.019	-7.955088	-.7190081
_cons	328.9135	87.48532	3.76	0.000	155.5204	502.3066

R2	0.5820
P	0.0000
AIC	1319.676
BIC	1333.357

Figure 5.15 Optimised linear regression models for Follow-up six-minute walk-test distance in those without (a) and with (b) lower limb loss

R2: Nagelkerke's R2, AIC: Akaike information criteria, BIC: Bayesian information criteria

5.4 Discussion

This large, unique study describes the progression and incidence of knee rOA and pain in a cohort of military personnel between 8 and 11 years following severe combat injury or matched deployment. It demonstrates novel findings related to rOA incidence in those with traumatic lower limb loss and the value of a pre-selected panel of Baseline variables to predict radiographic, symptomatic or functional change 3 years later.

5.4.1 Progression and incidence

Previous work within the cohort has demonstrated that Baseline knee rOA and pain were both significantly worse in the Exp-NA, Exp-A and Exp-K groups compared to the Unexposed group (138). In keeping with my hypothesis, this study has found that no Exposed-subgroup showed significantly different risk for rOA progression compared to the Unexposed group between the Baseline and Follow-up data ~3-years later. However, the Exp-K group showed a 2.52-times greater risk for knee Pain progression compared to the Unexposed group.

The lack of significantly different rOA progression between groups mirrors other cohort studies (123,207). In a cohort of over 500,000 individuals, the risk of future OA peaked for those with a traumatic knee injury compared to controls 5-years after injury, after which it decreased gradually but always remained elevated (123). Our findings also suggest that people with a traumatic knee injury have higher rates of rOA initially, but after 8-years it progresses at a similar rate to controls.

Despite no increased risk of rOA progression in the Exp-K group, they did have a 2.52 times greater risk for progression of significant knee pain. Earlier work showed that the Exp-K group had significantly worse KOOS Pain scores at Baseline (138), and to explain this, I hypothesise

that the Exp-K group may have undergone peripheral pain sensitisation. A meta-analysis of pain sensitisation in people with knee OA found that people with high symptom severity often had lower physical pain thresholds than people with low symptom severity (403). The median KOOS Symptoms score for the Exp-K group at FU1 was more severe than all other participants, potentially contributing to a lower pain threshold and exaggerating the risk of pain progression in the absence of rOA progression. The Exp-K and comparison groups were well matched demographically (Table 5.1), however, the measurement of pain is challenging, with more measures (such as quantitative sensory testing) required to understand why pain impacts one group over another (404,405). Another potential explanation is that the pain experienced in this group is the beginning of future OA disability. One would not expect to see end-stage disease or overt disability in a cohort so young; however, as the ADVANCE study progresses, it might become clear that this group experiences increased and earlier disability and end-stage disease compared to their peers. It could be that these pain scores are a possible sentinel event.

There was a 2.06 times greater risk for rOA incidence in the Exp-A group compared to the Unexposed group. I hypothesise that the Exp-A group, alongside the other Exposed sub-groups, experienced an initial accelerated period of knee OA following trauma, perhaps within a similar 5-year window observed in the UK Biobank study (94,123). Subsequently, unlike the rest of the Exposed group, altered biomechanics and subsequent mechanical overload subsequently become the primary OA pathomechanism, explaining increased rOA incidence. This finding is potentially supported by other studies demonstrating higher rates of OA in people with lower limb loss than the general population (393,406). The theoretical model in Figure 5.16 outlines the postulated differences within this sub-group. Further research is recommended to understand the contribution of unilateral/bilateral limb loss, level of limb loss, and the intact or

amputated side knee in people with unilateral transtibial limb loss, given the differences in their biomechanics of gait (407,408).

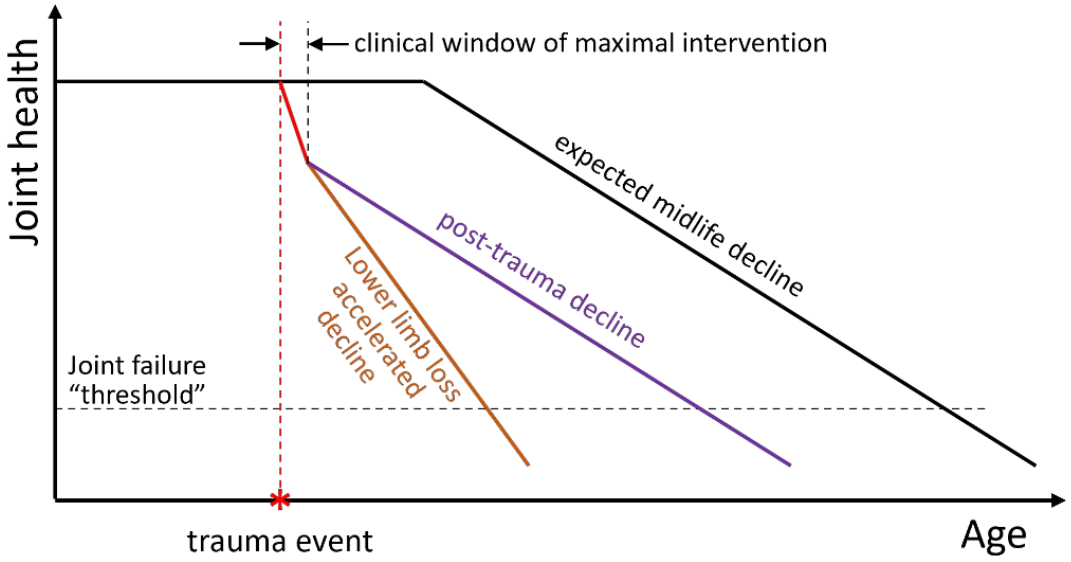


Figure 5.16 A theoretical model of Osteoarthritis disease trajectory depending on predominant aetiology

The increased risk of OA development in the early years infers a potential early 'clinical window of maximal intervention', supported by the negative correlation between time from injury and rOA development, as summarised in Figure 5.16. The figure shows the theoretical trajectories of different OA aetiologies: the gradual reduction of joint-health in those with expected midlife decline, a sharp decrease in those with a joint injury followed by a plateau and subsequent similar decline to expected midline, and finally, the steeper decline in those with a lower-limb amputation, initially due to the trauma event but followed by significantly altered biomechanics and mechanoinflammation (68,93). These findings highlight the need for secondary prevention and timely intervention which should commence soon after the injury or completion of rehabilitation/recovery pathways especially in high-risk populations (9,37).

However, despite increased rOA incidence in the Exp-A group, there was no matched increased risk of pain incidence. The dominant symptom of OA is pain, which heavily influences clinical decision-

making (68). Our findings suggest the potential for new rOA to occur, but with no/reduced associated pain, and therefore an opportunity for intervention might be missed. Clinically, new pain might rightly stimulate early investigations or appropriate early treatment interventions for knee OA, with the opposite also true. Interestingly, the inverse relationship was seen for the Exp-NA group; there was no increased risk for rOA progression or incidence but a 1.48-times increased risk for pain incidence. Other work in this cohort has shown that participants with lower limb loss reported less pain than those with non-amputation injuries (405). This is possibly due to this subgroup's improved mental health impacting how they experience and report pain (261), or perhaps these findings are giving an insight into differences in central or peripheral pain processing in those with lower-limb loss. Therefore, we recommend acute clinical sensitivity for signs of knee OA in people with lower limb loss, as they may experience and report pain differently, or indeed, later, by which time, opportunities for early intervention could be missed.

5.4.2 Predictive Modelling

The key to any potentially successful clinical intervention is identifying those who will develop knee rOA or pain in the future. Clinical interventions, centred holistically around patient education, self-management and access to expert healthcare professionals when needed, improve patient-related OA outcomes, especially when targeted to the right person at the right time (9,60). These include physical activity (including cardiovascular, strengthening and neuromuscular exercises), appropriate weight loss, suitable activity/job modification, and optimisation of nutrition and sleep, with pharmacological studies underway for disease-modifying therapies (9,52,60).

In this study, I used different models related to demographics, injury and function; joint-specific metrics; and molecular biomarkers to predict the incidence or progression of knee rOA or pain. Whilst overall, these combined models had reasonable AUROCs (between 0.58 to 0.80), the explanation of variance within the models was low (R^2 between 0.02 to 0.22) suggesting limited value for prediction. Perhaps unsurprisingly, the model with the best fit was the demographic model (Model 1) for rOA progression, with an AIC of 77.8 and BIC of 93.3. It is clear that, similar to other populations (123,388,390), there remain challenges for accurately predicting who will develop knee rOA or pain, especially when one notes the differing patterns seen across incidence and progression. This suggests different pathological progresses may be underway between the initial development and subsequent progression of OA, requiring different predictive models. It also suggests that these potential predictors were not appropriate for the prediction of the OA and pain outcomes.

Across the 20 potential predictor variables, age and JSN were associated with both incident and progressive OA, Baseline KOOS associated with new rOA and pain, as was the KHJP scale. From a

predictive point of view, within the models, age and JSN had higher odds for rOA incidence; time from injury, JSN and knee pain frequency to rOA progression; BMI, KOOS Symptoms and knee pain frequency to Pain incidence and KOOS Symptoms to Pain progression. Perhaps it is self-evident that age would predict the risk of rOA, radiographic change would predict radiographic change, and pain would predict pain, but it remains important to include demographic, objective (radiological) and subjective (patient-reported) measures in any future clinical risk prediction model. In addition, these measures could be used to identify or stratify those with, or at risk of, OA, and enable the creation of phenotypes for improved clinical care and targeted research (2). It was notable that BMI did not significantly affect rOA risk. This might be explained by military personnel, similar to other athletic populations, having a higher percentage of muscle compared to adipose tissue (409,410). The BMI scale 'over-scores' this, but a more sensitive measure, such as dual X-ray absorptiometry (DEXA), might able to differentiate better, with future work planned to investigate this.

From a molecular biomarker perspective, COMP was significant for rOA incidence, with adiponectin and CTX-II both having lower odds for pain incidence. The most significant result, the relationship of adiponectin to pain, is consistent with previous results and literature; however, given the weak nature of the correlation and limited OR, the clinical significance is uncertain (6,190). Two possible explanations might contextualise the limited biomarker performance. Firstly, the significant period of time between injury and sampling – Chapter Three articulated the challenges of chronic phase PTOA biomarkers, and perhaps those associated with structural or symptomatic change in the acute phase are less effective further from injury (Section 3.1.4). This suggests the current biomarkers may have initial value in determining elevated post-injury OA risk but may be less valuable when this risk reduces and the potential window of maximal clinical intervention closes, with alternative metrics or biomarkers required (52,57). Another explanation might be the early stage of OA under investigation, with only 26 knees having

KL3/4 at Baseline, rising to 31 by Follow-Up. It is possible that, given the small amount of severe disease, there is a limited systemic pattern, with the pathological signal still contained locally within the joint space, potentially only revealed by synovial fluid sampling or local serum sampling.

There was a different picture in those with lower-limb loss (Figure 5.4). Knee pain severity and frequency and KOOS Pain all correlated with incident Pain, with the latter also correlated with incident rOA. In addition, TNF-α correlated with incident rOA and leptin with rOA progression. However, no predictors for rOA incidence or progression were selected by LASSO, and those selected for incident Pain demonstrated non-significant OR. These results hint at the possible mechanoinflammation due to the ongoing biomechanical changes; however, they demonstrate that a different predictive paradigm is required for those with lower-limb loss.

5.4.3 Functional outcomes

Along with pain, impairment of function is the main reason that individuals with OA consult their healthcare professionals. It is this impairment which leads to future disability, and therefore, there is a need to identify any predictors of this.

Interestingly, there is an inconsistent correlation between radiographic features of OA and functional status (411). One study showed unadjusted correlations of 0.23-0.26 for OP and JSN to self-reported disability (412), and another reported increased severity of JSN increased the risk of functional deterioration (413), however, others showed no relationship between function to JSN and OP (414) or severity (415). It has previously been suggested that the correlation might be stronger in a community based cohort with early disease (411), a description which the ADVANCE cohort would fit. However, in this study, there was no statistically significant correlation between JSN and the functional outcomes of 6MWD or IPAQ (Figure 5.9), nor was it significant in any of the regression models displayed in Figures 5.10-5.14, suggesting that the radiographic picture alone does not explain the associated functional limitation. It is likely that an individual's function and associated disability are more readily described within the bracket of 'OA illness', much like pain, and this is why the hallmarks features of OA disease, i.e. structural change on radiographs, do not accurately reflect self-reported or objective function.

As discussed previously, 'OA illness' represents the impact of OA on an individual and is influenced by many factors, including pain perception, body mass, demography and social support (Section 1.1.4). Previous work has shown that age, BMI and knee pain intensity have been associated with a deterioration of function in those with OA (416). Age had no correlation or predictive value in this study, with body mass significantly correlated (negatively) to 6MWD however was non-significant as a predictor for either Follow-up 6MWD or decrease in

function (Figure 5.9). Infact, Model 1, comprised of demographic and injury-related variables, had the highest R2 (0.44) for Follow-up 6WMD, far higher than all other models (0.01-0.04). Although KOOS Pain, KOOS Symptoms and knee pain impact all had statistically significant associations with Follow-up 6WMD, only KOOS Pain offered predictive value. There are many other possible contributing factors, including multi-joint involvement or muscle strength, which this analysis did not account for and could be investigated in future ADVANCE visits.

The strongest association was seen between 6WMD at Baseline and 6WMD at Follow-up (0.56, $p<0.001$), which was also unsurprisingly the best performing predictor in regression modelling. The same pattern was seen in lower-limb loss sub-group, with Baseline 6MWD strongly correlated to, and a predictor of, Follow-up 6WMD. The relatively strong performance of this as a predictor is surprising given the sub-maximal nature of the test. Unsurprisingly, there was also a negative correlation to injury severity (0.693 and -0.52, both $p<0.001$). Perhaps again, similar to pain predicting pain, and radiographic change predicting radiographic change, this is self-evident, however, I think it demonstrates the importance of effective rehabilitation following injury. If individuals are able to reach higher functional status upon completion of their rehabilitation journey (417,418), which is the ambition of UK Defence Rehabilitation (242), then they will be in a better position to maintain it. In addition, it demonstrates there is a requirement for functional testing in the prediction and stratification of those with, or at risk, of OA.

From a molecular perspective, serum biomarkers offer a different way to stratify disease in OA, similar to other disease states, such as glycated haemoglobin in diabetes mellitus or B-type natriuretic peptide in heart failure (419,420). In the cohort, leptin was associated with Follow-up 6WMD, as was COMP in the lower-limb loss (-0.20 and 0.37, respectively, both $p<0.001$). In addition, leptin and COMP were both associated with Follow-up IPAQ in the lower-limb loss subgroup (-0.22

and 0.22, both p=0.03). However, only leptin had predictive value for Follow-up 6MWD, with no other biomarkers predictive of Follow-up IPAQ or functional change. Similar to the previous discussion, this performance might be related to the type of biomarker, time from injury or the early stage of OA within the cohort. Further work is required to understand the use of molecular biomarkers in the prediction of future functional status. However, this requires studies to have functional outcomes in the methodology. This, unfortunately, is not always the case with most OA studies utilising radiological progress or patient-reported pain measures as outcomes. As we have already seen, there are unreliable correlations between those and function (411,421), which is also demonstrated in this cohort, in the previous chapter, between KOOS and 6MWT (Section 4.3.6).

The ability to identify those who are more likely to have significant functional limitations should be prioritised as high as pain and radiological features for the individual. This also has a profound impact on society. As described in Chapter One (Section 1.2.1), the indirect cost of OA outstrips the direct medical cost, due to occupational restrictions and early medical retirement (25,68). This same picture is seen in active populations, such as athletes, sportspeople and the military, with a quarter of ex-Olympians developing knee OA (422), a 2-3x increased risk of OA in ex-footballers compared to the general public (207), and one-third of military medical retirements involving OA (225). The creation of optimised models for Follow-up 6WMD for both the whole cohort and for the lower-limb loss sub-group, offers a potential opportunity to stratify those based on future functional status. In these models, SES, KOOS Pain, leptin and Baseline 6MWD could explain over a third of variance in the whole cohort and time from injury, injury severity, body mass and Baseline 6MWD nearly two-thirds in those with lower-limb loss. Given the simplicity of all these measures, it would be practical to introduce them as a stratification tool, especially in those with lower-limb loss, however, validation is required in different populations prior to that.

What is of further interest are the variables included in the optimised models. Beyond injury-related and socioeconomic factors, pain, body mass and functional status all feature (the latter in both). These factors are amenable to interventions, therefore, potentially offering an insight into optimal clinical management. It is hoped, therefore, that this work will not only enable interventions to be targeted to those who are at higher risk, but also help shape what these interventions are. This are particularly important after injury, given the 4x risk of OA and 2.5x knee pain post-knee injury demonstrated over the last two chapters, consistent with other military studies (36,217). What these interventions could look like and other ways that predictive tools could be refined, given the limited performance of the candidate biomarker panel, will be discussed in the final chapter.

5.4.4 Limitations

This research utilises two-wave data from an ongoing longitudinal study. Only three or more waves of data can definitively confirm patterns in the data. These three wave data gives us the opportunity to meaningfully assess how radiological and symptomatic progression occur and correlate to each other. Our definition of rOA progression required knees that already had a KL score of 4 to be excluded because there is no way for that score to increase. The predictive models were very limited in their discrimination and predictive ability for OA and pain outcomes with very low R2 values, and this prevented the development of any optimised models for these outcomes. Additionally, only a single radiographic view is reported, which might underreport the rates of rOA, with radiographs also not able to identify subtle OA changes which might be detected on advanced imaging. With regards to comparability to other PTOA datasets, ADVANCE participants are likely to have sustained much more serious and complex comorbid injuries that may affect rOA and their perception of pain in immeasurable ways. In addition, only male participants were recruited into the ADVANCE study, which means these findings need to be investigated in a comparable female population. Finally, all molecular biomarkers were measured in a single form (serum), with floor and ceiling effects, which might affect their ability to detect changes.

5.5 Conclusion

Men with non-amputation combat trauma and knee-specific combat trauma had the same risk of rOA progression and incidence between 8- and 11-years post-injury as a control group. This suggests that a period of rapid PTOA progression has already occurred, and a window of opportunity for intervention has passed. However, men with lower limb loss had a higher risk of rOA incidence than the control group, perhaps due to continuous mechanical pathomechanism in addition to the primary trauma. Despite this, men with lower limb loss did not show an increased incidence of knee pain, and therefore, should be closely monitored for signs of knee OA and opportunities for intervention. Previous functional status was the best predictor of future function, with pain and body mass also predictive, therefore interventions should focus on these. Further work is required to identify predictors which can enable the early recognition of those at risk of new or progressive knee rOA or pain and function.

Chapter 6 : How can we improve the use of biomarkers?

In the previous two chapters, I have cross-sectionally and prospectively analysed a panel of serum candidate biomarkers to identify their value in identifying and predicting the development or progression of knee radiographic osteoarthritis (rOA), pain or function. The performance of these biomarkers was not as good as expected. I have discussed several reasons for these underwhelming results, including the single source of biomarkers sampled, length of time since injury, and significant floor and ceiling effects. In addition, there are study design factors which can influence analysis results, which were controlled for within the Armed Services Trauma Rehabilitation Outcome (ADVANCE) study methodology, including fasted venous sampling, on a seated, rested participant, and appropriate and timely sample processing and freezing. However, there are potential other methodological factors which might also influence the subsequent results of the analysis. This chapter will address some other potential influencing elements which could introduce bias into the results, including the location of the venous sampling and impact of physical activity on the concentration of serum biomarkers. A pilot study, the *Serum concentration of joint-specific biomarkers from the venous drainage of the lower limb may improve in the interpretation of acute and chronic degenerative knee and ankle joint disease* (SORE) study, was designed and performed to answer these questions. Documentation related to the SORE study, including the research ethics committee application (with protocol) and subsequent approval, and the participant consent form and information leaflet, can be found in Supplementary File 2.

6.1 Background

As a brief recap, the synovial whole-joint disease OA is an extremely common condition, affecting 600 million people globally, with a significant burden of pain and disability (68). OA is the endpoint of a series of interrelated and concurrent pathological mechanisms, including a loss of bone and cartilage turnover homeostasis, altered biomechanics, low-grade inflammation, aberrant healing after injury and metabolic dysregulation (21). It has a prolonged, asymptomatic, prodromal phase that lasts for years, allowing the opportunity to recognise molecular changes prior to radiological and symptomatic changes, improved drug-discovery trials and enhanced clinical care(23). Within the field of OA, there has been a particular focus on biomarkers for case identification, phenotyping and as an outcome measure for drug discovery studies (40,46,155,167,168).

Biomarkers, measurable indicators of biological states or conditions, have become integral to understanding, diagnosing, and treating diseases in contemporary medicine. The use of molecular biological markers (biomarkers) has allowed the prediction and stratification of clinical care, monitoring responsiveness to treatment and change on biological processes and has been a corner stone of modern clinical medicine and pathophysiological research (423,424). Serum measures of joint-specific molecules have been investigated in many conditions. Markers such as anti-CCP, CRP, and urate in inflammatory or crystal arthropathies reflect systemic dysfunction rather than joint-specific biology.

In recent years, there has been a concerted research focus on the identification and clinical validation of biomarkers as a proxy for underlying pathological tissue and joint changes, such as cartilage and collagen turnover (cartilage oligomeric matrix protein, COMP, or C-terminal cross-linked telopeptide of type-II collagen, CTX-II), inflammation (interleukin, IL-6 or tumour necrosis factor-alpha, TNF-α)

and adiposity and metabolism (leptin or adiponectin) (23,58,425). Progress is slow within the field, partly due to lack of standardisation in collection and analysis, and differences across populations, biological fluids, time from injury and study methodology (5,426), however, consensus is beginning to be reached (292,381). Research has focused on post-traumatic OA (PTOA) as a paradigm due to the clear initial event, younger age of the population and fewer co-morbidities (57,167,348).

The joint intra-articular micro-environment is created by the synovium, which consists of two layers: the outer subintima and the inner intima. The subintima contains loose extracellular matrix (ECM) components like type I collagen, adhesins, and fibronectin, as well as adipose and areolar tissues, and is highly vascularised with blood vessels, lymphatic vessels, and nerve fibres (92). Together, the ECM and resident monocytic cells form a three-dimensional structure that acts as an internal immune barrier within the synovial lining, helping to regulate inflammation (427). Understanding the characteristics of the synovial microenvironment is crucial for developing new therapies. Biomarkers measured directly from the knee joint via synovial fluid (SF), a hyperfiltration of serum with a maintained equilibrium of urea concentration (353), provide valuable insights into the local microenvironment at the time of collection (428).

However, frequent sampling of joint fluid can theoretically disturb joint homeostasis, trigger inflammation, and hinder the accurate interpretation of findings (429,430). Furthermore, detecting changes in SF is challenging due to uncertainty about how quickly the joint environment can shift with activity and loading and measurement errors associated with collecting and analysing fluid of varying viscosity (431). In addition, this procedure requires technical skill and is associated with several potential side effects, including pain and an increased risk of infection. Additionally, there is a risk of blood contamination during a traumatic tap, significant variability in biomarker concentrations due to

hyaluronan concentration, and challenges in accurately measuring temporal changes in concentration. Therefore, as an alternative, systemic measurement of biomarkers from the blood is performed, which is technically easier, with fewer side-effects; however, paired serum and SF biomarkers correlate poorly (Section 3.2.4), with no serum biomarker reliably reflecting the microenvironment of an affected joint (5).

The ability to study the joint environment without disturbing joint homeostasis would be ideal, and new imaging techniques have garnered great interest, particularly those capable of detecting early biochemical and microarchitectural changes before structural damage occurs. Beyond the synovium, techniques such as T2 relaxation time mapping and T1rho imaging have been validated for detecting early cartilage damage (4). Additionally, methods like sodium imaging and glycosaminoglycan chemical exchange saturation transfer show promise (432). However, the practical application of these techniques, especially for disease stratification, remains limited.

The key challenge is the potential error in assuming that serum biomarkers collected from the upper arm reflect concentrations near an affected joint in the lower limb. The serum from the antecubital fossa (ACF) represents the fraction of the blood that, after leaving pulmonary circulation, is pumped from the left ventricle into the aorta and reaches the arm via the brachiocephalic or subclavian arteries before flowing through smaller arteries and capillaries and entering the venous system of the arm. However, this is not the same in the lower limb, with the highly-vascularised synovial membrane providing effective plasma filtration and exchange with synovial tissue through an extensive network of capillaries, fed by fenestrated arterioles and venules in the synovium. These supply the capillary beds with blood flow, maintaining the pressure gradient and selective diffusion necessary for filtration, before the capillaries lead to venules which form the larger veins (353). Transudate of plasma from synovial tissue blood vessels supplemented

with high molecular weight saccharide-rich molecules, particularly hyaluronan (433). Importantly, this ultrafiltrate of plasma, is actively reabsorbed to maintain homeostasis (353), indicating that venous circulation from lower limb likely contains molecules that better reflect the knee, foot and ankle joint -specific processes than serum samples collected from upper limb.

International consortia between academia and pharmacological companies have identified a list of potential candidate biomarkers which require translation into the clinical and research sphere (50,86). During this process, there have been several challenges, including understanding the pathological process identified by the biomarker in question, the range of values perceived to be within acceptable limits, and the form of the biomarker under measurement. Work is underway to address these with animal and tissue models, normative population values, and multiple biomarker sources undergoing assessment (including serum, SF and urine) (176,292,380,434).

However, further challenges must be overcome, such as recent physical activity, time from injury and location of sampling. It is well-recognised in other fields that certain ‘experimental’ variables need to be controlled for in the clinical environment to ensure the laboratory result is not affected/biased (435). This is particularly relevant when concentration thresholds are used to categorise ‘normal v abnormal’, especially in the early stages of the disease process (436). Examples of this include early morning testing of hormone levels (to avoid the effect of circadian rhythm) or fasted sampling of blood glucose and lipids. For OA biomarkers specifically, previous work has demonstrated that load-bearing alters certain biomarker concentrations, likely in response to the physiological mechanical effects at a joint level, not necessarily an indication of a pathological process at work (437-439). Also, it appears that increased time from injury affects the value of the biomarkers measured, possibly demonstrating different processes during the post-injury healing and initial OA incidence and those during more well-

established OA disease (4,5). The evidence surrounding OA biomarkers becomes less convincing as the chronic phase of PTOA is reached a year or more after injury. In addition, when biomarkers are measured in the systemic circulation, such as when sampling occurs at the ACF, there is likely to be a dilutional effect, further impacting the concentration of the biomarker(s) in question. This is particularly relevant if the systematic concentration is far lower than the local concentration, as postulated previously in Chapter Five (Section 5.4.2).

Unpublished work has demonstrated that in those with Charcot-joint, levels of IL-6 were higher when measured in the venous system nearer the injured joint, compared to the ACF. This might offer an opportunity to measure serum biomarker concentrations without systemic dilution, which better represent the local environment. The aim of this pilot study is to understand the effect of two 'experimental variables', the location of sampling and non-weight bearing sub-maximal exercise intervention, on the concentrations of a panel of candidate serum OA biomarkers, across three groups (control, established OA and recent lower-limb musculoskeletal injury (MSKI)).

The hypotheses of this study are two-fold: firstly, serum concentration of biomarkers will be higher nearer to the joint than those measured in the systemic circulation, and secondly, precedent physical activity will influence biomarker concentrations. To test the first hypothesis, a new technique for the measurement of serum near the joint to improve the ability for sampling OA-related biomarkers was required, which, to my knowledge, has never previously been described. This chapter reports both the development and refining of a novel method of local serum collection and the results of the pilot study measuring the effect of sampling location and physical activity on serum OA biomarker concentrations.

6.2 Methods

6.2.1 Ethics and participants

The University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (UoN FMHS 170-1122) granted a favourable ethical opinion for the SORE study in April 2023 (Supplementary File 2.2). Study participation was voluntary, with written consent at least 24 hours after receiving the participant information leaflet (PIL) (Supplementary File 2.4). All researchers were trained in Good Clinical Practice and had appropriate phlebotomy and ultrasound skills. Chaperones were in attendance during sampling (due to full exposure of the lower limb). Sampling was performed at the Academic Unit of Injury, Recovery and Inflammation Sciences (IRIS), UoN, between June and October 2023.

Potential participants were identified and recruited through university clubs, local sports teams, and regional running events, using word of mouth, posters and targeted emails to selected local networks. After an expression of interest, potential participants were given a verbal explanation and a written PIL outlining the study and screened for eligibility. If interested and eligible, they were invited for a one-hour testing/data collection study visit, which was preceded by time for participant questions and written, voluntary, informed consent.

Photos of the prepared and dissected vasculature included in this chapter were provided for this specific purpose by the UoN Anatomy Suite and consent was obtained for their inclusion (Figure 6.2). The images of training and vessels by ultrasound, were taken for this purpose with the full, informed consent of the subject (Figure 6.3- 6.5).

6.2.2 Anatomical considerations

The greater saphenous vein (GSV) in the mid-thigh region (approximately 10–20 cm above the medial knee) was targeted for venous puncture (Figure 6.1). It courses posterior to the medial femoral condyle, ascending medially towards its eventual junction with the femoral vein at the saphenofemoral junction in the groin (Figure 6.2). The GSV runs along the medial aspect of the thigh, lying within the superficial fascia, and can be easily visualised with ultrasound (Figure 6.3). It is important to note that just posterior to the medial condyle, anterior to the GSV, lies the saphenous nerve, which can be difficult to distinguish from connective tissue (Figure 6.2). Above this region, the saphenous nerve diverges and follows a different course.

As a reference location for sampling, the most common site for venepuncture, the ACF, was chosen. The vessels here, as demonstrated in Figure 6.2, are typically the median cubital, cephalic, or basilic vein, and receive blood from the lower arm (including the joints), via a network of superficial and named smaller tributaries. The median cubital vein is the most prominent and runs from the cephalic vein below the lateral epicondyle of the humerus to the basilic vein above the medial epicondyle with an upward oblique course (Figure 6.2).

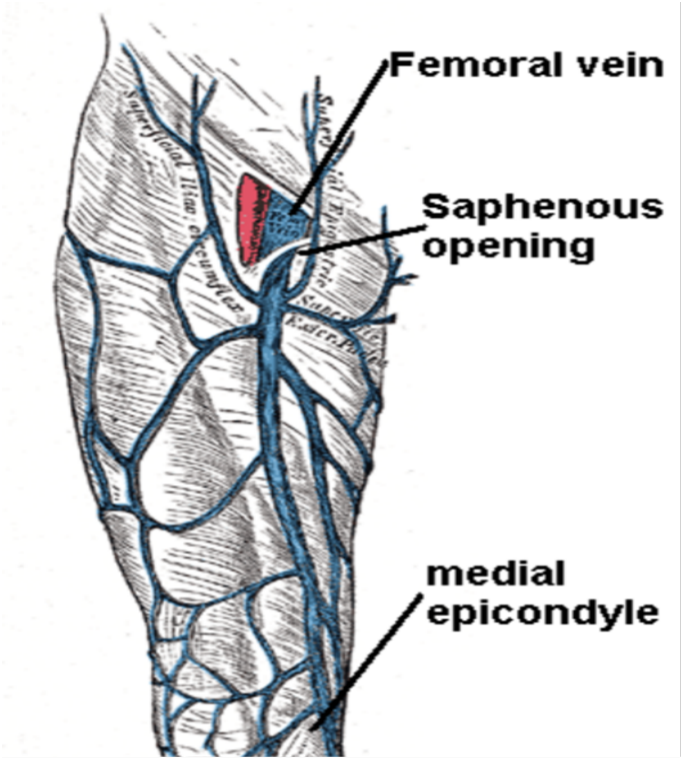


Figure 6.1 Anatomy of lower limb venous system.
Open source image, available in the public domain under a CC0.10 Universal Public Domain Dedication (440)

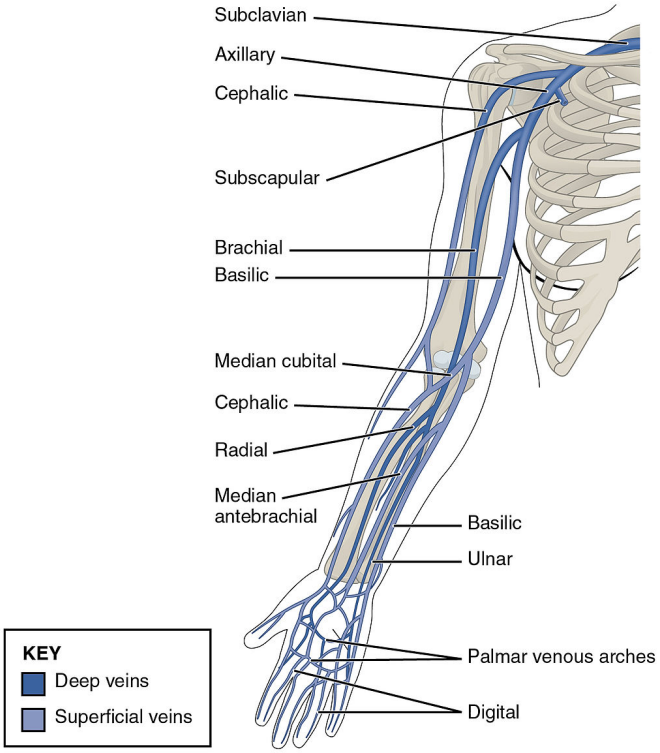


Figure 6.2 Anatomy of upper limb venous system.
Open source image, available in public domain under CC BY 3.0 (441)

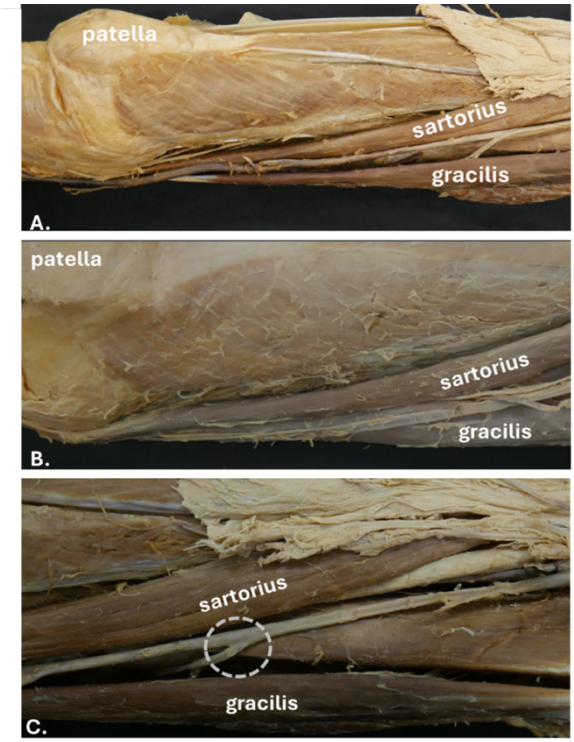


Figure 6.2 Position of the great saphenous vein (GSV), identified using the landmarks of the vastus medialis, sartorius, and gracilis muscles.
A and B illustrate its consistency in two different donors, while C exemplifies its connection with the deep venous system.
Consent for use from the University of Nottingham Anatomy Suite.

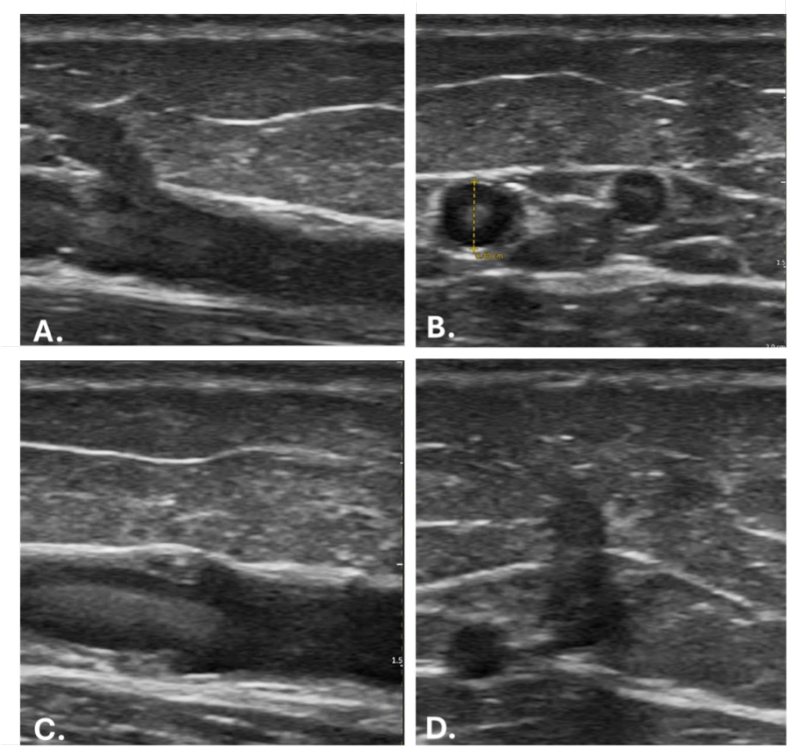


Figure 6.3 Ultrasound images of the great saphenous vein (GSV).
A. Longitudinal view showing the superficial cutaneous branch. B. Cross-sectional view illustrating the smaller branch. C. Longitudinal view representing venous valves. D. Connection between the GSV and the deep circulation. Consent for use gained from subject.

6.2.3 Preparatory phase

Prior to performing this procedure on participants, I (experienced in phlebotomy) underwent training on a gel-based model to improve the ability to perform venepuncture under direct ultrasound visualisation (Figure 6.4) (442). This was a model made from clear ballistic gel utilising a previously described method (443). The model contained a balloon partly filled with water, which aimed to replicate the images visible when performing ultrasound-assisted procedures (Figure 6.5). The intent of this training was to improve the ability to perform venepuncture under direct visualisation. Once the senior investigator (SK) was content that I was proficient, participant visits were scheduled.

The training criteria were as follows:

1. Ensure trainees could operate the ultrasound device and optimise images using gain, depth, and focus.
2. Develop the ability to follow small cylindrical structures with diameters ranging from 2 to 15 mm.
3. Achieve proficiency in needle placement and control of needle visualisation during advancement of the needle, both in-plane and out-of-plane.
4. Successfully and repeatedly demonstrated the ability to aspirate fluid within the simulated vessels.

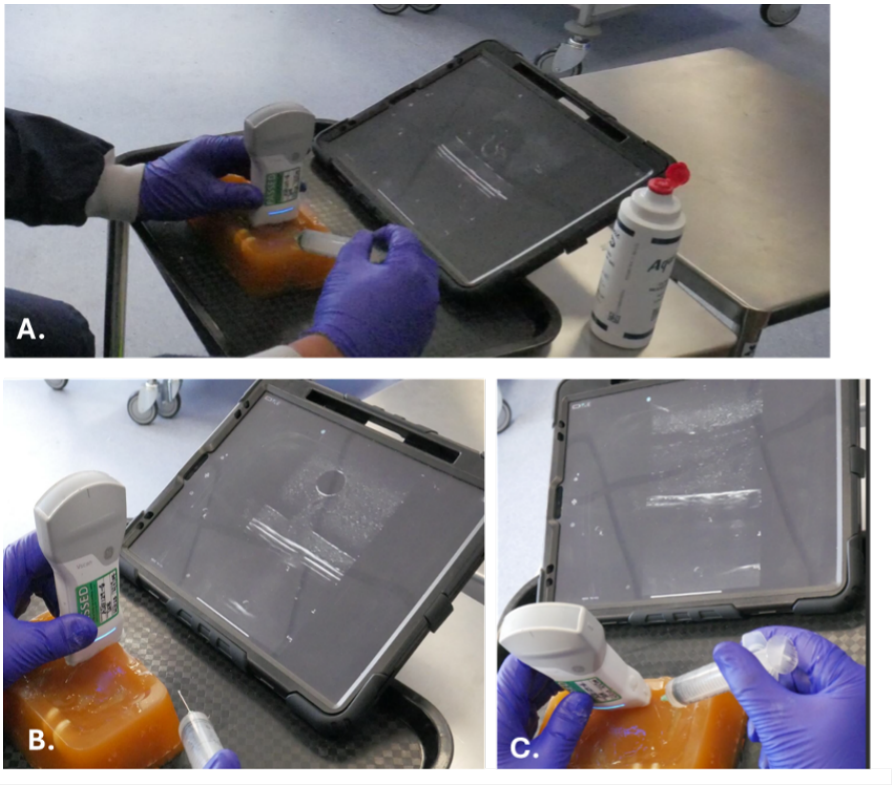


Figure 6.4 Set-up of the ultrasound gel training model for ultrasound-guided venepuncture

A. General set-up, B. Out-of-plane venepuncture, and C. In-plane venepuncture. Consent for use gained from subject.

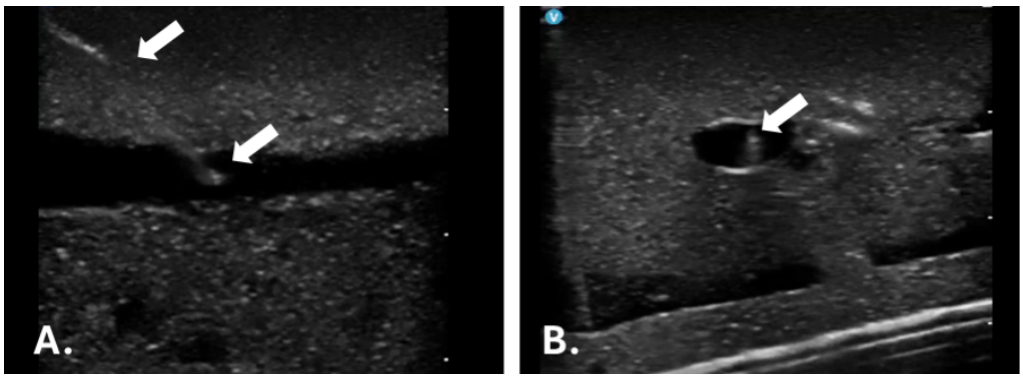


Figure 6.5 Ultrasound training in In-plane and Out-of-plane needle positioning and visualisation– images from the gel training model

A. Longitudinal view of the simulated vein in the gel model with in-plane needle placement (white arrows indicating the position of the needle). B. Cross-sectional view of the simulated vein in the gel model with out-of-plane needle placement (white arrows indicating the position of the needle

Consent for use gained from subject.

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I had limited prior experience using a handheld ultrasound device, with two dedicated practice sessions using the gel-based model sufficient to develop the necessary skills to perform ultrasound-guided venepuncture effectively. However, it is important to note that the level of training required may vary depending on the practitioner's initial familiarity with ultrasound technology. For practitioners who are entirely ultrasound-naïve, additional practice sessions may be necessary to ensure a thorough understanding of the device and its application.

The gel models were stored in a refrigerator between sessions, placed in a plastic container sealed with cling film to maintain hygiene and prevent contamination. Despite these precautions, one of the models developed visible mould on its top surface after three weeks of storage.

6.2.4 Study visit

Potential participants were invited to IRIS after they had expressed interest and been provided with a PIL. I identified an appropriate space and ensured the room was pre-booked and study visits co-ordinated to ensure multiple participants per session. Upon arrival, I re-explained the study, asked them about the PIL and checked for questions, and then undertook the consent process. After consent and an additional screening and eligibility questionnaire, basic demographic data (age, ethnicity, body mass) were collected, and any history of recent or chronic injury was recorded.

In brief, the protocol involved the collection of three serum samples from each participant, two from the ACF (‘Arm 1’ and ‘Arm 2’), and one from a vein near their knee (‘Knee’), with the physical activity performed between the two ACF samples. After the local sample (Arm 1) had been collected, a paired sample from the same side was drawn from a regional vein located near the joint (GSV). Following, the participant underwent a sub-maximal, non-load-bearing physical activity task. This was performed using a standardised protocol. The participant was asked to use a static cycle ergometer after adjusting saddle height, and after a 1-minute warm-up, they were asked to cycle constantly for 10 minutes aiming to maintain a rate of perceived effort (RPE) of 12 on the Borg scale (relating to ‘somewhat hard’) (444). Following the activity task, a second sample was drawn from the ACF (Arm 2) to measure the effect of precedent physical activity on biomarker concentration.

6.2.5 Positioning

In order to undergo sampling from the Knee, participants were positioned on a pre-cleaned examination plinth reclined at 45 degrees, which was covered in disposable blue roll. They were asked to wear shorts, with the shorts rolled up to fully expose the lower limb, and a foam roller placed under the knee to provide a mild degree of flexion and prevent unnecessary muscle contraction. The hip was mildly externally rotated and knee slightly flexed.

6.2.6 Technique

Once the participant was positioned, comfort levels were verbally assessed as they would need to remain in this position for upto 15 minutes. Once comfortable, the relevant anatomy was visualised. This was undertaken using a reusable rubber tourniquet applied over the upper third of the upper leg, with the lower limb venous vasculature identified using a hand-held ultrasound device (VScanAir™, General Electric, USA), which provided real-time images to a stand-alone portable device (iPad®, Apple, USA). Identification of the most appropriate vein, typically either great or small saphenous vein (GSV/SSV), and other relevant feature (depth from skin, diameter of vessel, presence of any anatomical variation), was done. The overlying skin was marked using a washable marker pen, and the tourniquet was loosened while the sampling pack was assembled (Box 6.1).

Box 6.1. Equipment required for novel lower-limb venepuncture

- Blue (23G) and green (21G) needles, depending on the vein's size and accessibility.
- 2 mL, 5 mL, and 10 mL capacity syringes, depending on required sample volume
- Vacutainer system was also used, when appropriate
- Hospital/department-approved needle/sharps container and clinical waste disposal
- A sterile pack containing drapes, ultrasound gel, a probe cover, and bands
- Long green 'Daisygrip venous tourniquet'
- 70% isopropyl alcohol wipes
- Sterile Chloraprep® Clear Applicator (2% Chlorhexidine Gluconate/70% Isopropyl Alcohol formulation)

- Sterile gloves
- Suggested (based on participants' comments): a warm pack and/or EMLA gel to minimize discomfort during the procedure

The procedure began with thorough handwashing using World Health Organization (WHO) techniques before donning double sterile gloves (445). To maintain a sterile field and prevent cross-contamination, sterile drapes, sterile ultrasound gel, and a sterile probe cover were used throughout the procedure. The injection site was cleansed with 70% isopropyl alcohol wipes, followed by the application of an antiseptic, 2% chlorhexidine gluconate and 70% isopropyl alcohol-containing cleaning product (Chloraprep™, Becton, Dickinson and Company (BD), UK).

The needle was placed under direct ultrasound guidance, using either an in-plane or out-of-plane approach depending on the vein's orientation (Figure 6.5). The needle was advanced carefully under visualisation to minimise the risk of complications. Three attempts were undertaken during sampling - if the third attempt was unsuccessful, the researcher terminated the procedure. After the procedure, pressure was applied to the puncture site to prevent bleeding, and the area was dressed appropriately. Once the sample had been collected, serum was transferred to analysis tubes, the participant checked for any post-procedure side effects, and equipment was tidied away after cleaning with alcohol wipes. All used equipment was disposed of following clinical waste protocols.

6.2.7 Biomarker analysis

Sera were centrifuged for 10 minutes at 3000rpm (Heraeus Biofuge Primo B, Heraeus) before the serum was extracted and aliquoted. Samples were frozen in cryovials at -80 in a monitored, temperature-controlled freezer (Model GGU 1500 Premium, Liebherr) before being transferred on ice to undergo analysis for a pre-selected panel of candidate biomarkers by Affinity Biomarker Labs (ABL, London, UK) using enzyme-linked immunosorbent assay (ELISA). The biomarkers chosen were high-sensitivity IL-1β, IL-6, CTX-II, COMP, N-propeptide of collagen IIA (PIIANP), and leptin, which were selected to offer insights into different pathological mechanisms and to allow comparison to the ADVANCE population analysed in Chapter Four and Five. The high-sensitivity IL-1β assay was chosen due to the significant floor effect seen previously. Each ELISA plate had two kit controls with three quality control samples, and underwent internal validation and quality control by ABL before release.

6.2.8 Statistical methods

The pre-specified minimal study sample size was 30, split into 10 participants per arm: control (CON), self-reported established knee OA (KOA) and recent (last 3 months) lower-limb injury (INJ) (Table 6.1). This was based on previously observed differences of IL-6 between local v central venous drainage in unpublished data in Charcot joint disease, showing a standard deviation (SD) of IL-6 of 18, and mean difference between paired samples (local vs ACF) of 25. Based on that, this study would require a sample size of 8 (number of paired serum samples) to achieve a power of 80% and a significance level of 5%.

Table 6.1 Definition of Collection Sites, Groups, Abbreviations, and Number of Samples Collected or Participants

		Abbreviation	Number
Serum Collection sites	Antecubital fossa *before* exercise	'Arm 1'	32
	Antecubital fossa *after* exercise	'Arm 2'	32
	Great saphenous vein above the knee *before* exercise	'Knee'	26
Participants subgroups	Control group	'CON'	12
	Knee osteoarthritis group	'KOA'	10
	Recent injury group	'INJ'	10

Data were imported into statistical software (Stata 18, StataCorp LLC, Texas), cleaned and checked for any missing data and significant outliers. The assumptions of normality were assessed, and parametric and non-parametric tests were used accordingly. During the initial condition analysis, normality tests were performed using the biomarker concentration levels, when paired data (location and exercise analysis), normality tests were performed using the difference between these paired data. Descriptive analysis was performed initially, reported in mean ± SD, or median (interquartile range, IQR), as appropriate.

Initially, univariate analysis was performed to assess the differences between conditions (CON/KOA/INJ) using analysis of variance (ANOVA) or the Kruskal-Wallis test using the initial ACF sample ('Arm 1'), i.e. Arm1: CON vs KOA vs INJ. Differences between the location of sampling were performed using 'Arm 1' and 'Knee' results, and

subsequently, differences pre- and post-exercise using ‘Arm 1’ and ‘Arm 2’, initially using the entire cohort, then stratified by condition (CON/KOA/INJ), were also performed, using paired Student t-test or Wilcoxon matched-pairs sign-rank test. In addition, the difference between the percentage (delta%) change between location and exercise intervention was calculated (|ARM1-Knee| & |ARM1-ARM2|), and differences were assessed by condition using the Kruskal-Wallis test or ANOVA. Significance was set at 0.05.

6.3 Results

Thirty-two participants were recruited into the study and underwent sampling by two researchers (OOS and SK), n=12 CON, n=10 KOA and n=10 INJ (Table 6.1). Participants had a median age 28.0 (25.0-43.0) (n=1 unrecorded), were 81% male (n=26/32) and had a median BMI of 23.9 (22.5-26.3) (Table 6.2). The ethnic background of participants was Indian (47%, n=15), Caucasian (34%, n=11), Black, Asian and Afro-Caribbean (3%, n=1 each), with 3 unreported (9%) (Table 6.2).

Table 6.2 Participant demographics

Demographic	**ALL (n=32)**	**CON (n=12)**	**KOA (n=10)**	**INJ (n=10)**	**p-value**
Age* Median (IQR)	28.0 (25.0-43.0)	26.5 (25.5-30.5)	52.0 (43.0-60.0)	25.5 (24.0-28.0)	<0.001
Sex (M/F)	26/6	9/3	10/0	7/3	0.18
BMI Median (IQR)	23.9 (22.5-26.3)	23.5 (21.9-25.2)	25.4 (23.5-27.1)	23.2 (19.6-25.2)	0.18
Ethnicity*					0.070
Caucasian	11 (34%)	5 (42%)	5 (50%)	1 (10%)	
Indian	15 (47%)	7 (58%)	1 (10%)	7 (70%)	
Black/Asian	3 (9%)	0 (0%)	2 (20%)	1 (10%)	
Not recorded	3 (9%)	0 (0%)	2 (20%)	1 (10%)	

**1 participant did not record their age and 3 did not record their ethnic background*
M: Male, F: Female, IQR: Interquartile range, CON: Control, KOA: Knee osteoarthritis, INJ: injury

The proportion of sampling procedures requiring multiple attempts remained similar throughout (first 16 participants, 50%, second 16 participants, 50%). From the successful procedures (n=26), fifteen were collected with one attempt (58%), eight after two attempts (31%), and five required three attempts (19%) (Table 6.3). Two participants underwent three unsuccessful attempts without collection, 6%. Four participants (12%) asked to terminate during the sampling procedure due to pain before successful completion (first attempt n=2, second attempt n=1, third attempt n=1) (Table 6.3). Aside from these, no major side effects or adverse events were encountered during or after the procedure.

Table 6.3 Number of attempts during successful procedures

Procedure attempt	Number (n=, %)
1	15 (58%)
2	8 (31%)
3	5 (19%)

6.3.1 Condition

The three condition groups were compared initially using Arm 1 values to ascertain any differences (Table 6.4, Figure 6.6). Across the three groups, IL-1β was seen to be significantly lower in those with KOA than those with a recent injury or control, p=0.004. IL-6, CTX-II, leptin, COMP and PIIANP were all not significantly different (Table 6.5).

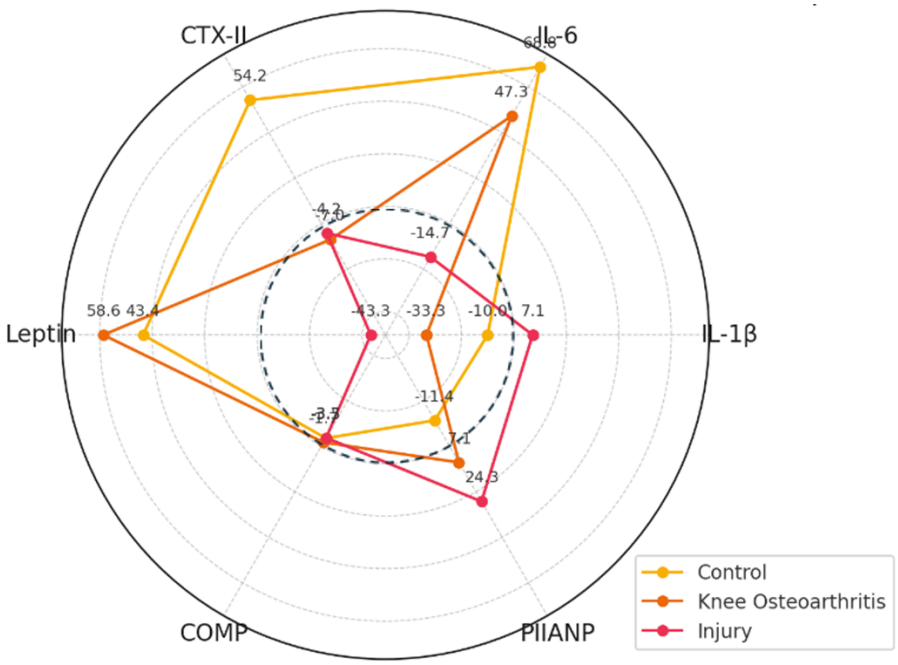

Figure 6.6 Radar map demonstrating the concentrations of serum biomarkers measured at the antecubital fossa stratified by condition (control, knee osteoarthritis, recent lower-limb injury).
The dashed circle represents the mean values for all the samples. The numbers represents percentage deviation of individual groups compared to the mean.
COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA

Table 6.4 Whole group and condition specific baseline serum biomarkers

	Total	**Control**	**OA**	**INJ**	**p-value**
	N=32	N=12	N=10	N=10	
IL-1β (ng/l) Median (IQR)	0.07 (0.04-0.14)	0.10 (0.05-0.20)	0.03 (0.02-0.06)	0.14 (0.07-4.62)	0.004
IL-6 (ng/l) Median (IQR)	0.96 (0.67-2.26)	1.38 (0.74-2.31)	0.93 (0.72-0.97)	1.91 (0.48-67.60)	0.420
CTX-II (ug/l) Median (IQR)	0.68 (0.37-1.09)	0.59 (0.34-1.16)	0.57 (0.27-0.99)	0.95 (0.46-1.10)	0.510
Leptin (ug/l) Median (IQR)	7.01 (2.37-20.62)	13.04 (3.30-41.67)	8.12 (5.26-10.65)	3.53 (1.95-11.33)	0.230
COMP (ug/l) Mean (SD)	173.06 (±47.76)	173.22 (±41.74)	192.91 (±56.34)	153.02 (±40.85)	0.180
PIIANP (ug/l) Median (IQR)	136.98 (88.78-204.04)	144.02 (97.58-177.99)	90.82 (67.05-123.66)	171.58 (132.91-388.92)	0.078

Collected from antecubital fossa before exercise (Arm 1). COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA. IQR: interquartile range, SD: Standard deviation

6.3.2 Location

Paired samples were taken from the arm (Arm 1) and leg (Knee) on the same side before exercise (Figure 6.7). Differences between these were assessed initially at a whole study population level and then stratified by condition (Table 6.6 and 6.7). Due to the technical difficulties of the novel technique employed, only 26 participants had blood drawn from their lower limbs (CON n=10, KOA n=10, INJ n=6).

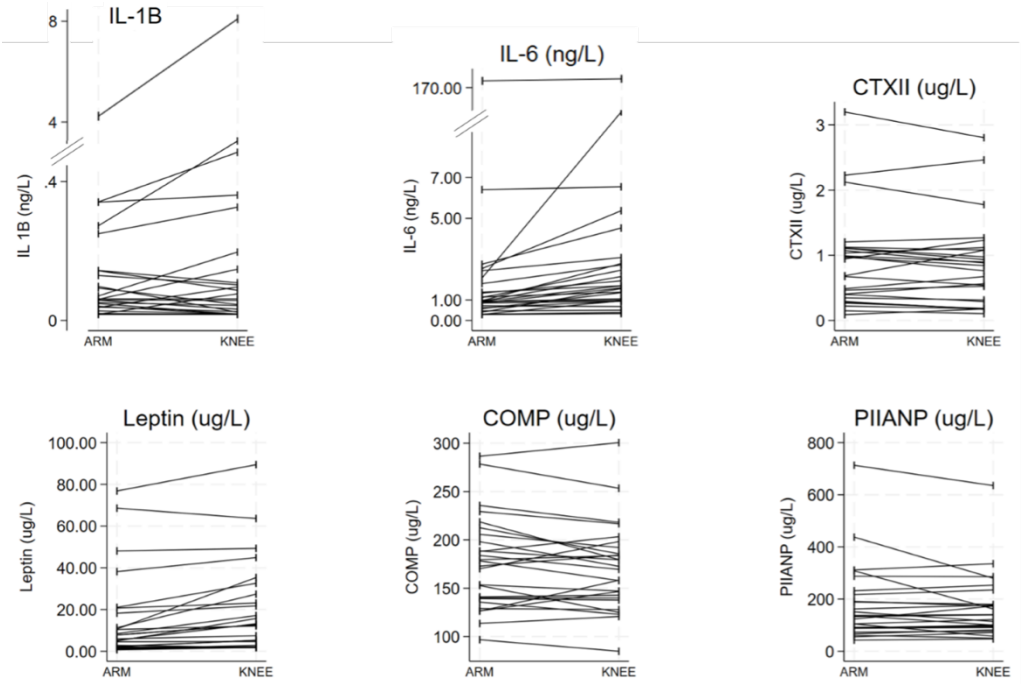

Figure 6.7 Line graph demonstrating serum biomarker concentration differences between location of sampling
COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA

At an all group level, significant differences in biomarker concentrations were observed based on the sampling location (Table 6.6). Both IL-6 and leptin concentrations were found to be 72–80% higher in serum collected around the knee compared to the ACF. No other significant differences were observed between biomarker concentrations.

Table 6.5 Whole group sampling location differences in serum biomarker concentrations

	Arm 1 n=32	Knee n=26	p-value
IL-1β (ng/L) Median (IQR)	0.07 (0.04-0.14)	0.06 (0.02-0.15)	0.670
IL-6 (ng/L) Median (IQR)	0.96 (0.67-2.26)	1.65 (0.97-2.79)	<0.001*
CTX-II (ug/L) Median (IQR)	0.68 (0.37-1.09)	0.80 (0.29-1.08)	0.377
Leptin (ug/L) Median (IQR)	7.01 (2.37-20.62)	12.65 (2.75-27.34)	<0.001*
COMP (ug/L) Mean (SD)	173.06 (±47.76)	171.16 (±45.36)	0.170
PIIANP (ug/L) Median (IQR)	136.98 (88.78-204.04)	130.22 (90.64-179.89)	1

Collected from antecubital fossa (Arm 1) and ipsilateral knee (Knee) prior to exercise. COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA. IQR: Interquartile range, SD: Standard deviation

When stratified for condition, four biomarkers exhibited differences between sampling locations (Table 6.7). IL-6 was seen to differ across all three conditions, increasing for CON and KOA and decreasing for INJ participants; leptin was significantly increased between locations in CON and KOA participants; PIIANP significantly increased between location in KOA individuals; and COMP significantly decreased between sampling locations in those with INJ (Table 6.7).

When the serum biomarker concentration percentage change was calculated between locations to see if the participant condition affected the joint response, only PIIANP differed (CON: 4.10%, (IQR: -5.9,23.17%); KOA: -4.04% (IQR: -9.01,-1.13); INJ 9.10%, (IQR: -7.69,17.06), p=0.007). No other biomarkers displayed any significant percentage change differences.

Table 6.6 Condition specific sampling location differences in serum biomarker concentrations

	Control			OA			INJ		
	Arm n=12	**Knee n=10**	**p-value**	**Arm n=10**	**Knee n=10**	**p-value**	**Arm n=10**	**Knee n=6**	**p-value**
IL-1β (ng/L) Median (IQR)	0.10 (0.05-0.20)	0.09 (0.06-0.33)	0.211	0.03 (0.02-0.06)	0.02 (0.02-0.02)	0.156	0.14 (0.07-4.62)	0.15 (0.04-0.36)	0.688
IL-6 (ng/L) Median (IQR)	1.38 (0.74-2.31)	2.33 (1.07-5.37)	0.002	0.93 (0.72-0.97)	1.37 (0.95-2.15)	0.004	1.91 (0.48-67.60)	1.63 (0.51-3.08)	0.031
CTX-II (ug/L) Median IQR)	0.59 (0.34-1.16)	0.91 (0.56-1.27)	0.695	0.57 (0.27-0.99)	0.53 (0.18-0.90)	0.193	0.95 (0.46-1.10)	0.91 (0.31-1.23)	0.313
Leptin (ug/L) Median (IQR)	13.04 (3.30-41.67)	18.70 (2.75-44.89)	0.027	8.12 (5.26-10.65)	12.88 (7.40-23.08)	0.002	3.53 (1.95-11.33)	2.00 (1.84-4.64)	0.219
COMP (ug/L) Mean (SD)	173.22 (±41.74)	167.08 (±38.37)	0.510	192.91 (±56.34)	189.55 (±55.84)	0.545	153.02 (±40.85)	147.30 (±25.12)	0.088
PIIANP (ug/L) Median (IQR)	144.02 (97.58-177.99)	127.61 (97.96-176.70)	0.322	90.82 (67.05-123.66)	97.26 (76.80-172.96)	0.004	171.58 (132.91-388.92)	213.19 (138.96-279.92)	0.313

CON: Control, KOA: Knee osteoarthritis, INJ: Injury COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA. IQR: Interquartile range, SD: Standard deviation

6.3.3 Exercise intervention

All 32 participants had a blood sample taken before and after the exercise intervention (Arm 1 and Arm 2, Figure 6.8). At the whole group level, three biomarkers were significantly different after the intervention (Table 6). These were IL-1β which significantly decreased, and CTX-II and COMP which significantly increased (Table 6.8). The three other biomarkers, IL-6, leptin and PIIANP, showed no differences at a group level following the exercise intervention.

There were further significant differences when the exercise intervention results were stratified by the participant condition (Table 6.7). Both IL-6 and CTX-II significantly increased in those with KOA post-exercise. COMP significantly increased in both INJ and CON, and IL-1β also significantly decreased in CON participants (Table 6.9). PIIANP and leptin displayed no difference following the exercise intervention at a condition level.

No significant differences were noted across the condition groups in any serum biomarker percentage change following the exercise intervention, suggesting all groups of participants responded to exercise uniformly.

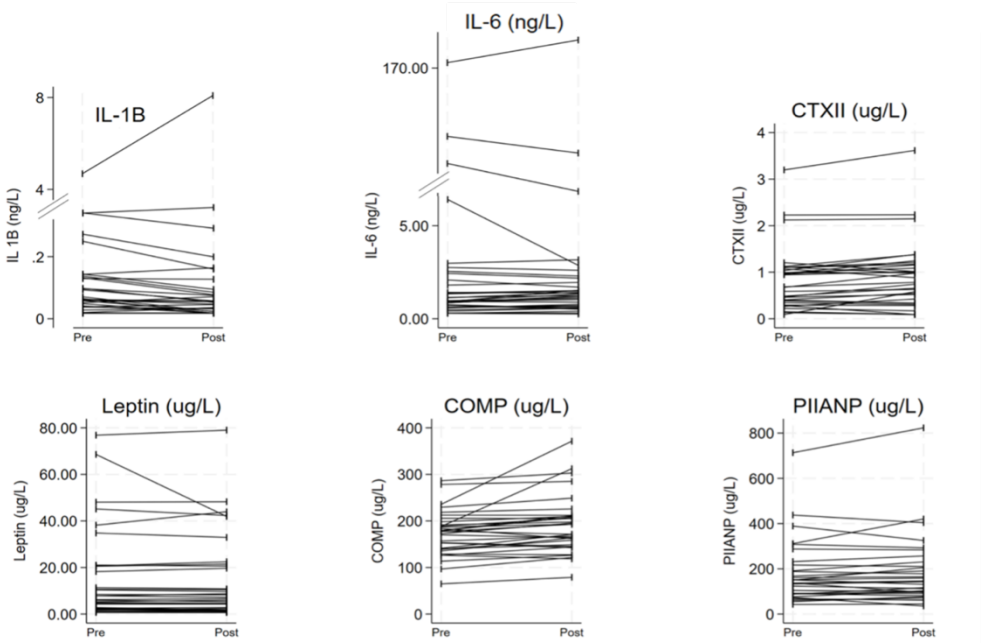

Figure 6.8 Line graph showing serum biomarker concentration differences before and after the sub-maximal exercise intervention

COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA

Table 6.7 Whole group serum biomarker concentrations before and after exercise intervention

	Pre-exercise n=32	**Post-exercise n=32**	**p-value**
IL-1β (ng/L) Median (IQR)	0.07 (0.04-0.14)	0.06 (0.02-0.14)	0.003
IL-6 (ng/L) Median (IQR)	0.96 (0.67-2.26)	1.29 (0.68-2.07)	0.494
CTX-II (ug/L) Median (IQR)	0.68 (0.37-1.09)	0.83 (0.38-1.16)	0.020
Leptin (ug/L) Median (IQR)	7.01 (2.37-20.62)	6.94 (2.10-21.04)	0.692
COMP (ug/L) Mean (SD)	173.06 (±47.76)	190.27 (±61.99)	0.005
PIIANP (ug/L) Median (IQR)	136.98 (88.78-204.04)	137.09 (93.28-220.46)	0.561

CON: Control, KOA: Knee osteoarthritis, INJ: Injury, COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA. IQR: Interquartile range, SD: Standard deviation

Table 6.8 Condition-specific serum biomarker concentrations before and after exercise intervention

	CON n=12			KOA n=10			INJ n=10		
	Pre-exercise	**Post-exercise**	**p-value**	**Pre-exercise**	**Post-exercise**	**p-value**	**Pre-exercise**	**Post-exercise**	**p-value**
IL-1β (ng/L) Median (IQR)	0.10 (0.05-0.20)	0.07 (0.04-0.16)	0.031*	0.03 (0.02-0.06)	0.03 (0.02-0.05)	0.081	0.14 (0.07-4.62)	0.09 (0.07-2.60)	0.646
IL-6 (ng/L) Median (IQR)	1.38 (0.74-2.31)	1.51 (0.94-2.12)	0.444	0.93 (0.72-0.97)	1.06 (0.77-1.25)	0.041*	1.91 (0.48-67.60)	1.85 (0.56-37.68)	0.609
CTX-II (ug/L) Median (IQR)	0.59 (0.34-1.16)	0.72 (0.31-1.13)	0.328	0.57 (0.27-0.99)	0.86 (0.42-1.09)	0.030*	0.95 (0.46-1.10)	0.95 (0.52-1.17)	0.420
Leptin (ug/L) Median (IQR)	13.04 (3.30-41.67)	14.19 (3.48-42.15)	0.569	8.12 (5.26-10.65)	7.73 (5.34-10.50)	0.432	3.53 (1.95-11.33)	3.52 (1.63-10.81)	0.322
COMP (ug/L) Mean (SD)	173.22 (±41.74)	184.27 (±40.36)	0.034*	192.91 (±56.34)	225.34 (±87.04)	0.065	153.02 (±40.85)	162.40 (±37.29)	0.049*
PIIANP (ug/L) Median (IQR)	144.02 (97.58-177.99)	138.89 (100.39-184.26)	0.408	90.82 (67.05-123.66)	85.77 (61.78-112.40)	0.248	171.58 (132.91-388.92)	218.05 (142.72-325.53)	0.331

Collected from antecubital fossa before (Arm 1) and after (Arm 2) exercise intervention. CON: Control, KOA: Knee osteoarthritis, INJ: Injury
COMP: Cartilage oligomeric matrix protein, CTX-II: C-terminal cross-linked telopeptide of type-II collagen, IL: Interleukin, PIIANP: N-propeptide of collagen IIA. IQR: Interquartile range, SD: Standard deviation

6.3.4 Comments from the researchers and participants:

Needle Positioning: "When inserting the needle toward the knee, it is recommended to rest the wrist on the leg for better control. This technique is likely to not create a pressure decrease observed when needle is inserted away from the knee, which facilitates fluid collection."

Success Rate: "The procedure tends to be more successful particularly when the needle is positioned higher and above the valve connecting the saphenous vein with deep circulation above the knee."

Saphenous Vein Caution: "The saphenous vein is located close to the nerve just above the knee, but it then diverges. Careful needle placement is necessary to avoid nerve irritation".

Application of topical anaesthetic: "Applying a heat pack and/or topical lidocaine gel before the procedure could be very helpful in reducing stress and discomfort."

6.4 Discussion

This pilot study aimed to describe the feasibility and acceptability of a novel venous sampling lower-limb technique, and test the hypothesis that the location of serum sampling and precedent physical activity would significantly alter serum concentrations of candidate OA biomarkers. It has several notable findings. This is the first description of such a technique, which might offer the ability to gain new insights into the microenvironment of the knee joint, and was largely successful to perform, and acceptable from a participant perspective.

Leptin and IL-6 concentrations differed between sampling locations, with PIIANP also differing when stratified by condition. A short volume of non-weight-bearing exercise altered the concentrations of IL-1β, CTX-II and COMP, with IL-6 varying within the KOA group. The discussion initially will focus on the development and refining of the technique, then describe the future clinical and research implications of the pilot study.

6.4.1 Development and refinement

Given the technical requirements for this procedure, it was important to undertake appropriate training beforehand. All the research team were experienced and proficient in phlebotomy, with one researcher (me!) only having limited experience using a handheld ultrasound device. Two practice sessions using the gel model was sufficient to develop these skills sufficiently, however, if future practitioners are ultrasound-naïve, this training may require further sessions. Throughout the development of the technique, the equipment used was refined. Different tourniquet models were used, including disposable thin rubber, reusable fabric and rubber tourniquets. Venepuncture in the lower limb presents unique challenges compared to the ACF, such as accommodating the limb's larger diameter and applying sufficient pressure to occlude deeper vessels. The disposable thin rubber tourniquet was variously too short and snapped under sustained pressure, and the fabric reusable tourniquet was impracticably short. The re-usable rubber tourniquet (daisygrip™, Tristel, UK) maintained the required increased pressure and had the length required for all participants' thigh diameters. It was cleaned between each use, which is essential for infection prevention. A further refinement might be using a blood flow restriction cuff, given its increased use in rehabilitation pathways (446).

Other equipment utilised and refined throughout this development was the needle and syringe combination. Initially, a butterfly system directly attached to the vacuum specimen tube (Vacutainer®, BD, UK) was trialled; however, often, the needle was not long enough to reach the subcutaneous or deeper vessels, and the pressure by the vacuum-tubes itself was too high for the small, fragile veins, causing vein collapse and reduced blood flow. As a result, the research team adopted the needle and syringe combination, which provided several advantages. The adjustable pressure allowed for gentler handling of fragile veins, and the longer needle offered better access to deeper vessels. Additionally, we observed improved dexterity and control with

this system, particularly beneficial when sampling from deep or small-calibre veins. This refined approach enhanced the precision and success rate of blood collection in challenging cases.

Despite its experimental nature, it was notable how acceptable and tolerable participants found this procedure. All participants had the technique explained in the PIL and initial brief, and once informed, no participants declined, suggesting this procedure was deemed acceptable before the start. During this pilot, there were 59 attempts across all participants, with four of them causing pain sufficient to request termination (7%), two cases of post-procedure bruising and no other side or post-venepuncture effects, demonstrating patient tolerability. Adverse reactions have been recorded in up to 49% of venepuncture procedures (447), with the most common side effect, pain, improved through the use of topical analgesia (448). However, the pain of this procedure is likely due to the regional anatomy – specifically, the proximity of the saphenous nerve to the GSV (449), with a more proximal approach for venepuncture likely to reduce the risk of severe pain.

The success rate of the procedure was high, with only two episodes of unsuccessful blood sample collection following three attempts (6%); however, half the participants underwent multiple attempts. There are two key reasons underpinning this, participant factors and operator factors. When the most suitable blood vessel was detected on ultrasound, those located deeper from the skin and those with the smallest calibre were typically harder to cannulate. During the early stages, vessels under 3mm had a lower success rate; however, by the end, those above 2.5mm were considered large enough. Whilst it is interesting to note that first-attempt success remained at approximately 50% through the study, it is likely that this would increase with more operator experience and competence, as potentially demonstrated by the anecdotal experiences related to vessel diameter. A further area that might improve rates for those with deeper vessels or high volumes

of subcutaneous adipose tissue is the use of a longer needle length, which was not available to the research team.

This novel technique for venepuncture nearer to the knee joint offers an opportunity to improve OA biomarker research across all elements of the BIPEDS taxonomy, especially for those with a lower-limb injury (57,348). It is likely that any biomarkers formed and/or released by the synovium or other components of the knee or ankle joint undergo dilutional effects in the systemic circulation, thus reducing their sensitivity (450,451). Differences were seen between the concentrations of biomarkers measured in GSV and ACF, and if thresholds of biomarker concentration are used to determine ‘normal vs abnormal’ and identify or define a diagnosis, then reduced concentration and sensitivity will cause false negative results. Furthermore, there is a poor correlation between paired serum and SF biomarkers, which might result from dilutional or perhaps degradation of the serum biomarker or indeed the influence of multiple joints (5). A further study undertaking paired local-serum and SF samples is required to assess if this novel technique improves correlation between serum and SF biomarkers.

6.4.2 Implication for future clinical and research work

Given the huge burden of OA morbidity and occupationally-related disability, molecular biomarkers are being investigated to see if they offer the ability to differentiate between good and poor joint health (both acute and chronic) (425). In non-inflammatory joint conditions, there are well-established processes in joint biology associated with cartilage, bone, or synovial inflammation that are clinically useful. An abnormal molecular pattern might be the first sign of OA development, especially in those with a traumatic injury at risk of PTOA (2,57). In this study, only IL-1β was seen to be lower in those with established OA, and no markers were different in those with a recent injury. IL-1β is known to be related to OA (285); however, it has not yet found a role as a potential OA biomarker (5,50,425), perhaps as a result of its non-specific nature as a pro-inflammatory cytokine. Despite that, this result is notable given the very modest sample size and is in part likely due to the high-sensitivity assay used, which should be considered when future studies are designed. This high-sensitivity assay was chosen deliberately, given the significant floor and ceiling effects encountered in ADVANCE population, as described in Chapter 4 and 5.

Whilst SF offers the best insight into the joint microenvironment, repeated synovial sampling can cause inflammation and disturb joint homeostasis. This study explores the idea that joint-specific biomarkers may differ in blood collected near the joint and improve test sensitivity. Sampling closer to the joint could provide a clearer view of the joint environment and improve test accuracy by minimising dilution in the bloodstream (12). We observed that leptin and IL-6 concentrations were 70-80% higher in the circulation near the joint compared to the upper arm, regardless of condition. When this analysis was stratified by condition, more differences were demonstrated, with IL-6, leptin and PIIANP all tending to be higher near the joint compared to the arm. These findings were in keeping with my hypothesis, based on the results in those with Charcot disease, and might reflect some mitigation

of systemic dilution effects. This might change our understanding of the microenvironment of the joint, but also the relative importance of these biomarker changes in the lower limb circulation. The mechanisms driving these discrepancies are not clear but potentially could be attributed to several factors, including greater adipose tissue presence around the lower limb joints, which produces more leptin, increased mechanical stress on weight-bearing joints like the knees, and heavy loading of muscles in weight-bearing limbs, with IL-6 being one of the first cytokines released in response.

These findings suggest that local venepuncture might offer an insight into the microenvironment and local inflammatory, metabolic and cartilage turnover due to injury and OA development (5,169,173,190). These findings require validation in other populations, with similar studies planned elsewhere (452), but offer an exciting new avenue for diagnostic and predictive biomarker studies. Earlier work in the thesis has demonstrated limitations for the use of serum biomarkers, perhaps, in part, due to the signal of disease remaining local to the joint in question – this method might help elicit more information.

Another proposition is that exercise can increase SF filtration, leading to a more distinct expression of OA or injury-related biomarkers in serum samples. The effect of exercise on serum biomarker concentration is important to understand for two reasons. Firstly, if the results of the biomarkers are negatively influenced by preceding or recent physical activity, especially if this is strenuous, then any clinical outcomes might be impacted by a potential false positive or false negative test result. Previous work has demonstrated that load-bearing activity can alter biomarker concentration (437-439), so in clinical settings, this needs to either be accounted for or the participants asked to refrain from activity for a period of time before the test. One previous study did not show an increase in biomarker concentration (453), which could have been a result of the sample size; however, a similarly sized study did show changes, but this followed a very different activity (454), so the intensity

and duration of load is also relevant. Secondly, if physical activity increases serum biomarker concentration, this might offer an additional way to increase test sensitivity (437-439,454). Not all individuals with or at risk of OA can weight bear pain-free. This study deliberately adopted a non-weight-wearing physical activity to determine if activity-related biomarker concentration changes were due to mechanical stress and to identify if this technique could be used as an alternative for those unable to fully weight bear.

Following the exercise intervention, two ECM turnover biomarkers, CTX-II and COMP, significantly increased, suggesting either increased cartilage turnover and cartilage matrix degradation or remodelling. These findings suggest that exercise might increase test sensitivity due to the potential reduction in systemic dilution as well as giving an insight into exercise-related joint changes. Further insights were detected during the condition analysis, with both IL-6 and CTX-II increased in those with OA (suggesting a potentially aberrant ECM and inflammatory response), COMP increasing in those with a recent injury and control participants, and IL-1β decreasing in control participants. This latter finding is supportive that exercise might have anti-inflammatory effects, as described in the literature, given the reduction in IL-1β, a pro-inflammatory mediator (455). The CTX-II and COMP findings complement those described previously (437-439); however, those related to IL-1β and IL-6 in this setting are novel, potentially giving us an insight into acute mechanoinflammation (93).

6.4.3 Strengths and limitations

This pilot study has identified some new findings which require external validation in much larger populations. The strengths are the paired samples for both interventions and well-trained, consistent research team (all venepuncture was performed by OOS or SK). In accordance with open data science principles, anonymized data and code are made available through the GitHub website (456). Limitations of this study include the small numbers of participants, and six missing samples from the knee, both of which might have contributed to type II error. Of the six missing knee samples, four of those were from the recent MSKI group, which meant that that sub-group, with only six samples, was unlikely to have adequate statistical power (based on the pre-study sample size of 8 pairs). Additionally, recruitment strategy and differences in age, sex, exercise levels, and BMI were not accounted for in this research due to the small sample size. Those factors, including participants' age could eliminate differences between groups but would not account for differences between the sample collection sites or the variations observed before and after exercise. Future studies comparing SF, femoral vein and venous dynamics, using advanced techniques like technetium-99m tracing, gamma scintigraphy, or single-photon emission computed tomography, could validate the use of the GSV for biomarker sampling.

6.5 Conclusion

In conclusion, this acceptable and well-tolerated novel technique for lower-limb venepuncture, with a small learning curve and easily available cheap equipment, might offer a way to provide insight into the joint microenvironment without the risks associated with SF sampling and, therefore, improve the use of serum biomarkers for OA. This unique pilot study has identified differences in serum biomarker concentration as a result of the location from which the venous sample was drawn and a ten-minute sub-maximal non-weight-bearing physical activity task. These findings have relevance for future clinical and research work but require validation in a larger, external population.

Chapter 7 : What other measures should be assessed?

It is clear that the panel of molecular biomarkers that I have studied in Chapters Four and Five are, in themselves, not able to fully identify nor predict the incidence or prevalence of knee radiographic osteoarthritis (OA), pain or function.

Chapter Three synthesised all the evidence for serum and synovial fluid (SF) and magnetic resonance imaging (MRI) biomarkers a year or more from injury, in the chronic phase of post-traumatic OA (PTOA). No studies were identified using plasma, urinary or other imaging markers. This review reported the findings of 18 papers involving 1629 participants aged mid-20s-30s, between one and 11 years from injury. These studies measured 38 serum biomarkers, 13 SF biomarkers and several MRI metrics, identifying multiple measures which were associated with either pain, function or structural change, with further work required to develop these metrics. The MRI markers demonstrated some changes, which were present after a year, and persisted for years after that – these included structural and compositional changes of bone and cartilage. Specifically, across the 11 studies, with 776 participants, the MRI studies showed structural changes, including the position of the tibia and condyle bone shape and height, especially around the medial femoral region. In addition, compositional changes suggestive of cartilage degeneration, were seen on T1rho and T2 relaxation time. It possible that in early PTOA, unlike iOA, the compositional changes, brought on by injury, precede the structural changes. However, still, similarly to molecular biomarkers, there are significant methodological challenges, including technological, study design and length of times from injury and follow-up.

However, in addition to molecular and imaging biomarkers, there are other variables which could be considered as potential identifiers or predictors of future OA disease and illness, such as an individual's biomechanical profile.

7.1 Introduction

Appropriate biomechanical load is required to maintain cartilage homeostasis; however inappropriate biomechanical load can contribute to a pathological tissue response and resultant mechanoinflammation (93,457-459). This can be either abnormal loads with normal anatomy/physiology (e.g. persistent overtraining) or normal loads with abnormal anatomy/physiology (e.g. post-injury) (458). Dynamic, functional tests of kinematics ('motion') and kinetics ('forces producing motion') objectively assess an individual using standardised movements. Biomechanical measures (including joint angles and moments) are used as a proxy for tissue-level injurious changes resulting from altered movement and muscle activation patterns, joint anatomy or muscle-tendon morphology (460). Joint kinetics can be assessed during rehabilitation following musculoskeletal injury using force plate technology (461), providing feedback on derivatives of muscle strength to guide clinical decision-making (462) and potentially providing insight on future PTOA prediction. Differences in gait patterns have been identified (408), as have relationships between knee moments and cartilage thickness (463), with knee adduction moment (KAM) or internal joint loading associated with OA progression (464,465) or cartilage health (465).

Following certain injuries, including ACLs, it can take 9-12 months to return to sport. An individual's injury will alter their biomechanical profile, potentially offering insight into long-term pathological changes. Subsequently, new or persistent symptoms might appear after the completion of rehabilitation. The 'chronic phase of PTOA' is defined as a year or more following injury (5) and demonstrates disease progression from acute (day-weeks) and sub-acute (weeks-months). Previous work from the authors reported chronic phase molecular, structural, and compositional joint changes (4,5), with a need identified

to understand the corresponding biomechanical changes and how they are measured to inform future research priorities and clinical service delivery, including prevention (8,9,60,61,135). The aims of this review are to identify the functional tasks utilised to assess knee kinematics and kinetics at least one year from significant injury, compared to uninjured contralateral knees, and healthy controls; describe the differences found between the injured and uninjured knee, and healthy controls; and report any features associated with the presence of PTOA.

7.2 Methods

A systematic review was conducted in line with PRISMA guidelines (297). Three databases (Medline, CINAHL from 2000, and EMBASE from 1974, to current) were searched on the 10th November 2023 by a specialist research librarian (VB) (search strategy in supplementary material). Searches utilised keywords and subject headings related to knee injury, kinematics, kinetics, assessment of neuromuscular performance, landing, hopping, jumping, cutting, squatting and OA (Supplementary File 3). Reference lists of papers were screened to identify additional relevant studies.

7.2.1 Study selection criteria and screening

Inclusion and exclusion criteria are listed in **Error! Reference source not found.**. To align with previous reviews (4,5), participant age was restricted to 18-45-year-olds (inclusive). Studies utilising fluoroscopy were excluded, as this cannot be used in everyday clinical practice, with a focus on functional tasks such as jumping, landing, changing direction and squatting. For a similar reason, studies including computational modelling, a commonly used method of biomechanical assessment, were excluded. There were no restrictions on participant ethnicity or sex. If participants underwent knee surgery, this was treated as new 'injury' to prevent confounding. Screening was performed using Rayyan (www.rayyan.ai) against pre-determined criteria (found in **Error! Reference source not found.**) drawn from the PICO analysis (supplementary material). Results were deduplicated, and an initial title/abstract sift was performed by two reviewers independently (CR and MDV), with a third (OOS) undertaking conflict resolution. Two reviewers (CR and OOS) performed a second full-text screen, with a third (MDV) for conflicts.

Table 7.1 Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
English and Italian	Animal or cadaveric studies

Full-text papers	Lack of comparator (control group or uninjured contralateral knee)
Human participants	Fluoroscopic assessment of kinematics
Participant age, inclusive of, 18-45 year old	Assessment of biomechanics via computer simulation
Knee injury or surgery at least one year prior	Assessment of gait, running, jogging, stair negotiation only
Assessment of functional tasks or performance tests	Assessment of muscle activity or muscle force production only
At least one knee biomechanics variable assessed (kinematics and/or kinetics)	Only reporting on Limb Symmetry Index

7.2.2 Data extraction

Data extraction was undertaken by two reviewers independently, with myself and one other reviewer combining results and resolving conflicts via discussion until consensus was drawn. Data extraction was done with a pre-prepared data extraction tool (Microsoft Excel, Microsoft, Redmond, WA, USA) and included, when reported:

- Publication: authors, year of publication, journal
- Population: sex, age, injury/surgery type and time, number of cases/controls
- Biomechanics: tasks performed, joints and variables assessed, technology and methods used

7.2.3 Risk of bias assessment

The same reviewers performed a risk of bias (RoB) assessment simultaneously with data extraction using the Newcastle-Ottawa Scale (NOS) (300). The NOS assesses three components: participant selection, case/control comparability, and outcome ascertainment, and has bespoke versions applicable to cohort, cross-sectional, and case-control studies.

7.3 Results

The initial database search yielded 2504 studies, with six more detected from reference lists. Thirty-three studies (466-498) were included in the review (**Error! Reference source not found.**, adapted from (297)). The most common reasons for exclusion were the participant's age and time from injury/surgery. Research papers with the same (485,488) or similar (470,471,474,477,481,483,484,487) cohort but exploring different tasks (470,471) or different aims (474,477,481,483-485,487,488) were retained.

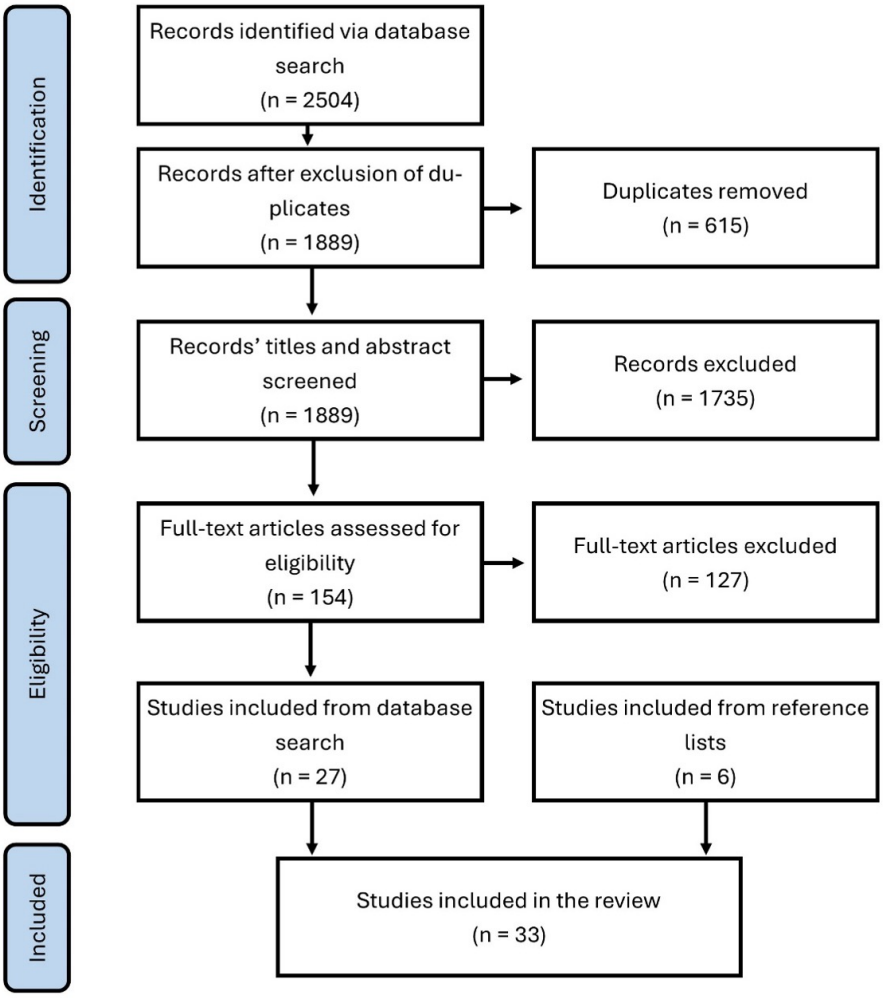

Figure 7.1 PRISMA flowchart of systematic review

7.3.1 Study characteristics

Six studies (18.2%) utilised a prospective or longitudinal design (474,477,480,484,487,491),
the remaining twenty-seven (81.8%) were cross-sectional (466,469-472,475,478,479,481-483,485,486,488-490,492,495). Eighteen studies (54.6%) were case-control (466,469-473,475,476,478-480,482,485-489,495), eight (24.2%) combined case-control and contralateral knee(467,468,473,481,484,487,492,498), and the remaining seven (21.2%) contralateral knee for comparison (474,477,483,490,491,493,497).

7.3.2 Participant characteristics

There were 737 injured knee participants (368 males, 348 females; 21 not reported(466)), and 514 uninjured controls (230 males, 273 females; 11 not reported(466,474)) included. The mean injured knee participants' age was 25.1 years (median age: 25.3 (interquartile range, IQR:19.2-31.5))(Table 2). All injured participants sustained an ACL injury (isolated ACL injury: n=8; combined ACL and other structures: n=8; not reported if isolated/combined: n=17), of which 98.4% (n=725) had surgery. Five longitudinal studies reported knee biomechanics at multiple time points post-surgery; only results at least one-year post-surgery were included(474,477,484,487,491). Post-injury/surgery time ranged between one and 13 years.

Table 7.2 Study characteristics

Study	N (case/control) Age cases, *years (SD)* Time from injury/surgery	Design, Comparator	Task(s) of interest	Biomechanical variables	Timing of biomechanical data extraction
Decker et al. 2002	22 (11 / 11) sex N/A 27.3 (SD not reported) >1 year post-ACLR	Cross-sectional, Control	LANDING Vertical drop landing (60 cm, bilateral)	Hip, knee, ankle angles at landing & peak angular velocity (sagittal plane), peak hip, knee, ankle internal moments, minimum internal joint powers, 1st & 2nd peak vGRF, vGRF loading rate	Initial contact to 100 ms post-initial contact (kinetics) - Initial contact to peak knee flexion angle (kinematics)
Rudroff 2003	40 (30M / 10M) ACLR (PT): 32.6 (4); ACLR (GS): 29.1 (6.7) 2 years post-ACLR	Cross-sectional, Control & Contralateral knee	LANDING Jump down (26 cm, bilateral); Vertical jump (single leg)	Knee angles (sagittal plane) at initial contact, and moment of stabilization (vGRF=body weight). Vertical jump only: peak vGRF	Initial contact and at time of stabilisation
Vairo et al. 2008	28 (5M 9F / 5M 9F) 22.5 (4.1) 21.4 (10.7) months post-ACLR	Cross-sectional, Control & Contralateral knee	LANDING Vertical drop landing (30 cm, single leg)	Peak hip, knee and ankle angles (sagittal plane), hip and net summated extensor moments, peak vGRF	At landing (not specified up until when)
Bjornaraa et al. 2011	34 (17F / 17F) 25.3 (6.0) 4.6 (2.7) years post-ACLR	Cross-sectional, Control	CUTTING Cutting after catching a ball (40° to the right or left, single leg)	Absolute knee displacement vector (triplanar), peak and average absolute knee velocities, time to peak vGRF	From movement start to 1 second afterwards
Delahunt et al. 2012 (a)	29 (13F / 16F) 23.7 (3.1) 4.4 years post-ACLR	Cross-sectional, Control	LANDING Diagonal jump landing (single leg)	Triplanar hip and knee peak angles, time-averaged movement profiles	Initial contact to 200 ms post-initial contact
Delahunt et al. 2012 (b)	28 (14F / 14F) 23.0 (3.4) 4.4 years post-ACLR	Cross-sectional, Control	LANDING Drop vertical jump (35 cm, bilateral)	Triplanar hip and knee peak angles and time-averaged movement profiles	Initial contact to 200 ms post-initial contact
Tsai et al. 2012	20 (10 F / 10F) 25.3 (2.4) 36.2 (18.5) months post-ACLR	Cross-sectional, Control	LANDING Drop vertical jump (25 cm, single leg)	Peak tibiofemoral compressive, anterior shear forces, peak knee angle (sagittal plane)	Initial contact to peak knee flexion angle

Webster et al. 2012	26 (15M / 11M) 27.0 (5.9) 67 (8.4) weeks post-ACLR	Cross-sectional, Control & Contralateral knee	LANDING Before and during fatigue protocol: Vertical drop landing (30 cm, single leg)	Initial contact hip, knee, ankle angle (sagittal plane), peak hip and knee angles (triplanar), peak ankle dorsiflexion angle, peak hip and knee flexion moment, peak knee adduction moment (external)	Initial contact to 100 ms post-initial contact
Oberlander et al. 2013	10 (10 / 0) sex N/A N/A, 28.0 (7.0) 6 & 12 months post-ACLR	Longitudinal, Contralateral knee	LANDING Hop for distance (single leg)	Trunk flexion, hip, knee, ankle external moments (sagittal plane), GRF moment arm at hip, knee, ankle, GRF, centre of pressure, centre of margin of stability	Initial contact to the point where all landing energy absorbed (divided in 5 phases)
Bell et al. 2014	106 (5M 50F / 7M 44F) ACLR (PT): 19.2 (1.9); ACLR (GS): 19.2 (1.7) 3.0 (1.5) years post-ACLR	Cross-sectional, Control	SQUATTING Squat (squat depth: as deep as possible maintaining balance, single leg)	Hip, knee angles (triplanar), trunk angles (sagittal, frontal), knee extension, valgus, internal rotation moments, hip extension, abduction, external rotation moments, summated hip & knee extension moments (internal)	All variables assessed at peak knee flexion
Holsgaard-Larsen et al. 2014	48 (23M / 25M) 27.2 (7.5) 26.5 (6.6) months post-ACLR	Cross-sectional, Control	JUMPING Countermovement jump (bilateral and single leg); Hop for distance (single leg)	Knee range of motion (sagittal), knee peak flexion at the deepest point of countermovement, peak & mean knee moment (sagittal plane)	Braking-propulsion phase of the countermovement jump
Oberlander et al. 2014	30 (18M / 12M) 26.0 (6.0) 6 & 12 months post-ACLR	Longitudinal, Contralateral knee	LANDING Hop for distance (single leg)	Knee peak external rotation. Maximal anterior translation of shank with respect to thigh. Maximal knee extension & adduction external moments, maximal ankle dorsiflexion external moment	Initial contact to 200 ms post-initial contact
Ortiz et al. 2014	31 (15F / 16F) 28.5 (4.6) Between 12 months and 5 years post-ACLR	Cross-sectional, Control	JUMPING Vertical drop countermovement jump (60cm, bilateral; 40 cm single leg)	Maximum dynamic knee valgus	Initial contact from drop landing to take-off
Pollard et al. 2014	20 (10F / 10F) 23.2 (3.4) 42.4 (41.8) months post-ACLR	Cross-sectional, Control	CUTTING 5 m run followed by a side step cutting manoeuvre (45° from the original direction, single leg)	Joint angles couplings (intralimb) average variability: hip rotation/knee abduction-adduction, hip flexion-extension/knee abduction-adduction, hip rotation/ankle inversion eversion, knee abduction-adduction/knee flexion-extension, knee abduction-adduction/ankle inversion-eversion, knee abduction-	0 to 40% of stance phase

				adduction/knee rotation, & knee flexion-extension/knee rotation	
Goerger et al. 2015	70 (17M 14F / 20M 19F) Dominant leg ACLR: 21.4 (0.8), non-dominant leg ACLR: 21.5 (0.8) Dominant leg ACLR: 1.83 (0.57) years post-ACLR, non-dominant leg ACLR: 1.89 (0.67) years post-ACLR	Prospective, Control	LANDING Forward jump landing vertical jump (from 30 cm height to half of participant's height distance, bilateral)	Knee & hip angles at initial contact (frontal plane), knee and hip peak angles (frontal and transverse planes); knee peak extension, hip peak flexion moments (internal); peak anterior tibial shear force	Initial contact and landing peak values
Lessi and Serrão 2017	40 (13M 7F / 13M 7F) 25.1 (4.2) At least 12 months post-ACLR	Cross-sectional, Control & Contralateral knee	LANDING Pre and post-fatigue protocol: Drop vertical jump (31 cm, single leg)	Peak contralateral pelvic drop, trunk flexion & ipsilateral lean, peak hip flexion, adduction & internal rotation, peak knee flexion & abduction	Initial contact to peak knee flexion angle
Pozzi et al. 2017	40 (3M 17F / 3M 17F) 22.0 (2.5) 58.4 (33.2) months post-ACLR	Cross-sectional, Control	LANDING Vertical drop landing (30 cm, single leg)	Hip, knee, ankle angle moments (internal), power (sagittal plane), L5-S1 marker displacement, total support moment (= ankle + knee + hip internal moment), joint contribution to total support moment, vGRF	25-50-75-100% of landing (= initial contact to the lowest vertical position of the L5-S1 marker)
Lessi et al. 2018	14 (7M 7F / 0) 23.3 (4.0) M: 21.1 (6.8); F: 24.2 (9.5) months post-ACLR	Cross-sectional, Contralateral knee	LANDING Pre and post-fatigue protocol: Drop vertical jump (31 cm, single leg)	Peak contralateral pelvic drop, trunk flexion, ipsilateral lean, peak hip flexion, adduction & internal rotation, peak knee flexion & abduction angles	Initial contact (2nd toe marker velocity = zero) to peak knee flexion angle
Shimizu et al. 2018	47 (17M 14F / 10M 6F) 31.5 (1.4) 6 months & 3 years post-ACLR	Prospective, Control & Contralateral knee	LANDING Drop vertical jump (30 cm, bilateral)	Peak knee flexion angle & peak sagittal & knee joint moment (external) impulses (sagittal plane). Peak ipsilateral vGRF & peak contralateral vGRF during first 50% of stance phase	Initial contact to toe-off
Alanazi et al. 2020	36 (8M 10F / 8M 10F) 26.1 (4.0) 5.0 (3.3) years post-ACLR	Cross-sectional, Control	LANDING Forward jump (80% max. long jump, bilateral); Forward jump to head a football (suspended from ceiling,	Peak hip & knee flexion, peak ankle dorsiflexion angles. Peak hip & knee extension, peak ankle plantarflexion moments (internal). Peak plantar pressure	Initial contact to peak knee flexion angle

			distance equivalent to 40% max. long jump, bilateral)		
Chang et al. 2020	30 (18F / 12F) ACLR passing functional test battery (ACLR-pass): 19.8 (1.0), ACLR failing a functional test battery (ACLR-fail): 20 (1.5) ACLR-pass: 35.1 (12.6) months post-ACLR; ACLR-fail: 34.0 (14.7) months post-ACLR	Cross-sectional, Control	LANDING Forward jump landing vertical jump (from 30 cm height to distance 50% of participant's height, bilateral) CUTTING Forward jump cut (bilateral jump over a hurdle at 25% of participant's height distance, single leg landing at 50% participant's height distance from start, cut 60° direction)	Knee flexion & valgus angles, knee extension and varus moments (internal), anterior tibial shear force, vGRF	Initial contact to peak knee flexion angle
Shimizu et al. 2020	50 (20M 16F / 9M 5F) 31.5 (7.6) 6 months, 1, 2, 3 years post-ACLR	Longitudinal, Control & Contralateral knee	LANDING Drop vertical jump (30 cm, bilateral)	Peak knee flexion angle and moment (external), ipsilateral and contralateral vGRF (first 50% stance)	Initial contact to toe-off
Alanazi et al. 2021	36 (8M 10F / 8M 10F) 26.1 (4.0) 5.0 (3.3) years post-ACLR	Cross-sectional, Control	LANDING Pre & post-high-intensity exercise: Forward jump to head football (suspended from ceiling, 40% max. long jump distance away, bilateral)	Peak hip & knee flexion, & peak ankle dorsiflexion angles. Peak hip & knee extension, peak plantar flexion moments (external)	Initial contact to peak knee flexion angle
Kuntze et al. 2021	96 (32M 16F / 32M 16F) 23.0 (18 to 26) 3 to 10 years from ACL injury	Cross-sectional, Control	LANDING Drop vertical jump (31 cm, bilateral)	Hip, knee & ankle ROM (sagittal plane) from initial contact to maximum joint excursion, hip & knee angles (frontal plane) at initial contact, peak knee flexion angle. Knee angles (frontal plane) rate of change (35% to 90% of support phase). Peak vGRF, peak mediolateral GRF at landing (0%-40%) & push-off (60%-100%)	Initial contact to toe-off
Larson et al. 2021	21 (21F / 0) Landing with extended knee: 19.5 (1.2); symmetric landing knee angle between knees: 19.4 (1.07)	Cross-sectional, Contralateral knee	LANDING Crossover triple hop for distance (single leg)	Hip, knee, ankle peak joint angles and ROM (sagittal plane), hip & knee peak extension moment, ankle peak plantarflexion moment (sagittal plane) (not specified if internal or external), peak vGRF	Initial contact to 150 ms from landing (second landing only)

	1 to 3 years post-ACLR				
Webster et al. 2021	14 (11M 3F / 0) 26.0 (6.0) 6 to 12 months and 3 years post-ACLR	Longitudinal, Contralateral knee	LANDING Hop to subject's leg length distance (single leg)	Knee flexion at initial contact, peak knee flexion & varus angle, hip & ankle peak flexion moments, knee peak flexion and adduction moments (external).	Initial contact and peak values from initial contact
White et al. 2021	30 (9M 6F / 6M 9F) 21.0 (3.0) 4.0 (3.0) years post-ACLR	Cross-sectional, Control & Contralateral knee	JUMPING, LANDING Triple hop for distance (single leg)	Peak hip, knee & ankle power and moments (not reported if internal or external) (sagittal plane); hip, knee, ankle power patterns, % of stance when peak power occurred	Peak joint angles to toe-off (concentric phase); initial contact to peak joint angles (eccentric phase) (second landing only)
Nawasreh et al. 2022	41 (40M 1F / 0) No OA group: 25.4 (4.3); OA group: 28.7 (6.2) 2.1 (0.4) years post-ACLR	Cross-sectional, Contralateral knee	LANDING Drop vertical jump (20, 30, 40 cm, bilateral)	Energy absorption contribution of involved & uninvolved hips, knees, ankles and feet	Initial contact to peak knee flexion angle
Rostami and Thomas 2022	24 (12M / 12M) 24.5 (2.3) 23.3 (7.0) months from ACL injury	Cross-sectional, Control	LANDING Pre & post-fatigue protocol: Drop forward landing (30 cm to force plate 20 cm away, single leg)	Initial contact hip & knee flexion angle, peak hip flexion & abduction angle, peak knee flexion, adduction & internal rotation angle. Peak hip flexion moment, peak knee flexion & adduction moment (external), peak vGRF	Initial contact and peak values from initial contact
Scarneo-Miller et al. 2022	46 (14M 9F / 14M 9F) 21.0 (3.0) 55.7 (37.4) months post-ACLR	Cross-sectional, Control	CUTTING Jump cut (landing with the opposite foot from 30 cm box to half subject's height; 60° cut to contralateral side, single leg) SQUATTING Squat (depth: sat on chair, single leg)	Trunk, hip, knee peak joint angle and displacement (triplanar), medial knee displacement	Jump-cut: Initial contact to take-off / Squat: peak joint angles
Sritharan et al. 2022	98 (42M 24F / 15M 17F) 28.2 (6.3) 17 (3) months post-ACLR	Cross-sectional, Control	LANDING Hop to the subject’s greater trochanter-to-floor distance (single leg)	Pelvis, lumbar spine, hip & knee angles patterns (triplanar), ankle dorsiflexion pattern. Lumbar spine, hip & knee moments patterns (triplanar), ankle dorsiflexion moment (internal) patterns	Initial contact to peak knee flexion angle

Ishida et al. 2023	26 (26/0) 23.1 (3.5) 5.5 (3.8) years from ACLR	Cross-sectional, Contralateral knee	SQUATTING Squat (depth: thighs parallel to the floor, bilateral)	Hip, knee, ankle internal moments (sagittal plane) & lower limb support moment (= peak hip extension + peak knee extension + peak ankle plantarflexion) & support moment ratios, peak vGRF	Peak values
Warathang asame et al. 2023	20 (10M / 10M) 30.8 (5.6) >2 years post-ACLR	Cross-sectional, Control & Contralateral knee	CUTTING 5 m run followed by side step cutting (45° from original direction, opposite direction to tested limb, single leg)	Hip, knee peak angle & moments (not reported if internal or external), hip knee, shank, thigh angular velocities (triplanar)	Initial 40% of stance (= initial contact to toe-off)

SD: standard deviation; N/A: not available; ACL: anterior cruciate ligament; ACLR: anterior cruciate ligament reconstruction; PT: patellar tendon; GS: gracilis-semitendinous; ROM: range of motion; M: male; F: female; GRF: ground reaction force; vGRF: vertical ground reaction force. All results reported as mean and standard deviation.

7.3.3 Functional tasks

Functional tasks were organised into four categories, including jumping (divided into two component parts: 1) landing, from initial contact (IC) with the ground after the jump and 2) jumping, during both the braking and propulsive phases of the jump), squatting, and cutting, with seven studies (21.2%) assessed multiple tasks (Table 7.2).

During these tasks, knee angles and excursions, knee moments, lower limb total support moments, knee power, ground reaction forces (GRF), knee angular velocity, and joint angles couplings (intralimb) average variability were measured (Table 7.2). In addition, three studies compared biomechanical characteristics to knee OA changes, including radiological (n=1)(493) and cartilage changes (n=2)(484,487).

7.3.3.1 Landing tasks

75.8% of studies assessed landing biomechanics (total n=25; n=14 single leg(468,470,472-474,477,481-483,490-492,494,496); n=11 bilateral(466,471,480,484-489,493), n=1both(467)). Landing tasks included landing from various heights (ranging from 20 to 60 cm): drop vertical jump (bilateral: n=5(471,484,487,489,493); single leg: n=3(472,481,483)), vertical drop landing from a box (bilateral: n=1(466); single leg: n=3(468,473,482)), bilateral forward jump from a box landing vertical jump (n=2(480,486)), single leg drop forward from a box landing (n=1(494)), bilateral jump down from a box (n=1(467)); landing from level ground during single leg hop (once: n=4(474,477,491,496); triple: n=1(492)), forward jump (bilateral: n=1(485)), forward jump to head a ball (bilateral: n=2(485,488)), diagonal jump landing (single leg: n=1(470)), single leg crossover triple hop for distance (n=1(490)).

7.3.3.1.1 Bilateral landing.

Of the 11 studies assessing knee biomechanics of the injured knee during bilateral landings, 10 explored knee kinematics(466,467,471,480,484-489), with most utilising control participants. When comparing injured knees to healthy controls, most reported no differences in peak knee flexion angles (KFA) (466,468,484-488), flexion-extension range of movement (ROM) (466,489) and angular velocities (466). However, one reported injured knees demonstrated a slightly smaller peak KFA (mean difference <10 degrees) than controls (471), and another reduced KFA when stabilising from landing (i.e., the instant vertical GRF (vGRF) equalled body weight), but only in those with a patellar tendon ACL reconstruction (ACLR), not a semitendinosus ACLR (467).

There were contrasting findings regarding altered knee kinematics in the frontal plane. Delahunt (471) found ACLR knees had a significantly smaller peak knee varus angle than controls during drop vertical jumps

(about 5 degrees mean difference), with IC occurring with an abducted (i.e., valgus) knee rather than a varus. Kuntze (489) found ACLR knees had greater knee valgus during the support phase of a drop vertical jump. Other studies did not identify any differences between groups in peak varus and valgus angles (480,486) or IC valgus angle (480,486,489).

Only one study (480) looked at peak knee internal rotation angle, reporting significantly smaller angles in injured knees compared to controls during landing from a box after a forward jump.

Three studies compared sagittal knee angles between injured and contralateral knees. Peak KFA was significantly, but slightly, smaller on the ACLR side (mean 3 degrees) one year (484), but not two (487) or three years post-surgery (484,487). Rudroff (467) reported the stabilisation KFA was slightly greater in the operated knee (less than 5 degrees).

Seven studies explored knee kinetics, including joint powers and joint moments. Some studies found a significantly lower external peak knee flexion moment (KFM) (484,487) or internal peak KFM (466) and KFM impulse in ACLR knees compared to controls, over one year(484), one and two years (487), and three years (484,487) post-surgery when drop-landing from a box (30-60cm). ACLR knees had a significantly smaller first peak of power absorption than controls (466). Nevertheless, several studies did not identify any differences between ACLR and controls in internal peak knee extension moments (KEM) during landing from a forward jump (480,485,486,488) in internal KEM and varus moments at IC, or in internal peak knee varus moment, pre or post-fatiguing protocol (486).

Additionally, it was found that the operated knee had a lower external peak KFM to the contralateral knee at one year (487), but not at two (487) or three years post-surgery (484,487). Similarly, Chang (486)

found no differences between ACLR and contralateral side in IC or peak KEM and varus internal moments. Nawasreh (493) dichotomised athletes into those with and without knee OA two years post-ACLR and examined the joints' contribution to the total lower limbs absorbed energy at landing; OA knees had a significantly smaller contribution than the hip on the affected side, compared both to the contralateral side and the participants with ACLR but without knee OA.

7.3.3.1.2 Single leg landing.

Thirteen studies out of fifteen analysing single-leg landing biomechanics explored knee kinematics after a knee injury(467,468,470,472,473,477,481-483,490,491,494,496). Compared to controls, ACLR knees had a significantly smaller peak KFA (470,472), a smaller KFA at ground contact (496) and in the early stages of landing (470,496), after diagonal jump landing (470), drop vertical jump (472), single hop (496). In contrast, other studies observed a larger KFA at peak vGRF (468), and a greater increase in peak KFA pre- to post-fatiguing protocol (about 2 degrees) compared to controls (494). Several studies found no differences between injured knees and controls in peak (473) or IC (468,473) KFA, before a fatiguing protocol (494), before and after a fatiguing protocol (481) (when landing from 30-31cm). Additionally, Pozzi (482) found no differences in KFA at 25-50-75-100% of the landing phase during vertical drop landing between ACLR and controls (30cm).

The studies looking at discrete peak values in the frontal and transverse plane found no differences in peak knee abduction (i.e., valgus)(473,481), adduction (i.e., varus) (494), or internal rotation angles (473,494) between injured and control knees. However, ACLR subjects had a significantly more valgus knee (about 3 degrees) in the late landing stages of diagonal jump landing (470), and a larger knee

internal rotation during mid-stance of the landing from a hop (496) compared to controls.

Compared to the contralateral knee, there was no difference in knee flexion-extension ROM (490), peak KFA (490) or pre- or post-fatiguing protocol (481,483), in KFA at ground contact (468,473) or at peak vGRF (468). Only one study found that the ACLR knee had a significantly smaller peak KFA than the contralateral three years post-surgery (about 4 degrees difference) (491). In the frontal plane, the ACLR knee had no differences to the contralateral in peak knee valgus (473) before and after a fatiguing protocol (481), or in peak knee varus three years after surgery (491). However, when investigating female athletes specifically, the knee valgus was significantly larger in the ACLR knee compared to the contralateral, but this difference was not observed in male athletes participating in the same study (483). Additionally, while Webster (473) found no differences between injured and contralateral knees in peak internal rotation, Oberländer (477) observed a significantly greater external rotation on the affected side (about 3 degrees) one year after surgery.

Nine studies explored differences in knee kinetics (473,474,477,482,490-492,494,496). Between injured and control knees, no differences were found in external peak KFM (473) pre- or post-fatiguing exercises (494). Conversely, ACLR had a reduced internal knee extensor moment compared to controls (496). Similarly, Pozzi (482) saw the internal knee moment was about 25% smaller than controls at 75 and 100% of the landing phase, with the injured knee contributing significantly less to the overall lower limb total support moment than controls. Additionally, the ACLR knees had smaller power absorption at 50 and 75% of the landing phase (482).

When considering the frontal plane, no differences were found in peak knee external adduction moment (473), pre- or post-fatiguing exercises (494) between injured knees and controls. However, Sritharan (496)

found that ACLR knees had lower internal peak knee abduction moments and lower knee abduction moments in the mid-phase of landing.

When compared to the contralateral side, the injured knee had smaller external peak KFM (473,477) and smaller KFM from the second half of landing (474) one-year post-surgery. Similarly, Larson (490) saw participants landing with an 'extended knee' (i.e., ≥10% reduction in ROM during landing for ACLR side compared to contralateral) had a smaller peak KEM on the affected side, but this was not observed in those who landed with a similar ROM between sides. However, while White (492) found smaller moments in the eccentric phase of landing on the operated side four years post-ACLR, Webster (491) did not find differences in peak KFM three years post-surgery. Additionally, White (492) found that the landing peak knee power absorption and power absorption pattern were reduced on the ACLR side.

In the frontal plane, the ACLR knee displayed a smaller external KAM one (473,477) and three years post-surgery (491) than the contralateral side.

7.3.3.2 Jumping tasks

Three studies (9.1% of the total) analysed the biomechanics of jumping (single leg only: n=1(492); bilateral and single leg: n=2(476,478)). All studies utilised controls, with one also using the contralateral knee(478). Tasks included countermovement jump (CMJ) and vertical drop CMJ (bilateral and single leg(476,478)), and single leg triple hop(492).

7.3.3.2.1 Bilateral jump.

One study (476) looked at knee flexion ROM and the transition flexion angle (i.e., at the deepest point of the CMJ), reporting increasing left-right knee asymmetry post-ACLR compared to controls. Within the ACLR group, injured knee ROM and KFA were only 3.5 degrees and 1.4 degrees smaller than the contralateral, respectively (476). Another study found no differences in peak knee valgus between injured knees and controls (478). When observing peak and mean knee moments in the braking-propulsion phase of the CMJ, the left-right knee asymmetry was no different between ACLR and reference groups (476).

7.3.3.2.2 Single leg jump.

Holsgaard-Larsen (476) reported a significantly greater asymmetry in knee flexion ROM in ACLR people (about 9 degrees), but not in the knee transition angle, with Ortiz describing no altered peak knee valgus angle in ACLR knees compared to controls (478). Holsgaard-Larsen (476) found that left-right symmetry in peak and mean knee flexion-extension moments were similar, with White (492) observing significantly smaller peak KEM in ACLR knees in the concentric phase of triple hops for distance. These values were comparable between ACLR and contralateral knees. No differences in knee peak power generation were found between ACLR and controls or between knees in people post-ACLR (492).

7.3.3.3 Cutting tasks

Five studies (15.2% of the total) explored knee biomechanics during cutting manoeuvres(469,479,486,495,498). All studies utilised a case-control approach, with one also using the contralateral knee (498). Tasks comprised cutting from a bilateral, still stance, after catching a ball (469), side-step cutting after a run (479,498), and jumping forward from a height and cutting (486,495). The cutting angles varied between 40 (469), 45 (479,498) and 60 degrees (486,495) from original direction.

While Scarneo-Miller (495) saw ACLR knees had significantly smaller KFA at IC compared to controls (mean 7 degrees), other studies did not observe any differences from controls at this instant (486), or in peak KFA, valgus, internal rotation angles (498), or peak KFA both pre- and post-fatiguing exercises (486). Additionally, no differences between control and ACLR knees were found in the medial displacement of the knee in the frontal plane(495). Pollard (479) saw that ACLR knees had significantly more movement variability during coupled knee valgus-varus and hip rotations, knee valgus-varus and knee flexion-extension, knee valgus-varus and knee internal-external rotation. Bjornaraa (469) assessed cutting motion of ACLR and control knees, from a static bilateral stance, observing a significantly smaller knee displacement, peak and average linear velocity of the knee, both when the ACLR knee was the leading and push-off leg.

The two studies exploring knee kinetics found no differences between ACLR and control knees in knee extension, adduction or abduction peak and IC internal moments (486,498), before or after a fatiguing protocol (486), or in peak external rotation moments (498).

Compared to the contralateral, no differences in peak KFA, peak valgus angle, peak internal rotation angle, knee extension, abduction or external rotation moments were found in ACLR knees (498).

7.3.3.4 Squatting tasks

Three studies (9.1% of the total) assessed squatting (bilateral: n=1(497); single leg: n=2(475,495)), two using controls (475,495) and one contralateral(497). The squat depth was thighs were parallel to the floor (497), as deep as possible whilst maintaining balance (475), or as if sitting on a chair (495).

7.3.3.4.1 Bilateral squat.

The only study (497) comparing ACLR to contralateral knee reported differences in KEM and the knee contribution to the lower limb support moment. It was found that subjects with quadriceps strength symmetry < 90% (based on isokinetic testing) had a smaller maximum internal KEM, and smaller knee contribution to the lower limb support moment on the injured side; however, those with a high quadriceps strength symmetry (≥ 90%) did not show any differences (497).

7.3.3.4.2 Single leg squat.

Following ACLR, there were no differences to controls in knee valgus angle (475), knee medial linear displacement (495), or internal rotation angle (475). However, ACLR knees showed a significantly smaller knee flexion displacement (average 9 degrees) and peak KFA vs controls (475). Additionally, internal KEM was significantly greater in the control group (475).

7.3.4 Associations with Osteoarthritis

Nawasreh measured hip, knee and ankle power (angular velocity) from 20, 30, and 40cm heights, having defined their population using the Kellgren-Lawrence, KL, grade classification into OA or no-OA (using KL2+ as cutoff) (493). One-third (31.7%) of participants had OA changes two years from surgery (those with radiographic change waited longer on average for surgery, but the wide SD meant this was a non-significant group difference). Individuals with KL2+ had less knee and more hip involvement for absorbing landing energy compared to contralateral and non-OA knees (493). Within two studies, Shimizu assessed peak KFA, external knee joint impulse from the sagittal plane, and vGRF using bilateral drop jump from 30cm and compared the results to cartilage composition (as measured by T1rho and T2) on MRI (484,487). At one, two and three years, there was no difference in peak vGRF with lower peak KFA at one year (which became similar by two years) between injured and non-injured knees, but there were significant increases in T1rho and T2 relaxation time (cartilage degeneration) three years from ACL-R.

7.3.5 Risk of Bias

As a recap from Chapter Three, the NOS has three versions for cohort, case-control and cross-sectional studies. The ratings for cohort and case-control studies are good/high (6-9 points), fair (3-5) and poor (0-2); and cross-sectional studies very good (9-10 points), good (7-8), satisfactory (5-6) and unsatisfactory (0-4). Across the 33 included studies, three (9%) were rated very good/high, 12 were good (36%), 15 satisfactory/fair (45%) and three unsatisfactory (9%) (Table 7.3).

Table 7.3 Risk of bias assessment

Study	Selection	Comparability	Outcome/ exposure	**Overall**	Rating	Study	Selection	Comparability	Outcome/ exposure	**Overall**	**Rating**
Decker 2002*	2	2	3	7	Good	Lessi 2018*	1	0	3	4	Unsatisfactory
Rudroff 2003^	1	1	2	4	Fair	Shimizu 2018^	1	1	3	5	Fair
Vairo 2008^	3	1	2	6	Good	Alanazi 2020^	2	2	0	4	Fair
Bjornaraa 2011^	1	1	2	4	Fair	Chang 2020^	0	2	1	3	Fair
Delahunt 2012 (a)*	2	2	3	7	Good	Shimizu 2020^	1	1	1	3	Fair
Delahunt 2012 (b)*	2	2	3	7	Good	Alanazi 2021^	3	2	0	5	Fair
Tsai 2012^	3	2	2	7	Good	Kuntze 2021^	2	2	1	5	Fair
Webster 2012*	5	2	3	10	Very Good	Larson 2021*	0	2	1	3	Unsatisfactory
Oberlander 2013^	3	2	3	8	Good	Webster 2021#	2	2	2	6	Good
Bell 2014*	4	2	3	9	Very Good	White 2021^	1	2	0	3	Fair
Holsgaard - Larsen 2014*	2	2	3	7	Good	Nawasreh 2022*	3	2	0	5	Satisfactory
Oberlander 2014^	2	2	1	5	Fair	Rostami 2022^	4	2	3	9	High
Ortiz 2014^	4	2	1	7	Good	Scarneo-Miller 2022^	1	2	0	3	Fair
Pollard 2014^	2	1	1	4	Fair	Sritharan 2022^	3	2	1	6	Good
Goerger 2015^	4	2	2	8	Good	Ishida 2023*	1	1	0	2	Unsatisfactory
Lessi 2017*	3	2	1	6	Satisfactory	Warathangasame 2023^	1	2	3	6	Good
Pozzi 2017^	2	2	1	5	Fair						

7.4 Discussion

This systematic review aims to identify functional tasks utilised to assess knee kinematics and kinetic at least a year from injury, report any differences between injured knees, uninjured contralateral knees and control participants following this timepoint and highlight any associations with PTOA. Across 33 studies and 1251 participants, 737 of whom sustained a traumatic knee injury, the majority assessed landing biomechanics, with the remainder examining jumping, cut or squat tasks. As early as a year from injury, there is an altered and sustained biomechanical pattern present in individuals sustaining significant knee injuries, however, given the heterogeneity of the included studies, it is difficult to identify the optimal assessments to conduct.

Biomechanical factors can contribute to both the initial injury and subsequent re-injury risk (68). Included studies postulated that this subsequent risk could be due to poor neuromuscular control or sub-optimal compensatory mechanisms following knee injury. Reporting the results by task allows patterns to emerge in the data regarding optimal assessment batteries or functional movement assessments. However, across all tasks analysed, there were contrasting findings on whether differences existed between injured, uninjured or control knees. This could be due to the different methods adopted across studies or a reflection of the limited discriminatory powers of those assessments used. Additionally, it was not clear if the observed biomechanical alterations were a consequence of the injury or if they were present before it, and contributing to the injury itself. Numerous investigations found no differences between injured and control/contralateral knees in KFA or sagittal ROM, knee angles in the frontal or transverse planes, and other investigations observed knee flexion differences resolving by three years. Other reports found no changes in knee moments in the frontal, or sagittal plane, or, when present, these normalised by three

years from the injury. This review found that when statistical differences between the injured and uninjured knees were noted, these were of a small magnitude with an unclear clinical significance, and more research is needed to determine if the observed differences may be clinically relevant. There was evidence that injured knees displayed a reduction in KFA, larger KFA at peak vGRF, larger increase in KFA after a fatiguing protocol (though not consistently), increased knee valgus angle (sometimes only observed in females). Contrasting findings were observed for the transverse plane, where studies found an increased or decreased knee internal rotation. Several studies found smaller knee moments in the sagittal and frontal planes, and a reduction in power absorption. These findings highlight the need for longer-term surveillance and 'recovery' programmes following acute rehabilitation targeting biomechanical retraining, such as appropriate movement patterns, strength, conditioning, and neuromuscular control (9,60,61).

In addition, if a 'high-risk post-injury' compensatory pattern can be reliably and consistently screened, then it could be used to identify those at risk of subsequent PTOA. The implications of varus or valgus malalignment for subsequent medial or lateral compartment OA are well-reported(499). The requirement for any future robust biomechanical screening programme would involve multiple movements and tasks in several planes, as well as functional assessments, as it is likely that individual movements or single joint angles will not capture the entire picture (492,496). It is unlikely a single task would be sufficient for a screen as suggested by one study (495), but it was interesting to note the findings of another, which used a functional test battery but could not differentiate injured from non-injured participants (486).

Specifically, from a PTOA prediction point of view, three papers aimed to assess the ability of biomechanical assessments to correlate or predict changes associated with OA changes (484,487,493). These studies demonstrated that peak KFA and vGRF from a drop jump could

not identify those with OA at two or three years, but that those with OA had decreased knee and increased hip energy absorption. This compensatory pattern is an important secondary pathology to identify and manage, with two further studies stating that persistent knee kinematic differences are likely to contribute to OA (474,477). Whilst increased and inappropriate knee loading alters cartilage structure and composition, the inverse is also true(499,500). Decreased knee loading impacts the health and integrity of the cartilage, thereby negatively influencing cartilage synthesis/catabolism and increasing the risk of PTOA (21,68); therefore, early recognition may prevent further structural and compositional joint change(4).

Work is required to understand the optimal biomechanical assessments to understand to identify pre-clinical OA or the differences between injured and uninjured knees that could potentially contribute to the development of OA following a knee injury. There was significant heterogeneity in methodology, including joints, tests, angles, phases, and box heights. Within the same functional task, there were substantial differences, such as drop jump height (between 20 and 60 cm), hop tests performed or assessment in one, two or three planes, with one study remarking that non-sagittal plane biomechanics were required to improve assessment (496). This is common across other areas of OA research (4,5), with work now underway to develop core outcome sets (382,501). The biomechanics field should adopt a similar approach, with early work by Osteoarthritis Research Society International underway (460).

In addition to core outcome sets, other future research directions must be considered. This systematic review has deliberately focused on the knee. However, work must look ‘beyond the knee’, with biomechanics likely influencing hip, ankle and spine OA (1,26). Future studies must also look ‘beyond the lab’, with portable technologies (such as force plates) able to ‘close the sophistication gap’ between high-end technology only available in specialist laboratories and patients who

could be assessed within a community healthcare setting. Finally, the use of machine learning to identify patterns, as introduced by Sritharan (496), might offer the potential for population-level assessments when study homogeneity has improved (460).

The key limitation of this study is the lack of a meta-analysis, which was due to the methodological discrepancies across studies. The vast heterogeneity of the included studies, from sample size to methodology to time from injury, meant that only broad conclusions could be made. Future data synthesis must consider heterogeneity and narrow search criteria accordingly. The time between search and publication was extended due to the time required for meaningful analysis and expert peer review. Finally, whilst RoB was performed to assess bias, the grading of the evidence wasn't performed, which was a deliberate choice given the 'breadth over depth' nature of this review. Strengths of the study included the number of studies and participants, the use of an expert research librarian, and multiple independent reviewers for screening, data extraction and RoB assessment.

7.5 Conclusion

This systematic review has demonstrated that changes in biomechanics are present as soon as a year post-injury and can persist for years afterwards. These aberrant biomechanical profiles could lead to re-injury and PTOA. There is significant heterogeneity in the field, and consensus needs to be established regarding a core outcome set of biomechanical assessments that should expand beyond the knee and the lab. Excitingly however, there is a potential for these biomechanical biomarkers to be combined with those described within the molecular (and radiological) research fields.

Chapter 8 : <u>What does the future hold?</u>

During Chapter One, I outlined the epidemiology of osteoarthritis (OA), highlighting that it is extremely common, on the rise, and has a substantial burden of symptoms and associated disability, before briefly introducing some of the classifications and categories, underlying pathophysiology and associated risk factors. The need for further research in the military population was discussed, given the implications of post-traumatic OA (PTOA) in this unique population, and the use of biological markers (biomarkers) to better characterise the pathophysiological process, create phenotypic categories, and develop effective pharmacological and non-pharmacological interventions was introduced. The aims and hypotheses of this PhD were summarised in Chapter Two prior to outlining the population of interest.

Chapter Three aimed to synthesise the evidence surrounding biomarkers, both wet (molecular) and dry (imaging) in the newly defined, chronic phase of PTOA (a year or more after injury). It gave indications about possible biomarkers of value, including molecular measures linked to pain and future, and bone changes linked to cartilage changes. Unfortunately, due to the methodological heterogeneity, these findings could not be meta-analysed, and consequently, the evidence provided was weak.

In Chapter Four, I sought to perform a cross-sectional analysis on a panel of pre-selected candidate serum biomarkers of OA representing a range of pathophysiological processes for their ability to differentiate those exposed to combat-trauma, those with radiographic OA (rOA), and those reporting knee pain within the population of the Armed Services Rehabilitation Outcome (ADVANCE) study. Subsequent analysis sought to understand their value in differentiating OA aetiology, painful rOA, injury patterns and individual radiographic components of knee OA. The key findings of this chapter were that cartilage oligomeric matrix protein (COMP) was elevated in those with a combat-injury, but

decreased in those with lower-limb loss, suggestive of the role of COMP in injury-healing and fibrosis and also the importance of the presence of healthy cartilage for the production of COMP; adipokines were statistically significant in those reporting pain and painful OA, suggesting a potential use for phenotyping; and there was no difference between those with idiopathic OA (iOA) and PTOA, which suggests a common mechanism and potential applicability of iOA study outcomes for individuals with PTOA (and vice versa). There were also weak correlations between leptin and pain, joint space narrowing (JSN), sclerosis and six-minute walk-test distance (6WMD), adiponectin to pain, OP and 6MWD, tumour necrosis factor-alpha (TNF-α) to 6MWD and N-propeptide of collagen IIA (PIIANP) to rOA – these findings were of uncertain clinical significance but do provide hypotheses for further analysis.

Chapter Five builds on this, using Follow-up 1 data from ADVANCE, with the aim to understand both the rate of incidence and progression of knee rOA and pain over 3 years, and the predictive nature of the candidate serum biomarkers for rOA, pain and function. Interestingly, there was no significant difference between the exposure groups from an epidemiological perspective, although there was a divergence within the lower-limb loss subgroup. Previous literature has postulated an increased risk of OA in the years immediately following injury, so these findings suggest that, within the ADVANCE cohort, this potential increased risk and ‘clinical window of maximal intervention’ has closed, with any future post-injury secondary preventative interventions required well before eight years. This finding also demonstrates a different pathophysiological mechanism in those with lower-limb loss (likely ongoing mechanoinflammation, secondary to altered biomechanics). From a molecular perspective, adiponectin was associated with incident pain and leptin to Follow-up 6MWD in the cohort with the lower-limb loss group removed, and leptin to rOA progression, and COMP to Follow-up 6MWD, and both weakly to international physical activity questionnaire (IPAQ), in those with lower-

limb loss. Disappointingly, the molecular biomarkers had no association with either rOA incidence or progression. The lack of correlation to rOA might be due to the predominance of early rOA, which might suggest that the optimal biomarkers might be found within or nearer the joint in question and are not widely represented in the systemic circulation.

As a result, Chapter Six aimed to propose a new methodology to help improve the use of molecular biomarkers in early OA. If some of the challenges relating to the serum biomarker performance could be due to the early-stage OA pathological signal remaining local to the joint and not widely within the systemic circulation, then any successful biomarkers need to reflect this. SF biomarker assessment is the gold-standard method of assessing the 'internal condition' of the joint, however, this is a technically challenging and invasive procedure, which is only typically performed in the clinical setting by specialists (including rheumatological and orthopaedic healthcare professionals). Therefore, this chapter aimed to develop a novel method of venous sampling near the knee joint to improve the collection of systemic biomarkers and reports the methodology and results from paired sampling from the systemic circulation. Furthermore, this chapter also assesses the impact of physical activity on the serum concentrations of biomarkers, which might also improve sensitivity of results. This pilot study showed that some, leptin and interleukin, (IL)-6, were found in different concentrations nearer to the joint and others, IL-1β, COMP and cross-linked telopeptide of type II collagen (CTX-II), were also influenced by physical activity, which might improve the ability to identify early joint changes, although, clearly, this work is only a pilot.

Chapter Seven aimed to also fill a research gap that evolved through the period of the PhD – namely, what other assessments should we be considering when developing predictive models in the future. There is a key role of biomechanics in the development of OA, and therefore, utilising these assessments is important. This chapter showed there was a distant biomechanical pattern observed within the participants

who had sustained injuries, including a reduction in knee flexion angle (KFA) and a larger KFA at peak vertical ground reaction force GRF and following a fatiguing protocol or an increased knee valgus angle. However, similar to Chapter Three, methodological differences prevented direct comparison between studies and precluded a meta-analysis, thus weakening the evidence, further amplified by contrasting findings observed in different planes of assessment.

In this final chapter, I plan to contextualise these results and describe their relevance and potential impact in two ways, initially by describing the research implications and future directions of my findings. Finally, to conclude this thesis, I plan to outline the clinical impact of the work and describe what could and should be established for those with, or at risk of, OA within the military.

8.1 What is the current evidence base for the use of biomarkers in the chronic phase of PTOA?

This thesis aims to articulate the utility of biomarkers, in particular molecular ones, for the identification or prediction of PTOA. It has focussed on the 'chronic phase', namely a year or more after injury, due to the clinical implications of this – either the completion of care pathways or the re-attendance of individuals with recurrent or chronic joint pain after an injury, as outlined in Chapter Three. As suggested in Section 1.5.2, the use of biomarkers during care pathway completion might be able to identify aberrant healing or ongoing inflammation, and therefore, stratify those who require intervention. The availability of data from the Armed Services Trauma Rehabilitation Outcome (ADVANCE) study, approximately 8 years from index injury, has provided a rich cohort to explore the utility of serum biomarkers supported by numerous reviews of evidence to understand how molecular, imaging and biomechanical biomarkers can be used to identify or predict PTOA.

However, it is not without its faults. The use of serum biomarkers to identify joint-specific OA changes is challenging, as seen by the as-yet inability to translate these markers into clinical practice. Unlike other systemic markers which demonstrate joint-organ dysfunction, such as the use of high-sensitivity Troponin to detect myocardial infarction, the use of OA molecular biomarkers in a systemic form (such as blood or urine) can be influenced by multi-joint disease, unrelated disease states, or even preceding exercise (as seen in Chapter Six, Section 6.3.3). Multi-site OA is likely to influence the concentrations of the biomarkers in question, however, is often overlooked when one only considers a single joint in isolation. This is likely to be less of an issue for PTOA, as the predominance of young and otherwise healthy individuals is likely to reduce the prevalence of multi-site OA, however, it is a consideration for idiopathic OA presenting in later life. This challenge is amplified by the methodological differences in biomarker studies (as highlighted in Chapter Three, Section 3.1.4), but also the

lack of robust and repeatable normative values to understand ‘what normal looks like’. Until there is clarity on what the biomarkers are measuring, how specific and sensitive they are to the joint in question, and the optimal way to measure them, the use of systemic molecular biomarkers is likely to be limited. Furthermore, given the dynamic pathophysiological process occurring in OA, then the use of a single snap-shot of time can create a false picture, and thus, multiple analyses over time might be a better way to understand how OA is developing and progressing.

A further difficulty is one that this thesis has likely fallen prey to. It is entirely possible, and indeed suggested in Chapter Five (Section 5.4.2), that those biomarkers felt to have value in early disease might not have value in more severe disease. To extend this further, it is possible that any changes that could have been identified during the pre-radiographic, pre-clinical initiation of OA are different to those found within established disease, which signify progression. I had hoped to mitigate this by using the methods outlined in Chapter Five (Section 5.2.2.3) to dichotomise groups by the presence or absence of OA changes at Baseline, to stratify the incidence and progression analyses. The differences noted in Figure 5.4 between those with incident and progressive rOA would support that. However, by aiming to identify any OA change, and thus combining Kellgren-Lawrence (KL) grade 1 or more, it is possible that I have combined these two categories and thus am unable to draw differences between them. I chose KL ≥1 as using a more severe category would not enable identification of early OA change, but perhaps it would have been more prudent to have KL 0, KL1, and KL ≥2 to further separate this question.

This leads into the next major limitation of this thesis, the use of radiographs to detect OA. A radiograph is a 2-dimensional representation of a 3-dimensional object (the joint), and thus is limited in its ability to identify subtle change and demonstrate the entirety of the joint in question. This is amplified by the use of a scoring system

developed in the 1950's (279), although the use of the more recent Osteoarthritis Research Society International atlas did not vastly improve the analysis (283). This speaks to the limitations of x-ray, which while very accessible, low-cost and minimally invasive, does not offer the same diagnostic ability of magnetic resonance imaging (MRI) (47). Whilst expensive and more difficult to obtain, MRI offers a wealth of different metrics, including specific tissue type or composite/multi-type scoring scales (as outlined in Chapter One, Section 1.4.2), offering the ability to detect very early and nuanced changes not visible on a radiograph. Over time, I suspect we will see a change in paradigm, with MRI imaging preferred over all others, and MRI-based imaging biomarkers offering diagnostic and predictive value (however, we are not there yet, as Chapter Three concludes, Section 3.1.4).

Strengths of this work are that it is the largest single molecular biomarker study, with a very unique cohort understudied in OA research. The participants are extremely well-understood, with frequency-matched comparisons on many measures, and this enables confidence in the results. The data that ADVANCE has already collected has many potential avenues to explore, and as the cohort ages, many new discoveries will be made. As already referred to, a single timepoint can create a false picture, so repeated molecular analyses are required within this cohort. These analyses should consider multiple sources, including urine, to assess the best performing biomarkers seen in other cohorts (52). The injury patterns are unique, providing new information and discovery, however, this can also be seen as a weakness given challenge of validation, as is the exclusion of any female participants. Work is required to identify equivalent populations with female participants to assess if the findings are sex-dependent or -influenced. Furthermore, the status of individuals prior to their deployments, and the gap between injury and Baseline, have uncertainties which can't be fully filled by self-reports and electronic health records.

8.2 Are any of the validated serum biomarkers associated with radiographic osteoarthritis, knee pain or function?

In the introduction of this chapter, I recapped the main findings of the cross-sectional analysis undertaken and reported in Chapter Four. No serum biomarkers were significantly different in those with rOA, but those with knee pain had higher leptin and lower adiponectin. Both adipokines correlated with pain, with leptin also correlated to JSN, sclerosis, adiponectin to osteophytes, and PIIANP to rOA, with TNF-α, leptin and adiponectin also all correlated to 6MWD. The use of other measures therefore is required, including alternative modalities such as radiological and biomechanical. However, there are more options within the serum itself, some simple and some more complex.

8.2.1 Easily accessible blood tests

In order to understand risk, one must consider adjacent factors, and earlier in the thesis (Section 1.3), I outlined the role of low-grade inflammation and metabolic factors in the development of OA. Therefore, would it be possible to use widely-used blood tests to identify those at risk of rOA? During ADVANCE study visits, these were collected, including serum cholesterol, glucose, high-sensitivity c-reactive protein (hsCRP) and glycated haemoglobin (HbA1c) (260).

In those with rOA (KL≥1) versus those without, serum cholesterol levels did not differ (5.02±1.19 v 4.98±0.96, p=0.62), nor did hsCRP (1.79±2.31 v 1.78±4.42, p=0.96), but serum glucose (5.14±1.70 v 4.94±0.74, p=0.01) and HbA1c (35.45±10.37 v 34.48±4.84, p=0.043) did. These latter two results do provide further evidence of the metabolic contribution toward OA development (58,187,459), especially as the differences remain (glucose 5.11±1.58 v 4.95±0.76, p=0.045 and HbA1c 35.54±10.34 v 34.52±4.98, p=0.047) when those who have sustained an lower-limb amputation are removed, given the body compositional changes that occur for those individuals (249,418).

These widely-used blood tests did not differ between those with and without pain; cholesterol 5.03±1.08 v 4.97±0.98, p=0.38, hsCRP 1.84±2.34 v 1.74±4.75, p=0.69, glucose 5.05±1.18 v 4.95±0.96, p=0.12, and HbA1c 34.87±5.55 v 34.57±7.11, p=0.46. This is a surprising result, given that pain can be modulated by so many factors, including body composition and adipose tissue, and highlights the challenges associated with the prediction and mechanisms of pain (114,187,390). This could signal alternative, non-metabolic or inflammatory pain mechanisms, perhaps such as peripheral or central sensitisation as postulated in Chapter Five, or perhaps could be related to the definition of pain used in this analysis.

Correlation analysis between these widely-used blood tests and the panel of candidate serum biomarkers demonstrated correlations between the majority of these, as demonstrated in Figure 8.1. Unsurprisingly, there were strong correlations between the inflammatory markers and adipokines to hsCRP given its role as an inflammatory marker (101,368), and again between the adipokines to glucose and HbA1c (114,118). The associations between the cartilage turnover markers to these routine markers is a possible further indicator of the link between OA pathophysiology and metabolic processes. When the widely-used blood tests were correlated to the outcomes of rOA, pain and 6MWD (Figure 8.2), only hsCRP were associated with 6MWD. It was interesting to note the lack of association and prediction between the inflammatory biomarkers and rOA in Chapter Four and Five, however, this new finding reinforces the role of inflammation in the presentation and impact of OA illness in individuals.

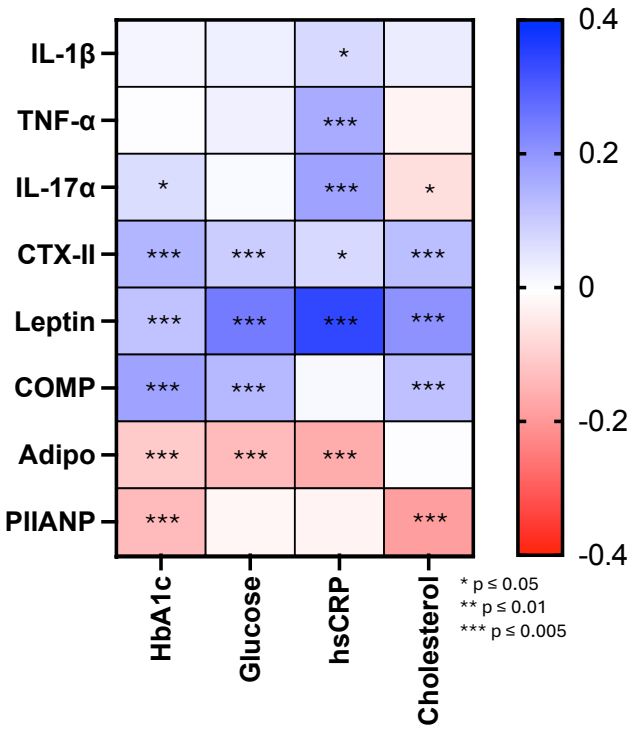

Figure 8.1 Correlation analysis between the panel of candidate serum biomarkers and widely-used blood tests

IL – Interleukin, TNF – Tumour Necrosis Factor, CTX-II – C-terminal cross-linked telopeptide of type II collagen, COMP – cartilage oligomeric matrix protein, PIIANP – N-propeptide of collagen IIA, Adipo – Adiponectin, HbA1c: Glycated haemoglobin, hsCRP: high sensitivity c-reactive protein

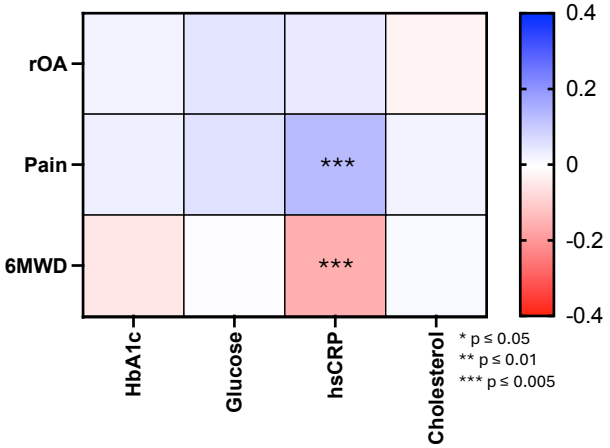

Figure 8.2 Correlations between knee radiographic osteoarthritis, knee pain and functional outcomes and widely-used blood tests

rOA – radiographic osteoarthritis, 6MWD – Six-minute walk-test distance, HbA1c: Glycated haemoglobin, hsCRP: high sensitivity c-reactive protein

8.2.2 Advanced blood tests

A more complex way to analyse further analyse the existing serum is through mass spectroscopy or aptamer binding to examine the 'omic' signature of individuals (502-504). This alternative method of biomarker detection, involving the analysis of thousands of molecules by measurement of the mass to charge ratio or aptamer affinity to binding sites, enables a deeper insight into the underlying pathways leading to disease process (296), and has been developed for OA-specific research over the last two decades, predominately to enhance drug-discovery trials (504-506). The ambition of this novel field is to find predictive molecules or molecular classes which would improve diagnosis, prognosis or therapeutic targeting (296). Using this approach, a panel as small as eight peptides offered the ability to discriminate OA from non-OA cases (506) and slightly larger one (including 11 peptides) could predict progression (507).

Alongside the analysis performed by Affinity Biomarker Labs (ABL) on the panel of eight serum biomarkers analysed in Chapter Four and Five, additional analysis was performed by SomaLogic, using the SomaScan 7k Assay (SomaLogic, Boulder, Colorado, USA). This panel, which provided over 7000 aptamers per individual, offers the opportunity to understand in more detail what has occurred at the molecular level. Within the BioMilOA study group, these aptamers have undergone initial cross-sectional analysis, led by the hypotheses generated within Chapters Four and Five, and involved untargeted and targeted analysis, with the latter focussing on the specific biomarkers which I identified from the literature (86,508-514). I will briefly summarise the key findings and implications of these analyses.

Initially, a comparison was performed between those with and without rOA (KL≥1), which showed 22 aptamers corresponding to 18 proteins were found to be downregulated; neurotrimin (NTRI); ecto-ADP-ribosyltransferase 3 (NAR3); opioid-binding protein/cell adhesion

molecule (OBCAM); netrin receptor UNC5D (UNC5H4); cell adhesion molecule 2 (Nectin-like protein 3); heparan-sulfate 6-O-sulfotransferase 3 (H6ST3); seizure 6-like protein (SEZ6L); myosin light polypeptide 4 (MYL4); limbic system-associated membrane protein (LSAMP); alpha-2-macroglobulin (a2-Macroglobulin); secretoglobin family 3A member 1 (Secretoglobin family 3A member 1); ectonucleotide pyrophosphatase/phosphodiesterase family member 5 (ENPP5); sodium/potassium-transporting ATPase subunit beta-2 (AT1B2); protein SCO1 homolog mitochondrial (SCO1); sex hormone-binding globulin (SHBG); apolipoprotein D (Apo D); potassium voltage gated channel subfamily E regulatory beta subunit 5 (KCE1L) and receptor-type tyrosine-protein phosphatase delta (PTPRD). When those with lower-limb loss were removed, only MYL4, UNC5H4 and Secretoglobin family 3A member 1 remained significantly different once adjusted for age, socio-economic status (SES), time since injury and ethnicity. When this was repeated in the lower-limb loss subgroup, there were no significant protein differences. A sensitivity analysis was performed, using KL≥2 as the cut-off, showing two proteins, UNC5H4 and LSAMP, were found to be decreased; however, once the lower-limb loss subgroup was excluded, these were no longer significantly different.

Following this, an analysis was undertaken between those with and without knee pain with the lower-limb loss subgroup excluded. This was in two phases, initially using targeted OA biomarkers identified from the literature and then in adipose tissue-related biomarkers, given the associations seen between adipokines and pain in Chapter Four and Five. During the OA-specific biomarker analysis, 10 proteins had increased concentrations, and two decreased, in those with knee pain compared to those without. These aptamers were Wnt-5a; adrenomedullin; activin A; serum amyloid P-component (SAP); leptin; A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS-4); interleukin (IL)-2: lipopolysaccharide-binding protein (LBP); R-spondin-2 and insulin, with adiponectin and SHBG both decreased in those describing knee pain. During the adipose tissue

analysis, two were higher; leptin and fatty acid binding protein for adipocyte (FABPA), and three lower; adiponectin, peroxisome proliferator-activated receptor gamma (PPARg) and CMRF35-like module 9 (CLM9), in those with knee pain, however, only adiponectin remained significant after adjustment for whole body fat. These findings were not replicated in the lower-limb loss subgroup, with no significant differences seen between those with and without rOA or knee pain.

Within the 7K assay platform, there were aptamers which corresponded to six of the validated biomarkers tested by ABL and analysed in the previous chapters. The correlation of these aptamers to the biomarkers is found below in Figure 8.3. Similar to the result in Chapter Four and Five, and described above, none were associated with rOA or pain. Whilst this result remains somewhat disappointing, it provides external validation of the findings previously described within this thesis.

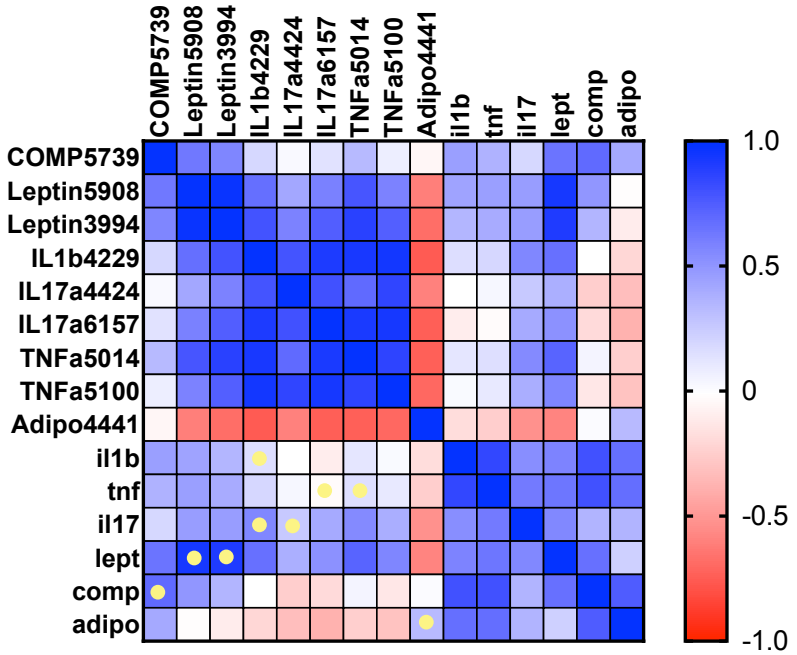

Figure 8.3 Correlation between validated biomarkers from Affinity Biomarker Labs and aptamers associated with the same protein performed by SomaLogic.
Yellow dot represents statistical significance (p<0.05)

Overall, this initial analysis of the proteome of ADVANCE individuals was unable to identify proteins associated with rOA, but did demonstrate that there was a proteomic fingerprint of pain. This

fingerprint confirmed that both leptin and adiponectin have a strong relationship to pain and painful rOA, and therefore could be utilised for phenotyping a ‘painful OA’ group, as well as targeting specific pathways (for example, anti-ADAMTS) (118,384,385). In addition, other proteins were seen to be upregulated in pain, including PPARg, which has previously seen to be involved in the inflammatory response and with cartilage degeneration (515), and CLM9, which is also implicated in the inflammatory response (516).

Further detailed cross-sectional analysis is planned, as is longitudinal analysis, to see if any aptamers might offer any predictive value for identifying changes in rOA or pain. It is possible, as previously mentioned, that given the vast majority (88%) of OA present in the ADVANCE cohort is KL grade 1 or 2, then the pathological signal is simply not within the systemic circulation yet, and perhaps proteomic analysis performed in samples collected using the methods outlined in Chapter Six might improve sensitivity. However, there is a promise for the role of these biomarkers in phenotyping, especially of a ‘painful OA’ category.

8.3 Do any of the validated biomarkers predict changes over three years in radiographic osteoarthritis, pain or function?

During Chapter Five, I aimed to develop predictive models, using a range of potential predictive variables from the Baseline ADVANCE study visit. For rOA, the individual factors with the highest odds ratio for incidence were age and JSN (unsurprisingly), rOA progression were shorter time from injury, JSN and knee pain frequency. For pain, the best predictors for incidence were body mass, KOOS Symptom score and knee pain frequency, with KOOS Symptom also strongest for pain progression. Overall, Baseline function best predicted Follow-up function, with KOOS Pain and body mass and SES also offering some predictive value. Overall, whilst associations were seen between the biomarkers, only leptin offered predictive value for future decline in function. The performance of the biomarkers should be contextualised, in that, unlike other cohort studies investigating OA, such as OA Initiative (OAI, participant age 45-79) or Multicenter OA Study (MOST, age 50-79), the ADVANCE population is far younger and therefore less likely to have severe or bilateral OA (of the 1145 participants at Baseline, only 30 individuals had KL 3 or 4, and only 27 had bilateral OA), so the presence of early signals are helpful and are worth monitoring as the cohort develops. It is also important to note, that alongside the probable lack of systemic signal, this cohort are not yet at risk of compensatory mechanisms (such as changes in gait or widespread pain), so a broader approach might be required.

8.3.1 Phenotyping and prediction

With all the above in mind, there is further work to do to understand the optimal variables for classification (or phenotyping) and for prediction. To develop this work further, I am planning future work to explore this further within the ADVANCE cohort.

A future study, provisionally-named Osteoarthritis in ADVANCE Longitudinal assEssment of biomArkers and Proteomics (OA LEAP), would exploit the first Follow-up visit of ADVANCE by utilising saved serum samples and other pre-collected variables, such as knee and hip radiographs, pain and disability related patient-reported outcome measures (PROMs), with functional testing. It could aim to understand how these change and relate to the underlying disease mechanisms identifiable in the molecular analysis, using the saved serum to repeat the same biomarker panel and proteomic analysis performed at Baseline (as reported in Chapter Four, Five and Eight). In addition, it could seek to undertake a focussed sub-group biomechanical and imaging assessment to include those with a lower-limb amputation, a specific knee injury and an increased number of matched controls to enhance understanding of the biomechanical and structural changes associated with PTOA, both from lower limb amputation and knee injury (and thus build on the results of Chapter Three and Seven). By pairing these assessments with a cohort-wide molecular analysis, specific biomarkers can be assessed for their value in recognising features of radiographic and MRI-related OA, functional changes on biomechanical assessment and the presence of knee pain. Furthermore, a longitudinal proteomic analysis will enhance our knowledge of the underlying molecular picture and mechanisms leading to OA, pain and disability. This will enhance the ability to phenotype any distinct sub-groups which may have subtle differences in pathology and/or response to treatment and mitigate the single point of time ‘snapshot’ issue previously outlined in Section 8.1.

To build upon the breadth of work covered in this thesis, the specific aims of OA LEAP would be to:

1. Compare serum biomarkers, knee and hip radiographs and PROMS cross-sectionally and longitudinally over time; 8 (Baseline), 11 (Follow-Up 1), and 14 (Follow-Up 2) years post injury/deployment, and as predictor variables in all ADVANCE participants with and without OA
2. Use proteomics to map the mechanistic pathways in all ADVANCE participants with and without OA
3. In a sub-set of the ADVANCE cohort containing participants who sustained (i) a knee injury, (ii) a lower-limb amputation, or (iii) no exposure to combat trauma, we will phenotype injury pattern in relation to OA using serum biomarkers, knee and hip MRI, gait biomechanics, knee and hip radiographs and PROMS
4. To develop a combined molecular, biomechanical and imaging predictive tool that can be tested in future prospective follow-ups of the on-going ADVANCE study.

To accurately be able to predict the risk of this complicated pathological process, a combined model with multiple variables might be required, rather than a single biomarker value. There is a precedent for this, with the FRAX tool widely used in clinical practice for the assessment of risk of fracture in an individual with low bone mineral density (BMD). A FRAX-like model might be possible for OA, such as the potentially-named ‘Future OA Risk score’ (FOAR-score), perhaps drawn from demographic and injury variables, PROMS, and supplemented by molecular, imaging and biomechanical biomarkers when available. The beauty of FRAX is its simplicity, so trying to make any potential ‘FOAR-score’ tool equally simple would be key to its success. Similar to BMD values improving the accuracy of the FRAX risk score, any biomarkers could improve accuracy, but as FRAX can generate an acceptable score without them, so should FOAR-score aspire to. A machine learning approach has been recently reported in the literature (517), including the accessible online tool, but requires a range of variables

not routinely collected in our population. I hope that the proposed OA LEAP study would be able to provide the results to enable the initial creation of a novel composite risk tool.

Of course, key to any screening or early identification programme, is the questions about what happens next. If a tool can be created, perhaps by harnessing the use of big data analytics, then a simple traffic light score could guide clinicians to their next steps. For the UK Defence Medical Services (DMS), a green score could trigger patient education and self-management resources, an amber could lead to an outpatient appointment or virtual programme to introduce a range of holistic measures and a red score could lead to a residential rehabilitation programme, aiming to thoroughly educate, demonstrate and empower individuals with the options they have to manage their condition, resources they can access, and healthcare professionals they can reach out to. There is precedent for this within UK Defence Rehabilitation, with the ASPIRE course designed for military personnel with axial spondyloarthropathy, with a similar approach also advocated for pre-arthritic hip pain management (518). Using ASPIRE as a model can help demonstrate the value of an equivalent course for OA to the organisation, clinicians and potential patients. However, any programme, virtual or residential, requires an understanding of what interventions could be offered and what their evidence is. The final section in this thesis will outline some clinical interventions that could already be utilised and some areas in which further work is required.

8.3.2 Validation

The ADVANCE cohort will further enhance our knowledge. However, this cohort is a very specific and, while over its time course, it will be able to hopefully answer some critical questions regarding the long-term outcomes following combat injury, the applicability of the findings of this thesis also need to be validated in a non-combat injury scenario. It is clear that there is a need for better research for military personnel who have or are at risk of OA, especially within the UK Armed Forces, addressing some of the limitations raised in Section 8.1. A new cohort should be developed, to assess if the findings of ADVANCE are translatable to a different injury type (such as anterior cruciate ligament, ACL or meniscus) and at an earlier timepoint in their patient journey.

This proposed future cohort study would allow the utility of each biomarker discussed across the previous pages and chapters (molecular, imaging, biomechanical) to be explored in a population which more accurately currently represent the injury burden of the Armed Forces, ideally over a period of years. A proposed study outline is found at Figure 8.4 (Surveillance and Targeted interventions for OA in Military Personnel, STOMP), demonstrating the outcome variables of interest and proposed follow up times. If the follow-up period extends to the five-years suggested by UK BioBank, then the proposed 'clinical window of maximal intervention' can be better demarcated. I am not oblivious to the challenges of an extended follow-up, and there would be definite logistic challenges, especially regarding cost, sample size and participant retention. Consequently, primary outcomes should be set earlier, perhaps at a two-year follow up point.

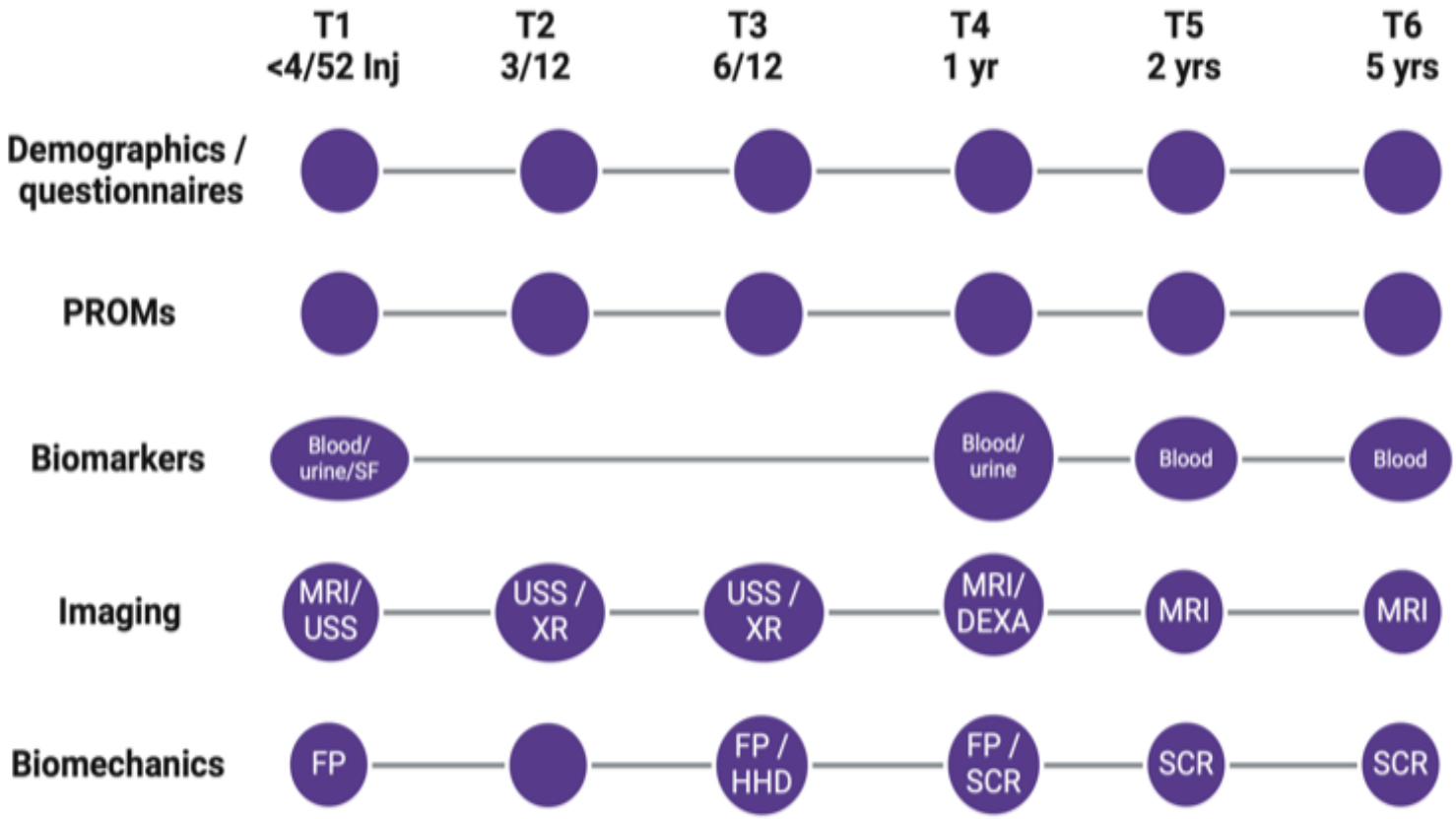

Figure 8.4 A proposed study outline for the Surveillance and Targeted interventions for Osteoarthritis in Military Personnel study
PROMs: Patient reported outcome measures, SF: synovial fluid, MRI: Magnetic resonance imaging, USS: Ultrasound scan, XR: X-ray, DEXA: dual energy x-ray absorptiometry, FP: Force plate, HHD: Handheld dynamometer, SCR: Soldier conditioning review

The aim of a study like STOMP would be to validate findings from this thesis and related work but also understand how pathophysiological changes are identified during earlier phases of injury and recovery to improve biological understanding of OA disease. This kind of study could test both the two pathological hypotheses raised in this thesis, as mentioned in Chapter Three and Four. Firstly, it is possible that compositional changes in the cartilage as a result of injury and healing precede structural changes in PTOA, unlike iOA, and then, secondly, that these differences converge into a common mechanism within a few years, thus closing the potential time for optimal clinical intervention. As the design of this proposal is an observational study, there would be no interventions involved, however, the influence of the existing care pathway for knee injuries (including ACL specific programmes) on clinical and patient-focussed outcomes will be reviewed. A key priority during the study design phase would be, unlike the vast majority of studies reported in Chapter Three and Seven, is the utilisation of a common core outcome set, such as those advocated by the Outcome Measures in Rheumatology-Osteoarthritis Research Society International (OMERACT-OARSI) initiative (382). Unless we all speak the same language, we will continue to struggle to leverage our knowledge.

Within the UK DMS, there is a wealth of clinical and academic staff and resources, and if such a proposed study could demonstrate a tangible impact on the organisation, such as improvement in employability, deployability or retention, then these networks can be leveraged. As a result of our unique population, there is an opportunity to maximise routine clinical care with extended appointments to collect additional data for research purposes using pre-existing infrastructure. Thus, the cost of such a study might not as high as the putative cost of existing cohorts which required de novo investment. Given the occupational impact of ACL injuries in the US military (230), and long-term risk (37), there is likely a need to develop better knowledge to improve care,

which is the ambition of Defence Rehabilitation (239,242). A proposed study like STOMP could fill in the gaps regarding different injuries, timepoints and use of multiple biomarkers (including molecular across different sample types, MRI, biomechanical and patient-reported), thus also adding valuable information for the development of a proposed FOAR-Score.

In addition, it is critical that future studies, like STOMP, address the limitations in the work undertaken thus far, such as those outlined in Section 8.1. As mentioned, the proposed study would hopefully address the time from injury and injury profile but also address the potential lack of sensitivity of molecular biomarkers by measuring SF and urinary markers in addition to samples collected from the venous circulation. Further to that, the lack of females in the ADVANCE cohort means that the findings have a significant sex bias; therefore, this should be urgently addressed in the design of a future study. Given the high rates of ACL injuries in females and increased rates of OA (104), their inclusion is a matter of priority. I had hoped to recruit a significant number of females into the pilot study reported in Chapter Six, but unfortunately, there was still a significant sex bias (81% male). In addition, given the stark age-related increase in OA in females, future studies should ensure that the age range of recruitment is kept as broadly as possible. To mitigate the implications of this on any future clinical care pathway development, I have established a collaboration with ‘Project ACL’ to improve the identification of sex-specific risk factors and personalised preventative strategies with the ambition to transfer knowledge from their work into our population. In addition, I assisted with a review of hormone replacement therapy for the incidence and prevalence of hip, knee and hand OA, to understand if this could be introduced to improve management options, however, similar to earlier reviews, the results remain inconclusive (111).

8.4 Does a novel methodological approach demonstrate any difference in serum biomarkers concentrations depending on sampling location or physical activity?

Despite over two decades worth of research, there are no clinical OA biomarkers yet. This thesis has discussed some of the outstanding hurdles to overcome, such as the stage and severity of OA, the possible different aetiology, or, in the case of PTOA, the time elapsed since the initial traumatic injury. It has also explored two of the key challenges, that of incongruence between local and systemic circulation and the role of physical activity on the concentration of serum biomarkers. In order to overcome these challenges, a pilot study was instigated to establish the need for further research.

The novel sampling method employed in this pilot study offered insight into the dilution of serum biomarkers within the circulation, as demonstrated by differing concentrations nearer to the joint than in the systemic circulation (Section 6.4.2), with obvious relevance for any thresholds established for a normal reference range, diagnosis or to judge the effectiveness of interventions (434). Furthermore, the use of physical activity potentially offers a way to enhance the sensitivity of serum biomarker collection, with the opposite also true, if physical activity affects the concentrations of biomarkers, then this needs to be accounted and controlled for, asking participants (and, hopefully in the future, patients) to not exercise prior to sampling.

8.4.1 Sampling location

Synovial sampling is the gold standard of investigation to measure the local environment of the joint, and identify early pathological changes which might contribute to OA development. However, the lack of correlation between paired serum and SF samples has been well demonstrated (302,305,309,312), and this, allied to the technical challenges of synovial sampling, has perhaps contributed to no SF biomarkers being represented on the FNIH Biomarkers Consortium study and therefore being investigated for clinical use (50,52,519). Having the ability to detect local changes of biomarkers in the systemic circulation, prior to systemic dilution, might negate some of the issues outlined above.

Earlier, unpublished work by Pearson et al. also demonstrated the effect of serum sampling location in a different population. In 33 individuals undergoing foot surgery, 17 of which had Charcot neuro-osteoarthropathy (CN) with 16 non-diabetic controls with elective surgery booked, there were higher levels of interleukin (IL)-6 and cross-linked C-telopeptide of type I collagen (CTX-I) in the dorsal vein of the affected foot, than the unaffected foot and antecubital vein (IL-6: 9.6 (6.0,13.1) v 6.3 (4.4,8.1) p=0.02, and 5.4 (3.4,7.5) p<0.001 pg/ml and CTX-I: 0.9 (0.7,1.1) v 0.6 (0.4,0.7) p=0.004 and 0.5 (0.4,0.6) p<0.001 μg/ml, respectively). Levels of IL-6, CTX-I and osteoprotegerin (OPG) were also seen to be significantly different in the peripheral circulation of those with CN compared to the control population. In addition, tumour necrosis factor (TNF), IL-1β and soluble receptor activator of nuclear factor- kappaB ligand (sRANKL) displayed no differences.

When one relates the findings of my pilot study with Pearson's earlier work, it is clear that the location of sampling is relevant. It is interesting to note that my work demonstrated that leptin and IL-1β were significant different between the antecubital vein and saphenous vein when all 32

participants were analysed. Further differences visible within the sub-groups, with IL-6, PIIANP, leptin and COMP all differing between location, depending on their condition. Therefore, I believe this method of sampling, near to the affected joint in question, does have potential for future research, however, work is required. It is an invasive procedure, not perhaps as invasive as an arterial blood gas or femoral vein sample, but not one which is likely to be adopted outside of a specialist service. Whilst on the surface this might be off-putting, I believe that this procedure is comparable to a lumbar puncture, which is widely used in the appropriate specialist settings, but with this new potential procedure requiring far lower training, skill and specialist equipment.

8.4.2 Precedent physical activity

The impact of physical activity or exercise on serum biomarker concentrations is relevant for the reasons outlined above. Either it can improve sensitivity and therefore test performance, or it needs to be controlled for, with individuals asked to undertake an ‘exercise fasting’ regime prior to sampling to avoid false positives or negatives.

Previous work has been tried to understand the physiological effect of exercise, with theory being that joint-bearing or impact leads to biomarker concentration alteration due to mechanical pressure and loading (437,439,452). Walking, running, jumping and strenuous endurance activity all lead to significant differences in biomarker concentration (437,438,454,520), which would be in keeping with that hypothesis. However, I sought to understand if the same effect would be seen in a non-weight bearing activity, to see if it was purely a mechanical effect or if a different activity would elicit a similar response, to enable us to better understand the effect and how we can utilise this in the future. Furthermore, if there was a true exercise effect which was modality-agnostic, this would be helpful for the significant proportion of individuals who struggle with weight-bearing activity due to pain or other symptoms.

In my study, both COMP and CTX-II increased in the cohort following activity, which is in line with the effect previously reported (437,520,521). Further effects were seen when examined by condition, with IL-6 and CTX-II increased within the KOA group, but not COMP, again, a finding previously seen (520). COMP was seen to increase in those with an acute injury and in the control group, similar to previous literature, suggesting that it is not a purely mechanical response, given our non-weight bearing exercise modality.

It is reasonable to say that we need to consider the presence of preceding physical activity or exercise when sampling, given the effects can last for hours (520,521) – this would be particularly relevant if someone walked or cycled to their appointment. Furthermore, it is also reasonable to explore sub-maximal activity prior to sampling to enhance sensitivity, although again, similar to sampling location, this would need to be done in an appropriate setting.

8.5 Clinical translation, implementation and impact

As a clinician, I am keen to translate the progress so far and develop OA specific care pathways within the military environment to meet the clear unmet needs of those with, or at risk of, this disease within the military environment. Through the period of my PhD, I have repeatedly presented interim findings to military audiences to raise awareness, and in the last year, have delivered clinical teaching to different professional groups, including medical, nursing, physiotherapy, exercise rehabilitation instructors and other members of the DMS. I am often greeted with the response of 'but we can't do anything for OA', so this following section, whilst not directly related to molecular biomarkers, aims to give a flavour of what we can, and should, be doing now, and what I hope to introduce more formally in the future, using this thesis as a foundation.

Professional or tactical athletes (including military, firefighters, or law enforcement) have higher rates of OA than age-matched general population peers due to occupationally-related factors (2,237,238), as well as age, sex, genetics, body composition, inflammation, activity, and previous injury (21,84). Pooled prevalence estimates suggest that 30% of former athletes have OA (137), compared to 16% of the general population (66), with tactical athletic populations at an increased 2-3x risk (218). Whilst OA incidence (and prevalence increases with age), there is not an insubstantial amount in younger populations, with one study suggesting 10% of OA is diagnosed under 35, and an additional 20% between 35-44 years old (522). These figures are highly representative of the UK Military population, with the mean age of Regular Service Personnel 31 and Reserve Service Personnel 38 years old (523).

The initial presentation is often insidious, episodic, and diverse (including pain, swelling, stiffness, and crepitus) (21,27) and

subsequently is ignored or downplayed in populations that are used to managing discomfort or pride themselves on physical robustness. This approach can lead to delayed presentation or health-avoidant behaviour if individuals are worried about the impact on their job-role or career. Subsequent disability can impair the ability to perform functional tasks (including load carriage or prolonged marching/patrolling) or meet physical employment standards, leading to medical downgrade or premature retirement on medical grounds (2,36,242). Diagnosing OA in the early stage is a challenge (524), with an OARSI working group established to improve this (525) (which I have linked into, to offer the ADVANCE cohort as a validation population for their new tools). Early identification and screening, potentially using molecular biomarkers as discussed in the previous chapters, is only relevant if treatment options are available, so the next section will outline some of the current options.

Identifying OA risk factors has enabled opportunities for preventative interventions to minimise their impact, potentially reducing OA development or progression, with a research focus on preclinical risk stratification (4,5,60,84,135,292). As a reminder from Chapter One (Section 1.1.3), primary prevention aims to reduce the overall OA risk in healthy individuals; secondary requires identifying those at risk or with preclinical changes to reduce progression; and tertiary strategies aim to improve function and reduce disability in diagnosed OA.

8.5.1 Prevention

As a result of the poor long-term outcomes of OA and the challenges of its long-term management, I believe the paradigm for OA management, especially in the military, must change from treatment to prevention. Most military researchers have focussed on identifying musculoskeletal injury (MSKI) risk factors and developing mitigation strategies, with ongoing debate surrounding which risk factors to prioritise (238-240,526), acute rehabilitation interventions and occupational return, rather than the long-term outcomes of MSKI (229,239). It is unknown if adequate treatment of MSKIs can reduce the risk or delay the onset of subsequent PTOA, but it is likely, given common risk factors between the two, including muscular weakness, joint mal-alignment and overloading (240,257).

Primary prevention strategies for MSKI implemented in recent years, aim to reduce the volume of injuries, especially those sustained in recruit/basic training or during regular physical activity (526,527). These interventions are multimodal, focussed on increasing physical robustness with low-level physical training for periods prior to more arduous programmes (conditioning), improving overall health by smoking restriction, sleep optimisation and high-quality nutrition within the workplace (physical health), and the education of military commanders on injury risk and load management to prevent overtraining (leadership) (237,526,528).

However, given that not every injury can be prevented, there is also a requirement for secondary prevention in those with an MSKI. Civilian international research groups are also focused on secondary prevention strategies. Two groups, the OPTIKNEE (portmanteau of ‘optimise’ and ‘knee’) consensus group and OA Action Alliance (OAAA), recently provided evidence-based recommendations for preventing OA following a knee injury (60,61).

In 2022, the OPTIKNEE group published their consensus statement (60) supported by systematic reviews and meta-analyses on risk factors (37), rehabilitation (529), patient-report outcome measures (530), strength-based outcome measures (531), functional outcome measures (532), imaging and molecular biomarkers (533), and long-term outcomes for individuals (including quality of life, occupation, activity and disease burden) (534). The OAAA produced secondary prevention recommendations for clinicians intended to reduce the risk of OA following an ACL injury (61). The audience for this consensus group was primarily clinicians (unlike OPTIKNEE, who also targeted researchers), with ACL injury chosen as it is the most common knee injury known to lead to PTOA (535). Subgroups were formed to draft recommendations supported by an evidence base (61). These consensus processes aimed to produced patient-centred recommendations to guide clinicians and researchers, focussing on measures of daily living (including symptom burden, function and quality of life), with each stage meticulously listed to improve methodological robustness.

ACL injuries are the most common traumatic knee injury in the military, and evidence-based treatment approaches that optimise return to duty and mitigate long-term consequences are needed. More than 50% of soldiers are unable to return to unrestricted duty after surgery in the short term, and almost none are fit for duty by five years after surgery (228). Clearly, there is room to improve the evidence-based management of knee joint injuries in the military, which will also likely serve as secondary prevention for long-term injury-related adverse health effects, such as PTOA, with clinical training packages such as free online Arthritis Training, Learning And up-Skilling for health professionals (ATLAS) package from the Arthritis Education Consortium (https://arthritiseducation.mygo1.com), required alongside cultural,

organisational and contextual nuances that prevent and impede the implementation of treatment recommendations.

There is a clear applicability of the evidence-based consensus statements from OPTIKNEE and OAAA for Service populations. Both sets of recommendations target a younger population (OAAA explicitly used a cut-off of ≤40 years), reflecting the most common age for knee injuries and the significant impact of PTOA in the working-age population (60,61). This younger age closely matches that represented in the military, in contrast to a large proportion of the overall OA research performed in populations aged 60 or more. Therefore, most of the lifespan-based consensus recommendations promoted by OPTIKNEE and OAAA are likely to be translatable to a military population, with a finite amount of tailoring to adjust to the nuance of Service life, to account for factors such as including time away from home, short notice orders and the uncomfortable occasional organisational requirements for training or deployment taking a higher priority than recovery programmes. However, work is required to translate these, as summarised in Figure 8.5 (which builds on Figure 1.1).

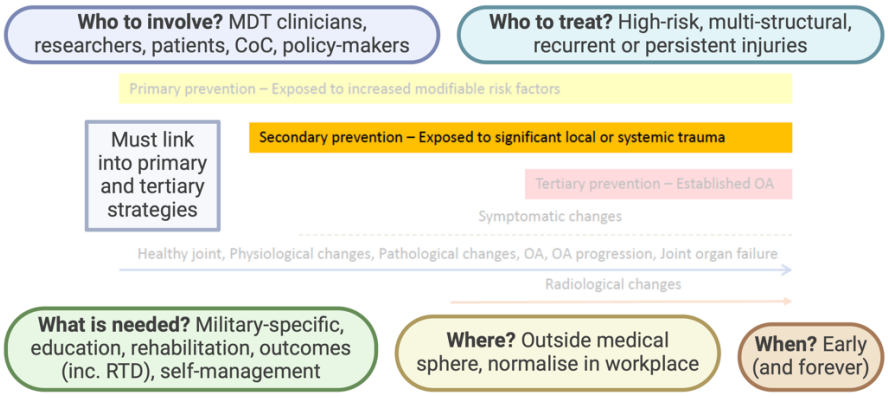

Figure 8.5 Key priorities for post-traumatic osteoarthritis prevention in the military
MDT: Multidisciplinary, CoC: Chain of command, RTD: Return to duty, OA: Osteoarthritis
Created in BioRender.

8.5.2 Management

There are challenges to military personnel receiving optimal OA care, including frequent postings/changes to duty stations, temporary remote duty assignments, and short-notice deployments. A recent health needs assessment performed within UK Defence agreed that injury prevention and recovery should move under the chain of command and not be siloed under the medical team (536). Stakeholder engagement should occur to ensure all who need to be involved are and that conflicting priorities can be met – lessons can be learnt from other industries on successful strategies (537).

These organisational challenges are compounded by a need for widespread knowledge of the best treatment approaches and self-management techniques among healthcare professionals and patients. In the short-term, clinicians with knowledge gaps can be signposted to education programmes such as ATLAS, before appropriate education and training across organisations has been performed. Within military care pathways, rehabilitation for knee joint injuries, for those who receive it, is often suboptimal and underdosed (229). One study showed that only 17.9% of nearly 3000 meniscal injured (with or without concurrent ACL injury) patients in the military received any exercise therapy within 60 days of the initial injury (227). For other knee injuries, data from a large cohort of 74,408 patients seeking care in the US Military Health System found that > 60% did not receive any supervised exercise therapy in the 2-years after the initial injury (229). Even when military personnel receive regular evidence-based physical therapy, notable deficits in patient-reported outcomes may last for years after injury and surgery (538).

As discussed previously within the thesis, there is a discrepancy between OA radiographic changes and the clinical picture experienced (21,84), ‘OA disease’ vs ‘OA illness’. This discrepancy can be partly

explained by the influence of individual factors, including peripheral and central pain sensitisation, understanding, co-existing mental health disease and body mass (84). Therefore, any strategies to improve OA must focus on disease and illness, using a combination of the medical and biopsychosocial care models tailored to the individual's life and occupational demands in a multi-disciplinary setting (84,539). This section aims to introduce some general and specific preventative strategies for OA to guide clinicians in developing individualised care for a younger, physically-active population, with a manuscript prepared and published in response to demand from clinicians (9). Further challenges exist, given the likely different needs between those with and at risk of OA – ideally, there should be different approaches for these groups, however, as this thesis has outlined, this is currently not feasible, and therefore, a pragmatic approach is required until this changes, and is reasonable given the overlap in strategies.

8.5.2.1 General strategies

There are diagnostic challenges for tactical athletes. A diagnosis of OA should be considered in those suffering recurrent early symptoms (pain, stiffness, crepitus, limitations) with a history of a traumatic injury or in those with persisting symptoms (with no trauma), even if they are younger than 45 (27). A recent study demonstrated that those under 35 wait nearly a decade for a diagnosis, compared to those over 65 who only wait for a year (522).

Prevention strategies should aim to improve joint 'healthspan', not just 'lifespan', through a multi-modal approach, ideally within a multi-disciplinary team, empowering individuals to live well with their condition (27,242,540). The UK National Institute of health and social Care Excellence (NICE) recommends, as front-line treatment, education, exercise and weight loss (when appropriate), which are also very relevant for military individuals.

8.5.2.2 Education

The foundation should be appropriate and thorough education, tailored and specific self-management strategies, and access to healthcare resources when required (Figure 8.6). As you can see, the figure builds on that presented in Chapter One (Figure 1.2), to highlight that the risk factors are central to the development of OA and therefore interventions must focus on them, but this is a condition where the individual with, or at risk, of OA must take control.

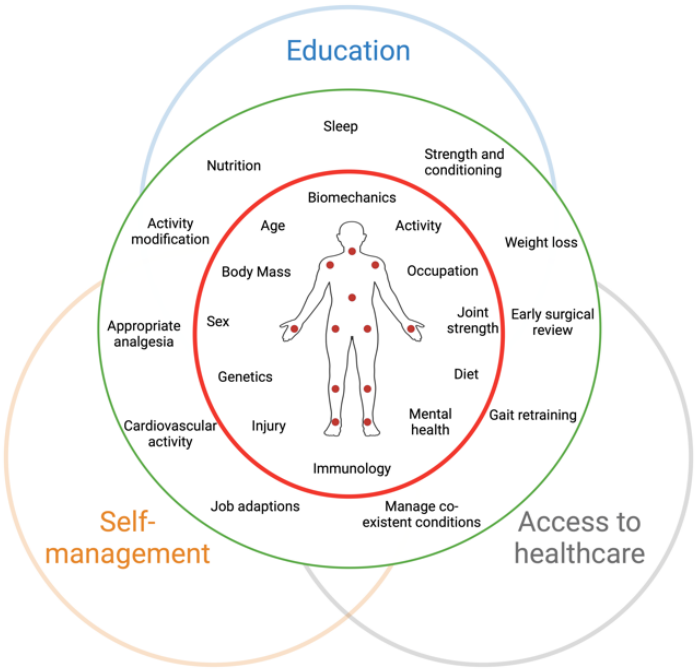

Figure 8.6 Osteoarthritis risk factors and prevention strategies underpinned by person-centred care.
Created in BioRender.

Giving individuals the correct tools to make individualised decisions is key; an example might be regarding their recreational activities – high-intensity running, such as sprinting, or collision sports, like football, may lead to a symptomatic flare in diagnosed OA, but evidence shows that moderate intensity activities are beneficial (including long-distance running which is not associated with an increased prevalence/progression of OA (21)). Therefore, outlining this would empower an individual to make an appropriate risk-benefit decision and either choose alternative activities (such as cycling or swimming) or continue their preferred activity due to its perceived benefits (e.g. cardiovascular fitness, personal goals, mental health benefits) (Table 8.1) (21,84). In addition, liaison with employers regarding occupational demands and risks might improve an individual's ability to remain in full-time employment, through job-role or workplace adaptions (237,238).

Table 8.1 Higher risk activities for osteoarthritis development and progression with possible alternatives

Activities to review	Possible alternatives

Contact and Collision sports, high-impact activity; football, American football, rugby, basketball, tennis, skiing	Lower impact; Cycling, swimming, walking (slow and brisk) Flexibility based: yoga, pilates, tai chi

In order to provide effective treatment, there is a need to provide a timely diagnosis, as introduced at the start of this section. The diagnosis must be explained in a manner that individuals can understand and retain, with third-sector organisations offering supportive resources (including www.versusarthritis.org and www.freefromkneepain.org). These should be discussed with the individual so they can understand how it will likely impact them and, importantly, what they can do about it. Understanding the needs of the population is important, such as occupational implications but perhaps more important, literacy. The average reading age of those recruited into the Army as soldiers is 11 years old (541). If explanations and health literacy education are done correctly, they can promote self-control of the disease (‘participatory discourse’) from passively being controlled by the disease (‘impairment discourse’) (542).

Communication is key. Words used by healthcare professionals influence the individual's perception of their condition – words such as ‘progressive, degenerative, wear and tear’ are more likely to have a negative impact than phrases like ‘dynamic processes of your joints adapting to how you use them’ (375,542). The effect of words in other areas of medicine and society is well established (543-545). Harmful perceptions should be challenged, such as ‘the joint breaking down’ or ‘exercise wears away the joint’, especially when these lead to avoidance behaviours (375). Objective medical records and PROMs can enable a pattern to be recognised that otherwise might be missed or downplayed. Finally, there should also be an understanding that this is a journey in partnership – hopefully, initial curiosity might lead to supported activity/behaviour modification, with the end goal of empowerment over impairment.

8.5.2.3 Physical activity

Regular physical activity should be the cornerstone of self-management and rehabilitation strategies (242,546). Not only do exercise-based treatments facilitate improvement in joint health and associated morbidity, but they also benefit cardiovascular health, with poorly managed OA associated with increased mortality (21,41). Physical activity is a more inclusive term than exercise, especially for individuals who have developed avoidance behaviours, and encompasses cardiovascular-based and 'strength and conditioning' categories (60,84). New activity programmes should be graduated, address potential barriers, and follow generic principles regarding symptom-titration and pacing to avoid symptom flares and 'boom and bust' behaviours (547,548).

Cardiovascular-based activities should be stratified based on their load-bearing properties and the risk or presence of OA. For those at risk, activity modification should be considered, especially if they are occupationally-related, such as an infantry soldier carrying heavy weight across undulating terrain. Those with confirmed OA should reduce, if possible, high-impact activities and switch to alternatives, like swimming or cycling, depending on individual preferences and risk-benefit ratios (Table 8.1) (21). Initiatives like Moving Medicine can help introduce physical activity conversations into routine consultations (www.movingmedicine.ac.uk). Options such as activity programmes (including Versus Arthritis' "Let's Move with Leon" or OA Action Alliance's "Remain in the Game"), with access to appropriate analgesia, can enable individuals to self-manage during both background 'maintenance' and breakthrough 'flare control', and demonstrate improved pain and functional scores (549).

Physical exercise should also underpin rehabilitation and recovery programmes. Rehabilitation should be focussed on pre-determined

outcomes, tailored to individual requirements, measured with goal setting and regularly reviewed in a supportive environment. Injuries such as ACL rupture carry a high risk of subsequent OA, so ACL rehabilitation programmes should instigate 'joint healthspan maintenance programmes' following return-to-play programmes, combining resistance-based neuromuscular exercises and plyometrics (60,84). Concurrent analgesia should be used to avoid limitations secondary to pain, with blood flow restriction demonstrating potential as an adjutant training modality (446).

8.5.2.3.1 Evidence for this population

To understand what evidence there was for specific programmes for a younger adult population with, or at risk of OA, I performed a narrative review, using an umbrella review methodology, with a pooled sub-group analysis of studies involving younger participants (as per PRISMA guideline) (297,550,551). Fifty systematic reviews (with or without meta-analysis) published over the last ten years investigating the impact of exercise on OA were assessed, from a range of journals published in different countries with different impact factors (to ensure wide scope of potential studies and to minimise potential publication bias). All studies included in the reviews were assessed, with those performed in participants aged over 18 and under 50 extracted. Across the 1046 studies included in the 50 systematic reviews, involving approximately 246859 participants, five met the inclusion criteria (552-556). Of these, one study was purely for those with patellofemoral pain syndrome (554), and another in French (553). Three studies were included (552,555,556), and their findings described below (Table 8.2).

Table 8.2 Studies included for the exercise therapy in osteoarthritis scoping review

Author, year	Participant, n=, age	Exercise Intervention	Outcome Measures

Roos, 2005 (552)	45 45.8 ±3.3	Supervised, weight bearing strength, neuromuscular control Three times weekly, 4 months	PROMs: KOOS MRI: Cartilage composition Physical: Strength, aerobic capacity, functional (hop, jump, rising tests)
Mecklenburg, 2018 (555)	162 46 ±12	Sensor-guided body weight strengthening (squats and lunges), thigh stretches, aerobic activities, three sessions each over 12 wks	PROMs: KOOS pain and physical function, pain and stiffness VAS Subjective: Understanding of condition and need for future surgery
Nambi, 2020 (556)	60 22.2 ± 1.6	Knee movement and function via virtual reality or sensor motor training programmes	PROMs: WOMAC Molecular: Inflammatory (TNF-a, IL-2,4,6) and bone markers (BMP 2,4,6,7)

The exercise interventions in these studies were very diverse, and difficult to categorise in a homogenous fashion, but all reported benefits in a variety of outcomes post exercise therapy. While Roos and Dahlberg (552) implemented weight bearing strengthening exercises as their intervention, this appeared more in line with the control intervention for Nambi *et al.* (556), and was also relatively similar to the Mecklenburg *et al.*'s intervention involving squats and lunges (555). The duration of each intervention varied widely, from 4 (556), to 12 (555), and 16-18 weeks (552), and replication of exercise dosages within these studies would be challenging. Despite Roos and Dahlberg (552) providing sample exercises, dosages (sets, reps, intensity etc.) are not described, with different exercise dosages being found to influence strength, muscle architecture, and pain response (557,558). Mecklenburg *et al.* (555) refer readers to a methods paper for further details on their exercise therapy prescription (559). However, despite descriptions of particular stretches and strengthening exercises, there are no details on exercise dosage (559).

It is obvious there is a significant bias in the published literature toward an older adult population. Traditionally, there has been a perception that OA is an inevitable part of the process of ageing, and therefore the focus has been on those in their late-middle age or older. Unfortunately, this perception extends into the clinical environment as well, with those

aged under 35 waiting nearly 10 years for a diagnosis, compared to under a year for those over 65 (522). The majority of early-onset OA cases are secondary, due to a joint injury, with PTOA estimated to compromise of 12% of all OA cases (35,66), with a corresponding research focus required to slow or stop the progression of OA and subsequent disability. Recent recommendations have suggested specific exercise interventions (60,61), but studies are required to demonstrate their efficacy and effectiveness. Future studies should include specifics on exercise prescription parameters to allow beneficial exercise therapy programmes to be prescribed by practitioners in an evidence-based manner, as the lack of detail and direction regarding implementation diminishes the value of any findings (560).

There is light on the horizon, with relevance for military individuals. Two new randomised cohort trials, SUpervised exercise-therapy and Patient Education Rehabilitation (SUPER-Knee) and Stop OsteoARthritis (SOAR), have been designed to answer these questions (561,562). SUPER-Knee seeks to investigate the impact of a 4-month supervised strengthening and neuromuscular programme with education v minimal intervention (no supervision) over a four-month period in those aged 18-40 years, for pain, function and quality of life (561). SOAR plans to deliver a series of educational, group and individual exercise sessions online and in person over 8 weeks and explore potential improvement in strength, function and quality of life (562). These studies will enable exploration of the value of exercise intervention and delivery methods in this population, and their findings will be directly applicable to the military population.

Until that time, a pragmatic approach, such as non-medical programmes such as ‘Couch to 5K’ or group-based activities might support individuals who haven’t been physically active for some time. Regular activities to build regional muscle strength, flexibility, and range of motion should be employed to improve and maintain function (60).

Home-based programmes can be utilised to improve accessibility with good adherence seen for these (563), with gym-based programmes potentially reserved for active rehabilitation or other healthcare-guided interventions. Programmes should be progressive, joint-specific, and focused, with Versus Arthritis offering ‘off-the-shelf’ adaptable programmes and daily 15-minute Remain in the Game injury prevention exercises, from OA Action Alliance, summarised in Table 8.3.

Table 8.3 Summary of OA Action Alliance ‘Remain in the Game’ Injury prevention home exercises

Type	Examples
Balance	Transverse twisting hop, Russian hamstring curl, forward hop with stabilisation
Strength	Single leg squat, side plank, forward lunge
Plyometric	Squat jump, scissor jump, ice skaters
Agility	Single leg line hops, diagonal run with a cut, carioca
Flexibility	Quadricep hip flexor stretch, hip gates, calf and hamstring stretch

8.5.2.4 Weight management and nutrition

Adipose tissue generates and releases cytokines and other mediators, contributing to OA progression and is independently associated with the disease, as outlined in Section 1.3.3 (114,564). The early analysis performed in adipose-tissue markers, reported in Section 8.2.2, also demonstrated the molecular relationship to pain, a relationship well-established in the literature. A higher body mass can experience a heightened perception of pain and reduced quality of life, thereby leading to worse OA illness (84). Therefore, weight loss (for those overweight) should be undertaken, and must be sustainable and appropriate. It should involve dietary changes, not just diet – although a reduction in ultra-processed foods (UPF) is likely beneficial; those with high levels of UPF in their diet are 40% more likely to have symptomatic OA (552-556).

Specifically, from an injury prevention perspective, ensuring any nutritional deficiencies are addressed, such as Vitamin D (when deficient) for the prevention of overuse injuries (565,566), or protein supplementation during arduous training blocks to improve muscle and bone metabolism and repair (567,568). In addition, weight loss must be combined with resistance training, and measured using body composition metrics, to avoid sarcopenia – muscle mass is key for strength and function, and also protective in reducing inflammation (569,570). As an example, unsupervised caloric restriction leads to muscle breakdown to meet energy requirements, with any subsequent ‘rebound’ weight gain disproportionately adipose tissue, thereby worsening body composition and potentially leading to sarcopenic obesity and worsening OA (120). In addition, poorly managed OA can worsen cardiovascular disease mortality due to increased physical inactivity and associated ongoing inflammation and metabolic dysregulation (41), as demonstrated by increased hsCRP and HbA1c noted earlier (Section 8.2.1).

The impact of body mass within this cohort, as reported in Chapter Four & Five, on OA and pain has been interesting. I used a body shape index (ABSI) to adjust for body mass during the cross-sectional analysis, given the limitations of the body mass index (BMI) in athletic populations. However, given the widespread use of the BMI measure, I felt it would be beneficial for other research groups to adopt this metric for the predictive analysis. During this latter analysis, it only significantly contributed to pain incidence, with a minimal effect (OR 1.08). It is likely that this results from the lack of nuance of this measure, especially for a military population, therefore, work is planned to explore this further, using the result of dual energy x-ray absorptiometry (DEXA) to more accurate form categories. This planned work will also explore those with sarcopenic obesity, to identify the influence of this on OA and pain (120).

8.5.2.5 Additional interventions

8.5.2.5.1 Pharmacological

There are currently no disease-modifying drugs, with analgesia the main pharmacological intervention for OA. Pain is the thing that brings people to healthcare professionals. Explaining when pain or symptoms are likely, such as after specific activities or if commencing a new programme, can improve adherence. For example, it is important to explain that pain is likely at the start of a new activity/rehabilitation programme but will decrease over time, so analgesia will be required initially but can subsequently be reduced/stopped – armed with that knowledge, individuals can self-manage appropriately. For those with chronic or regular OA-related pain, the use of resources such as the Pain Toolkit can help reframe why and how pain features in their lives (www.paintoolkit.org).

Access to analgesia is important; however, it should not be the only method of controlling pain. Utilising combination analgesia, aiming for the lowest dose for the shortest time, including topical and oral formulations, access to breakthrough medication, and regular analgesia reviews can improve outcomes (27). A sensible approach would involve a combination of topical non-steroid anti-inflammatories and/or capsaicin cream, oral paracetamol and COX-2 inhibitors, and sparing use of intra-articular corticosteroid, depending on individual co-morbidities, whilst avoiding opioids to prevent secondary conditions such as opioid-induced hyperanalgesia (27,540).

There are many pharmacological options in widespread clinical use at present, with multiple National or professional organisations offering evidence-based recommendations for their use. A simplified version of the recommendations from the American College of Rheumatology (ACR), European Society of Osteoporosis, OA and MSK Diseases

(ESCEO), OARSI, American Academy of Orthopaedic Surgeons (AAOS) and NICE are summarised in Figure 8.8 (27,571-574).

Supplement / Topical	Vitamin D	Fish Oil	Chondroitin	Glucosamine	Topical NSAIDs	Topical Capsaicin
ACR 2019						
ESCEO 2019	N/R	N/R				N/R
OARSI 2019		N/R				
AAOS 2021	N/R	N/R	N/R	N/R		N/R
NICE 2022	N/R	N/R				N/R

Oral	Paracetamol	Oral NSAIDs	COX-2 Inhibitor	Weak Opioids	Strong Opioids	SNRI
ACR 2019			N/R			
ESCEO 2019						
OARSI 2019						
AAOS 2021						N/R
NICE 2022						N/R

Intra-articular	Corticosteroid	Hyaluronic Acid	Platelet Rich Plasma
ACR 2019			
ESCEO 2019			N/R
OARSI 2019			N/R
AAOS 2021			N/R
NICE 2022			N/R

Figure 8.7 Osteoarthritis pharmacological management recommendations
ACR: American College of Rheumatology, ESCEO: European Society of Osteoporosis, OA and MSK Diseases, AAOS: American Academy of Orthopaedic Surgeons OARSI: Osteoarthritis Research Society International, NICE: National Institute for Health and Care Excellence, N/R: No Recommendation, NSAID: Non-steroidal anti-inflammatory, SNRI: Serotonin and Noradrenaline reuptake inhibitor

Future avenues of pharmacological therapy include the use of medications used elsewhere for inflammatory disease, such as methotrexate (575,576), or those targeting metabolic processes, such metformin (577,578) or the novel glucagon-like peptide-1 (GLP-1) inhibitors (579,580), all of which show promise. Further research is required to refine which patient groups might benefit most, with the use of phenotyping and biomarkers integral to this.

8.5.2.5.2 Surgery

It is beyond the scope of this chapter, or indeed thesis, to discuss the full nuance of surgical interventions for OA. In brief, surgical interventions, considered after all conservative measures have been utilised, are either joint stabilising, such as osteotomy or unicompartmental arthroplasty and therefore tertiary prevention, or a definitive, in the form of a total arthroplasty (581). The former is preferable for young, active individuals, especially as arthroplasty lifespan is reduced in younger populations (36,581). Procedures such as arthroscopic lavage or debridement are no longer recommended (27,581). Surgical decisions should be made in collaboration with the individuals to improve specific symptomatic and functional outcomes, with pre-habilitation often improving post-surgical outcomes. This discussion must include both surgical and patient expectations (as 20% of arthroplasties don't improve outcomes) and the potential iatrogenic complications of surgery (581).

8.5.2.5.3 Sleep

The relationship between sleep and pain is well studied, with poor sleep linked to worse pain (582,583), and worse OA disease due to disruption of circadian rhythm impacting articular cartilage and bone health, and the possible mediating effect on tissue homeostasis, as seen in animal and human studies (21,584-586).

Certain professions, including tactical athletes, have sleep patterns deleterious to overall health (582,587). Good core sleep patterns should be established and optimised to reduce the negative effects of sleep disruption related to shift work or night duty (588,589). Most people require 7-9 hours in a cool, dark, quiet environment with a regular pattern (such as 2200 to 0600), with sensible advice such as "10-3-2-1" aiming to improve sleep hygiene, depending on the

individual (avoid caffeine, stop eating, stop working, avoid screens, 10,3,2,1 hours before bed, respectively) (21,582). Optimisation of sleep would benefit OA illness and OA disease.

8.5.2.5.4 Optimisation of concurrent conditions

Undermanaged co-existent disease can worsen OA. The impact of mental health conditions on OA illness is clear, but metabolic (including diabetes mellitus), endocrine and rheumatological diseases can also impact OA disease (21). In addition, poorly managed OA can worsen cardiovascular disease mortality due to increased physical inactivity and associated ongoing inflammation and metabolic dysregulation (41).

8.6 Conclusion

This thesis aimed to demonstrate the positive value of a panel of serum biomarkers to predict rOA, pain and function in line with the overarching hypothesis in Section 2.2.1. It was unable to do so, and perhaps biomarkers in isolation might not be the panacea, especially in the earliest stages of this disease. However, in combination with other metrics, biomarkers might offer the potential to improve risk stratification and patient phenotype to improve clinical interventions. The most important next steps are trying to untangle the biological reasons as to why certain people do, and don't, develop PTOA, but to not hold off providing treatment that we know can make a difference whilst we untie this Gordian knot, and there are many options available.

One of the most striking findings of this thesis is that there may be no difference between iOA and PTOA. Prior to the analysis, my hypothesis was that there would be a different molecular pattern given the different aetiology – whilst these changes might have been prior to radiographic change, OA is a dynamic and not static process, therefore I expected to see a residual signal. However, following the analysis, I have postulated an increased risk in the years immediately after an injury, before settling to the same risk and rate of expected mid-life OA. There may be, therefore, the same pathomechanism underpinning these processes, suggested by the molecular results, with hope for good, evidence-based interventions offering value now, while further work is performed to understand what great evidence-based interventions might look like. I am a pragmatist, so will settle for good, which, ultimately, is better than none. Clinicians managing OA can sometimes feel helpless, especially if they frame OA as an irreversible, progressive, age-related condition.

An improved understanding of OA pathophysiology enables specific interventions for modifiable risk factors which, by combining the medical and biopsychological models of care, can targeting OA disease and OA

illness. I hope that biomarkers (molecular, imaging, biomechanical, patient-reported, or a combination) can highlight those who would benefit from early intervention and prevention. A preventative approach should be adopted in partnership with the individual, empowered to actively manage OA through education and self-management, with access to healthcare services when needed. Expert communication is required to educate, identify unrealistic expectations, challenge barriers, and provide a *raison d'etre* for ongoing joint maintenance. Clinicians should feel confident there is plenty to offer individuals to slow or prevent OA progression. Work is underway to identify those at high risk, hoping to further stratify those with or at risk of OA for targeted intervention, but until then, pragmatic criteria, such as traumatic joint injury or persistent symptoms, should enable appropriately tiered prevention, personalised to an individual's wants, needs, and occupation.

In order to translate these research findings and recommendations into our population, there is a need to form internal coalitions. I have established a Military OA Group, to work through the gaps in education, training and care pathways. Whilst good patient education resources exist, such as those by Versus Arthritis, there is a need to develop military-specific ones, with details on military-specifics risks and mitigations, impact for bespoke trades/job roles, and possible implications for medical deployability and employability. Furthermore, clinician education, centred on the same, would help extend the programme from ATLAS to encompass military clinician needs. A care pathway needs to be developed, underpinned with best practice guidance (BPG) and medical policy – this is the core remit of Defence working groups. During the last year, I have been directly recruited into the Traumatic Knee Injury and Return to Work working groups, offering me the unique opportunity to shape BPGs and relevant medical policy from acute injury, through the end stage of rehabilitation, to ongoing

maintenance and secondary prevention, shaped, in part, through the findings of this PhD thesis.

Knowledge is not linear. It is progressive, sometimes slow, sometimes exponential. There is a zeitgeist in the field of PTOA detection and prevention, with a corresponding wave of momentum across the globe. It is my responsibility to build on the giants who have gone before, and throw my modest findings on the gravel path for the next person to build upon.

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Supplementary Material

Supplementary File 1 – Search strategy for Chapter Three

Medline search.

Medline (Ovid MEDLINE® Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE®	
#########	https://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=N&PAGE=main&SHAREDSEARCHID=3buR0
1	Osteoarthritis, Knee/
2	(osteoarthr* adj5 knee*).ti,ab,kw.
3	Osteoarthritis/
4	exp Knee/ or exp Knee Joint/ or exp Knee Injuries/ or Arthroplasty, Replacement, Knee/ or Kn
5	knee*.ti,ab,kw.
6	4 or 5
7	3 and 6
8	1 or 2 or 7
9	("post trauma*" or post-trauma* or posttrauma*).ti,ab,kw.
10	(osteoarthrit* adj5 trauma*).ti,ab,kw.
11	9 or 10
12	exp Biomarkers/
13	(biomarker* or biological marker* or biochemical marker*).ti,ab,kw.
14	serum marker*.ti,ab,kw.
15	inflammat* marker*.ti,ab,kw.
16	catabolic marker*.ti,ab,kw.
17	anabolic marker*.ti,ab,kw.
18	metabol* marker*.ti,ab,kw.
19	laboratory marker*.ti,ab,kw.
20	clinical marker*.ti,ab,kw.
21	(surrogate endpoint* or surrogate end point*).ti,ab,kw.
22	Urine/
23	exp Blood/
24	Synovial Fluid/
25	(urine or blood or serum or plasma or synovial fluid).ti,ab,kw.
26	exp Magnetic Resonance Imaging/

27	(magnetic resonance or MRI*).ti,ab,kw.
28	exp Ultrasonography/
29	(ultrasound* or ultrasonograph* or ultrasonic).ti,ab,kw.
30	imaging.ti,ab,kw.
31	USS.ti,ab,kw.
32	or/12-31
33	8 and 11 and 32
34	exp animals/ not (exp animals/ and exp humans/)
35	33 not 34

Embase search

Embase 1974 to present	
08/11/2022	https://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=N&PAGE=main&
1	knee osteoarthritis/
2	(osteoarthr* adj5 knee*).ti,ab,kw.
3	osteoarthritis/
4	exp knee/ or exp knee injury/
5	knee*.ti,ab,kw.
6	4 or 5
7	3 and 6
8	1 or 2 or 7
9	("post trauma*" or post-trauma* or posttrauma*).ti,ab,kw.
10	(osteoarthrit* adj5 trauma*).ti,ab,kw.
11	9 or 10
12	biological marker/
13	(biomarker* or biological marker* or biochemical marker*).ti,ab,kw
14	serum marker*.ti,ab,kw.
15	inflammat* marker*.ti,ab,kw.
16	catabolic marker*.ti,ab,kw.
17	anabolic marker*.ti,ab,kw.
18	metabol* marker*.ti,ab,kw.
19	laboratory marker*.ti,ab,kw.
20	clinical marker*.ti,ab,kw.
21	(surrogate endpoint* or surrogate end point*).ti,ab,kw.
22	urine/
23	exp blood/
24	synovial fluid/
25	(urine or blood or serum or plasma or synovial fluid).ti,ab,kw.

26	exp nuclear magnetic resonance imaging/
27	(magnetic resonance or MRI*).ti,ab,kw.
28	exp echography/
29	(ultrasound* or ultrasonograph* or ultrasonic).ti,ab,kw.
30	imaging.ti,ab,kw.
31	USS.ti,ab,kw.
32	or/12-31
33	8 and 11 and 32
34	(exp animal/ or nonhuman/) not exp human/
35	33 not 34

Cochrane search

Date Run: 08/11/2022 10:03

https://www.cochranelibrary.com/advanced-search/search-manager?search=7062384

ID	Search
#1	MeSH descriptor: [Osteoarthritis, Knee] this term only
#2	(osteoarthr* NEAR/4 knee*):ti,ab,kw
#3	MeSH descriptor: [Osteoarthritis] this term only
#4	MeSH descriptor: [Knee] explode all trees
#5	MeSH descriptor: [Knee Joint] explode all trees
#6	MeSH descriptor: [Knee Injuries] explode all trees
#7	MeSH descriptor: [Arthroplasty, Replacement, Knee] explode all trees
#8	knee*:ti,ab,kw
#9	#4 or #5 or #6 or #7 or #8
#10	#3 and #9
#11	#1 or #2 or #10
#12	(post-trauma* or posttrauma*):ti,ab,kw
#13	(osteoarthrit* NEAR/4 trauma*):ti,ab,kw
#14	#12 or #13
#15	MeSH descriptor: [Biomarkers] explode all trees
#16	(biomarker* or biological NEXT marker* or biochemical NEXT marker*):ti,ab,kw
#17	(serum NEXT marker*):ti,ab,kw
#18	(inflammat* NEXT marker*):ti,ab,kw
#19	(catabolic NEXT marker*):ti,ab,kw

#20	(anabolic NEXT marker*):ti,ab,kw
#21	(metabol* NEXT marker*):ti,ab,kw
#22	(laboratory NEXT marker*):ti,ab,kw
#23	(clinical NEXT marker*):ti,ab,kw
#24	(surrogate NEXT endpoint*):ti,ab,kw
#25	("surrogate end" NEXT point*):ti,ab,kw
#26	MeSH descriptor: [Urine] this term only
#27	MeSH descriptor: [Blood] explode all trees
#28	MeSH descriptor: [Synovial Fluid] this term only
#29	(urine or blood or serum or plasma or synovial NEXT fluid):ti,ab,kw
#30	MeSH descriptor: [Magnetic Resonance Imaging] explode all trees
#31	(magnetic NEXT resonance or MRI*):ti,ab,kw
#32	MeSH descriptor: [Ultrasonography] explode all trees
#33	(ultrasound* or ultrasonograph* or ultrasonic):ti,ab,kw
#34	imaging:ti,ab,kw
#35	USS:ti,ab,kw
#36	#15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or # or #29 or #30 or #31 or #32 or #33 or #34 or #35
#37	#11 and #14 and #36

Trial registries

ClinicalTrials.gov	https://clinicaltrials.gov/ct2/results?cond=Post-Traumatic+Osteoarthritis+of+Knee&term=&cntry=&state=&cit
Condition or disease	Post-traumatic osteoarthritis of knee
Results	30
Searched 8/11/22	
WHO ICTRP	
Condition	post-traumatic osteoarthritis
Results	50
Searched 9/11/22	

Supplementary File 2 – The SORE study documentation

SF 2.1 Study ethics application

Faculty of Medicine & Health Sciences (FHMS) Research Ethics Committee

Application for approval of all studies involving **Healthy Human Volunteers only conducted by Staff and Students of the University of Nottingham**

1 Title of Project: Serum concentration of joint-specific biomarkers from the venous drainage of the lower limb may improve in the interpretation of acute and chronic degenerative knee and ankle joint disease.
Short title: SORE

2 Names, Qualifications, Job Title, Work Address, telephone and e-mail of Researchers:

Chief Academic/Supervisor:

Dr Stefan Kluzek FRCP FFSEM D.Phil
Associate Professor in Sport and Exercise Medicine
Consultant in Sport and Exercise Medicine
NUH Deputy Director for VA Centre for Sport, Exercise and Osteoarthritis
Stefan.kluzek@nottingham.ac.uk
GCP Trained, Medical trained (including phlebotomy and advanced life support)

Lead researcher:

Dr Richard Pearson
Assistant Professor
Senior Tutor, PGR School of Medicine
University of Nottingham
Richard.pearson@nottingham.ac.uk
GCP Trained, Laboratory trained

Other key researchers/collaborators:

Dr Oliver O'Sullivan MRCP
Academic Unit of Injury, Recovery and Inflammation Science
ADMR, DMRC Stanford Hall, Loughborough
Oliver.o'sullivan@nottingham.ac.uk
GCP Trained, Medical trained (including phlebotomy and advanced life support)

. **3 Type of Project**: (Please select one or more from list below and delete as appropriate)
Pilot study
Blood samples only

4a Summary of Experimental Protocol

Background
Biological markers (biomarkers) can indirectly measure pathological processes, including those associated with acute and chronic joint injury. These can be measured in serum and plasma with ultrasound scans, with multiple biomarkers associated with changes to bone, collagen, and cartilage following traumatic or inflammatory processes. (Garringa 2021) Given the tests' experimental nature; there are currently multiple variables unaccounted for in their sampling. These variables could potentially influence the biomarker results independently of any pathological changes, and their impact is yet to be fully understood. Unpublished data demonstrate a difference in concentration of specific inflammatory - related biomarkers in samples taken close to the affected joint, rather than in the more distal circulation. (Pearson, personal communication) Other biomarkers have been seen to be raised following physical activity.(Andersson 2006, Dreiner 2022)

To understand variability and improve standardisation in future studies, there is a need to perform a study to investigate any changes in certain markers, depending on the location, and prior physical activity of the individual. We hypothesise that the concentration of serum biomarkers associated with joint injury and chronic osteoarthritis is different in venous drainage of the affected lower limb when compared to more distal concentration and therefore is more suitable for differentiation between central and localised processes associated with joint-related disability.

Aims
To compare the concentration of serum biomarkers associated with lower limb joint injury and chronic osteoarthritis between the venous drainage of the afflicted lower limb and more distal concentrations at rest and following a lower limb loading regimen.

Experimental protocol and methods
This study will aim to compare three different groups; those with a chronic musculoskeletal injury (MSKI), those with previously diagnosed osteoarthritis (OA) and a comparison group with no joint injury, in order to see if experimental variables, namely, location of sampling and precedent physical activity alter biomarker concentration. This study will act as a pilot study to guide further work.
Using lower limb joint pathology, predominantly knee and ankle, blood samples will be drawn from a regional vein located near the joint, including the Great Saphenous Vein (GSV) and Small Saphenous Vein (SSV). These samples will be taken using a tourniquet and ultrasound guidance. A paired sample will be taken from the contralateral antecubital fossa. This will allow a comparison between serum from venous drainage of the afflicted limb and more distal venous circulation. A concurrent measurement of joint structure and identification of co-existing effusion using ultrasound will be performed using a standardised protocol. Following this, if able, individuals will be asked to row on an Concept 2 indoor rowing machine for ten minutes until a rate of perceived exertion (RPE) of 7 is reached. Further blood samples will be taken at this point, allowing the effect of precedent physical activity on biomarker concentration to be measured.

Measurable endpoints/statistical power of the study
Levels of serum biomarkers will be analysed, which are likely to include IL17, TNFa, IL-1b, Adiponectin, Leptin CTXII, CPII, COMP and C2M. Serum will be analysed via ELISA or MAGPIX at David Greenfield Human Physiology Lab, School of Life Sciences, QMC and Clinical Sciences Building, School of Medicine, City Hospital; additional analysis will be outsourced to external service providers where commercial kits can't be purchased.

Key references

Garringa 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 Rheumatology 2021;3(9):648-658

Andersson ML. et al. Serum levels of Cartilage Oligomeric Matrix Protein (COMP) increase temporarily after physical exercise in patients with knee osteoarthritis. BMC Musculoskelet Disord 2006;7:98 https://doi.org/10.1186/1471-2474-7-98
Dreiner M. et al. Relationship between different serum cartilage biomarkers in the acute response to running and jumping in healthy male individuals. Sci Rep 2022;12:6434 https://doi.org/10.1038/s41598-022-10310-z

4b Lay Summary of project

This study is designed to see if small blood proteins can be used to detect joint problems after an injury or accident. Unfortunately, these tests can sometimes be influenced by outside reasons which haven't been accounted for by the researchers. This study aims to explore if any of these outside reasons are likely to affect the overall test results greatly. The reason to do this study is to hopefully get new information to help doctors and other people to discover which people might be at risk of joint pain and damage in the future before it is a problem.

5 Location of Study:

Queens Medical Centre, Nottingham; City Hospital, Nottingham

6 Duration of study: 2 year

Proposed starting date: Jan 2023 Proposed finishing date: Dec 2024

7 Description and number of volunteers to be studied:

There will be three groups investigated in this study, those with existing joint disease (osteoarthritis), those with a chronic musculoskeletal injury (>3 months following lower limb injury) and a control population with no injury.

Each group will have ten individuals, including both males and females.

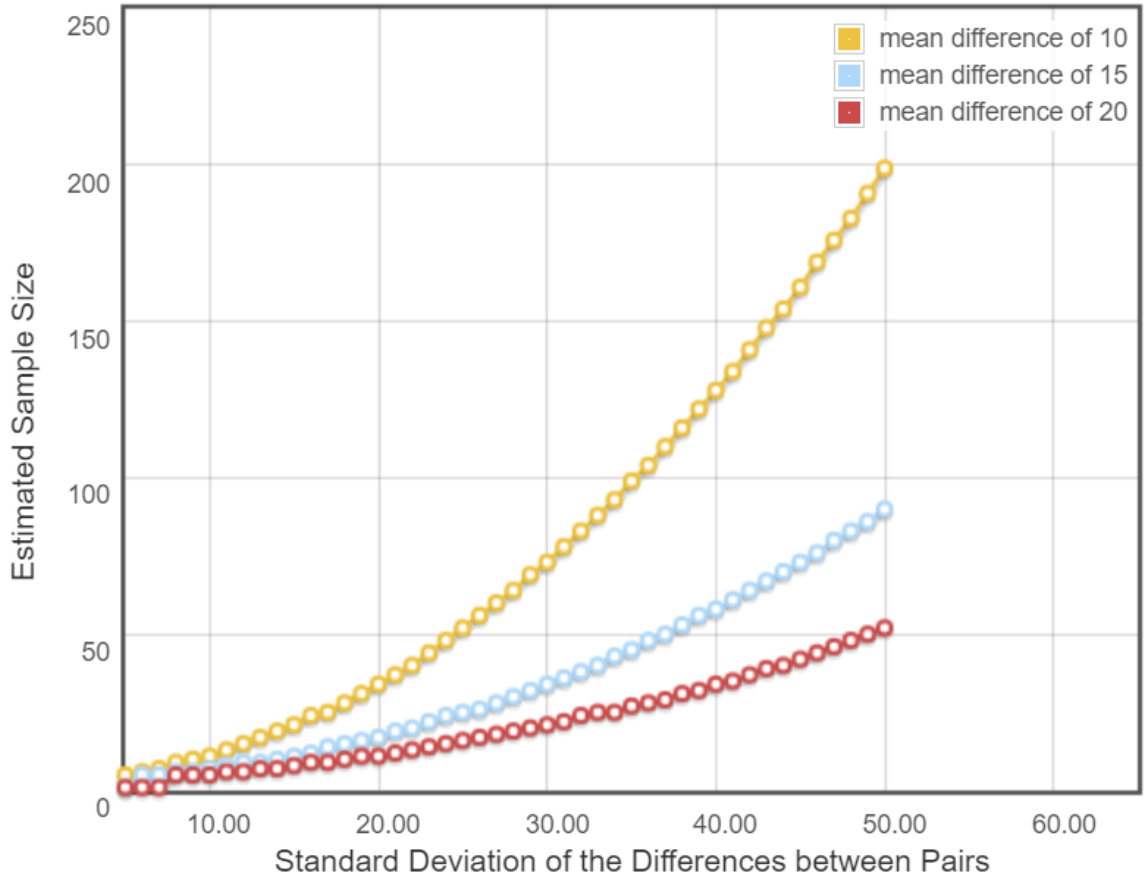
7a How was the sample size decided upon?

Total sample 30 volunteers; 10 in each group (established OA, chronic knee or ankle injury and healthy volunteers)

This pilot project will estimate the standard deviation (SD) between different conditions and the paired delta difference, allowing sample size calculations for future investigations;

Based on previously observed differences in Charcot joint disease comparing IL-6 serum concentration between local venous drainage of the affected lower limb vs. more peripheral venous drainage and published data on SD of ~IL-6 in osteoarthritis, a required sample size of paired serum samples was calculated as an initial estimation.

Previous studies showed a standard deviation of IL-6 to be around 18, and the mean difference between paired samples (local venous drainage of the affected lower limb vs more peripheral) was 25. Based on that, this study would require a sample size of 8 (number of pairs) to achieve a power of 80% and a level of significance of 5% (two sided), for detecting a mean of the differences of 25 between pairs, assuming the standard deviation of the differences to be 18.



Reference:
Liao Y, et al. Interleukin-6 signalling mediates cartilage degradation and pain in posttraumatic osteoarthritis in a sex-specific manner. Sci Signal. 2022;15(744):eabn7082. doi: 10.1126/scisignal.abn7082.
Dhand NK, Khatkar MS. Statulator: An online statistical calculator. Sample Size Calculator for Comparing Two Paired Means. Accessed 22 November 2022 at http://statulator.com/SampleSize/ss2PM.html

7b Studies involving NHS Patients Staff, organisations, Services

Does the study involve any premises, services staff who hold a contract with a hospital, Primary Healthcare or Social Care Trust? No

8 Will written consent be obtained from all volunteers? Yes

9 Will an inconvenience allowance be offered? No

10 Will a medical supervisor be present: Yes Dr Stefen Kluzek or Dr Oliver O'Sullivan

11 If the procedure involves any intervention or treatment (blood sampling, biopsy, i.v injections, manipulation, ECG monitoring) Yes

12 Does the study involve the exposure of the patient to radioactive materials? No

13 Does the study involve the exposure of the patient to X-rays ? No

14 <u>Will participant's General Practitioners be told about the study?</u> No
If no please justify: No intervention, blood test analysis only

15. Has external funding for the research been secured? Yes/No

Will there be any material benefits from the study for the Department or individual investigator? (E.g. equipment, research salaries, consumables etc)
Yes
If yes please specify in general terms what the benefits will be: Department knowledge in laboratory techniques when running in house sampling techniques, including ELISA. Individual investigator training benefits, including research methodology, statistical analysis training and data handling.

16a <u>Drugs or other substances to be administered (including placebo and comparators</u>)

N/A

16b Will any drug used be stored in the Pharmacy and dispensed to a prescription written in red? No

17 <u>Does the project involve painful or invasive procedures on volunteers?</u> No

17a Does the study involve the use of any medical device without a CE Mark, or a CE marked device which has been modified or will be used outside its intended purposes? No

19 <u>Will blood samples or other specimens be required?</u> Yes - 4 x 5ml sample bottles (total 20ml)

After appropriate screening, consent and recruitment, blood samples in an appropriate clinical environment, by sufficient trained individuals, using approved equipment and services as found in University of Nottingham guidance. Certain blood samples, including those taken from veins in the lower limb, will be taken under ultrasound guidance for safety. During the consent process, and repeated immediately prior to venepuncture, the key risks and side effects will be explained, as will the destruction/storage of blood samples (as appropriate). Management of possible side effects, including excessive bleeding or fainting, will be pro-actively planned for when entering appropriate clinical environment. Adverse or unforeseen events will be reported to departmental safety officer and FHMS REC.

20 <u>How will the subjects be chosen?</u>

Participants will be selected based on the initial diagnosis (chronic lower limb MSKI, or previously diagnosed OA) or control population. Patients over 18 will be eligible. Patient diagnosis (MSKI or OA) will confirmed using the patients' health record (with their consent).

21 <u>Describe how and by whom possible participants will first be approached?</u>

Participants with established osteoarthritis or recent chronic knee or ankle injuries recruited directly through other studies (SALI cohort/ running through study/ BRC inflammatory response) who agreed to be contacted for future research will be contacted through agreed means (postal mail and/or e-mail) and invited to participate in future studies. Those with MSKI will be recruited throughout the chronic phase of their injury (3 months or more) so that they may exercise. Moreover, the study will be advertised by study posters of study through public notice boards. Advertisements will also be posted on social media, and through the mailing lists which distribute research information amongst its members. The posters will have an email address to contact to register interest.

22 Data Storage and Data management

Study data will be held securely on paper and, on an electronic database satisfying NHS and UoN security requirements at the University of Nottingham. Access to the database will be restricted to authenticated, named users logged into either the NHS or UoN network. The data collected will strictly only be used in this research and other relevant research.

In line with the principles of GCP / UK Clinical Trial Regulations Guidelines, at the end of the trial data will be securely archived at each participating centre for a minimum of 7 years.

Study data will be entered and maintained on an Excel spreadsheet. The participants will be identified by a unique study specific number and/or code in any database. Their name or any other identifying detail will NOT be included in any study data electronic file. Any documentation with identifiable (personal) data will be stored securely and separate from the research data.

23 Please describe the methods of analysis by which the data will be evaluated to meet the study objectives.

Laboratory based analysis techniques will be employed, including ELISA and MAGPIX, with the results of paired sampling undergoing statistical testing in order to identify SD and paired delta difference.

18 What ethical problems do you foresee in this project?

The only invasive procedure is that of venepuncture, which is a relatively minor intervention. Asking participants who are injured to exercise might give an ethical concern, which we have aimed to mitigate by asking those in the chronic phase of injury (3 months or beyond), rather than the acute phase.

19 What are the possible limitations of the proposed design of this study?

Due to the nature of a pilot study, the numbers are deliberately small. This may prevent a statistically significant result being identified.
Participants with a MSKI may not want to perform physical activity, preventing full engagement with the study. We hope this will be mitigated by using a non-weight bearing exercise (rowing) and recruiting participants in the chronic phase (>3 months) when they should be returning to activity.

DECLARATION: I will inform the Faculty of Medicine and Health Sciences Research Ethics Committee as soon as I hear the outcome of any application for funding for the

proposed project and/or if there are any significant changes to this proposal. I have read the notes to the investigators and clearly understand my obligations as to the rights, welfare and dignity of the subjects to be studied, particularly with regard to the giving of information and the obtaining of consent.

Signature of Lead Investigator: **Date:**

SF 2.2 Ethical approval

University of Nottingham
UK | CHINA | MALAYSIA

Faculty of Medicine & Health Sciences
Research Ethics Committee
Faculty Hub
Room E41, E Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH
Email: FMHS-ResearchEthics@nottingham.ac.uk

28 April 2023

Dr Oliver O'Sullivan
PhD Student
Academic Unit of Injury, Recovery and Inflammation Science (IRIS)
School of Medicine
C Floor, West Block
QMC Campus
Nottingham University Hospitals
Nottingham, NG7 2UH

Dear Dr O'Sullivan

Ethics Reference No: FMHS 170-1122 **– please always quote**	
Study Title: Serum concentration of joint-specific biomarkers from the venous drainage of the lower limb may improve in the interpretation of acute and chronic degenerative knee and ankle joint disease. (SORE)	
Chief Academic/Supervisor: Dr Stefan Kluzek, Clinical Associate Professor in Sport and Exercise Medicine, IRIS, School of Medicine/Consultant in Sport and Exercise Medicine, VA Centre for Sport, Exercise and Osteoarthritis, Nottingham University Hospitals Trust.	
Lead Researcher/student: Dr Oliver O'Sullivan, PhD Student, IRIS, School of Medicine	
Other Key investigators: Dr Richard Pearson, Assistant Professor, IRIS, School of Medicine	
Proposed Start Date: 01/01/2023	**Proposed End Date:** 01/01/2024

Thank you for responding to the comments made by the Committee at the meeting held on 09 December 2022. The following revised documents were received:

- FMHS REC Application form version 1.1 17.03.2023
- SORE pre-screening questionnaire dated 17.03.2023
- SORE consent form v1.1 17.03.2023

These have been reviewed and are satisfactory and the project is given a favourable research ethics opinion.

A favourable research ethics opinion is given on the understanding that:

1. The protocol agreed is followed and the Committee is informed of any changes using a notice of amendment form (please request a form).
2. The Chair is informed of any serious or unexpected event.
3. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

Dr John Williams, Associate Professor in Anaesthesia and Pain Medicine
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

SF 2.2 Consent form

Title of Study:
Serum concentration of joint-specific biomarkers from the venous drainage of the lower limb may improve in the interpretation of acute and chronic degenerative knee and ankle joint disease (SORE)

FHMSREC Reference:

Please Initial or Tick Boxes

- **The nature, aims and risks of the research have been explained to me. I have read and understood the Participant Information Sheet** *(version 1.0)* **and understand what is expected of me. All my questions have been answered fully to my satisfaction.** ☐
- **I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately without having to give a reason. I also understand that I may be withdrawn from the study at any time by the research team.** ☐
- **I understand that the screening process to decide if I am suitable to be selected as a participant may include completing a medical screening questionnaire and/or a physical examination, and I consent to this.** ☐
- **I understand that, in the unlikely event of detecting abnormal clinical findings, I will be informed, and referrals will be made to the appropriate clinical teams.** ☐
- **I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as confidential and handled in accordance with the provisions of the Data Protection Act 2018.** ☐
- **This consent is specific to the particular study described in the Participant Information Sheet and shall not be taken to imply my consent to participate in any subsequent study or deviation from that detailed here.** ☐
- **I understand that my data will be linked, kept securely, and not passed on any further or used for any other reason. I consent to this.** ☐
- **I understand that the research team may like to contact me again to continue to study, or to give me information about other similar studies that may be relevant to me. I consent to this.** ☐
- **I agree to participate in this study** ☐

Participant's Statement:

I ….....

agree that the research project named above has been explained to me to my satisfaction, and I agree to take part in the study.

Signed : Date :

Investigator's Statement :

I .....

confirm that I have carefully explained the nature, demands and any foreseeable risks of proposed research to the Participant.

Signed : Date :

Contact Details of Chief Investigator :

Name: Dr Stefan Kluzek

Address: Academic Unit of Injury, Recovery and Inflammation Sciences, School of Medicin

E-mail: Stefan.kluzek@nottingham.ac.uk

SF 2.4 Participant Information Leaflet

Study Title: Serum concentration of joint-specific biomarkers from the venous drainage of the lower limb may improve in the interpretation of acute and chronic degenerative knee and ankle joint disease (**The SORE study**)

PARTICIPANT INFORMATION SHEET

Research Ethics Reference: FMHS 170-1122
Version 1.2 Date: 28/06/2023

We would like to invite you to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. One of our team will go through the information sheet with you and answer any questions you have. Please take time to read this carefully and discuss it with others if you wish. Ask us anything that is not clear.

What is the purpose of the research?
You are invited to take part in a research project about changes to your joints and how we can better understand them. It is running at the University of Nottingham. The study aims to understand the how proteins in the blood might show joint damage. We would like to know if these small proteins are different if blood samples are taken from different parts of the body or if they change following exercise.

Why have I been invited to take part?
You have been chosen either because you have had an recent injury, a diagnosis of osteoarthritis or someone without any joint problems. We would like to invite approximately 30 people into this study, ten with a recent injury, ten with osteoarthritis, and ten without any known joint problems.

Do I have to take part?
It is up to you to decide if you want to take part in this research. We will describe the study and go through this information sheet with you to answer any questions you may have. If you agree to participate, we will ask you to sign a consent form and will give you a copy to keep. However, you would still be free to withdraw from the study at any time, without giving a reason, simply let the research team know.

What will happen to me if I take part?

A researcher will contact you to go over the information sheet, explain the procedures, and go through a pre-screening with you to check if it is safe for you to participate. If you agree to take part in the study, you will be asked to attend a single visit at the School of Medicine at the University of Nottingham. Please bring with you a pair of shorts to wear for the study visit.

Upon arrival, we will talk you through the study procedures and give you chance to ask any questions. At this time, you will be asked a few questions about yourself and your medical history. If you are still happy to take part, then you will then be asked to sign a consent form.

Once the research team have checked that you can perform the study, a blood sample will be taken from the inside of your elbow. Another blood sample will be taken from your leg, in the middle of your thigh. A small needle will used to take the blood tests and a tourniquet will be applied during this process.

The second part of the study will measure the changes following physical activity, so if you are able, we will ask you to sit on a seated cycling machine for approximately ten minutes until you are slightly out of breath and your heart is beating a bit harder. Once you have reached this point, we will repeat the blood tests from your arm and from your leg.

Finally, we will use a handheld ultrasound scanner to look at your knees and take some measurements. This will not hurt and will involve some cold gel being applied to the skin.

Once those tests have been finished, you are free to leave. The entire visit will last approximately 45-60minutes.

<table>
<tr><th colspan="2">Test</th></tr>
<tr><td colspan="2">Blood Tests
To measure approximately ten small proteins in the blood. This will require approx.. 4 small (one teaspoon each) bottles of blood, and is taken using a needle.</td></tr>
<tr><td>Cycling machine
We will ask you to sit on an indoor cycling bike and cycle for approximately ten minutes until you are slightly out of breath and your heart is beating faster.</td><td>  </td></tr>
<tr><td>  </td><td>Ultrasound scan
A handheld ultrasound scanner will be used to take some measurements from your knees. This will be painless and involve some gel placed on the skin.</td></tr>
</table>

1. What are you measuring?

We are measuring some small proteins in the blood which have been seen to raised in people with joint changes. We would like to know if these proteins are present in higher levels in different parts of the body or following physical activity. These proteins appear when the structure of the joint (cartilage or bone) changes, and if there is any inflammation present.

2. Are there any risks in taking part?

Disadvantages include the uncomfortable sensation when blood is being taken. Uncommonly, this can cause pain, bleeding, and rarely, infection. Finally, when performing exercise tests on an exercise bike, there is a very small risk of a medical problem, and there will be fully trained medical people present for that unlikely situation.

3. Are there any benefits in taking part?

There are no direct benefits for you as this is early research. We hope in the future that we will be able to improve the care for anyone with osteoarthritis, and this study is an important step toward that.

4. Will my time/travel costs be reimbursed?

Unfortunately, participants will not receive an inconvenience allowance to participate in the study Travel expenses will be offered for any visits incurred as a result of participation.

5. What will happen to any samples I give?

We would also like to seek your consent so that any remaining samples may be stored and used in possible future research – this is optional (please indicate you agree to this on the consent form). The samples will be stored with a code unique to you and securely at the University of Nottingham under the University's Human Tissue Research Licence (no 12265).

Some of these future studies may be carried out by researchers other than the current team, including researchers working outside the University. Any samples or data used will be anonymised, and you will not be identified in anyway. If you do not agree to this any remaining samples will be disposed of in accordance with the Human Tissue Authority's codes of practice.

6. What happens to the data provided?

Information will be collected from you at your visit regarding some basic personal information and some details about your medical history. This data will be stored securely on a computer with a password only known to the study team. After the study has finished, all the personal data will be deleted and only anonymous research data will be kept.

All research data and records will be stored for a minimum of 7 years after publication or public release of the work of the research.

We would like your permission to use anonymised data in future studies, and to share our research data (e.g. in online databases) with other researchers in other Universities and organisations both inside and outside the European Union. This would be used for research in health and social care. Sharing research data is important to allow peer scrutiny, re-use (and therefore avoiding duplication of research) and to understand the bigger picture in particular areas of research. All personal information

that could identify you will be removed or changed before information is shared with other researchers or results are made public.

Data sharing in this way is usually anonymised (so that you could not be identified)

7. What will happen if I don't want to carry on with the study?

Even after you have signed the consent form, you are free to withdraw from the study at any time without giving any reason. Any personal data will be destroyed. If you withdraw we will no longer collect any information about you or from you but we will keep the anonymous research data that has already been collected and stored as we are not allowed to tamper with study records. This information may have already been used in some analyses and may still be used in the final study analyses.

8. Who will know that I am taking part in this research?

Data will be used for research purposes only and in accordance with the General Data Protection Regulations. Electronic storage devices will be encrypted while transferring and saving of all sensitive data generated in the course of the research. All such data are kept on password-protected databases sitting on a restricted access computer system and any paper information (such as your consent form, contact details and any research questionnaires) would be stored safely in lockable cabinets in a swipe-card secured building and would only be accessed by the research team. Under UK Data Protection laws the University is the Data Controller (legally responsible for the data security) and the Chief Investigator of this study (named above) is the Data Custodian (manages access to the data). You can find out more about how we use your personal information and to read our privacy notice at: https://www.nottingham.ac.uk/utilities/privacy.aspx/

Designated individuals of the University of Nottingham may be given access to data for monitoring and/or audit of the study to ensure we are complying with guidelines. With your consent, we will keep your personal information on a secure database in order to contact you for future studies.

9. What will happen to the results of the research?

The research will aim to be published in medical journals and will contribute toward the thesis of members of the research team. On successful submission of the thesis, it will be deposited both in print and online in the University archives, to facilitate its use in future research.

10. Who has reviewed this study?

All research involving people is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests.

11. Who is organising and funding the research?

Dr Stefan Kluzek, an Associate Professor at the University of Nottingham, is organising the research and it is funded by the University of Nottingham.

12. What if there is a problem?

If you have a concern about any aspect of this project, please speak to the researcher Dr Oliver O'Sullivan or the Principal Investigator Dr Stefan Kluzek, who will do their best to answer your query. The researcher should acknowledge your concern and give you an indication of how he intends to deal with it.

If you remain unhappy and wish to complain formally, you can do this by contacting the FMHS Research Ethics Committee Administrator, Faculty Hub, Medicine and Health Sciences, E41, E Floor, Medical School, Queen's Medical Centre Campus, Nottingham University Hospitals, Nottingham, NG7 2UH or via E-mail: FMHS-ResearchEthics@nottingham.ac.uk.
Please quote ref no: FMHS FMHS 170-1122

13. Contact Details

If you would like to discuss the research with someone beforehand (or if you have questions afterwards), please contact:

Dr Oliver O'Sullivan
Academic Unit of Injury, Recovery and Inflammation Sciences
Email: oliver.o'sullivan@nottingham.ac.uk

Supplementary File 3 – Search strategy for Chapter Seven

This search was conducted on 10 November 2023

The following databases were searched:-

Medline on EBSCO platform
CINAHL Complete on EBSCO platform
EMBASE on Ovid platform

using the search criteria given after the results.

Search strategies

Number of articles retrieved

Database	Total retrieved
Medline	1460
CINAHL	497
EMBASE	547

Total retrieved from all databases	2504

Limiters
English language
Italian language
Publication year 2000 - current

Medline search strategy

Search ID#	Search Terms	Search Options	Results
S29	S14 AND S22 AND S25	Limiters - Date of Publication: 20000101-20231231; Language: Italian	0
S28	S14 AND S22 AND S25	Limiters - Date of Publication: 20000101-20231231; English Language	1,460
S27	S14 AND S22 AND S25		1,582
S26	S13 AND S21 AND S25		13,843
S25	S23 OR S24		8,244,939

S24	AB “Neuromuscular Performance” OR function* OR land* OR jump* OR hop* OR squat* OR run OR running OR Cutting OR assessment OR Assessments OR test OR testing OR “test batteries” OR “Peak Force” OR “Rapid Force” OR “Rapid Force Production” OR “Force production”		7,624,465
S23	TI “Neuromuscular Performance” OR function* OR land* OR jump* OR hop* OR squat* OR run OR running OR Cutting OR assessment OR Assessments OR test OR testing OR “test batteries” OR “Peak Force” OR “Rapid Force” OR “Rapid Force Production” OR “Force production”		1,683,685
S22	S18 OR S19		336,948
S21	S18 OR S19 OR S20		1,297,148
S20	AB Biomechanics OR biomarkers OR “biological markers” OR kinetic OR kinetics OR kinematic OR kinematics OR force OR “joint angle*” OR “Joint moment*” OR “joint power” OR “peak angle*”		1,182,467
S19	TI Biomechanics OR biomarkers OR “biological markers” OR kinetic OR kinetics OR kinematic OR kinematics OR force OR “joint angle*” OR “Joint moment*” OR “joint power” OR “peak angle*”		302,779
S18	S15 OR S16 OR S17		55,243
S17	(MM "Kinetics")		1,407
S16	(MM "Biomarkers")		46,497
S15	(MM "Biomechanical Phenomena")		7,348
S14	S4 OR S12		81,208
S13	S4 OR S11		215,877
S12	S5 AND S8		60,424

S11	S7 AND S10		207,386
S10	S8 OR S9		414,101
S9	AB “Lower limb” OR “lower limbs” OR “lower extremity” OR “Lower extremities” OR leg OR knee OR “anterior cruciate ligament” OR ACL		367,503
S8	TI “Lower limb” OR “lower limbs” OR “lower extremity” OR “Lower extremities” OR leg OR knee OR “anterior cruciate ligament” OR ACL		170,413
S7	S5 OR S6		3,266,841
S6	AB Injur* OR ligament OR tendon OR bone OR cartilage OR rupture OR reconstruct* OR “post-traumatic osteoarthritis” OR “posttraumatic osteoarthritis” OR Osteoarthritis OR menisc* OR rehab* OR fracture* OR pain		2,881,536
S5	TI Injur* OR ligament OR tendon OR bone OR cartilage OR rupture OR reconstruct* OR “post-traumatic osteoarthritis” OR “posttraumatic osteoarthritis” OR Osteoarthritis OR menisc* OR rehab* OR fracture* OR pain		1,398,508
S4	S1 OR S2 OR S3		46,180
S3	(MM "Anterior Cruciate Ligament Injuries")		9,821
S2	(MM "Osteoarthritis, Knee")		24,339
S1	(MM "Knee Injuries")		15,438

CINAHL search strategy

Search ID#	Search Terms	Search Options	Results
S39	S14 AND S24 AND S32	Limiters - Published Date: 20000101-20231231; Language: Italian	0

S38	S14 AND S24 AND S32	Limiters - Published Date: 20000101-20231231; English Language	497
S37	S13 AND S24 AND S32	Limiters - Published Date: 20000101-20231231; English Language	1,488
S36	S13 AND S23 AND S31	Limiters - Published Date: 20000101-20231231; English Language	6,759
S35	S14 AND S24 AND S32		507
S34	S13 AND S24 AND S32		1,533
S33	S13 AND S23 AND S31		7,107
S32	S28 OR S29		392,483
S31	S28 OR S29 OR S30		1,481,913
S30	AB “Neuromuscular Performance” OR function* OR land* OR jump* OR hop* OR squat* OR run OR running OR Cutting OR assessment OR Assessments OR test OR testing OR “test batteries” OR “Peak Force” OR “Rapid Force” OR “Rapid Force Production” OR “Force production”		1,291,669
S29	TI “Neuromuscular Performance” OR function* OR land* OR jump* OR hop* OR squat* OR run OR running OR Cutting OR assessment OR Assessments OR test OR testing OR “test batteries” OR “Peak Force” OR “Rapid Force” OR “Rapid Force Production” OR “Force production”		391,956
S28	S25 OR S26 OR S27		3,138
S27	MM "Squatting"		664
S26	MM "Hopping"		186
S25	MM "Jumping"		2,398

S24	S20 OR S21		80,686
S23	S20 OR S21 OR S22		188,094
S22	AB Biomechanics OR biomarkers OR “biological markers” OR kinetic OR kinetics OR kinematic OR kinematics OR force OR “joint angle*” OR “Joint moment*” OR “joint power” OR “peak angle*”		144,414
S21	TI Biomechanics OR biomarkers OR “biological markers” OR kinetic OR kinetics OR kinematic OR kinematics OR force OR “joint angle*” OR “Joint moment*” OR “joint power” OR “peak angle*”		56,031
S20	S16 OR S17 OR S18 OR S19		37,958
S19	MM "Kinematics"		4,801
S18	MM "Kinetics"		1,382
S17	MM "Biological Markers"		22,342
S16	MM "Biomechanics"		10,259
S15	S5 OR S14		42,772
S14	S6 AND S9		33,613
S13	S5 OR S12		89,402
S12	S8 AND S11		85,258
S11	S9 OR S10		148,836
S10	AB “Lower limb” OR “lower limbs” OR “lower extremity” OR “Lower extremities” OR leg OR knee OR “anterior cruciate ligament” OR ACL		120,041
S9	TI “Lower limb” OR “lower limbs” OR “lower extremity” OR “Lower extremities” OR leg OR knee OR “anterior cruciate ligament” OR ACL		76,969
S8	S6 OR S7		865,131
S7	AB Injur* OR ligament OR tendon OR bone OR cartilage OR rupture OR reconstruct* OR “post-traumatic osteoarthritis” OR “posttraumatic osteoarthritis” OR		679,832

	Osteoarthritis OR menisc* OR rehab* OR fracture* OR pain		
S6	TI Injur* OR ligament OR tendon OR bone OR cartilage OR rupture OR reconstruct* OR “post-traumatic osteoarthritis” OR “posttraumatic osteoarthritis” OR Osteoarthritis OR menisc* OR rehab* OR fracture* OR pain		464,754
S5	S1 OR S2 OR S3 OR S4		22,669
S4	(MM "Meniscal Injuries")		1,067
S3	(MM "Anterior Cruciate Ligament Injuries")		4,688
S2	(MM "Osteoarthritis, Knee")		12,800
S1	(MM "Knee Injuries") OR (MM "Knee Injuries, Articular Cartilage")		5,210

EMBASE search strategy

Database: Embase <1974 to 2023 November 09>
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1 *knee injury/ (7983)
2 *anterior cruciate ligament injury/ (4442)
3 *knee osteoarthritis/ (29771)
4 *knee meniscus rupture/ or *knee meniscus/ (8894)
5 1 or 2 or 3 or 4 (49048)
6 (Injur* or ligament or tendon or bone or cartilage or rupture or reconstruct* or post-traumatic osteoarthritis or posttraumatic osteoarthritis or Osteoarthritis or menisc* or rehab* or fracture* or pain).ti. (1630233)
7 (Injur* or ligament or tendon or bone or cartilage or rupture or reconstruct* or post-traumatic osteoarthritis or posttraumatic osteoarthritis or Osteoarthritis or menisc* or rehab* or fracture* or pain).ab. (3949748)
8 6 or 7 (4281525)
9 (Lower limb or lower limbs or lower extremity or Lower extremities or leg or knee or anterior cruciate ligament or ACL).ti. (188030)
10 (Lower limb or lower limbs or lower extremity or Lower extremities or leg or knee or anterior cruciate ligament or ACL).ab. (471679)
11 9 or 10 (510956)
12 8 and 11 (273138)
13 6 and 9 (73790)
14 5 or 12 (281172)
15 5 or 13 (93221)
16 *biomechanics/ (32840)
17 *biological marker/ (123213)

18 *kinetics/ (15410)
19 *kinematics/ (8859)
20 *joint angle/ (130)
21 16 or 17 or 18 or 19 or 20 (179248)
22 (Biomechanics or biomarkers or biological markers or kinetic or kinetics or kinematic or kinematics or force or joint angle* or Joint moment* or joint power or peak angle*).ti. (280922)
23 (Biomechanics or biomarkers or biological markers or kinetic or kinetics or kinematic or kinematics or force or joint angle* or Joint moment* or joint power or peak angle*).ab. (1188870)
24 22 or 23 (1269596)
25 21 or 24 (1331894)
26 21 or 22 (378348)
27 *jumping/ (2127)
28 (Neuromuscular Performance or function* or land* or jump* or hop* or squat* or run or running or Cutting or assessment or Assessments or test or testing or test batteries or Peak Force or Rapid Force or Rapid Force Production or Force production).ti. (1924808)
29 (Neuromuscular Performance or function* or land* or jump* or hop* or squat* or run or running or Cutting or assessment or Assessments or test or testing or test batteries or Peak Force or Rapid Force or Rapid Force Production or Force production).ab. (9613586)
30 27 or 28 or 29 (10221209)
31 14 and 21 and 30 (3871)
32 15 and 21 and 30 (1433)
33 15 and 26 and 28 (600)
34 limit 33 to (english language and yr="2000 -Current") (547)
35 limit 33 to (italian and yr="2000 -Current") (0)